Absence of genotoxic impact assessed by micronucleus frequency in circulating lymphocytes of workers exposed to cadmium

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Abstract

Purpose - Cd is considered as a genotoxic carcinogen for which a threshold can be identified. This threshold has, however, not been established and the shape of the relationship between Cd exposure and genotoxic effects is unknown. The aim of the present study was to analyse the shape of the dose-response relationship for the genotoxic effects of Cd in occupational settings.

Methods - The study has a cross-sectional design and includes 60 healthy male and female workers with known Cd exposure selected from two plants manufacturing or recycling nickel-Cd batteries. The frequency of MN was measured in circulating lymphocytes, and related to internal Cd doses (Cd-B, Cd-U). Determinants of MN frequency were traced by multivariate regression analysis.

Results - Cd exposure covered a wide range as measured by Cd-B (0.02-1.26 µg/dL), Cd-U (0.26-15.80 µg/g creat) and seniority in the plant (1-42 years). Gender was the only parameter significantly associated with MN frequency, women having on average 8.5 additional MN/1000 BN cells compared to men. Cd-B, Cd-U or Ni-U did not influence MN frequency when adjusted for gender and other potential confounders.

Conclusion - This finding is consistent with the existing knowledge on the mechanisms governing the genotoxic activity of Cd, which are all non-stochastic and thresholded. The

threshold for systemic genotoxic effects of Cd is thus beyond the range of internal exposure considered in the present investigation.

Introduction

Occupational exposure to cadmium (Cd) occurs in production and refining facilities (smelters), Ni-Cd battery manufactures and recycling plants, pigment manufacture and formulation plants, Cd alloy production, mechanical plating, zinc smelting, brazing with Cd-containing silver alloy solders, and in polyvinyl chloride producing factories. Cd and its compounds are classified as carcinogenic to humans (group 1) based on experimental and epidemiological studies identifying increased incidence of lung cancer after prolonged inhalation of cadmium dusts. Increased risks of prostate and kidney cancer have also been reported (IARC 2012).

The genotoxic activity of Cd has been investigated experimentally and different mechanisms relevant for the carcinogenicity of Cd have been identified (reviewed in (Filipic 2012; Hartwig 2010; Hartwig 2017; Henkler et al. 2010; Joseph 2009; Koedrith et al. 2013; Koedrith and Seo 2011; Luevano and Damodaran 2014) . While point mutations and chromosome alterations (chromosomal aberrations, sister chromatid exchanges) are recorded after exposure, Cd does not induce direct DNA damage. Cd can increase reactive oxygen species generation, which in turn induces oxidative DNA damage and can interfere with cell signalling. Cd also interferes with major DNA repair pathways, induces dysregulation of cell proliferation and epigenetic modulations of gene expression (Beyersmann and Hartwig 2008; Filipic 2012). All these mechanisms are non-stochastic in nature, implying that a threshold exists below which no genotoxic effect is expected. Cd should, therefore, be considered as a "genotoxic carcinogen for which a threshold can be identified" (SCOEL category C carcinogen; (Bolt and Huici-Montagud 2008). The threshold for genotoxic effects of Cd has, however, not been established, and the shape of the relationship between Cd exposure and genotoxic effects is unknown. Early epidemiological data suggest that chromosomal aberrations (CA), but not micronuclei (MN) appear in blood lymphocytes of workers with high Cd exposure, i.e. urinary Cd (Cd-U) >10 µg/L ((Forni 1994; Forni et al. 1990).

The modern version of the micronucleus (MN) assay, the cytokinesis-block micronucleus (CBMN) assay in human circulating lymphocytes has become one of the most widely used methods for measuring structural and numerical chromosomal changes in human cells *in vitro* and *in vivo* (Fenech et al. 2011; Kirsch-Volders et al. 2011). MN are small extranuclear bodies

mainly resulting from acentric chromosomal fragments lagging behind during anaphase. Most mechanisms underlying Cd genotoxicity can be captured by the CBMN assay. Several experimental studies have shown that Cd increases the frequency of MN as measured by the CBMN assay *in vitro* (e.g. (Fowler et al. 2010; Rozgaj et al. 2002).

