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RESEARCH ARTICLE

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Exposure assessment and cytogenetic biomonitoring study of workers occupationally exposed to extremely low-frequency magnetic fields

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Abstract

Human cytogenetic biomonitoring (HCB) has long been used to evaluate the potential effects of work environments on the DNA integrity of workers. However, HCB studies on the genotoxic effects of occupational exposure to extremely low-frequency electromagnetic fields (ELF-MFs) were limited by the quality of the exposure assessment. More specifically, concerns were raised regarding the method of exposure assessment, the selection of exposure metrics, and the definition of exposure group. In this study, genotoxic effects of occupational exposure to ELF-MFs were assessed on peripheral blood lymphocytes of 88 workers from the electrical sector using the comet and cytokinesis-block micronucleus assay, considering workers' actual exposure over three consecutive days. Different methods were applied to define exposure groups. Overall, the summarized ELF-MF data indicated a low exposure level in the whole study population. It also showed that relying solely on job titles might misclassify 12 workers into exposure groups. We proposed combining hierarchical agglomerative clustering on personal exposure data and job titles to define exposure groups. The final results showed that occupational MF exposure did not significantly induce more genetic damage. Other factors such as age or past smoking rather than ELF-MF exposure could affect the cytogenetic test outcomes.

KEYWORDS

cluster analysis, exposure groups, genotoxicity tests, occupational exposure data, occupational magnetic field exposure

1 | INTRODUCTION

Although exposure to extremely low-frequency magnetic fields (ELF-MFs) below the exposure limits does not induce any acute effects (EU recommendation, 1999; ICNIRP, 2010; The European Parliament and the Council of the European Union, 2013), the potential long-term effects of this type of exposure, for example, the induction of DNA damage or cancer, remain controversial. Evidence from epidemiological studies indicating that chronic (long-term) exposure to ELF-MFs with an estimated daily average exposure above 0.3–0.4 μT may increase the risk of childhood leukemia (Ahlbom et al., 2000; Greenland et al., 2000). However, no causal relationship has been established and the association has not been confirmed by the experimental studies. Consequently, more studies on long-term exposure to ELF-MFs below the exposure limits are needed to unravel this controversy.

In studies on long-term occupational exposure to ELF-MFs, exposure assessment method remains the biggest challenge. This process is complicated by some of the characteristics of exposure: (1) regardless of occupation, everyone is exposed to some degree of ELF-MF due to the ubiquitous use of electricity in modern civilization, and (2) exposure may vary greatly over time and space depending on the use of electric power and the distance to the power sources. To address these concerns, personal MF measurement is often employed to monitor workers' exposure. This method is considered the gold standard for exposure assessment, as it can continuously record the workers' actual MF exposure over a long period, as well as ensure that the matched control population is only exposed to background level of MF. Personal monitoring can also provide information on the workers' residential exposure level, which is an important confounding factor when assessing the effect of long-term occupational exposure to MF (Gobba et al., 2011; Lewis et al., 2016; Magne et al., 2020; Savitz et al., 2006; Touitou et al., 2022). Nevertheless, it is worth noting that this method often results in a large data set that is difficult to summarize. Also, precautions in the measurement procedure must be taken in order to collect accurate and representative exposure data.

On top of that, occupational MF exposure assessment is also challenged by a lack of clear guidelines for grouping workers into exposure groups. In many previous studies, exposure group classification was not accompanied by a robust dosimetry evaluation but relied on superficial job titles. This is problematic as numerous studies showed that relying solely on job titles can lead to the misclassification of workers into exposure groups (Gobba et al., 2011; IARC, 2002). In studies that relied on ELF-MF measurements, occupational exposure was summarized using a single measure of central tendency, for example, time-weighted average and exposure groups were defined using one or several cutoff values (Gobba et al., 2009, 2011; Hosseinabadi et al., 2019; Juutilainen & Kumlin, 2006; Juutilainen et al., 2000). Although this method has been applied in several studies, its validity remains questionable due to the uncertainties related to exposure metrics and cutoff values. In the absence of a generally accepted biophysical mechanism for the biological effects of ELF-MFs, it is unclear which exposure metrics are related to biological responses (Hansen et al., 2000; IARC, 2002; Verrier et al., 2005; Villeneuve et al., 1998). Moreover, this method is complicated by the limited understanding in whether or not ELF-MF exposure below exposure standards can actually induce genetic damage and, if so, whether any threshold exists. In the published studies, the cutoffs to divide the study population appeared relatively arbitrary, making it difficult to compare the study results.

Highlights

- The study results indicated that exposure assessment had better rely on the workers' actual exposure data.
- Electrical workers in this study were exposed on average to quite low levels of 50 Hz magnetic fields at work. But there was wide variability between workers. This highlights the value of using cluster analysis on workers' actual exposure data to define exposure groups.
- Occupational exposure to 50 Hz magnetic fields did not significantly increase the level of DNA or chromosomal damage on workers' blood samples.

As genetic instability is considered a hallmark of cancer (Negrini et al., 2010), genetic biomonitoring studies measuring the level of DNA and chromosomal damage have been widely used to evaluate the biological effects of ELF-MF exposure. To date, several reviews of studies investigating the biological effects of occupational exposure to ELF-MFs were performed, but the results remain controversial. For instance, a recent review on the long-term health effect of occupational exposure to ELF-MFs below exposure limits showed no clear association between exposure and the occurrence of cancers or other disorders (Maffei, 2022; Rijs & Stam, 2019). A review focusing solely on cytogenetic biomonitoring studies pointed out that although the majority of reported studies suggested an association between MF exposure and genotoxic effects, most of the studies were hampered by methodological shortcomings (small study population, ambiguous exposure assessment, different test protocols), preventing any firm conclusions made (Maes & Verschaeve, 2016).

In the present study, we investigated the potential genotoxic effects of long-term occupational exposure to 50 Hz MF using personal exposimetry and two genotoxicity tests on workers' peripheral blood lymphocytes (PBLs). We considered long-term exposed workers are those who had worked on-site for at least one year (Touitou et al., 2022). In addition, a new grouping method that may circumvent the need to select specific exposure metrics or cutoff values to define exposure groups was introduced. For this purpose, personal exposure monitoring was performed on 88 workers in the electrical sector. Then cluster analysis was used to classify them into exposure groups. Since cluster analysis could simultaneously consider various exposure summary statistics, it could be a promising approach to group workers with the same exposure characteristics into clusters.

2 | MATERIALS AND METHODS

This study was approved by the ULB-Erasme ethical committee (CCB: B406201837179; November 06, 2018).

2.1 | Participants and study protocol

The recruitment was focus on workers from ELIA (Belgium's high voltage transmission system operator), Sibelga (an electricity supplier in Brussels, Belgium), and Ores (an electricity supplier in Wallonia, Belgium). The invitation for participation was delivered via email and the company intranet. In this study, the investigators had no knowledge on the participants' identity nor their exposure status. To do so, each participant was assigned by a code, that is, ELIA_xxx, SIB_xxx or ORES_xxx for ELIA, Sibelga and Ores workers, respectively. These codes were selected by the occupational physicians who were in charge of collecting samples and were only communicated to the investigators at the end of the study when all the genotoxicity data were collected.

Workers were first recruited based on their job descriptions (i.e., office workers and technicians) following the predefined inclusion/exclusion criteria. Office workers (control subjects) were only exposed to a background level of 50 Hz MF as the general public, while the technicians (exposed subjects) were exposed to various MFs at the workplace. The latter's jobs involved all stages of the electricity transmission and distribution processes: operation of 380/220k high-voltage grid, maintenance in the high-voltage stations of the protection equipment, testing of new installations, switching on high-voltage installations, and so on. The inclusion criteria were workers aged between 20 and 65 years old, no current smoking and technicians who had at least one year of occupational exposure to 50 Hz MF. Exclusion criteria were lacking either exposure measurement or blood sample for genotoxicity analysis, past and/or present residence under a power line, a history of malignancy, or genetic diseases. Other exclusion criteria related to the actual MF exposure level, namely average residential exposure higher than 0.1 μT and control subjects with occupational exposure higher than 0.1 μT, were also applied in the later stage of this study (after having

TABLE 1 Characteristics of the office and technician groups.

Characteristics	Office workers	Technicians
Group size	36	43
Average age (years)	37.72 (25–54)	37.12 (21–60)
Gender	29 male, 7 female	43 male
Months of working on site	0–216[a]	21–360
Ratio of time duration of working on site (%)	0–20	30–100
Average number of medical X-rays in the last 5 years (min–max)	1.19 (0–5)	1.2 (0–4)
Past smoking	4	5

[a]Most office workers did not work on-site, except for E022 had 216 months of working on-site, E041 and E057 had 21 and 18 months of working on-site, respectively. Over the years, their work evolved from on-site work to office work.

information on each workers' actual MF exposure level).

