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Genetic damage in mammalian somatic cells exposed to extremely low frequency electro-magnetic fields: A meta-analysis of data from 87 publications (1990–2007)

VIJAYALAXMI[1] & THOMAS J. PRIHODA[2]

[1]*Department of Radiation Oncology, and* [2]*Department of Pathology, University of Texas Health Science Center, San Antonio, TX 78229, USA*

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Abstract

Purpose: A meta-analysis was conducted to obtain a 'quantitative' estimate of the extent of genetic damage in mammalian somatic cells exposed to non-ionizing radiation emitted from extremely low frequency electro-magnetic fields (ELF-EMF) and to compare with that in unexposed control cells.

Methods: The methods used for the meta-analysis were recommended in several standard text books. Three specific variables related to ELF-EMF exposure characteristics were examined in the meta-analysis: (i) frequency (Hz), (ii) flux density (mT), and (iii) in occupationally exposed individuals.

Result and conclusions: (1) The difference between ELF-EMF-exposed and control cells as well as the 'effect size' due to ELF-EMF exposure were biologically small (although statistically significant) with very few exceptions. (2) At certain ELF-EMF exposure conditions there was a statistically significant increase in genetic damage assessed from some end-points. (3) The mean indices for chromosomal aberrations and micronuclei end-points in ELF-EMF-exposed and control cells were within the spontaneous levels reported in historical database. (4) Considerable evidence for publication bias was found in the meta-analysis.

Keywords: *Meta-analysis, electromagnetic fields, DNA strand breaks, chromosomal aberrations, micronuclei, sister chromatid exchanges*

Introduction

The possible effects of exposure to non-ionizing radiation emitted from extremely low frequency electro-magnetic fields (ELF-EMF) on the genetic material (DNA) are very important. Damage in the DNA of somatic cells can lead to the development of cancer or cell death. Hence, during the last several decades, researchers have used recently developed experimental techniques as well as classical cytogenetic methods to determine the extent of genetic damage in mammalian somatic cells exposed in vitro and/or in vivo to ELF-EMF in the frequency range of 0–5000 Hz. The data were published in peer-reviewed scientific journals. Vijayalaxmi and Obe (2005) reviewed the literature published during 1990–2003 and made a 'qualitative assessment' of the data reported in 63 investigations. The extent of genetic damage in all these publications was determined using one or more end-points, namely, single- and/or double-strand breaks in the DNA evaluated using the comet assay in which the comet tail length was measured in microns (SBM) and the comet tail moment expressed as a ratio (SBR, derived from the amount of DNA in comet head and comet tail), and the incidence of chromosomal aberrations (CA), micronuclei (MN) and sister chromatid exchanges (SCE). The conclusions from 29 investigations (46%) did not indicate significantly increased genetic damage in ELF-EMF-exposed cells as compared with that in sham- and/or un-exposed cells while the results from 14 studies (22%) have suggested such an increase in the former as compared with the latter cells. The observations from 20 other studies (32%) were inconclusive. Similar conclusions were drawn in earlier reviews (McCann et al. 1993, 1998, Murphy et al. 1993, Moulder 1998). The details presented in these publications revealed several

Correspondence: Vijayalaxmi, PhD, Department of Radiation Oncology, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA. Tel: +1 (210) 692 7874. Fax: +1 (210) 949 5085. E-mail: vijay@uthscsa.edu

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differences among the investigations including ELF-EMF exposure conditions, experimental protocols, etc. Any and/or all of these variables could have contributed to the controversial observations in the existing literature (Vijayalaxmi and Obe 2005).