The aim of the present study was to analyse the shape of the dose-response relationship for the genotoxic effects of Cd in occupational settings. Blood and urine were collected in a selected sample of workers with known exposure to Cd. Upon occupational exposure, cadmium in blood (Cd-B) reflects both the body burden and recent exposure, whereas Cd-U is mainly reflecting the Cd body burden (Lauwerys and Hoet 2001). The frequency of MN in circulating lymphocytes was measured, and related to internal Cd doses (Cd-B, Cd-U) to test the hypothesis of a threshold for the genotoxic effects of Cd.

Materials and methods

Study sample and experimental design

The study has a cross-sectional design and includes 60 healthy male and female workers with known Cd exposure selected from two plants manufacturing or recycling nickel-Cd batteries. Subjects were recruited on a voluntary basis by the local occupational health service between September and October 2017 during the regular annual medical check-up. They were a priori selected based on their urinary cadmium (Cd-U) results measured at the previous medical check-up, i.e. in 2016 or 2015. As the study aims at characterizing the dose-response relationship, individuals were selected to cover a broad and balanced range of exposure levels (Cd-U from <1 up to >10 µg/g creatinine). The study sample is, therefore, not representative of the plant populations. The study included individuals with low exposure (Cd-U≤ 2 µg/g creatinine), not different from the general population not occupationally exposed to Cd ((Hoet et al. 2016; Nisse et al. 2017). The objective was to recruit a total of 60 workers with varying Cd exposure levels, based on a statistical power analysis. In this analysis, MN frequencies as well as Cd internal doses from seven studies (Forni 1994; Hamurcu et al. 2001; Iarmarcovai et al. 2008; Kasuba et al. 2010; Kasuba et al. 2012; Palus et al. 2003; Wegner et al. 2004) were used to calculate the sample size by simulation. Assuming that Cd-U lies on a continuous scale, a linear regression model was created on a logarithm scale. In a simulation analysis, 1000 data sets were generated for each sample size (from n=3 to n=60). The power was calculated for each sample size as the proportion of statistically significant results (p-values <0.05). A sample size n ≥ 60 was needed to reach a power of ≥ 95%.

The study was approved by the Ethics Committee of the Faculty of Medicine at the Université catholique de Louvain (B403201630100), and the protocol complies with requirements of national and international regulations. Each worker gave written consent for voluntary participation in the study and was interviewed on age, smoking habits and years in the plant since hiring (seniority). Blood and urine samples were collected to measure Cd internal doses (Cd-U and Cd in whole blood (Cd-B)). Genotoxic effects were assessed in peripheral lymphocytes with the CBMN assay. Peripheral blood samples were collected by venipuncture under sterile conditions in certified trace element-free tubes (BD Vacutainer) : (i) in heparin tubes (one per worker) for the CBMN assay and (ii) in EDTA tubes (one per worker) for blood Cd (Cd-B) and for analysing glutathione S-transferase (GST) M1 and T1 polymorphisms which

could influence MN levels. Urine was collected in metal-free containers (Novolab) for the determination of Cd-U and Ni-U.

Blood and urine samples were collected at the beginning of the week (max. 10 individuals per week), coded anonymously and air-shipped at ambient temperature to reach the laboratory at the Ghent university. Blood cultures for MN analysis were set up within a maximum of 24h. A temperature logger and a radiation dosimeter were included in each shipment of heparin tubes to verify that transport conditions could not have induced MN. EDTA tubes and urinary samples were kept at 4°C in Ghent before transfer to the Louvain center for Toxicology and Applied Pharmacology (LTAP, UCL) for the other analyses.

Cytokinesis-block micronucleus assay (CBMN)

The CBMN assay was performed as previously described (Vral et al. 2011). Each blood sample was cultured in duplicate. Per culture, heparinised blood (0.5 ml) was added to 4.5 ml of pre-warmed RPMI-1640 supplemented with L-glutamine (Gibco), 10% of foetal calf serum (Gibco) and antibiotics (50U/ml penicillin and 50 mg/ml streptomycin; Gibco). Lymphocyte division was stimulated by the addition of 100 µl of phytohaemagglutanin (PHA – stock solution 1 mg/ml (Gibco)) and cytokinesis was blocked after 23 h by adding 6 µg cytochalasin B/ml (Sigma-Aldrich). Seventy hours post PHA-stimulation, cells were harvested, submitted to a cold hypotonic shock with 7 ml of KCl (0.075 M, Merck) and fixed in a methanol: acetic acid: ringer solution (4:1:5) (Merck). After overnight storage at 4°C, cells were further fixed 3 times in methanol: acetic acid solution (4:1). A suspension of cells (40 µl) was dropped onto clean slides and stained with acridin orange (10 µg/ml) (Sigma-Aldrich). Micronuclei were scored in cells that underwent a single division, identified as binucleated (BN) cells. Duplicate slides (one per culture) were made for each sample, coded and 1000 BN cells per slide were scored under a fluorescence microscope. In total, 2000 BN cells per blood sample were scored by 2 readers. This protocol was successfully applied by the Ghent laboratory in several intercomparison programmes on the CBMN assay (Depuydt et al. 2017; Romm et al. 2013).