At first, 216 workers showed their interest in participating in this biomonitoring study. These workers were screened and only those who fitted the inclusion criteria were recruited and contacted for a medical appointment to collect samples. Finally, 96 workers in total showed up at the appointment for data collection. Following the inclusion/exclusion criteria set at the beginning of the study, 88 workers were retained and made up the study population. By applying additional exclusion criteria related to the actual MF exposure data, 9 workers (8 office workers and 1 technician) were excluded from analysis and the final study population ended up with 79 workers (36 office workers and 43 technicians) (Supporting Information S5: Table 1). The characteristics of the workers in the office and technician groups are illustrated in Table 1. The average age and number of X rays examinations are comparable between the two job groups. In the technician group, the total number of months of working on-site (meaning working closely with the power lines or transformers) ranged from 21 to 360. The percentage of worktime on-site was above 75% for almost 75% of workers. Four office workers and five technicians (11.11 and 12.5%, respectively) are past smokers. Most of them declared having stopped smoking more than 10 years ago, except for two workers who quit smoking 3 years ago.

After recruitment, informed consent was obtained from all subjects at the beginning of the study. Besides, all participants were asked to fill in a questionnaire (Carrano & Natarajan, 1988) pertaining to their working history and possible confounding factors, including lifestyle (i.e., past smoking and alcohol habits), medical history, and X-ray examinations.

2.2 | MF measurements

The magnetic flux density (B) was recorded by a wearable personal exposimeter, the Emdex II (Enertech Consultants Inc.; sensitivity: 0.01–300 µT; sampling rate 30 s) (Magne et al., 2017). For measurement, the values representing the MFs both in broadband (40–800 Hz) and 50 Hz harmonics (in the range from 100 to 800 Hz) were recorded. Although the fundamental component could be calculated from the broadband and harmonics values, there are cases where the calculated fundamental magnitude may be in error in excess of the EMDEX II's operating specifications due to the accuracy limits of the broadband and harmonic bandwidth. To avoid these additional approximations, especially for our workers in the electricity sector, as the harmonic values are assumed to be low, broadband measurement is preferred for ELF-MF exposure measurements. Before data collection, all exposimeters were checked for the accuracy of measurements by the Department of Electrical Engineering and Computer Science, University of Liège. The 50 Hz MF values were recorded in the instrument's memory. After recording, these data were retrieved using the EMCALC2013 software (Enertech).

Measurements were performed for at least three consecutive full days during a typical work week in order to cover the between-day and within-day variations in residential and occupational exposure levels (Feychting et al., 2005; Gobba et al., 2011; Touitou et al., 2013; WHO, 2007). At work, participants were asked to wear the device in a pouch around their hip (Bowman et al., 1998; Cutler et al., 1999). Wearing the EMDEX at the hip level provides a good estimate of body exposure (Delpizzo, 1993). Besides, we observed that this position is less perturbing to the technicians, ensuring that the protocol was respected. Participants were instructed to wear the EMDEX as much as possible at home. Otherwise, they could leave it in its pouch and place it far from any electrical devices or transformers, for example, the power unit of a PC or an alarm clock, to prevent exposure measurement bias (Decat et al., 2009; Magne et al., 2020).

During the MF measurement period, participants were invited to complete an activity logbook on a 15 min schedule with the codes from 1 to 6, corresponding to (1) office work, (2) on-site work, (3) lunch/pause, (4) commute/leisure activities (elsewhere), (5) at home, and (6) sleep (Supporting Information S1: Figure 1). These logbooks made it possible to distinguish between working and non-working periods and check location-dependent exposure profiles.

2.3 | Evaluation of genetic damage

Workers' whole blood samples were collected in sodium heparin tubes (6 mL Vacuette®). After collection, all blood samples were transported to the lab and handled immediately to avoid further DNA damage. The genetic damage in PBLs was evaluated using the comet and the cytokinesis-block micronucleus (CBMN) assay. Both tests were performed according to standard protocols, with slight modifications (Bajpayee et al., 2013; Bolognesi & Fenech, 2013).

2.3.1 | Comet assay

After blood collection, two PBL samples/subject were prepared using Ficoll-Paque™ Plus density-gradient medium, following the manufacturer's protocol. Then, 50 µL of isolated PBL was mixed with 600 µL 0.8% low melting point agarose, of which 75 µL was loaded on two Superfrost Plus® microscopic slides (two slides/sample) that were precoated with 1% normal melting point agarose. After gel solidification, the slides were immersed in a 4°C lysing solution (10% dimethyl sulfoxide, 1% Triton X-100, NaCl 2.5 M, EDTA 100 mM, TRIS 10 mM, NaOH added freely until the solution reached pH 10) for about 18 h. Subsequently, the slides were placed in a freshly prepared denaturation buffer (EDTA 1.27 mM, NaOH 0.3 M, pH > 13) for 40 min to allow DNA unwinding, followed by electrophoresis at a voltage gradient of 1 V/cm for 20 min. Next, the slides were removed from the alkaline buffer, rinsed three times with a neutralizing buffer (TRIS, pH 7.5), and fixed in ice-cold ethanol for 10 min. For DNA visualization, slides were dried overnight and stained with nucleic acid gel stain (GelRed® 3x). The scoring of 200 comets/slide was done automatically with a fluorescent microscope (AxioImager. Z2) linked to an image analysis system (Metafer4, version 3.8.5; Metasystems). The comet result was described as the percentage of DNA in the comet tail (tail intensity) of the first 50 cells per slide (200 cells per person). Subsequently, the median tail intensity was calculated, and the overall level of DNA damage was expressed as the mean of comet scores.

2.3.2 | CBMN assay

Two lymphocyte cultures/subject were prepared by adding 0.5 mL sodium heparinized blood to 5 mL RPMI 1640 culture medium supplemented with 15% decomplemented fetal bovine serum, 2% phytohemagglutinin (for mitogenic stimulation), 1% gentamicin, and 1% GlutaMAX™ Supplement. The blood cultures were then incubated at 37°C in a humidified incubator in an atmosphere of 5% CO_2 for 44 h. Next, 6 µg/mL of cytochalasin B was added to each culture and the incubation was continued for the next 28 h. At 72 h, the lymphocytes were collected and treated with a hypotonic solution (5 mL, 0.075 M KCl). The cells were then fixed two times with acetic acid-methanol solution (1:3, 5 mL) supplemented with three droplets of 37% formaldehyde at the first fixation. After fixation, two to three drops of the cell suspension were loaded on precleaned slides (three slides/culture). Cells were stained with Vectashield® mounting medium with 4′,6-diamidino-2-phenylindole and 2000 binucleate cells per sample (4000 cells per person) were automatically scored using a fluorescent microscope (AxioImager. Z2) linked to an image analysis system (Metafer4 system). The presence of micronucleate cells was confirmed visually following the scoring criteria adopted by the HUMN project (Bonassi et al., 2001). The micronucleus (MN) frequency was expressed as the number of micronucleate cells per 1000 binucleate cells. Acridine orange staining was also performed to visually check the quality of the cells and to calculate the cytokinesis-block cell proliferation index.

2.4 | Data analysis

2.4.1 | Summary of MF exposure data

The EMDEX measurement was recorded every 30 s for at least three days, resulting in around 8000 exposure values for each participant. The exposure metric to summarize personal exposure was selected by considering the distribution of the occupational exposure data (Shapiro–Wilk tests and *q*–*q* plot). Using the selected metric, workers' residential and occupational exposure levels were summarized. The occupational exposure data were aggregated according to Activities 1 to 3. Residential exposures were all other nonoccupational exposures (exposures at home, during sleep, and elsewhere); it was, therefore, aggregated according to Activities 4 to 6. The Wilcoxon rank-sum test was used to compare the residential and occupational exposure levels between job groups.