Meta-analysis is widely used in biomedical research, especially when the outcomes of the observations in different investigations are controversial. If considered separately, any one study may be either too small or too limited in scope to arrive at a generalized and unequivocal conclusion. Analyses of the combined data from all such related studies represent an attractive alternative to strengthen the evidence from any individual study. The importance of precise methods used in analyzing the data to draw inferences from heterogeneous but logically related studies has been emphasized by Armitage (1984). A meta-analysis was conducted using all SBM, SBR, CA, MN and SCE data published in peer-reviewed scientific journals during the years 1990–2007 to obtain a 'quantitative' estimate of the extent of genetic and epigenetic damage (theoretically, it may well be that ELF-EMF exposure per se is not genotoxic, but that such exposure could enhance the damage induced by other biological, chemical and/or physical genotoxic agents, i.e., ELF-EMF+ known genotoxic agents) in mammalian somatic cells exposed in vitro and/or in vivo to ELF-EMF. Such analyses could also help in better understanding of the genotoxic/carcinogenic potential of ELF-EMF. The objectives were to: (i) Obtain a good 'quantitative' estimates of the damage reported in ELF-EMF-exposed cells compared with that in sham- and/or un-exposed control cells, (ii) study the correlation between certain specific ELF-EMF exposure characteristics (see below) and increased genotoxicity which is larger than the random variability, (iii) examine whether the damage indices in ELF-EMF-exposed cells were within the spontaneous levels reported in historical database, (iv) use multiple regression analysis to determine the combined effects of ELF-EMF characteristics (each adjusted for the others) on genotoxicity, and (v) test for heterogeneity of residual variability to indicate if other factors that were not considered in the meta-analysis could explain the effects reported in the publications. A similar meta-analysis was conducted using the genetic damage indices, reported from 1990–2005, in mammalian somatic cells exposed in vitro and in vivo to radiofrequency radiation (300 MHz to 300 GHz), and the conclusions were published recently (Vijayalaxmi and Prihoda 2008).

Materials and methods

The methods employed for the meta-analysis have been recommended in several standard textbooks: (i) *Statistical Methods for Meta-analysis* (Hedges and Olkin 1985), (ii) *Practical Meta-Analysis* (Lipsey and Wilson 2001), (iii) *Methods of Meta-Analysis: Correcting Error and Bias in Research Findings* (Hunter and Schmidt 2004), (iv) *How to Report Statistics in Medicine: Annotated Guidelines for Authors, Editors, and Reviewers* (Lang and Secic 2006) and (v) Cochrane Handbook for Systematic Reviews of Interventions 2006. A total of 87 papers were retrieved from peer-reviewed scientific publications. Each publication was examined in detail by both authors: the results reported as numbers in the Tables were documented as such while the Figures were 200% enlarged to enter the data (nearer to the actual numbers) in an Excel spreadsheet (Microsoft, WA). The quality of investigations, i.e., whether or not the researcher(s) have included sham-/unexposed-/positive-controls, 'blind' evaluations, provided detailed description of dosimetry, experimental protocols, data collection procedures, appropriate statistical analyses, and conclusions from results in the text, tables and figures being consistent were assessed and agreed upon by both authors. This information was not intended to 'rank' the publications either to exclude or include the data in meta-analysis. All data recorded from each publication in the Excel spread sheet was checked and re-checked (to ensure no errors were made during the recording process) before subjecting the data to meta-analysis.

For the same genotoxicity end-point, researchers have examined different numbers of cells in the same or different experiments. For example, investigators have reported the incidence of MN recorded in a total of 500, 1000 or 2000 cells. In such instances, Fleiss et al. (2003) have suggested a method using 'raw' data to calculate the mean, standard deviation (SD) and variance from varying number of cells examined in different experiments (when averaging over experiments and cells within experiments). However, it is almost impossible to obtain the raw data from individual investigators. Hence, for each end-point, a standardized 'unit' was obtained and used as a more homogeneous measure. The standardized unit for CA was in 100 cells, i.e., if an investigator has reported the incidence in 200 cells, it was divided by two to obtain the unit as CA/100 cells. Similarly, the standardized units were MN/1000 cells and SCE/cell. The results reported for DNA single- and double-strand breaks were pooled to obtain a standardized unit for the comet tail length in microns (SBM) and comet tail moment as radio (SBR) (the 'tail factor' used in some investigations was included in this category). The 'units' in ELF-EMF-exposed were integrated to obtain overall pooled mean and SD to designate to the 'ELF-EMF-exposed group' while similar data in controls were assigned to the 'control group'. These are the

'descriptive' data for standardized units from which the meta-analysis was conducted.