Every week, a control was introduced to verify the proficiency of the CBMN procedure. For this, a second heparin tube collected from a worker with low Cd exposure in 2015-16, was exposed to ionizing radiation (between 2.5 and 4 Gy) and processed for the CBMN assay in parallel with other blood tubes of the week. The frequency of MN measured in these positive controls was 400-900 MN/1000 BN cells.

Analytical methods

The biological samples were analyzed for metal content in the laboratory of the Louvain centre for Toxicology and Applied Pharmacology (Université catholique de Louvain, Belgium). In all urine samples, 24 trace elements (including Cd and Ni) were quantified by means of inductively coupled argon plasma mass spectrometry (ICP-MS) on an Agilent 7500ce instrument. Briefly, urine specimens (500 µl) were diluted quantitatively (1+9) with a HNO_3 1%, HCl 0.5% solution containing Sc, Ge, Rh and Ir as internal standards. Cd was analyzed using no-gas mode, and helium mode was selected to quantify Ni. Six trace elements (including Cd) were quantified in all blood samples by means of ICP-MS on an Agilent 7500cx instrument. Briefly, whole blood specimens (500 µl) were diluted quantitatively (1+9) with a 1-butanol (2%w/v), EDTA (0.05%w/v), Triton X-100 (0.05%w/v), NH_4OH (1%w/v) solution containing Sc, Ge, Rh and Ir as internal standards. Cd was analyzed using no-gas mode. With both validated ICP-MS methods, the laboratory has obtained successful results in external quality assessment schemes organized by the Institute for Occupational, Environmental and Social Medicine of the University of Erlangen, Germany (G-EQUAS program), and by the Institut National de Santé Publique, Québec (PCI and QMEQAS programs). The laboratory is also ISO15189 certified for Cd-U, Ni-U and Cd-B measurements.

Creatinine was measured by a modified Jaffé reaction using a C502 module on a Cobas 8000 analyser (Roche diagnostics).

GST (*M1* and *T1*) polymorphisms were assessed based on a Multiplex Ligation-dependent Probe Amplification (MLPA) technique according to the manufacturer's protocol using SALSA MLPA P128-C1 Cytochrome P450 probemix (Lot C1-1014, MRC-Holland). Two exons for each gene (exon 3, 5 and 1, 5 for *GSTM1* and *GSTT1* respectively) were simultaneously checked to confirm gene deletion.

Statistical analyses

Statistical analyses were conducted with SPSS statistics version 25. Descriptive statistics include arithmetic mean, standard deviation, median and range. The normality of the distribution was evaluated for each continuous variable with the Kolmogorov-Smirnov test. Differences between two groups were assessed with Chi-square or Mann-Whitney tests. Bivariate analyses between continuous variables were assessed with the Spearman

correlation test. Multivariate regression analyses were carried out (enter procedure, p_{in} : 0.05, p_{out} : 0.10) to identify the contribution of independent variables (log-transformed continuous variables except for age) on the frequency of MN (dependent variable). A p-value ≤ 0.05 was set as level of statistical significance.

Reporting

This manuscript follows the STROBE recommendations for reporting cross-sectional studies (von Elm et al. 2014).

Results

Characteristics of the study population.

A total of 60 male and female workers were recruited (Table 1). These workers were on average 50 years old, women being significantly older than men. Their employment in the Cd plant ranged from 1 to 42 years, with no difference in seniority between genders. Current smokers (last 3 months) were statistically more frequent in men than women.

	Total	Men	Women	p-value*
Number	60	38	22	
Age (years)				
mean ± SD	48.20 ± 9.32	44.95 ± 8.56	53.82 ± 7.93	<0.001
median	50	45	56	
range	28 - 62	28 - 61	36 - 62	
Seniority (years)				
mean ± SD	16.03 ± 11.62	15.18 ± 11.24	17.50 ± 12.39	ns
median	12.5	10.5	22.5	
range	1 - 42	1 – 42	2 - 41	
Smoking habits				
current smokers	17 (28%)	14 (37%)	3 (14%)	<0.05
non-smokers	43	24	19	

Table 1 : Characteristics of the study population

*: Mann-Whitney or Chi-Square test.