2.4.2 | Defining exposure groups: two methods

The first method was based solely on the job titles, resulting in two groups of workers: the office and the technician groups. The second method consists of the combination of both workers' actual exposure and job titles. At first, hierarchical clustering (Norusis, 2010; Yim & Ack Baraly, 2015) using Ward's method (SPSS [package 26]) was used to group workers with similar exposure characteristics into a group. It was performed on a matrix of 79 × 10 real data, each representing subjects (row) and resulting data when 10 different exposure metrics were calculated on the occupational exposure data (10 predictor variables) (column). The list of predictor variables used for the cluster analysis is provided in Table 2. Ward's method was preferred because it generally creates more evenly sized clusters (Esztergár-Kiss & Caesar, 2017). The Ward's linkage between two clusters (e.g., Clusters A and B) is a measure of dissimilarity between two clusters and is computed as the emerging cost (the increase in the sum of squares) when fusing two clusters into a single cluster, following equation below (Shalizi, 2009):

$$\Delta(A, \quad B) = \sum_{i \in A \cup B} \|\vec{x}_i - \vec{m}_{A \cup B}\|^2 - \sum_{i \in A} \|\vec{x}_i - \vec{m}_A\|^2 - \sum_{i \in B} \|\vec{x}_i - \vec{m}_B\|^2 = \frac{n_A n_B}{n_A + n_B} \|\vec{m}_A - \vec{m}_B\|^2.$$

where:
Δ: merging cost of combining Clusters A and B,
$\vec{m}_j$: center of Cluster j,

TABLE 2 List of predictor variables (exposure metrics) for cluster analysis.

Predictor variables	Abbreviation	Description
Arithmetic mean	AM	Average of exposure
Geometric mean	GM	Average of exposure
Median	Med	Average of exposure
Arithmetic standard deviation	Amsd	Variability of exposure data
Geometric standard deviation	Gmsd	Variability of exposure data
Maximum	Max	Peak of exposure
Percentage of time higher than 1 µT	P1	Duration of exposure at a given exposure level
90th percentile	P90	High exposure percentile within the data set
Rate of change[a]	RC	Variability of exposure
Standardized rate of change[a]	sRC	Temporal stability of exposure variation

[a]Exposure metric "Rate of change" reflects the intermittence of magnetic field exposure data, meaning the amount of change in the flux density for data discretely sampled over time (Wilson et al., 1996). It is calculated by the following equation:

$$\mathrm{RC} = \sqrt{\frac{\sum_{t=1}^{n-1}(B_{t+1} - B_1)^2}{N-1}},$$

$$\mathrm{sRC} = \frac{\mathrm{RC}}{\mathrm{Amsd}},$$

where B_t is the magnetic field at time t.

n_j: number of points in Cluster j.

As agglomerative hierarchical clustering starts with each individual as a single cluster, the sum of squares begins at zero. Then, two clusters are merged when their sum of square value is the lowest (smallest merging cost). This process is repeated until there is only a single cluster left. The optimal number of clusters is determined by visually examining the dendrogram. Then discriminant analysis (Wilks' λ test) was also performed to test how well each metric separates workers into groups.

After having three well-separated clusters, the partition of job titles in each cluster was examined. In the end, only office workers in the low-exposed cluster and technicians in the high-exposed cluster were used in subsequent analyses. From this point, we refer to the newly formed groups: the "lower-exposed group" and "higher-exposed group."

2.4.3 | Association between occupational MF exposure and genetic damage

Statistics were performed using the statistical software Stata/SE 15. In the first analysis, data from the whole population were treated as one big data set. Then, the Spearman correlation coefficient test was used to seek the correlation between genetic damage parameters (tail intensity and MN frequency) in PBL and the median level of occupational exposure to ELF-MF, considering a significance level of 0.05. In the second analysis, exposure groups were defined using the two above-mentioned methods. Then, genetic damage data between two groups (office workers vs. technicians or lower-exposed group vs. higher-exposed group) were compared. As the comet and CBMN data distribution are normal, a t-test was used to compare the genetic damage between groups. The statistically significant level was set at 0.05.

2.4.4 | Analysis of genotoxic outcomes in relation to main confounders of human biomonitoring study

In a human biomonitoring study, numerous confounders (e.g., sampling time, age, gender) could affect the study results. Consequently, an analysis of the relationship between the results of genotoxic tests on workers' blood samples and these confounders (predictor variables) is needed to determine the potential for confounding if any effects are observed.

As the results of the comet assay and CBMN assay on workers' blood samples were normally distributed (Supporting Information S2: Figure 2), linear regression analysis (both simple and multiple regression) was used to determine the degree to which the independent variables predict the dependent variables (cytogenetic test outcomes). At first, simple regression was performed to determine the connection between the dependent variables and each of the independent variables. Then, variables that had a high potential of affecting the test outcomes (i.e., resulting $p < 0.2$ in simple regression analysis) were selected to perform the multiple regression analysis. All the regression analysis was done with Stata 17 software and the list of considered independent variables is presented in Table 3.

TABLE 3 List of variables considered for the regression analysis.

Types of variables	Variable name	Description
Confounders (predictor variables)	Sample origin (companies)	Identity of workers (from Elia, Sibelga, or Ores companies)
	Sampling times	Time of blood sampling and MF measurement
	Gender	Male or female
	Age	Participant age
	Smoking	Illustrate whether participant used to smoke or not
	X-rays	Number of X-rays examination in the last 5 years
Variables under hypothesis	Job title	Classification of workers in job groups (i.e., office worker and technician)
	Med123	MF exposure at work
	Exposure duration	Number of months of working on-site (occupational exposure duration)
Response (dependent variables)	Tail intensity	Percentage of DNA damage in blood sample
	MN frequency	Level of chromosomal damage in blood sample

Abbreviation: MF, magnetic field.

3 | RESULTS

3.1 | Summary of MF exposure data

3.1.1 | Personal ELF-MF exposure data

The obtained personal exposure data demonstrated that workers' MF exposure varied significantly during the measurement period (Figures 1 and 2). When looking at group level, office workers were exposed to a low level of MF both at work and at home (Figure 1). In the technician group, the residential MF exposure levels were low, but the occupational MF exposure levels were higher than those in the office group. Occupational exposure data varied considerably among technicians. For instance, the exposure profile of technician A (median exposure = 0.14 μT) was characterized by many high peaks, especially on Day 1 of measurement (Figure 2a). Technician B, on the other hand, showed much lower exposure levels (median exposure = 0.8 μT), but at work, he often exposed to a constant level of MF at around 1 μT (Figure 2b). When looking at individual level, the MF exposure of each technician varied significantly between and within days of measurement (Figure 2). There were some peaks of exposure, but only for a short period. Several technicians were considered highly exposed based on their job description but their actual exposure was quite low compared to other technicians (Figure 2b).

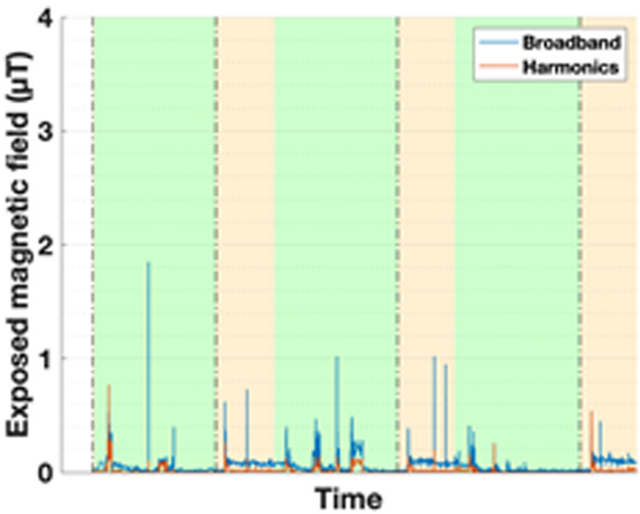

FIGURE 1 Example of an exposure profile of a typical office worker. Magnetic field exposure levels were monitored using an Emdex II device every 30 s for at least 3 working days. The green and yellow parts illustrate exposure outside work and exposure at work, respectively.

Shapiro–Wilks test on the retrieved personal MF exposure data showed that the data were not normally distributed ($p > 0.05$). This result was confirmed using a histogram (data not shown). The normal quantile plots also did not allow retrieving any normally distributed transformations (Supporting Information S4: Figure 3). Our exposure data were thus neither normally nor, e.g., log-normally or squared distributed. Hence, the median of occupational exposure data (Med123) was chosen as the most suitable exposure metric to estimate workers' occupational exposure data (Decat et al., 2009).