Meta-analysis

Several variables in the experimental protocols used by different investigators in different countries were identified and discussed in an earlier review by Vijayalaxmi and Obe (2005). It is beyond the scope and goals of this meta-analysis to determine the effect of all such variables. Only three specific variables related to ELF-EMF exposure characteristics were selected to determine their 'potential' influence on various genotoxicity end-points (based on 50 Hz used in Europe/Asia and 60 Hz used mainly in USA, and flux density exposure limits suggested by international organizations): (i) Frequency (Hz), (ii) flux density (mT) and (iii) occupational exposure (electric train drivers, power-line inspectors and maintenance personnel, and also individuals employed in high voltage electric supply substations). The frequency of ELF-EMF was sub-classified: (a) $\leq$50 Hz and (b) $\geq$60 Hz. The flux density was sub-classified: (a) 0.0–0.5 mT, (b) > 0.5–1.0 mT, (c) > 1.0–5.0 mT and (d) > 5 mT. The issue related to epigenetic effects of ELF-EMF exposure was investigated in rodent and human cells which were treated in vitro with a known genotoxic agent prior/during/after ELF-EMF exposure: The data reported in a total of 33 publications were also subjected to meta-analysis. The Statistical Analysis System (SAS 2006) Version 9.1 for Windows was used for all analyses described below.

Magnitude of weighted difference between ELF-EMF-exposed and controls (E–C)

The existence of variability in the data obtained from one experiment to another experiment and from one laboratory to another laboratory is well known. One of the goals of the meta-analysis was to take this variability into consideration to summarize the descriptive data. Fixed-effects models described by Lipsey and Wilson (2001) were first used to calculate the magnitude of difference between ELF-EMF-exposed and control (E–C). These models assume a single 'fixed' effect that every study will approximate within each sub-group. This conservative approach provides very 'narrow' confidence intervals (CI) and is more likely to find significant differences between ELF-EMF-exposed and control groups (as compared with random effects models which yield 'wider' CI and would not find such differences). Furthermore, the variability in the results obtained from different experiments and from different laboratories was taken into consideration to provide a 'weight' which is based on the sample size and variance in ELF-EMF-exposed and controls in each publication (Lipsey and Wilson 2001). Separate statistical analyses were performed for each genotoxicity end-point because of the differences in their standardized units and for the interpretation of the units. The method used to obtain a quantitative estimate of E–C was described in detail in the supplementary information provided in an earlier publication (Vijayalaxmi and Prihoda 2008).

Effect size (ES) or standardized mean difference (d)

Another method regularly used in the meta-analysis is to determine 'unit-less' measures called 'effect size' (ES) between ELF-EMF-exposed and controls in each publication and for each endpoint. The ES was calculated using the random-effects models suggested by Hunter and Schmidt (2004). These models are recommended by the National Research Council (1992) and are more accurate than the traditional random effects models, and have several advantages: they allow for the possibility that p values vary from one study to another, make fewer assumptions, are more conservative, and use weighting by sample size (which is critical for meta-analysis). The method also corrected for 'bias' in the estimated ES and provided 'weights' for the data in each publication. The method used to obtain the ES was described in detail in the supplementary information provided in an earlier publication (Vijayalaxmi and Prihoda 2008).

Multiple regression analysis

The meta-analysis considered the influence of several sub-groups in ELF-EMF exposure characteristics on each end-point. The % contribution of each sub-group for the outcomes in E–C and ES were examined using the standard output of weighted multiple regression analysis with adjustments for meta-analysis as described in Hedges and Olkin (1985). Nine predictor variables in ELF-EMF exposure characteristics [ELF-EMF *frequency sub-groups* (*$\leq$50 Hz and $\geq$60 Hz*) *as 1 predictor variable*; flux *density sub-groups* (0.0–0.5 mT, > 0.5–1.0 mT, > 1.0–5.0 mT, <5 mT and occupationally exposed *individuals*) *as four predictor variables*; *interaction of Hz and flux density as four predictor variables*], adjusted for each other, provided 'weighted' regression coefficients and sums of squares for E–C (Lipsey and Wilson 2001) and for ES (Hunter and Schmidt 2004). The weighted regression coefficients and sums of squares for each predictor variable, for residual variability and for total variability in the regression were obtained from SAS software (SAS 2006). The standard error (SE) of these regression coefficients from SAS was adjusted (Hedges and Olkin 1985) for the weighted meta-analysis of

sub-group effects. The % variance due to the predictor variables was calculated from each of the weighted sums of squares as % of their total. The % contribution of each sub-group to the effect/outcome observed on E–C and ES on each genotoxicity end-point was estimated. The interaction effects are to test if the effect of Hz is the same or different at different flux densities. If there was no interaction, then the effect of Hz would be the same regardless of the flux density.