Biological parameters of exposure

The biological parameters measured in blood and urine reflected, as expected, a wide range of exposure to Cd (Table 2). Women had significantly higher Cd-B values than men; no gender difference was noted for Cd-U expressed in µg Cd/L or after adjustment for urine dilution (µg Cd/g creatinine). As those workers were potentially exposed to nickel compounds, this element was also measured in urine (Ni-U). Ni-U also showed a wide range of distribution and no gender difference was observed.

	Total	Men	Women	p-value*
Cd-B (µg/dL)				
mean ± SD	0.44 ± 0.33	0.35 ± 0.28	0.61 ± 0.34	0.001
median	0.37	0.20	0.55	
range	0.02-1.26	0.02 – 1.15	0.18 -1.20	
Cd-U (µg/L)				
mean ± SD	4.99 ± 5.19	4.72 ± 5.03	5.45 ± 5.55	ns
median	3.06	2.70	4.22	
range	0.22 – 21.38	0.24 – 21.38	0.22 – 19.87	
Cd-U (µg/g creat)				
mean ± SD	4.53 ± 3.93	3.75 ± 3.49	5.87 ± 4.33	ns
median	3.67	3.36	5.22	
range	0.26 – 15.80	0.26 – 15.80	0.26 – 13.31	
Ni-U (µg/g creat)				
mean ± SD	4.64 ± 8.85	3.81 ± 9.00	6.09 ± 8.58	ns
median	2.04	1.86	2.85	
range	0.86 – 55.53	0.86 – 55.53	1.46 – 35.22	

Table 2 : Biomarkers of Cd exposure
Cd-B, cadmium in whole blood; Cd-U, cadmium in urine; Ni-U, nickel in urine
* : Mann-Whitney test.

Cd-B and Cd-U (µg/L or µg/g creat) were not significantly different between smokers and non-smokers (Additional table).

	Non-smokers	Smokers	p-value*
Cd-B (µg/dL)			
mean ± SD	0.43 ± 0.31	0.48 ± 0.38	
median	0.37	0.38	
range	0.02-1.15	0.10-1.26	0.799
Cd-U (µg/L)			
mean ± SD	4.76 ± 5.34	5.56 ± 4.91	
median	2.55	3.13	
range	0.22-21.38	0.46-16.19	0.305
Cd-U (µg/g creat)			
mean ± SD	4.50 ± 4.01	4.61 ± 3.83	
median	3.55	3.82	
range	0.26-15.80	0.63-12.75	0.812

Additional table : Exposure biomarkers according to smoking status.
Smokers were defined as current (last 3 months) smokers.
* : Mann-Whitney U test

Micronuclei in circulating lymphocytes

The frequency of MN in circulating lymphocytes among the 60 workers ranged between 10 and 38 MN/1000 BN (median : 22.5). When grouping workers by increasing Cd-U values (Figure 1), the MN frequency did no vary significantly with Cd-U (trend test, p=0.459).