3.1.2 | Level of residential and occupational exposure to ELF-MF in the whole study population

Except for one office worker and one technician, the remaining study population had average (median) MF exposure at home below 0.1 μT. Concerning occupational MF exposure, office workers were exposed to much lower and less varied MF levels than technicians. However, seven office workers were exposed to more than 0.1 μT MF at work. Of these, six workers (prevention advisor, project leader, head safety) were classified as office workers, but their tasks also required their presence on-site (exposure at the

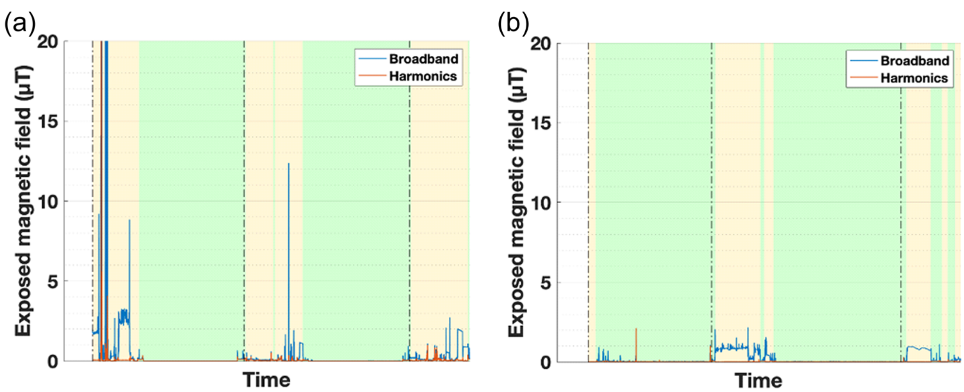

FIGURE 2 Examples of exposure profiles from two technicians (a, b). Magnetic field exposure levels were monitored using an Emdex II device every 30 s for at least 3 working days. The green and yellow parts illustrate exposure outside work and exposure at work, respectively.

secondary workplace). The remaining worker was indirectly environmentally exposed to MF as his office turned out to be below a 220 kV line.

In the whole sample, the mean occupational exposure level (mean Med123) was 0.15 µT and the median Med123 was 0.06 µT (5%–95% percentiles 0.01–0.61 µT). Regarding occupational exposure in each job group, Median Med123 in the Office and Technician groups were 0.02 µT (min–max = 0–0.08 µT) and 0.16 µT (min–max = 0.02–1.31), respectively (Table 4). Wilcoxon rank-sum test on Med123 data showed a significant difference in the MF exposure level in the technicians compared to office workers ($p < 0.0001$).

3.2 | Defining exposure groups using MF exposure data

The cluster analysis considered all exposure metrics applied to the Med123 data set, resulting in three clusters (low-, medium-, and high-exposed clusters) of 29, 37, and 13 workers, respectively (Figure 3). The summary of exposure levels according to predictor variables in each cluster is shown in Table 5 and clearly illustrates that exposure was well-differentiated among the three clusters. This observation was also confirmed by the Wilks' λ test, which indicates that the mean scores in each of those predictor variables significantly differed between clusters (Supporting Information S6: Table 2).

Table 6 describes the repartition of job titles into clusters determined by cluster analysis. Cluster analysis classified most of the office workers into the low-exposed group. On the other hand, technicians were distributed mostly into the medium-exposed group ($n = 26$) and high-exposed groups ($n = 13$). The classification of 4 technicians in the low-exposed group implies that the exposure levels of the so-called "exposed group' in this study were quite low.

3.3 | Association between MF exposure in the occupational setting and genetic damage on peripheral blood lymphocytes

Detailed information on each participant's MF exposure level, level of DNA damage (expressed as tail intensity [%]), and level of chromosomal damage (expressed as MN frequency) are described in Supporting Information S7: Table 3. Considering the whole study population, the mean tail intensity and MN frequency were 2.7% (min–max: 1.35%–4.77%) and 5.03 (min–max: 1.13–11.75), respectively. These values were

TABLE 4 Summary of residential and occupational MF exposure levels in the two job groups.

	Job groups	n	Mean	Median	Min	Max
Residential exposure	Office workers	36	0.02	0.01	0.00	0.08
	Technicians	43	0.02	0.01	0.00	0.08
Occupational exposure	Office workers	36	0.02	0.02	0.00	0.08
	Technicians	43	0.25	0.16	0.02	1.31

Abbreviation: MF, magnetic field.

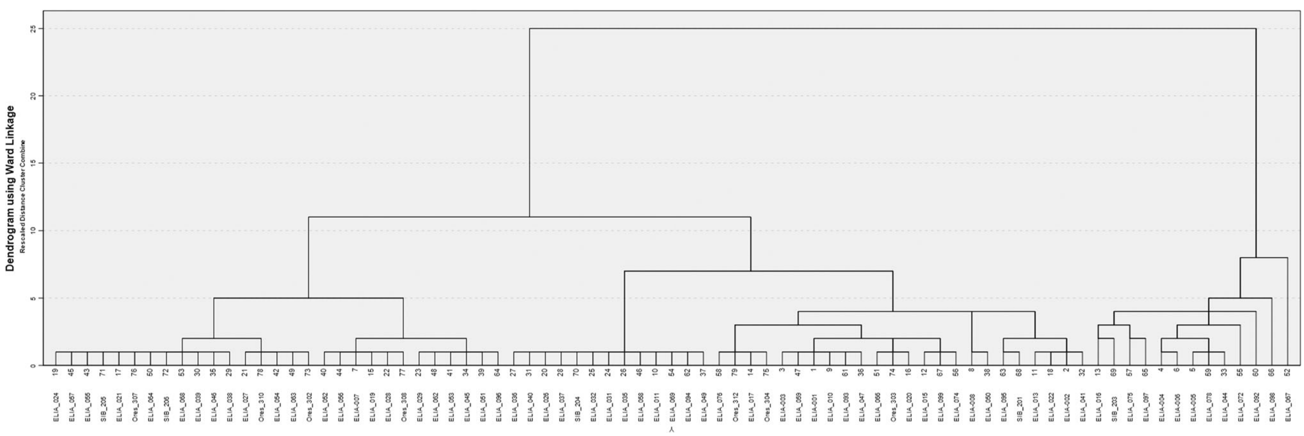

FIGURE 3 Dendrogram using Ward linkage showing the results of cluster analysis with three separate clusters (from left to right are low-, medium-, and high-exposed clusters).

TABLE 5 Summary of average occupational exposure level (expressed in μT) in low, medium, and high exposed clusters classified by cluster analysis according to different metrics.

Exposure metrics	Low-exposed cluster		Medium-exposed cluster		High-exposed cluster	
	Mean	SD	Mean	SD	Mean	SD
AM13	0.05	0.04	0.35	0.26	1.51	0.42
Med13	0.03	0.02	0.15	0.18	0.40	0.35
GM13	0.03	0.02	0.12	0.11	0.42	0.35
Amsd13	0.10	0.10	0.83	1.21	4.51	5.17
Gmsd13	2.84	1.22	4.61	2.22	7.05	5.23
Max13	2.50	3.87	10.39	13.44	86.80	107.29
P9013	0.09	0.11	0.82	0.76	3.87	2.24
P113	0.16	0.30	6.77	6.44	32.92	15.33
RC13	0.09	0.13	0.35	0.38	2.83	2.78
sRC13	0.84	0.22	0.47	0.25	0.69	0.20

TABLE 6 The repartition of job titles in three newly-formed clusters.

Clusters	*n*	Job titles
1. Low-exposed	29	25 Office workers 4 Technicians
2. Medium-exposed	37	11 Office workers 26 Technicians
3. High-exposed	13	13 Technicians

all in the same range of the background tail intensity and MN frequency obtained in the general public (Fenech et al., 2003; Milić et al., 2021) and our laboratory's own historical control data.

In order to investigate the link between occupational MF exposure (Med123) and the level of genetic damage, Spearman's correlation was applied. Spearman's correlation coefficient between comet assay result (tail intensity) and Med123 data of the whole study population was $r = 0.1219$ ($p = 0.2878$), indicating no association between tail intensity results and MF exposure (Figure 4). There was also no significant association between CBMN assay results (MN frequency) and MF exposure ($r = 0.0620$, $p = 0.5870$) (Figure 5).

When considering only data of the technician groups, Spearman's rank correlation coefficient also revealed no correlation between occupational MF exposure and the results of the comet assay ($r = 0.0484$, $p = 0.7611$) and CBMN assay ($r = -0.0370$, $p = 0.8136$) (Figures 6 and 7).

For group analysis, levels of genetic damage among groups with different MF exposure levels were compared.

