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ORIGINAL ARTICLE

Urinary 6-sulfatoxymelatonin excretion in humans during domestic exposure to 50 hertz electromagnetic fields

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Abstract

OBJECTIVES. Exposure to extremely low frequency electromagnetic fields (ELF-EMF) has been suggested to suppress melatonin secretion, which might result in higher cancer risks because of its missing oncostatic action. We investigated the effects of residential exposure to ELF-EMF on the excretion of urinary 6-sulfatoxymelatonin (6-OHMS), the major melatonin metabolite, as an indicator of nocturnal melatonin secretion.

METHODS. 6-OHMS was measured in two spot urine samples, collected at 22.00 h and 08.00 h, in 29 men and 22 women. Spot ELF-EMF measurements were conducted at the centre and the four angles of the living room, the bedroom, and the kitchen of study subjects at low current configuration (all lights and appliances turned off), and they were repeated immediately at high current configuration (all lights and appliances turned on).

RESULTS. Risk of a reduced 6-OMHS nocturnal secretion was elevated for daily alcohol intake (OR = 6.4; 95%C.I. 1.4,33.1), and body mass index (BMI) above the median (OR = 2.2; 95%C.I. 0.5,9.6). Risk of disrupted rhythm of 6-OHMS excretion was moderately elevated for domestic ELF-EMF exposure above the upper tertile at low current configuration (OR = 2.6; 95%C.I. 0.4,15.7).

CONCLUSION. Alcohol consumption, BMI, and gender seem to affect nocturnal melatonin secretion, while an effect of residential exposure to ELF-EMF is uncertain. Future studies should properly account for the effect of such variables, when addressing the hypothesis of disturbances in melatonin secretion as a plausible explanation for the reported excess risk of several tumoral diseases associated with low level ELF-EMF exposure.

Introduction

Despite considerable methodological advances, two decades of extensive epidemiological research into a possible causal link between exposure to electromagnetic fields (EMF) and cancer have not resolved this conceivably important public health issue. Stevens et al. [22,23] suggested that extremely low frequency (ELF) EMF and/or light may have melatonin-mediated effects on cancer. Interestingly, further to providing a biologically plausible etiologic link between ELF-EMF and cancer, alterations in melatonin levels may be an intermediate endpoint for epidemiological studies "… that respond over short periods of time rather than long periods" [19]. The aim of our study was to explore effects of residential exposure to ELF EMF on the rhythm and level of 6-hydroxymelatonin sulfate (6-OHMS) urinary excretion in men and women, in a Mediterranean area with smaller seasonal differences in hours of daylight compared to the areas previously covered by similar studies. 6-OHMS is the major metabolite of melatonin, and its levels are strongly correlated with blood melatonin concentration [16]. We explored its use as a biomarker of alterations in melatonin secretion as a response to domestic ELF-EMF exposure, to assess its validity as a surrogate endpoint for future studies on the melatonin hypothesis.

Material and methods

We asked to the first one hundred individuals participating in a population based case-control study of lymphoma in the Sardinia region of Italy, to participate in this side study to test the relation between 6-OHMS concentration in a spot urine sample and residential ELF-EMF exposure. Subjects were incident lymphoma cases or population controls, aged 25–74 years. Controls were a random sample of the resident population, frequency matched to cases by age, gender and province of residence. However, in the first months of the case-controls study, participating subjects were mainly cases. All subjects were interviewed in person at their residence by a specially trained interviewer, and signed an informed consent form. The questionnaire included information on demographics, education, health history, occupational history, and diet. The study was approved by the Ethical Committee of the University Polyclinic, University of Cagliari. None of the co-authors is or has been involved in activities generating conflict of interest, that would prejudice their impartiality in the matters discussed in this paper. Fifty one subjects, 29 men and 22 women accepted participation in this side study. Study subjects were instructed to collect an urine spot sample at their residence in a plastic vial with a screw cap, late at night (around 22.00 h), and early in the morning after (around 08.00 h), and preserved it refrigerated up to delivery to the personnel of the Occupational Health Section of the Department of Public Health, University of Cagliari, the same day. Thirty subjects provided both samples, and 21 only the morning sample.