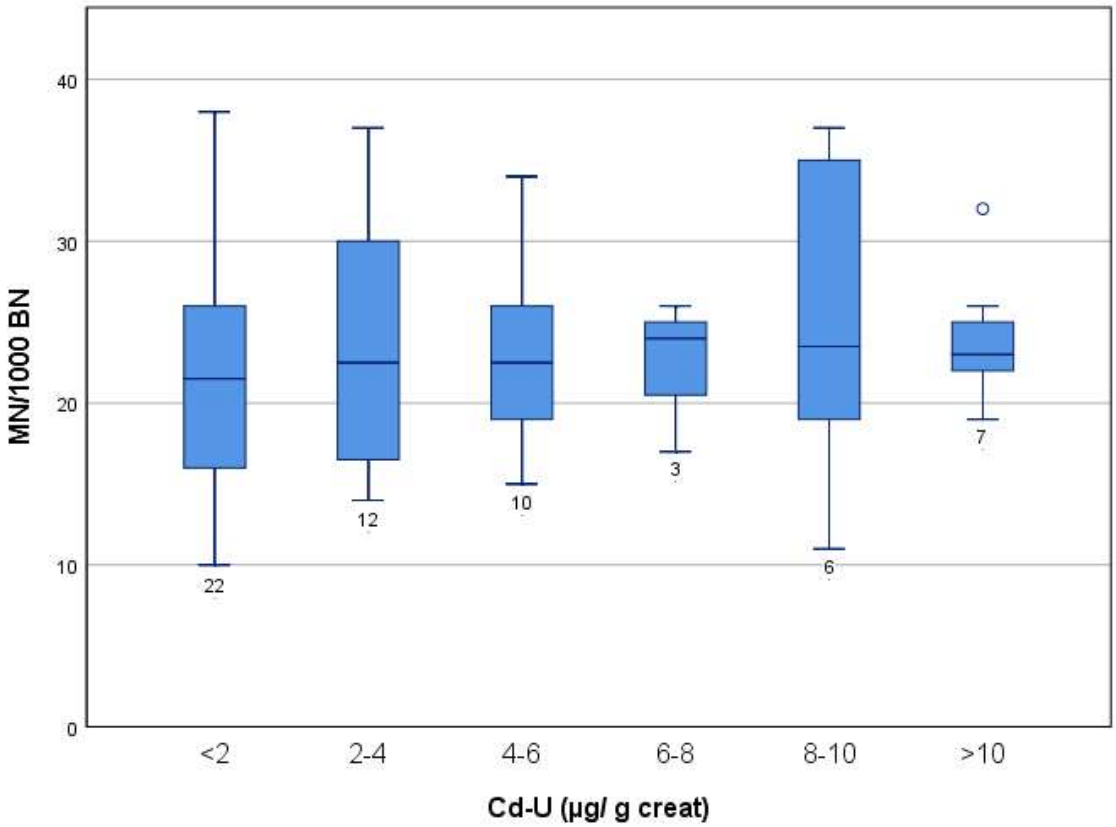

Figure 1 : Frequency of micronuclei in circulating lymphocytes as a function of cadmiuria. Whiskers represent median, first and third quartiles, minimum and maximum values.

Bivariate analyses

Categorical variables - MN frequency was significantly higher in women than in men; no significant difference was noted between current smokers and non-smokers (Table 3).

MN (/1000 BN)	Total	Men	Women	p-value*
mean ± SD	23.32 ± 7.21	20.58 ± 5.985	28.05 ± 6.779	0.001
median	22.50	20.5	27	
range	10 - 38	10 – 37	14 – 38	
		Non-smokers (n=43)	Smokers (n=17)	
mean ± SD		23 ± 6.817	24.118 ± 8.291	ns
median		22	24	
range		11 - 37	10 – 38	

Table 3 : Micronucleus frequency in circulating lymphocytes according to gender or smoking status.
MN number per 1000 binucleated cells (BN)
Smokers were defined as current (last 3 months) smokers.
* : Mann-Whitney U test

Continuous variables – Bivariate correlation analyses indicated a significant association between MN frequency and Cd-B. As expected Cd-U (µg/L or µg/g creat) was significantly associated with Cd-B and age. Cd-B was significantly associated with age (Table 4).

		MN /1000BN	Cd-U (µg/L)	Cd-U (µg/g creat)	Cd-B (µg/dL)	Ni-U (µg/g creat)	Age	Seniority
MN /1000BN	R	1.000	0.097	0.134	**0.300**	0.094	0.240	0.104
	p-value	.	*0.460*	*0.309*	*0.020*	*0.475*	*0.065*	*0.428*
Cd-U (µg/L)	R		1,000	**0.817**	**0.705**	-0.115	**0.256**	0.036
	p-value		.	*0.000*	*0.000*	*0.382*	*0.048*	*0.785*
Cd-U (µg/g creat)	R			1,000	**0.818**	0.082	**0.556**	0.031
	p-value			.	*0.000*	*0.533*	*0.000*	*0.813*
Cd-B (µg/dL)	R				1,000	0.079	**0.503**	0.027
	p-value				.	*0.548*	*0.000*	*0.839*
Ni-U (µg/g creat)	R					1,000	0.213	0.034
	p-value					.	*0.102*	*0.798*
age	R						1,000	0.050
	p-value						.	*0.705*

Table 4 : Bivariate correlations between continuous variables.
R : Spearman correlation coefficient; statistically significant coefficients are identified in bold.