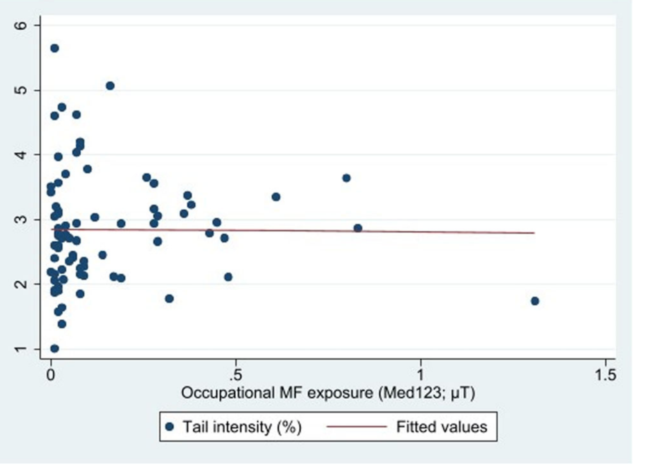

FIGURE 4 Scatterplot on occupational exposure data (Med123) and tail intensity (%) of the whole study population ($n = 78$). MF, magnetic field.

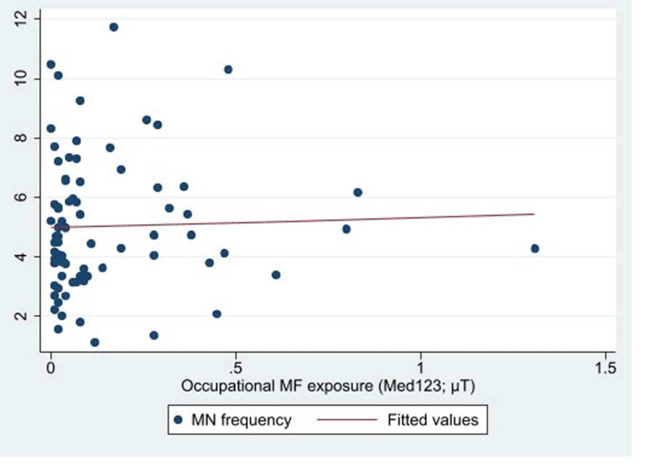

FIGURE 5 Scatterplot on occupational exposure data (Med123) and MN frequency of the whole study population ($n = 79$). MF, magnetic field.

Considering different job groups, there was no difference in the level of genetic damage between the office and the technician groups (Table 7). No significant difference in tail intensity was observed in isolated lymphocytes between the office and technician groups, $t(76) = -0.6667$, $p = 0.5070$. Also, there was no significant difference in MN frequency in technicians compared to office workers, $t(77) = -1.1748$, $p = 0.2437$.

Furthermore, the tail intensity was not statistically increased in the higher-exposed group compared to the lower-exposed group, $t(36) = 0.9690$, $p = 0.3390$. There was also no difference in the MN frequency between the former and the latter group, $t(36) = 0.2484$, $p = 0.8053$.

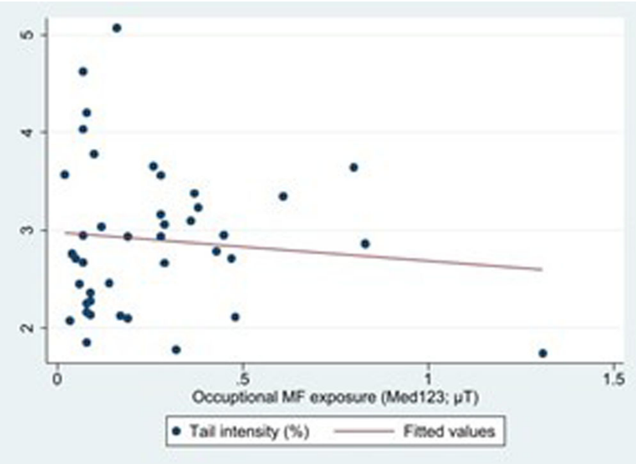

FIGURE 6 Scatterplot on occupational exposure data (Med123) and tail intensity (%) of the technicians ($n = 42$). MF, magnetic field.

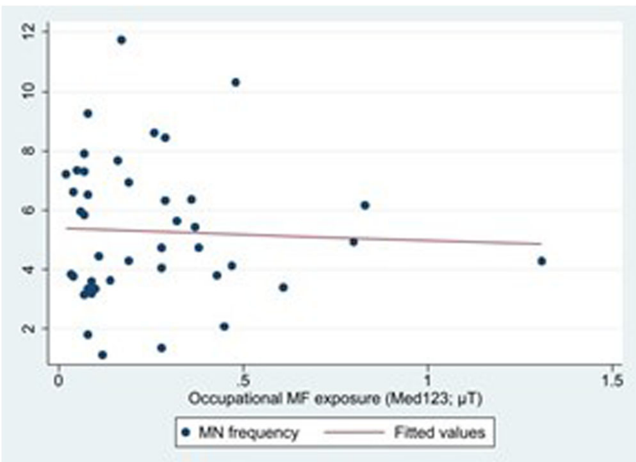

FIGURE 7 Scatterplot on occupational exposure data (Med123) and MN frequency of the technicians ($n = 43$). MF, magnetic field.

3.3.1 | Analysis of genotoxic outcomes in relation to main confounders of the human biomonitoring study

Simple linear regression was used to examine the linear relationship between the independent variables (age, exposure duration, gender, job title, smoking, sample origin, sampling times, occupational exposure to MF [Med123], X-rays examination) and the genotoxicity test outcomes (tail intensity and MN frequency data). The results of the simple regression analyses on the comet and the CBMN results are presented in Tables 8 and 9. In general, age had a significant effect on the results of the comet assay ($\beta = -0.0216$, $p = 0.043$) and the CBMN assay ($\beta = 0.1304$, $p = 0.0000$). Smoking was also associated with altered DNA damage in the comet assay ($\beta = 0.6030$, $p = 0.017$) and chromosomal damage in the CBMN assay ($\beta = 1.3577$, $p = 0.0379$).

Sample origin and sampling time also could significantly affect the results of both the comet and the CBMN assay. With regards to the sample origin variable, the overall regression was statistically significant ($F(2,75) = 19.61$, $p = 0.0000$ for comet assay and $F(2,76) = 9.86$, $p = 0.0002$ for CBMN assay). The overall regression was also statistically significant when examining the effect of the sampling time variable on the comet assay result ($F(3,74) = 14.13$, $p = 0.0000$ and the results of CBMN assay ($F(3, 75) = 8.96$, $p = 0.0000$). In contrast, other variables including exposure duration, gender, job title, MF exposure at the workplace, and number of X-rays examination over the past 5 years did not significantly predict the results of the comet assay and CBMN assay.

In order to better understand the results of regression analysis, a two-sample t test was used to compare the test outcomes between groups that differed in smoking status while one-way analysis of variance (ANOVA) was used to evaluate the effects of age, sample origin, and sampling times on the test outcomes. In each ANOVA test, Turkey's multiple comparison tests were applied to determine the specific mean value that was statistically different from

TABLE 7 Level of genetic damage across different investigated groups.

	Groups based on job titles			Groups based on cluster analysis of MF exposure data and job titles		
Cytogenetic results	Office workers	Technicians	p	Low-exposed group	High-exposed group	p
Tail intensity (%)	2.66 (1.01–5.66)	2.83 (1.74–5.07)	0.5070	2.57 (1.01–4.74)	2.79 (1.74–5.07)	0.3390
MN frequency	4.49 (1.57–10.49	4.74 (1.13–11.75)	0.2437	4.50 (1.57–10.49)	5.43 (1.80–10.31)	0.8053

Note: Data are presented as the median (min–max) of tail intensity (%) and MN frequency. *T*-test was used to compare group means with significant level set at 0.05.
Abbreviation: MF, magnetic field.

TABLE 8 Results of the simple regression analyses on the comet results.

Independent variables	Coefficient	95% confident interval		*t*	*p* > |*t*|
Age	−0.0216	−0.0425	−0.0007	−2.06	0.0430
Exposure duration	−0.0002	−0.0025	0.0022	−0.13	0.8950
Gender					0.6311
Female	0	0	0	0	
Male	−0.1658	−0.8510	0.5193	−0.48	
Job titles					0.5070
Office workers	0	0	0	0	
Technicians	0.1313	−0.2609	0.5236	0.67	
Smoking					0.017
No smoking	0	0	0	0	
Past smoking	0.6030	0.1109	1.0952	2.44	
Sample origin					0.0000
ELIA	0	0	0	0	
Sibelga	2.0488	1.3933	2.7043	6.23	
ORES	−0.0428	−0.6046	0.5190	−0.15	
Sampling time					0.0000
Spring 2019	0	0	0	0	
Winter 2019	−0.4126	−0.9407	0.1156	−1.56	
Spring 2021	1.9988	1.3461	2.6515	6.10	
Spring 2022	−0.0928	−0.6531	0.4675	−0.33	
Occupational MF exposure (Med123)	−0.0414	−0.9262	0.8433	−0.09	0.9260
X-rays examination	0.0634	−0.1030	0.2299	0.76	0.4500

the other means. All the tests were performed using the GraphPad Prism 9 software and the level of statistical significance was set at 0.05.