As highest plasma melatonin concentrations are reached between midnight and 04.00 h [10], we measured the main melatonin metabolite, 6-hydroxymelatonin sulphate (6-OHMS), in the urine of study subjects at 22.00 h., as a surrogate of day time plasma melatonin levels, and the morning after at 08.00 h, as a surrogate of night time melatonin plasma levels. Urinary 6-OHMS excretion was measured with a competitive ELISA assay (DRG Diagnostics, Marburg, Germany). Fifty µl of 1:51 solutions of the samples were added with 50 µl of melatonin sulphate peroxidase-conjugate, and 50 µl of antiserum from rabbit. After incubating for 120 minutes at room temperature in a orbital shaker, samples were washed four times with 250 µl phosphate buffer, added with 200 µl of a 1:31 tetramethylbenzidine and hydrogen peroxide solution, and allowed to react for 30 minutes at room temperature. One hundred µl of 1M sulphuric acid were subsequently added as the stopping solution. Readings at 450 nm wavelength within 1 hour were compared to the standard curve in ng/ml. The intra-assay coefficient of variations were 5.4% and 3.8% for 10 control samples of 7.27 and 45.2 6-OHMS ng/ml, respectively. The inter-assay coefficients of variation were 8.7% and 8.5% for another 10 control samples of 6.21 and 41.48 6-OHMS ng/ml, respectively. Recovery rate was 99–110% at concentrations ranging 41.6–87.2 ng/ml. The lowest detectable level was 1 ng/ml in the undiluted sample.

ELF-EMF exposure was assessed using a standard portable programmable instrument (Emdex II, Ampere SpA, Milan, Italy), which captures the magnetic flux density of three axes and computes the resultant on the range 0–300 Hertz, with a 1–3% range of error. Spot measurements were made for 15" each in the centre and the four angles the living room, kitchen, and bedroom of the residence home of study participants, first with all lights and appliances turned off (low current configuration), and afterwards with all lights and appliances, as allowed by the electric power supply, turned on (high current configuration). For each home, we calculated the mean of ELF-EMF readings across all positions.

Due to the heavily skewed distribution of the means of ELF-EMF readings, their median and interquartile (I.Q.) range were used as the summary statistics for the total study population and subgroups, and comparisons across subgroups were performed using non parametric tests (Mann-Whitney test for independent series), or the Student's *t* test when appropriate. In the univariate regression analysis, urinary 6-OHMS was tested against age, BMI, weight and height separately, and years of education, using the Pearson's correlation coefficient or the Spearman's correlation coefficient, as appropriate. Multivariate analysis was also performed with unconditional logistic regression analysis, to model the odds ratio (OR) for 6-OHMS excretion in the morning urine sample equal or below the lowest tertile, and for a dis-

rupted (less than 50% increase in the morning urine or reversed ratio) rhythm of urinary 6-OHMS excretion, as a function of covariates showing an association in the univariate analysis, and ELF-EMF exposure above the upper tertile at low and, alternatively, high current configuration, adjusting by age (continuous), and gender. ORs, and the respective 95% confidence intervals (95% C.I.) with the Wald method, were calculated using the GMBO program included in the Epicureâ software. Two-tailed tests were calculated to assess the probability associated with the null hypothesis.

Results

Mean age of study participants was 56.6 years (*sd* 13.3) (Table 1). BMI was smaller among women, who tended to be more educated than men. They less frequently drank alcohol beverages (daily alcohol intake: women: 6/22, rate 0.27; men: 18/29, rate 0.62) or smoked (current smokers: women: 6/22, rate 0.27; men: 11/29, rate 0.38). However, six out of seven study subjects taking medication previously suggested to influence 6-OHMS levels were females. Blood draw was more frequent in spring for women, and in summer and autumn for men.