Heterogeneity

The meta-analysis considered several related studies. The degree of heterogeneity among the results from such studies can influence the overall conclusions. This was examined in the weighted multiple regression analysis (Hedges and Olkin 1985) using the random error for testing heterogeneity of effects to verify the validity of the models used for both E–C and ES. The residual weighted sums of squares were used in the Chi-square 'goodness of fit' hypothesis with appropriate degrees of freedom (Hedges and Olkin 1985). When the hypothesis was not rejected, the regression model was considered as adequate. Also, when the goodness of fit gave significant results, the data indicated heterogeneity in E–C and ES values obtained for each end-point, i.e., factors which were not considered in this meta-analysis had an influence on the differences between ELF-EMF-exposed and control conditions. Such data were further examined: (i) to explain which sub-group ELF-EMF exposure characteristic contributed to the heterogeneity, (ii) to compare minimum and maximum effects with those in controls, and (iii) interpret the magnitude of heterogeneity.

Publication 'bias'

Publication 'bias' refers to the fact that studies with statistically significant results, even with small sample size, are more likely to be published than those without statistically significant results (Dickersin 1990). When the data with 'no' publication bias were presented in a Figure, studies with small sample size would have the same mean ES (as in those with large sample size) but, indicate a greater variability with wider dispersion of low and high ES values around the mean ES. In contrast, if there is a publication bias, the smaller ES in studies with small sample size would be disproportionately absent since such studies will fail to accomplish statistical significance ($p < 0.05$). Sterne and Egger (2001) have suggested that the graphs/figures to represent publication bias should generally use standard error (SE) as the measure of study/sample size for the vertical axis. Hence, this method was used to assess the publication bias in ELF-EMF research investigations.

Historical database

To provide a proper perspective in the evaluation of potential 'adverse' effects of ELF-EMF exposure, the genotoxicity indices reported in ELF-EMF research investigations were compared with the 'spontaneous' indices in normal cells published in a large historical database. A simple descriptive meta-analysis was performed by pooling the spontaneous incidence of CA, MN or SCE reported in normal cells in several studies in which large sample size was used (Lloyd et al. 1980, Vijayalaxmi and Evans 1982, Fenech and Morley 1985, Obe 1986, Rudd et al. 1988, Bender et al. 1988, 1989, 1992, Bonassi et al. 1995, 2001, Bolognesi et al. 1997, Stephan and Pressl 1999, Fenech et al. 2003, Hagmar et al. 2004, Neri et al. 2005, Rossner et al. 2005) and weighted by the sample size and variance. The spontaneous indices obtained for each end-point was compared with those in ELF-EMF-exposed and controls in the meta-analysis database.

Results

There were a total of 87 peer-reviewed scientific publications during 1990–2007 (Tables I and II). The geographical distribution showed that a great majority of the publications were from Europe followed by the USA: consequently, large numbers of investigations were conducted using ELF-EMF exposure at 50 Hz. Only two studies have compared the effect of two different frequencies (32 Hz and 50 Hz, and 50 Hz and 60 Hz). With respect to flux density, 1 mT was predominantly used by the researchers. Other studies compared the effect of more than one flux density. Seven studies were conducted in human volunteers exposed to ELF-EMF. Investigations were conducted both in vitro and in vivo conditions in humans and experimental animals. The issue related to epigenetic effects of ELF-EMF exposure (± physical and chemical mutagens) was addressed in vitro using human and rodent cells and the observations were reported in a total of 33 publications. A great majority of the researchers have used only one genotoxic end-point while 4 studies have compared the data obtained in all four different end-points. Twenty five different tissue/cell types have been used to examine the effect of ELF-EMF exposure.

DNA single- and double-strand breaks

The results of the meta-analysis of the weighted mean E–C for SBM and SBR, presented in Table III,

Table I. List of ELF-EMF publications in chronological order.