Figure 8 illustrates the effects of smoking status on the results of the comet and CBMN assay. The *t* test result showed a significant increase in tail intensity ($t(76) = 2.441$, $p = 0.0170$) and MN frequency ($t(77) = 2.113$, $p = 0.0379$) for the group of past smoking compared to the group of no smoking (Figure 8).

One-way ANOVA was conducted to investigate the effects of age on the test results for different age groups: 21–30 years; 31–40 years; 41–50 years; >50 years. The distribution of tail intensity and MN frequency over different age groups is illustrated in Figure 9. Although our data depicted a downward trend in tail intensity when the age increased, there were no statistically significant differences between group means as determined by one-way ANOVA on tail intensity data ($F(3, 74) = 1.347$, $p = 0.2658$). Posthoc comparisons using the Tukey's honestly significant difference (HSD) test also indicated no significant difference in the mean scores for the tail intensity between age groups.

One-way ANOVA on the results of the CBMN assay, on the other hand, revealed a significant difference in mean MN frequencies between age groups ($F(3, 75) = 11.99$, $p < 0.0001$). Pairwise comparisons of the means using the Tukey's HSD test indicated that the MN frequency observed in the oldest age group (>50 y) was significantly higher than in the younger age groups (p values were 0.0096, <0.0001, <0.0001 when compared to age groups 41–50, 31–40, and 21–30 years, respectively). There was also a significant difference in MN frequency between the groups aged 41–50 and 21–30 years ($p = 0.0064$). However, MN frequency in the group 31–40 years did not significantly differ from the groups aged 21–30 and 41–50 years.

One-way ANOVA revealed that there was a statistical difference in the mean tail intensity ($F(2, 75) = 19.61$, $p < 0.0001$) and mean MN frequency ($F(2, 76) = 9.860$, $p = 0.0002$) between at least two groups of workers from different companies. Tukey's HSD test

TABLE 9 Results of the simple regression analyses on the CBMN results.

Independent variables	Coefficient	95% confident interval		t	$p > \|t\|$
Age	0.1304	0.0838	0.1770	5.57	0.0000
Exposure duration	0.0058	−0.0002	0.0117	1.94	0.0560
Gender					0.4639
Female	0	0	0	0	
Male	−0.6516	−2.4142	1.1110	−0.74	
Job titles					0.2437
Office workers	0	0	0	0	
Technicians	0.5902	−0.4101	1.5905	1.17	
Smoking					0.0379
No smoking	0	0	0	0	
Past smoking	1.3577	0.0779	2.6375	2.11	
Sample origin					0.0002
ELIA	0	0	0	0	
Sibelga	−0.4688	−2.3268	1.3892	−0.50	
ORES	3.4827	1.8907	5.0746	4.36	
Sampling time					0.0000
Spring 2019	0	0	0	0	
Winter 2019	1.7849	0.3210	3.2488	0.018	
Spring 2021	−0.2557	−2.0654	1.5541	0.779	
Spring 2022	3.6958	2.1426	5.2490	0.000	
Occupational MF exposure (Med123)	0.3293	−1.9514	2.6099	0.29	0.7750
X-rays examination	−0.0745	−0.4986	0.3497	−0.35	0.7280

Abbreviations: CBMN, cytokinesis-block micronucleus; MF, magnetic field.

for multiple comparisons found that the mean tail intensity was significantly different between Sibelga and ELIA ($p < 0.0001$, 95% confident interval [95% CI]: −2.836 to −1.262) and between Sibelga and Ores ($p < 0.0001$, 95% CI: 1.098 to 3.085). It also revealed a significant difference in MN frequency between Ores and ELIA ($p = 0.0001$, 95% CI: −5.393 to −1.572) and between Ores and Sibelga ($p = 0.0035$, 95% CI: −6.768 to −1.135) (Figure 10a,b).

The same results were observed when performing one-way ANOVA to compare the effect of sampling times on the comet and CBMN assay results (Figure 10c,d). Statistical differences in group mean tail intensity (F (3, 74) = 14.13, $p < 0.0001$) and MN frequency (F (3, 75) = 8.964, $p < 0.0001$) were detected.

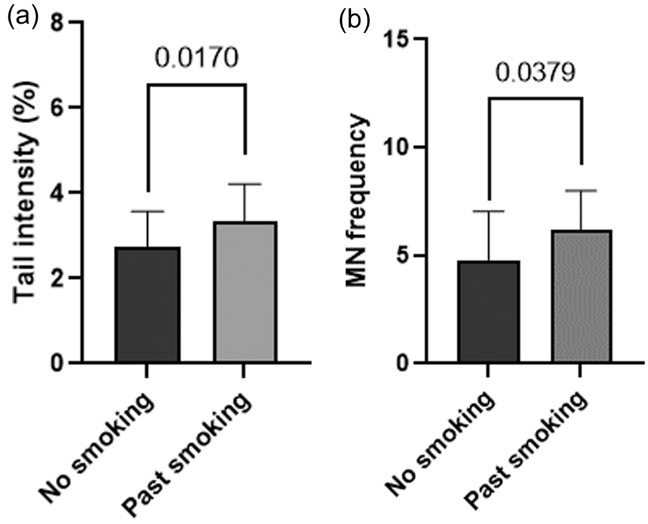

FIGURE 8 Effects of smoking habit on the results of the comet assay (a) and the cytokinesis-blocked micronucleus assay (b). Data are presented as mean ± SD. Statistical significance was at 0.05.

Tukey's HSD test for multiple comparisons indicated a significant difference in tail intensity in samples collected in Spring 2021 compared to samples collected in different periods, in particular in Spring 2019 ($p < 0.0001$, 95% CI: −2.860 to −1.138), Winter 2019 ($p < 0.0001$, 95% CI: −3.464 to −1.358), and Spring 2022 ($p < 0.0001$, 95% CI: 1.010 to 3.173). Regarding multiple comparisons of MN frequency data, there were significant differences in mean MN frequency between samples collected in Spring 2022 compared to Spring 2019 ($p < 0.0001$, 95% CI: −5.744 to −1.647) and Spring 2021 ($p = 0.0049$, 95% CI: −6.952 to −0.9507).

3.3.2 | Results of multiple regression analysis

Multiple linear regression was used to test if age, smoking, sampling origin, and sampling time significantly affected the comet assay result. The overall regression was statistically significant ($R2 = 0.4221$, $F(5, 72) = 10.52$, $P = 0.0000$). Considering the effects of sample origin and sampling time, significant results were only observed in one of the comparisons (Table 10). Smoking still had a significant effect on the comet results ($\beta = 0.5222$, $P = 0.017$), but not age ($\beta = -0.0158$, $P = 0.103$) (Table 10). In particular, though simple regression showed that age had a borderline significant effect on the comet results, this effect was no longer observed in the multiple regression analysis. Based on this result, we can conclude that smoking is the main variable that affected the comet assay results. This observation could also be seen in the interaction plot for comet results (Figure 11). Figure 11a clearly showed that tail intensities in the past smoking groups

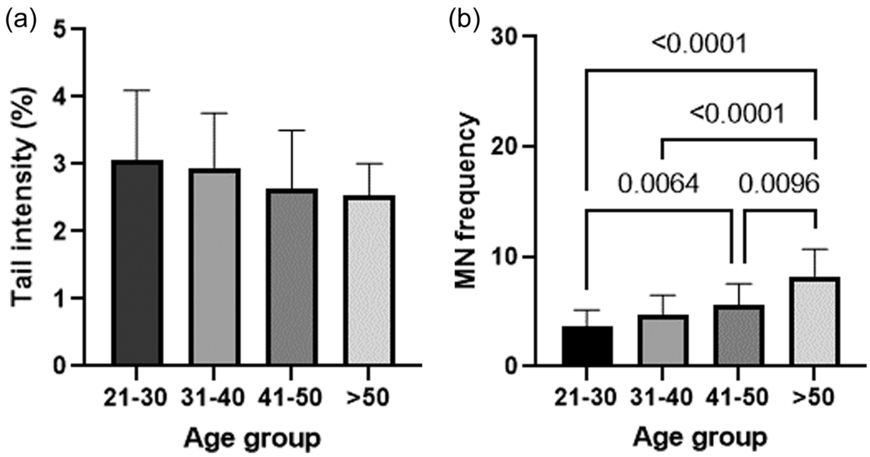

FIGURE 9 The distribution of tail intensity (a) and MN frequency (b) among different age groups. Data presented as mean ± SD. Statistical significance was at 0.05.