The median urinary 6-OHMS concentration on morning urine samples was 44.5 ng/ml (I.Q. range 15.9 – 60.0). Values did not differ by gender (Mann Whitney test: 0.10, p =0.32). 6-OHMS concentration in both the night and morning urine spot samples, as well as the ratio between morning and night concentrations, did not show a correlation with age. However, a modest inverse correlation of morning concentrations with age was observed among women (Pearson's correlation coefficient = –0.317; 0.10>p>0.05), similar to that reported in a Swedish study among patients of both genders (r = – 0.31) [1]. Education, smoking, taking medications, health status, and season of urine sampling did not affect 6-OHMS excretion (Table 2). Height showed a positive correlation with 6-OHMS excretion in the morning urine among men, but not among women, while weight and BMI showed a modest negative correlation among women. Readings were significantly lower among daily alcohol drinkers (Mann Whitney test: –2.55, p =0.011). Only two men worked on night shifts. However, due to the study protocol, both had a day work shift the day before blood draw. The urinary 6-OHMS morning excretion was below the lowest quartile for one man, and above the median for the other man. Neither provided the night urine sample, and therefore we couldn't assess the morning/night ratio of 6-OHMS excretion in these men. Another subject had been working with a schedule including night shifts up to 2 years before blood draw. He had a 6-OHMS concentration below the lowest quartile in the morning urine, and showed a reversed morning/night ratio of 6-OHMS excretion. We did not exclude these subjects from the analyses.

The median ELF-EMF intensity at the residence of study subjects was 0.028 μT (I.Q. range 0.004–0.083) for low current configuration, with only 5/51 homes (rate: 0.10) with an average intensity equal or greater than 0.2 μT. The median ELF-EMF intensity at high current configuration was about twice (0.057 μT, I.Q. range 0.023–0.148), and 6/51 homes (rate: 0.12) had an average intensity equal or greater than 0.2 μT. The distributions of the average ELF-EMF intensity at both high current and low current configurations were skewed to the right (not shown in the tables), and strongly correlated to each other (Pearson's correlation coefficient = 0.895, p < 0.001). The median ELF-EMF exposure at either low or high current configuration did not differ by health status in both genders (not shown in the tables). In the correlation analysis, residential ELF-EMF intensity at either low and high current configuration did not affect morn-

Table 1. Selected characteristics of the study population.

	Men (N =29)	Women (N =22)	Total (N=51)
Age (*mean* ± *sd*)	57.2 ± 12.4	55.9 ± 14.7	56.6 ± 13.3
Height (*mean* ± *sd*)	1.71 ± 0.05	1.58 ± 0.06	1.66 ± 0.09
Weight (*mean* ± *sd*)	75.3 ± 11.9	57.2 ± 11.6	67.7 ± 14.8
BMI (*mean* ± *sd*)	25.7 ± 3.6	23.0 ± 4.7	24.6 ± 4.3
Education (years: *mean* ± *sd*)	9.2 ± 4.8	10.3 ± 6.7	9.6 ± 5.6
Domestic ELF-EMF exposure			
Low current configuration (mT: *median, IQ range*)	0.015 0.001 – 0.037	0.038 0.008 – 0.129	0.029 0.004 – 0.079
Urinary 6-OHMS excretion			
Morning, *n*	29	22	51
ng/ml: *median (IQ range)*	37.0 (17.4–48.5)	49.0 (19.0–60.0)	44.5 (17.2–60.0)
Night, *n*	19	11	30
ng/ml: *median (IQ range)*	18.0 (9.3–44.3)	19.0 (5.4–24.0)	18.5 (7.2–32.2)
Morning/night ratio: n, mean (sd)*	19 3.0 (3.0)	11 8.9 (12.3)	30 5.3 (8.26)
Flat/reversed rhythm: *n (rate)*	9 (0.50) ǂ	4 (0.36)	13 (0.45)

Note: ǂ rate was calculated over 18 men who provided both morning and night urine spot samples. * $t = 3.87$; $p < 0.01$

Table 2. 6-OHMS excretion in the morning by gender and selected variables.