#	First author	Year	#	First author	Year	#	First author	Year
1	Frazier	1990	30	Lai	1997b	59	McNamee	2002
2	Garcia-Sagredo	1990	31	Scarfi	1997a	60	Nakahara	2002
3	Garcia-Sagredo	1991	32	Scarfi	1997b	61	Robison	2002
4	Khalil	1991	33	Scarfi	1997c	62	Zeni	2002
5	Livingston	1991	34	Singh	1997	63	Cho	2003
6	Scarfi	1991	35	Simko	1998a	64	Ding	2003
7	Fiorani	1992	36	Simko	1998b	65	Hone	2003
8	Ciccone	1993	37	Singh	1998	66	Ivancsits	2003a
9	Hintenlang	1993	38	Svedenstal	1998	67	Ivancsits	2003b
10	Khalil	1993	39	Ahuja	1999	68	Pasquini	2003
11	Scarfi	1993	40	Pacini	1999	69	Verheyen	2003
12	Skyberg	1993	41	Scarfi	1999	70	Lai	2004
13	Valjus	1993	42	Simko	1999	71	Lloyd	2004
14	Zwingelberg	1993	43	Svedenstal	1999a	72	Stronati	2004
15	Fairbairn	1994	44	Svedenstal	1999b	73	Testa	2004
16	Nordenson	1994	45	Yaguchi	1999	74	Zmyslony	2004
17	Scarfi	1994	46	Kindzelskii	2000	75	Ivancsits	2005
18	Antonopoulos	1995	47	Maes	2000	76	Luceri	2005
19	Cantoni	1995	48	Miyakoshi	2000	77	McNamee	2005
20	d'Ambrosio	1995	49	Yaguchi	2000	78	Moratti	2005
21	Galt	1995	50	Zmyslony	2000	79	Scarfi	2005
22	Paile	1995	51	Abramsson-Zetterberg	2001	80	Winker	2005
23	Tofani	1995	52	Heredia-Rojas	2001	81	Wolf	2005
24	Cantoni	1996	53	Jajte	2001	82	Yokus	2005
25	Okonogi	1996	54	Nordenson	2001	83	Udroiu	2006
26	Ahuja	1997	55	Othman	2001	84	Villarini	2006
27	Jacobson-Kram	1997	56	Simko	2001	85	Erdal	2007
28	Lagroye	1997	57	Skyberg	2001	86	Miyakoshi	2007
29	Lai	1997a	58	Ivancsits	2002	87	Wahab	2007

Table II. Publications characteristics.

Year:	1990 – 2; 1991 – 4; 1992 – 1; 1993 – 7; 1994 – 3; 1995 – 6; 1996 – 2; 1997 – 9; 1998 – 4; 1999 – 7; 2000 – 5; 2001 – 7; 2002 – 5; 2003 – 7; 2004 – 5; 2005 – 8; 2006 – 2; 2007 – 3	87
Countries:	Austria – 5; Belgium – 2; Canada – 2; Egypt – 1; Finland – 2; France – 1; Germany – 6; India – 3; Italy – 24; Japan – 7; Jordan – 2; Mexico – 1; New Zealand – 1; Norway – 2; Poland – 3; South Korea – 1; Spain – 2; Sweden – 7; Turkey – 2; UK – 2; USA – 11.	87
ELF-EMF frequencies:	16 Hz – 1; 50 Hz – 65; 60 Hz – 16; 100 Hz – 1; 4400 Hz – 2; 32 and 50 Hz – 1; 50 and 60 Hz – 1	87
Flux density: 0.0– > 5.0 mT	1 Flux density – 54; 2 different flux densities – 6; 3 different flux densities – 14; 4 different flux densities – 1; 5 different flux densities – 3; 6 different flux densities – 1; 8 different flux densities – 1; Occupational – 7;	87
Studies: In vitro-Human; In vitro-Rodent; In vivo-Human; In vivo-Rodent In vitro-Human ± Mutagen; In vitro-Rodent ± Mutagen In vivo-Rodent ± Mutagen	1 study – 78; 2 different studies – 8; 4 different studies – 1	87
Genotoxicity end-points: DNA strand breaks; Chromosomal aberrations Micronuclei; Sister chromatic exchanges	1 end-point – 72; 2 different end–points – 11; 3 different end–points – 2; 4 different end–points – 2	87
Cell types (*):	1 cell type only – 79; 2 different cell types – 5; 3 different cell types – 2; 6 different cell types – 1	87

(*): Freshly collected and cultured human cells: Human amniotic cells; Human blood lymphocytes; Human blood neutrophils; Human glioma cells; Human lymphoblastoid cells; Human melanocytes; Human monocytes; Human skeletal muscle cells; Human skin fibroblasts; Human tumor cells. (*): Freshly collected and cultured rodent cells: Chinese hamster lung cells; Chinese hamster ovary cells; Mouse blood lymphocytes; Mouse bone marrow cells; Mouse brain cells; Mouse liver cells; Mouse red blood cells; Mouse skin cells; Rat brain cells; Rat blood lymphocytes; Rat bone marrow cells; Rat granulosa cells; Rat lung cells; Rat skin fibroblasts; Syrian hamster embryo cells.