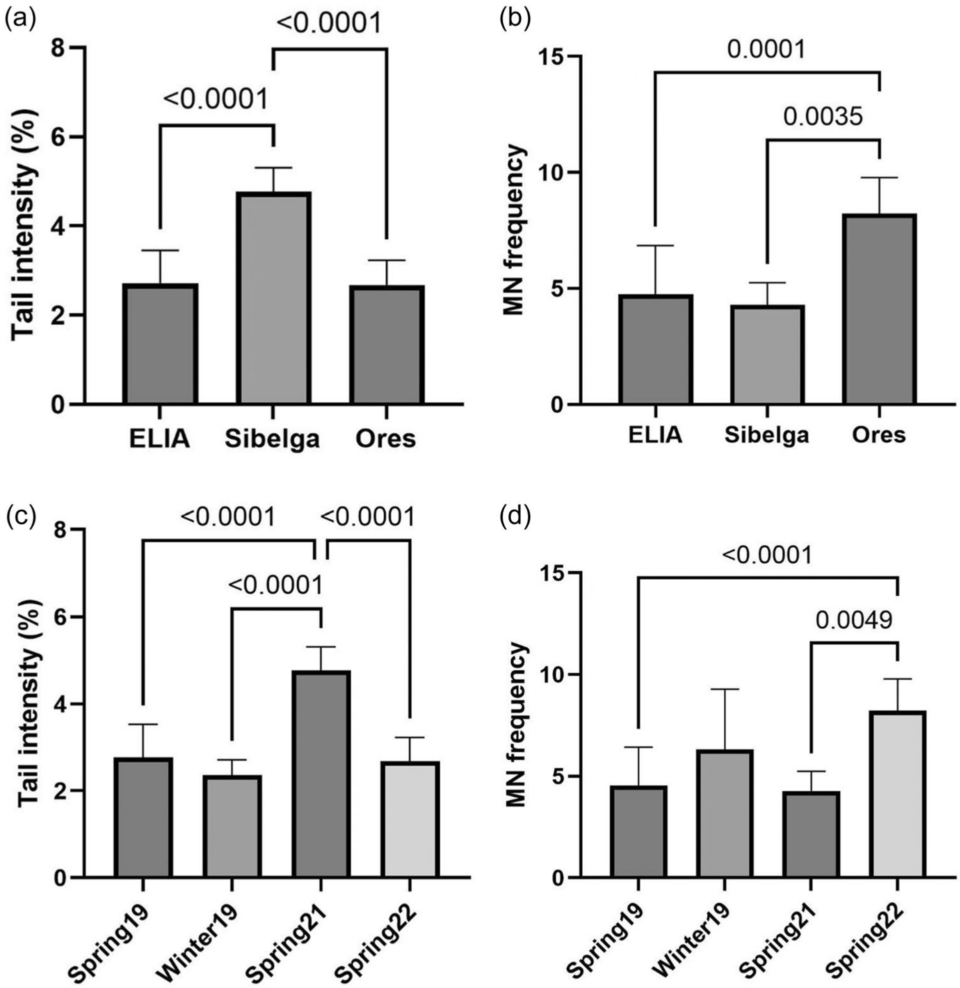

FIGURE 10 Effects of sample origin (companies) and sampling time on the results of the comet assay (a, c), and the cytokinesis-blocked micronucleus assay (b, d). Data are presented as mean ± SD. Statistical significance was at 0.05.

TABLE 10 Results of the multiple regression analyses on the comet and CBMN results.

Independent variables	Analysis on comet data: Coefficient	95% confident interval		t	p > |t|	Analysis on CBMN data: Coefficient	95% confident interval		t	p > |t|
Sample origin										
ELIA	0					0				
Sibelga	0 (omitted)[a]					0 (omitted)				
ORES	0 (omitted)					0 (omitted)				
Sampling time										
Spring 2019	0	0	0	0	0	0	0	0	0	0
Winter 2019	−0.2572	−0.7989	0.2845	−0.95	0.347	0.7797	−0.8863	2.4456	0.93	0.354
Spring 2021	1.7997	1.1514	2.4480	5.53	0.000	0.0376	−1.6970	1.7722	0.04	0.966
Spring 2022	0.1041	−0.6767	0.4685	−0.36	0.718	2.7142	1.2090	4.2194	3.59	0.001
Age	−0.0158	−0.0348	0.0033	−1.65	0.103	0.0952	0.0450	0.1455	3.78	0.000
Smoking					0.017					0.476
No smoking	0	0	0	0		0	0	0	0	
Past smoking	0.5222	0.0964	0.9480	2.44		0.4145	−0.7400	1.5690	0.72	
Exposure duration						0.0007	−0.0055	0.0069	0.23	0.821

Abbreviation: CBMN, cytokinesis-block micronucleus.
[a]Omitted because of the collinearity.

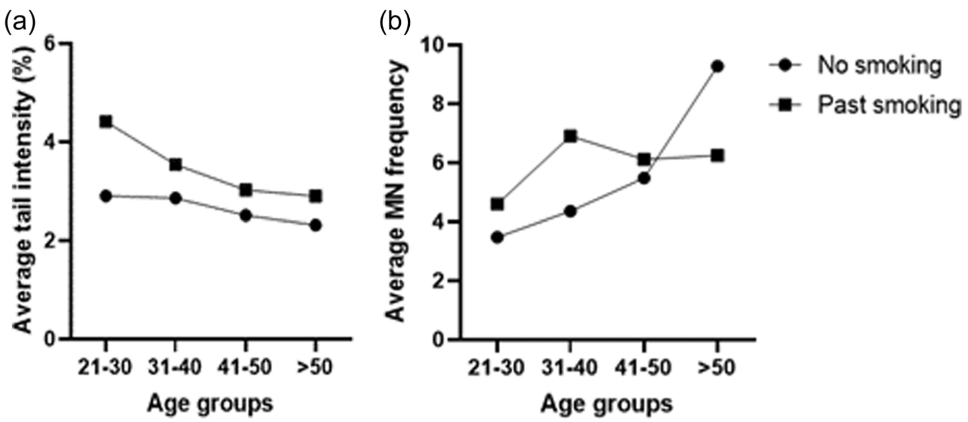

FIGURE 11 Interaction plots for comet assay results (a) and cytokinesis-block micronucleus (CBMN) assay results (b) showing the effects of age and smoking on the test outcomes.

were always higher than those values in the non-smoking groups in all studied age groups.

A reverse result was obtained when multiple linear regression was used to investigate the relationship between age, exposure duration, smoking, sample origin, sampling time, and MN frequency (Table 10). In this analysis, the results indicated that age significantly affected the CBMN results ($\beta = 0.0952$, $p = 0.0000$). However, smoking had no significant effect on the test outcome ($\beta = 0.4145$, $p = 0.4760$). Based on the observed results, we included an interaction term to model possible variation in the effect of smoking status for different values of age. Our model suggests that there was indeed an interaction between smoking status and age on the CBMN results ($p = 0.017$) (Table 11). The interaction plot indicated that MN frequency increased considerably with age in the non-smoking group. The past smoking group had higher MN frequencies compared to the non-smoking group in most of the age groups, except for the group >50 years.

TABLE 11 Linear regression output on the effects of smoking and age on the CBMN results.

Independent variables	Coefficient	SE	95% confident interval		t	p
Smoking	6.5071	2.4457	1.6349	11.3793	2.66	0.0100
Age	0.1522	0.0260	0.1005	0.2039	5.86	0.0000
Smoking × Age	−0.1428	0.0583	−0.2590	−0.0266	−2.45	0.0170

Abbreviation: CBMN, cytokinesis-block micronucleus.

In the past smoking group, we only observe a significant increase in MN frequency between groups aged higher than 30 years and groups aged lower than 30 years.

4 | DISCUSSION

This biomonitoring study aimed to investigate the potential genotoxic effect of long-term occupational exposure to 50 Hz MF on PBLs of workers from the electrical sector. The evaluation of the genotoxic effects via the comet and the CBMN assay was accompanied by the quantitative measurement of their actual MF exposure.