	Men *N corr. coeff.* ‡	***Women*** *N corr. coeff.* ‡	***Total*** *N corr. coeff.* ‡
Age	29 –0.095	22 –0.317	51 –0.135
Height	29 0.373*	22 0.042	51 0.215
Weight	29 0.263	22 –0.315	51 0.130
BMI	29 0.116	22 –0.320	51 0.010
Education	29 0.310	22 0.201	51 0.232
	Men N median (I.Q. range)	Women N median (I.Q. range)	Total N median (I.Q. range)
Smoking			
Non smokers	18 37.0 (16.1–49.0)	16 49.0 (23.0–61.5)	34 44.7 (17.2–60.0)
Current smokers	11 38.0 (21.5–47.7)	6 46.5 (20.0–58.7)	17 41.5 (19.2–56.2)
Alcohol			
Non drinkers	11 49.0* (45.2–77.2)	13 48.0 (38.0–60.0)	24 48.0** (41.0–68.5)
Social drinkers	– –	3 55.0 (52.5–57.5)	–
Daily alcohol intake	18 22.0* (15.0–40.7)	6 20.0 (9.5–55.7)	24 22.0** (14.0–42.1)
Use of medication			
no	27 39.0 (14.7–64.5)	15 48.0 (31.5–60.0)	42 44.0 (21.0–60.0)
yes ‡‡	1 16.0 –	6 39.0 (14.7–64.5)	7 17.0 (14.7–63.0)
Season blood draw			
Spring	5 15.5 (13.0–27.0)	8 54.0 (32.7–61.5)	13 38.0 (15.5–60.0)
summer	9 46.0 (21.0–69.0)	5 46.8 (12.0–50.0)	14 46.3 (15.7–64.2)
autumn	9 40.0 (31.0–45.0)	3 44.0 (25.7–74.5)	12 40.5 (29.0–45.7)
winter	6 50.0 (14.8–60.0)	6 57.5 (32.5–60.0)	12 55.0 (19.9–60.0)
Night shift work			
Current (*n, range*)	2 14.0 – 48.0	0 –	–
In the past 2 years (*n, value*)	1 10.0	0 –	–
Health status			
Lymphoma patients	21 37.0 (17.4–46.2)	18 49.0 (19.0–60.0)	39 43.0 (17.2–53.7)
General population group	8 50.0 (20.9–93.7)	4 52.0 (34.2–62.0)	12 52.0 (20.9–71.7)

Notes: ‡ Pearson's correlation coefficients.
I.Q. range = interquartile range. ‡‡ β blockers, calcium channel blockers, antianxiety drugs, nonsteroidal anti-inflammatory drugs. Daily drinkers vs non drinkers:
* men: Mann Whitney test = 2.55; p = 0.011; ** total: Mann Whitney test = 2.73; p = 0.006

Table 3. Median and interquartile (I.Q.) range of 6-OHMS excretion in the morning urine, by quartiles of residential ELF-EMF exposure.

ELF–EMF (μT)	Men N median I.Q.range	Women N median I.Q.range	All N median I.Q.range
Low current configuration			
≤ 0.0045	9 40.5 (30.0–49.1)	4 60.0 (49.2–62.0)	13 43.2 (30.0–60.0)
0.0046 – 0.028	9 34.0 (18.0–50.0)	5 38.0 (14.0–46.8)	14 36.0 (15.1–49.2)
0.029 – 0.083	7 46.0 (18.5–47.3)	4 10.0 (7.2–20.0)	11 23.0 (12.5–46.3)
≥ 0.084	4 17.5 (13.0–33.0)	9 60.0 (50.0–80.0)	13 55.0 (26.0–69.0)
High current configuration			
≤ 0.0227	9 32.5 (24.7–45.0)	4 38.5 (14.6–62.0)	13 32.5 (17.7–60.0)
0.0228 – 0.057	9 45.0 (21.0–46.7)	4 49.0 (32.0–60.0)	13 45.0 (21.0–48.0)
0.058 – 0.147	6 34.5 (17.4–131)	6 45.4 (20.0–71.7)	12 45.0 (14.6–86.2)
≥ 0.148	5 21.0 (14.0–69.0)	8 52.5 (42.5–61.5)	13 50.0 (21.0–66.0)