Table III. Meta-analysis of the pooled E-C[a] and ES[b] data for DNA strand breaks evaluated as comet tail length in microns (SBM) and comet tail moment expressed as ratio (SBR).

End Point	ELF-EMF Exposure	Group	Pooled data N	Pooled data Mean (SD)	E–C Total N	E–C Mean (SE)	ES Mean (SE)
SBM	Hz and Flux	Control	545	4.8 (5.8)			
		ELF-EMF-exposed	544	2.8 (10.9)	1089	1.07 (0.06)***	0.81 (0.08)***
SBM	≤50 Hz	Control	95	1.0 (3.8)			
		ELF-EMF-exposed	94	0.8 (4.3)	189	0.81 (0.06)***	1.34 (0.21)***
SBM	≥60 Hz	Control	450	30.9 (6.1)			
		ELF-EMF-exposed	450	42.9 (11.8)	900	3.34 (0.18)***	0.73 (0.08)***
SBM	0.0–0.5 mT	Control	269	6.0 (7.7)			
		ELF-EMF-exposed	268	2.1 (15.2)	537	0.35 (0.07)***	1.32 (0.12)***
SBM	>0.5–1.0 mT	Control	132	13.5 (2.9)			
		ELF-EMF-exposed	132	14.3 (3.2)	264	0.57 (0.25)***	0.26 (0.14)*
SBM	>1.0–5.0 mT	Control	108	2.6 (2.7)			
		ELF-EMF-exposed	108	5.6 (2.7)	216	1.39 (0.15)***	0.50 (0.16)***
SBM	>5.0 mT	Control	36	1.7 (1.1)			
		ELF-EMF-exposed	36	6.4 (1.1)	72	4.40 (0.15)***	1.84 (0.45)***
SBR	Hz and Flux	Control	1575	3.8 (0.8)			
		ELF-EMF-exposed	1575	5.4 (3.4)	3150	2.68 (0.00)***	0.57 (0.04)***
SBR	≤50 Hz	Control	1305	3.9 (0.9)			
		ELF-EMF-exposed	1305	5.7 (3.8)	2610	2.74 (0.00)***	0.75 (0.05)***
SBR	≥60 Hz	Control	270	0.8 (0.3)			
		ELF-EMF-exposed	270	0.7 (0.3)	540	−0.08 (0.01) ns	−0.21 (0.10)*
SBR	0.0–0.5 mT	Control	187	3.5 (0.4)			
		ELF-EMF-exposed	187	8.8 (0.9)	374	3.83 (0.01)***	−0.21 (0.14) ns
SBR	>0.5–1.0 mT	Control	1274	3.9 (0.9)			
		ELF-EMF-exposed	1274	5.0 (3.8)	2548	2.61 (0.00)***	0.71 (0.05)***
SBR	>1.0–5.0 mT	Control	104	0.4 (0.3)			
		ELF-EMF-exposed	104	0.4 (0.3)	208	0.00 (0.01) ns	−0.01 (0.16) ns
SBR	>5.0 mT	Control	10	1.8 (1.5)			
		ELF-EMF-exposed	10	2.1 (1.4)	20	0.30 (0.27) ns	0.17 (0.61) ns

[a]E–C: Magnitude of weighted difference between ELF-EMF-exposed and control groups based on sample size and variance; [b]ES: Effect size. N: Sample size (i.e., number of experimental units); SD: Standard Deviation; SE: Standard Error; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; ns: Not significant.

indicated significant increases in mean ± SE values in ELF-EMF-exposed as compared with controls ($p < 0.001$). There were no significant differences between ELF-EMF-exposed and control groups for SBR at ≥60 Hz and at > 1.0 mT ($p > 0.05$). The weighted mean E–C for SBM and SBR ranged between 0.35 and 4.4 and −0.08 and 3.83, respectively. Also, see Table X for further results. A significantly increased ES was evident for SBM at all exposure conditions (Hz and flux densities, $p < 0.05$). Similarly, a significantly increased ES was evident for SBR at Hz and flux, ≤50 Hz and >0.5–1.0 mT ($p < 0.05$). On the other hand, a significantly decreased (negative) effect was observed for SBR at ≥60 Hz ($p < 0.05$) while the effect was not significant at 0.0–0.5 mT and >1.0 mT flux densities.