Multivariate regression analyses

Multivariate regression analyses were conducted to disentangle the influence of Cd-B, gender and possibly age on the frequency of MN, (Table 5). Variables identified in bivariate analyses or known to potentially influence MN frequency (GST deficiency which can result in increased susceptibility to genotoxic damage (Kirsch-Volders et al. 2006), smoking (Bonassi et al. 2003)) were introduced in a multiple regression model. The possible influence of Ni exposure was also tested. Gender was the only parameter significantly associated with MN frequency, women having on average 8.5 additional MN/1000 BN cells compared to men. Cd or Ni exposure did not influence MN frequency when adjusted for gender and other potential confounders.

	B	p-value.	VIF
constant	**23.046**	0.004	
log Cd-B	1.684	0.554	1.664
gender	**8.575**	<0.001	1.542
GSTT1 status	0.246	0.926	1.059
GSTM1 status	1.133	0.511	1.078
age	-0.065	0.585	1.789
smoking	3.443	0.088	1.178
log Ni-U	-1.469	0.492	1.134

Table 5 : Multiple regression analysis tracing the determinants of the MN frequency in circulating lymphocytes (MN/1000 BN). Significant determinants are identified in bold (ANOVA, F : 3.394, p=0.005; adjusted r^2 of the model : 0.221). Gender was coded 1 for women and 0 for men; GST status was coded 1 for the presence of the allele, 0 for homozygote absence of the allele, smoking was coded 1 for current smokers (3 months) and 0 for non-smokers. VIF, variance inflation factor.

The influence of Cd-B and possibly age on MN frequency recorded in bivariate analyses was, therefore, reflecting a gender effect as women were older, and had higher Cd-B values than men. In order to visualize the gender effect on MN, we plotted the MN frequency as a function of Cd-B for men and women separately (Figure 2). When considering Cd-B values >0.15 µg/dL (minimum value in women, 0.18 µg/dL, Table 2), women had statistically higher MN frequencies than men (median 27 vs 21, respectively; Mann-Whitney U-test p=0.001) at similar Cd-B levels.

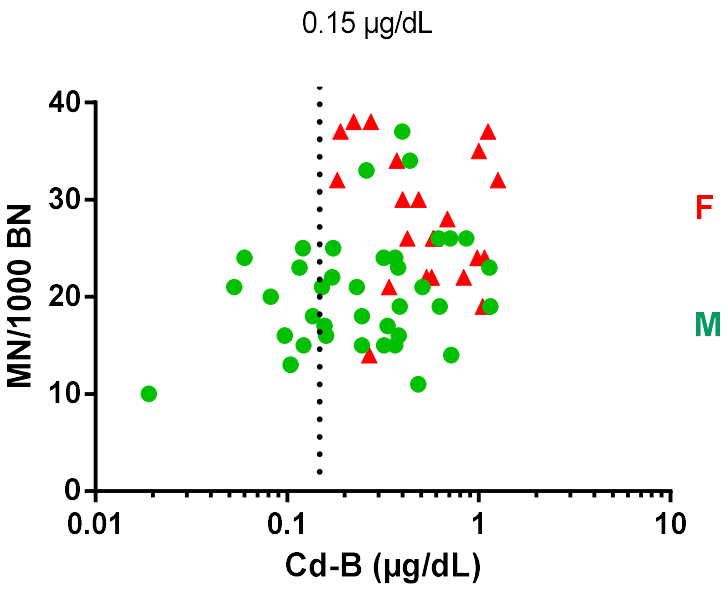

Figure 2 : Relationship between cadmium in blood (Cd-B) and frequency of micronuclei in circulating lymphocytes (MN/1000 BN) in men (M) and women (F).

Discussion

The possible genotoxic impact of occupational exposure to Cd was assessed in a sample of workers *a priori* selected to cover a wide range of Cd exposure as measured by Cd-B (0.02-1.26 µg/dL), Cd-U (0.26-15.80 µg/g creat) and seniority (1-42 years). Cd exposure did not influence the MN frequency in circulating lymphocytes. Ni exposure did not affect MN frequency either.