Considering the individual MF exposure data, we observed a considerable variation in MF exposure between and within days of measurement. There was also a notable difference in exposure levels between workers in the same job group, especially in the technician group. This observation could be explained by the fact that MF is ubiquitous, and the exposure depends largely on the distance to the MF sources. Besides, except for some peaks of high exposure, workers were often exposed to a quite low level of MF. We observed only a few workers who had some peaks of exposure (e.g., higher than 300 µT), a relatively high level but far below the 1000 µT reported in the low action level of the 2013/35/EU directive. Still, those high measurement points were observed briefly and did not represent much over the measurement period (3 days).

We chose the median as exposure metric because our obtained exposure data followed neither normal nor log-normal distribution. This observation was in contrast with previous studies in which the exposure data were often assumed to be log-normally distributed (Behrens et al., 2004; Patterson et al., 2005; Seixas et al., 1988; Vila et al., 2017). However, this is not the first time this kind of distribution has been reported (Choi et al., 2018; Decat et al., 2009). Although choosing a specific exposure metric to represent average exposure is common in MF exposure studies, no exposure metric has been confirmed relating to a biological response yet. Until now, numerous metrics have been proposed to summarize occupational exposure (IARC, 2002; Verrier et al., 2005; Villeneuve et al., 1998). Among them, metrics describing average exposure were in favor and were considered to be strongly correlated with biological effects (Verrier et al., 2005). However, we should keep in mind that the use of some average values, for example, arithmetic mean might lead to the overestimation of exposure as it is very sensitive to extreme values.

In our study, the average levels of occupational MF exposure in both job groups (office workers: 0.02 µT [0–0.08 µT]; technicians: 0.16 µT [0.02–1.31 µT]) were quite low compared to previous HCB studies. For example, Zendehdel et al. (2019) reported median MF exposure levels of 11 (4–50) µT and 0.85 (0.4–25.5) µT in the groups of utility workers and support personnel (control subjects), respectively. In another study by Touitou et al. (2013), the geometric mean of individual exposures ranged from 0.1 to 2.6 µT and from 0.004 to 0.092 µT for the exposed (technicians who worked at substations) and control subjects, respectively. The low exposure levels observed in our study population might be due to the nature of the work performed by the subjects included in our study. For example, for some technicians, their work did not require them to spend all day close to energized conductors. In addition, due to the company's strict safety procedures avoiding unnecessary exposure from energized equipment (working-off voltage, safe approach distances, limiting exposure time), the workers' actual exposure to MF could be significantly reduced. Besides, the use of the median, which is insensitive to extreme values, as the measure of central tendency might also explain our results. Lastly, previous studies often relied on spot measurements to assess the workers' exposure, which might overestimate workers' actual exposure levels.

It is undeniable that job title is a useful indicator of exposure, especially in the study's recruitment stage (Kelsh et al., 2000; Sorahan & Swanson, 2017). Job title can reflect the worker's MF exposure situations and provide information on confounders at the workplace, for example, heavy metal exposure. Nevertheless, considering the summarized personal exposure data, we found evidence showing that relying solely on job titles could lead to the misclassification of seven office workers (accounting for 19.44% of office workers) into exposure groups. These workers were exposed to higher than 0.1 µT MF on average at work and therefore were excluded from this study. As the average daily MF exposure is about 0.1 µT in the adult population, we used this value to define the exclusion criterion (Brix et al., 2001; Magne et al., 2020). Using this criterion, we also excluded two more workers (one office worker and one technician) because their average residential exposure was higher than 0.1 µT.

Exposure group classification relying solely on actual MF exposure data also raised several concerns. For instance, the use of personal monitoring as the gold standard has to be tempered as some errors may occur, for example, the selection of untypical days for measurement, the sampling rate, and the way workers wear the exposimeter. Moreover, it gives no idea of past exposure, which is necessary for epidemiological studies and studies on the effects of accumulative MF exposure. In addition, it should be emphasized that collecting exposure data from three typical consecutive days, although already quite long compared to many studies, might still not correctly reflect the workers' actual exposure. In this case, repetition of exposure measurement is a must for accurately assessing the workers' exposure (Foliart et al., 2002). Lastly, obtaining realistic cutoff values for exposed groups based on the scientific literature is a huge challenge since each publication provides different values. Percentile-based cutoff values could finely divide the study population into equal groups. However, precautions must be taken since percentiles are subject to change depending on the inclusion/exclusion of participants in the study.

From our perspective, when being applied separately, both job title and exposure data have strengths and limitations. Therefore, we proposed using both personal exposure data and job titles to define exposure groups. In fact, the strategy of using additional information to job titles (i.e., specific work location, load data in relevant years) to lower the risk of job title-based group misclassification was already applied in previous studies (Renew et al., 2003; Sorahan & Mohammed, 2014; Sorahan & Swanson, 2017; Thériault et al., 1994). In this study, we performed hierarchical agglomerative cluster analysis on personal occupational exposure data when 10 different exposure metrics were applied. The list of exposure metrics was selected based on the results of previous studies (Verrier et al., 2005). The obtained results confirmed that it is possible to use cluster analysis to group workers with the same exposure features. Regardless of the overlap in exposure values among clusters, there was a clear difference between the low- and high-exposed clusters. Then, by considering the partition of job titles in the newly formed clusters, we obtained two new groups with 28 office workers in the low-exposed cluster and 13 technicians in the high-exposed cluster. This new set of subjects, though smaller than the original one, consists of two well-differentiated groups. Apparently, the reduction of the study population is the main limitation of this proposed method. However, this problem can be addressed by recruiting more subjects, especially those exposed to a higher level of MFs. To date, we only found two instances in the literature where cluster analysis was applied (Tognola et al., 2019; Verrier et al., 2005). Although Verrier et al. (2005) used cluster analysis to identify a combination of exposure metrics to represent chronic ELF-MF exposure, Tognola et al. (2019) applied this method to investigate the link between ELF-MF residential exposure patterns and environmental variables. To the best of our knowledge, no previous study applying cluster analysis in defining occupational exposure groups has been published so far.

In this study, the effects of other confounding factors, such as age, gender, smoking, sampling time, sample origin, and number of X-rays were also taken into consideration. At first, regression analysis of the association between the tail intensity and MN frequency data and each of the confounding variables indicated that age, past smoking, sampling time, and sample origin might have a significant effect on the test outcomes. It is worth noting that though we found statistically different results, all the obtained genotoxicity damage data were at the same levels of the baseline of tail intensity and MN frequency observed in the general public (Fenech et al., 2003; Milić et al., 2021). Also, the weight of the significant results may be lessened due to the inequality in sample size between study groups. For example, there were only 14 workers who used to smoke compared to 65 nonsmoking workers. Considering sample origin, we recruited three groups of 67, 5, and 7 workers from ELIA, Sibelga, and Ores, respectively. As the recruitment of workers from the two latter companies was carried out during the Covid-19 period, it was a huge challenge to recruit and organize an appointment with participants. Consequently, we ended up with a small number of participants from these two companies.

Age seemed to have consistently significant effects on both comet and CBMN test results. The effects of age on comet and CBMN outcomes were well described in literature (Fenech & Bonassi, 2011; Milić et al., 2021). In particular, Milić et al. (2021) observed a small variation in tail intensity between age groups. The higher tail intensity was associated with a higher age group in some datasets. Fenech and Bonassi (2011) also reported that the baseline MN frequencies in subjects aged higher than 40 years were higher than those aged less than 40 years (7 vs. 6.3). In this study, we also observed a positive correlation between age and CBMN frequency and a negative correlation between age and tail intensity. In human biomonitoring studies, human peripheral blood samples should ideally be collected from young individuals (~18–35 years of age) (OECD 487, 2016). However, to enlarge our study population, we decided to recruit workers aged between 18 and 65 years. Age was therefore considered as a variable in our analysis. Regarding the effects of smoking, we could find several studies reporting the effects of smoking on DNA damage in literature (Hoffmann et al., 2005; Söylemez Yeşilçimen et al., 2012). In this study, a *t* test on tail intensity/MN frequency between no smoking and past smoking groups indicated that the latter group had a significantly higher level of DNA and chromosomal damage than the former group. However, the significant effect of past smoking on the CBMN results was not detected in the multiple regression analysis.

In conclusion, our results suggest that exposure groups should be defined based on both personal exposure data and job titles. In the end, there was no evidence of an association between occupational exposure to 50 Hz MF and increased DNA or chromosomal damage in workers.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

ETHICS STATEMENT

This research was conducted in strict adherence to ethical principles and guidelines. The study protocol underwent a thorough review and received approval from the Ethics Committee of ULB-Erasme on the 6th of November 2018 (CCB B406201837179).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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