Table 4. Residential ELF-EMF exposure metrics by rhythm of 6-OHMS excretion.

Rhythm of 6-OHMS excretion	N median I.Q. range
Low current configuration	
Regular rhythm	18 0.037 (0.006–0.158)
Disrupted rhythm	12 0.043 (0.006–0.134)
High current configuration	
Regular rhythm	18 0.062 (0.025–0.176)
Disrupted rhythm	12 0.078 (0.035–0.224)

ing 6-OHMS levels, nor night 6-OHMS levels, nor the morning/night 6-OHMS ratio (not shown in the tables). Results by gender were likewise. However, men with the highest residential ELF-EMF exposure at low and high current configuration had apparently lower 6-OHMS concentrations in the morning urine (Table 3), although numbers were too small for any inference to be drawn (upper quartile versus lower: Mann-Whitney test: –0.35, $p = 0.726$; upper quartile versus lower: Mann-Whitney test: 0.95; $p = 0.342$).

Both night and morning urine samples were available for 30 subjects, 19 men and 11 women. As expected, urinary 6-OHMS concentration reproduced that of melatonin secretion as an integrated summary measure of plasma levels in the preceding hours (Table 1). Therefore, we observed the highest concentration in the morning samples, reflecting the top plasma concentration at night time. The morning/night ratio was higher among women (about 9-fold on average) than men (about 3-fold) ($t = 3.87$; $p < 0.001$). Twelve subjects showed a disrupted rhythm (i.e. a morning/night flat or reversed ratio) of 6-OHMS excretion. Subjects with a disrupted rhythm of 6-OHMS excretion, owed their alteration not only to a significant reduction of 6-OHMS excretion in the morning urine (= a reduced melatonin secretion at night time; Mann-Whitney test = 2.34; $p = 0.019$), but also to a significant increase in the 6-OHMS concentration in the bedtime urine sample (=an increased melatonin secretion at day time; Mann-Whitney test = 13.38; $p = 0.0001$). The median ELF-EMF residential exposures at either low and high current configurations were similar between subjects with regular 6-OHMS excretion rhythm and subjects with disrupted 6-OHMS excretion rhythm (Table 4).

Factors showing an association with low 6-OMHS excretion in the morning or a disrupted rhythm of 6-OMHS excretion were reciprocally adjusted for in the multivariate analysis (Table 5). After adjusting for age and gender, BMI above the upper tertile, and domestic ELF-EMF exposure above the upper tertile, did not show a significant association with a reduced 6-OHMS melatonin concentration in the morning urine or a disrupted rhythm of 6-OHMS excretion. The odds ratio for ELF-EMF exposure above the upper tertile at low current configuration was about 2.5-fold for a disrupted rhythm of 6-OHMS excretion, but statistical power was not enough for any inference to be drawn in this regard. Daily alcohol intake was strongly associated with low 6-OHMS melatonin concentration in the morning urine, with a significant more than 6-fold excess risk. However, no association of daily alcohol intake was observed with a disrupted 6-OHMS excretion rhythm.

Discussion

While the evidence for light's pivotal role in regulating of melatonin secretion is robust and unequivocal [4, 10,20], it is still unclear whether EMF in other parts of the spectrum modulate melatonin secretion significantly. Reduction of pineal melatonin synthesis, NAT activity, and serum melatonin levels were observed in relation to exposure to 60-Hz power-frequency electric fields in Sprague-Dawley rats [27], although results were not always consistent [10]. Also for magnetic fields results varied inconsistently by rodent species, sexual maturity (in hamsters), and the physical characteristics of the magnetic field [10].