Women usually have higher biomarkers of Cd exposure than men because of an increased gastro-intestinal uptake linked to a lower iron status in women (Berglund et al. 1994). It is not immediately clear why, in the present study sample, this was reflected in Cd-B but not Cd-U values (expressed in µg/L or µg/g creat). Similar contrasts have been reported in other studies (CDC 2018; Garner and Levallois 2017). No statistically significant effect of smoking on Cd-B or Cd-U was recorded in this sample probably because the impact of occupational exposure was prominent. Cd-U values are generally expressed in µg/g creat; here, we also presented values expressed in µg/L because the normalization to creatinine is gender sensitive (women excrete less creatinine than men). The value of creatinine normalization of urinary Cd values has also been questioned, especially in the general population (Hoet et al. 2016).

Earlier epidemiological studies did not allow drawing a clear conclusion on the genotoxic activity of Cd, as reviewed previously (Nersesyan et al. 2016; Verougstraete et al. 2002). Three studies reported inconsistent genotoxic damage in workers exposed to Cd. Forni (1994; 1990) reported increased chromosomal aberrations (CA) but not micronuclei (MN) in circulating lymphocytes of workers exposed to Cd in an alloy production plant (n=40; Cd-U, 1.5-31.6 µg/L) as compared to controls (n=40; Cd-U not available). The CA levels did, however, not correlate with Cd-U. Abrahim et al. (2011) reported increased sister chromatid exchanges (SCE) and CA in circulating lymphocytes collected from workers in a Cd electroplating plant in Egypt (n=40; Cd-U, 18.47 ± 4.84 µg/L) compared to controls (n=40; Cd-U, 4.16 ± 2.32 µg/L). SCE, but not CA, levels correlated with Cd-B and Cd-U. Palus et al. (2003) reported increased MN and SCE in blood lymphocytes of workers from a battery plant in Poland (n=22; Cd-B, 5.4-30.8 µg/L) compared to controls (n=52; Cd-B, 0.2–5.7 µg/L). The level of genotoxic damage in exposed workers was, however, not correlated with Cd-B in bivariate analyses. Others studies reported no increased genotoxic damage in the blood of workers exposed to Cd, possibly due to low Cd exposure or low study power (Verougstraete et al. 2002).

The present study has several strengths compared to previous works, including an *a priori* power calculation, a careful selection of healthy workers covering a large array of Cd exposure levels documented by seniority, Cd-B and Cd-U, the use of the CBMN assay, the inclusion of positive controls, the documentation of and adjustment for potential confounders through multivariate statistical analyses.

We found in preliminary bivariate analyses that the levels of MN measured in the 60 workers were related to Cd-B, but not to Cd-U. Multivariate analyses revealed that this association was confounded by the influence of gender as women in the study samples had higher Cd-B values than male workers. It is well-known that women, independently of Cd exposure, have higher MN frequencies in circulating lymphocytes, notably after 45 years of age (Gajski et al. 2018; Kirsch-Volders et al. 2010; Thierens et al. 2000). This is often explained by a random loss of the inactive X chromosome (Fenech 2007). More than half of the female workers in the study sample were older than 45 years, thus explaining the apparent effect of Cd-B on MN levels. Figure 1 clearly shows that women had higher MN frequency values than men at similar Cd-B levels.

It has been suggested that the CBMN assay is not sufficiently sensitive to detect the impact of occupational exposure to genotoxicants (Speit 2013), and this might contribute to explain the absence of effect in the present investigation. While this criticism is subject to debate (Kirsch-Volders et al. 2014), it is important to note that it was mainly expressed for direct genotoxic compounds (e.g. alkylating agents, styrene oxide or formaldehyde), in part because DNA damage might be repaired before MN are expressed. Here we investigated the impact of a metallic compound, which mainly acts indirectly by inhibiting DNA repair. The question of the sensitivity of the CBMN assay thus appears less relevant for the present investigation.

It should also be considered that, given its capacity to inhibit DNA repair, the extent of DNA damage caused by Cd exposure may depend on co-exposure to other genotoxins (Hengstler et al. 2003; Nersesyan et al. 2016). In the present investigation, we did not record higher MN frequencies in smokers than in non-smokers, which is not consistent with depressed repair capacity at the levels of Cd exposure considered here.

In conclusion, we did not record a dose-response relationship for MN frequency in circulating lymphocytes of workers with a wide range of occupational exposure (up to 1.25 µg/dL Cd-B and 20 µg/L Cd-U). This finding is consistent with the existing knowledge on the mechanisms

governing the genotoxic activity of Cd, which are all non-stochastic and thresholded. The threshold for systemic genotoxic effects of Cd is thus beyond the range of internal exposure levels considered in the present investigation.

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