Table 5. Odds ratios for selected covariates associated with 6-OHMS excretion below the lowest tertile in the morning urine, and flat/reversed rhythm of 6-OHMS excretion: results from the logistic regression analysis adjusted by age and gender.

Covariates	6-OHMs below lowest tertile in the morning urine cases/ctrls OR 95% C.I.	Disrupted rhythm of 6-OHMS excretion cases/ctrls OR 95% C.I.
ELF-EMF at low current configuration		
BMI above the upper tertile (26.2)	9/10 2.2 0.5 – 9.6	3/7 0.2 0.02 – 1.7
Daily alcohol consumption	13/11 6.9 1.4 – 33.1	6/6 1.5 0.2 – 9.9
ELF-EMF above upper tertile (0.038 μT)	5/13 0.8 0.2 – 3.6	7/7 2.6 0.4 – 15.7
ELF-EMF at high current configuration		
BMI above the upper tertile (26.2)	9/10 2.3 0.5 – 9.7	3/7 0.2 0.03 – 1.8
Daily alcohol consumption	13/11 6.8 1.4 – 33.3	6/6 1.3 0.2 – 8.9
ELF-EMF above upper tertile (0.080 T)	6/12 1.0 0.2 – 4.2	6/7 1.2 0.2 – 7.0

Exposure of lambs to a 4 microtesla EMF for one year did not change their nocturnal secretion of melatonin or age at onset of puberty or oestrus [11]. Baboons exposed to a 100 microtesla EMF for 6 weeks did not suffer alterations of melatonin plasma concentrations during the experiment. However, when the type of exposure changed to a rapid field onset/offset, a 15% drop in melatonin plasma concentration was observed [17,18]. Nocturnal acute exposure to either continuous or intermittent 50-Hz linearly polarised 10 mT magnetic fields did not affect melatonin secretion in healthy human volunteers [21]. On the other hand, when exposure was prolonged up to 4 nights, a possible cumulative effect of magnetic field exposure on the stability of individual melatonin measurements over time appeared [7]. Men working more than 2 hours per day in a substation and 3-phase environment, with exposure to 50–60 Hz magnetic fields, showed a magnetic-field dependent reduction in the adjusted mean nocturnal and post-work urinary (6-OHMS) levels [2]. Women occupationally exposed to a ELF-EMF flux density above 1 μT showed a decreased 6-OHMS excretion on Friday compared to the reference group [8]. Therefore, circular or elliptical magnetic field polarisation, or other factors linked to substations and 3-phase electricity, and elevated ELF-EMF exposures seem associated with magnetic field induced melatonin suppression.

6-OHMS is the major metabolite of melatonin, and its levels are strongly correlated with blood melatonin concentration [16]. A Canadian study of women living in proximity of a 735 kV power line did not exhibit a change in the 6-OHMS night-time urinary concentration [12]. However, body mass index (BMI) and age were significantly associated with decreased 6-OHMS concentrations, and the trend of decreasing 6-OHMS with age and BMI was more pronounced among women living near power lines. On the other hand, among women from State of Washington (U.S.A.), lower nocturnal 6-OHMS levels were associated with more hours of daylight, older age, higher BMI, alcohol consumption, and regular use of β-blockers, calcium channel blockers, or psychotropic medication [3]. Women using these medications had a significant inverse slope in log 6-OHMS night time concentration by night time EMF level in the bedroom.

In the present study, in which urinary 6-OHMS excretion was measured, low intensity residential ELF-EMF exposure had no apparent effect on nocturnal melatonin secretion, while the observed association with a disrupted 6-OHMS excretion rhythm was uncertain. 6-OHMS concentration in the morning urine was not affected by age, education, or season of urine sampling. Consistently with another report [3], daily alcohol intake was strongly associated with reduced concentrations of 6-OHMS. Only one man and six women reported taking medications which reportedly affect melatonin secretion [3]. We did not find a decrease in 6-OHMS excretion among subjects regularly taking such medications. Subjects working in night shifts were too few for any interpretation of the observed reduction in 6-OHMS concentration in the morning urine. In the multivariate analysis, risk of low nocturnal 6-OHMS secretion was moderately elevated for BMI above the upper tertile, and risk of disrupted 6-OHMS rhythm was elevated for ELF-EMF exposure above the upper tertile at low current configuration. Although not statistically significant, these findings are in agreement with other reports [3,12]. The reported seasonal changes in melatonin secretion came from studies conducted in upper northern latitudes [3,13,15] with greater seasonal differences in hours of day light compared to the Mediterranean area. In fact, latitude [26], and length of the natural photoperiod [9] appear to be the major determinants of shifts in the melatonin biorhythm.

We made assumptions, which must be considered as potentially affecting the results and their interpretation:

6-OHMS measurements were not taken during the day time of EMF measurements for practical reasons. Therefore, we had to assume that the ambient EMF intensity in the home of study subjects did not change day by day. As we tested EMF with all electrical appliances turned off, and verified that this was the main source of inter-individual variation in EMF-exposure (not shown in the tables), we are confident that our assumption was an acceptable approximation. External sources of ELF-EMF were considered in the questionnaire and double checked by the interviewer. None of the study subjects lived in proximity of electric power lines or active transforming substations.

We made only spot ELF-EMF measurements, using the average as a surrogate for exposure. Long term measurements in the bedroom or personal monitoring have been considered to be a better indicator. However, it has been shown that spot measurements are well correlated with the average of spot measurements in pre-defined points of the houses. Indeed, the average of the spot measurements of a residence resulted in least exposure measurement error [6], and spot measurements are better accepted by voluntary participants in epidemiological studies. The measured ELF-EMF levels were very low. However, as far as we know, a threshold for disturbances in melatonin secretion is not defined, and elevated childhood [14] and adult [25] leukemia risks were reported at levels within the range measured in the present study.

We assumed urinary 6-OHMS concentration in a morning urine sample to be representative of nocturnal melatonin secretion [16]. Such an assumption holds true if the subject did not waste her/his own urine during the night of participating in the study, which we cannot be certain of. We asked for two separate samples, one at night before going to bed, at approximately 22.00 h. or later, and another from the first urine of the following morning, at approximately 08.00 h or earlier, to increase the compliance of the study subjects. Subjects voluntarily accepted participation, which did not include any reward or compensation.

We also assumed similar patterns of sunlight and artificial light exposure among study participants. While this assumption is reasonably acceptable for sunlight, as all participants lived in the same area, artificial light exposure at night time is more a cause of concern. However, bedroom nocturnal light exposure and light use at night did not affect 6-OHMS concentration in the morning urine in a Canadian study [12]. Nonetheless, as visible light unequivocally affects melatonin synthesis [4,5,24], future studies on residential ELF-EMF exposure and melatonin secretion should carefully register sunlight and artificial light exposure in the hours preceding urine sampling.

The above cited study conducted among Canadian women also had information on other indicators, such as caffeine consumption in the last 24 hours and number of hours between evening and morning urination, which we did not consider in our study. Neither one showed any effect of 6-OHMS concentration [12].

In conclusion, our study of low level residential ELF-EMF exposure did not show a significant effect on the rhythm of urinary 6-OHMS concentration and on its concentration in the morning, as reflecting nocturnal peak melatonin secretion. Limitations highlighted in the discussion and in the statistical power of the present study impose caution in interpreting our results. However, future studies addressing the melatonin hypothesis as a possible and plausible explanation for the excess cancer risk reportedly associated with ELF-EMF exposure, should properly consider the effects of other variables such as gender, BMI and alcohol consumption.

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