Chromosomal aberrations

The results of the meta-analysis of the weighted mean E–C for CA, presented in Table IV, indicated significant increases in mean ± SE values in ELF-EMF-exposed as compared with controls ($p < 0.01$); the only exception was at the flux density of >5.0 mT ($p > 0.05$). The weighted mean E–C for CA ranged between 0.12 and 0.89 (i.e., an increase of <1.0 CA in 100 ELF-EMF exposed cells). Also, see Table X for further results. The ES for CA was significant at all exposure conditions (Hz and flux densities, $p < 0.01$), the only exception being at flux density 0.0–0.5 mT.

Micronuclei

A majority of investigators in ELF-EMF research have used MN as a genotoxicity end-point to assess the damage. Consequently, the consolidated sample size is the largest among all of the end-points investigated, as given in Table V. The results of the meta-analysis of the weighted mean E–C for MN, indicated significant increase in mean ± SE values in ELF-EMF-exposed as compared with controls ($p < 0.05$) at all exposure conditions; the only exception was in cells from occupationally exposed individuals. The weighted mean E–C for MN ranged between 0.07 and 3.71 (i.e., <4.0 MN in 1000 ELF-EMF-exposed cells). Also, see Table X for further results. The ES for MN was significant at all

Table IV. Meta-analysis of the pooled, E–C[a] and ES[b] data for chromosomal aberrations/100 cells (CA).

End Point	ELF-EMF Exposure	Group	Pooled data N	Pooled data Mean (SD)	E–C Total N	E–C Mean (SE)	ES Mean (SE)
CA	Hz and Flux	Control	262	3.4 (0.9)			
		ELF-EMF-exposed	344	4.7 (1.7)	606	0.61 (0.03)***	0.67 (0.10)***
CA	≤50 Hz	Control	170	1.7 (0.8)			
		ELF-EMF-exposed	252	1.6 (1.9)	422	0.22 (0.07)***	0.59 (0.11)***
CA	≥60 Hz	Control	92	4.5 (0.9)			
		ELF-EMF-exposed	92	5.4 (0.9)	184	0.74 (0.04)***	0.91 (0.19)***
CA	0.0–0.5 mT	Control	48	0.5 (0.8)			
		ELF-EMF-exposed	48	0.8 (0.8)	96	0.12 (0.09)**	0.33 (0.24) ns
CA	>0.5–1.0 mT	Control	43	4.4 (1.0)			
		ELF-EMF-exposed	43	4.7 (1.1)	86	0.31 (0.07)***	0.96 (0.27)***
CA	>1.0–5.0 mT	Control	75	4.1 (0.4)			
		ELF-EMF-exposed	75	5.6 (1.6)	150	0.88 (0.04)***	1.49 (0.24)***
CA	>5.0 mT	Control	16	0.8 (0.4)			
		ELF-EMF-exposed	16	1.2 (4.1)	32	0.89 (0.50) ns	1.30 (0.45)**
CA	Occupational	Control	80	2.1 (1.1)			
		ELF-EMF-exposed	162	1.9 (1.7)	242	0.52 (0.19)***	0.37 (0.13)**

[a]E–C: Magnitude of weighted difference between ELF-EMF-exposed and control groups based on sample size and variance; [b]ES: Effect size; N: Sample size (i.e., number of experimental units); SD: Standard Deviation; SE: Standard Error; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; ns: Not significant. The indices reported in the historical database: CA – mean 1.5/100 cells (SD = 3.7, $n = 15{,}594$).

Table V. Meta-analysis of the pooled, E–C[a] and ES[b] data for micronuclei/1000 cells (MN).

End Point	ELF-EMF Exposure	Group	Pooled data N	Pooled data Mean (SD)	E–C Total N	E–C Mean (SE)	ES Mean (SE)
MN	Hz and Flux	Control	828	9.5 (3.8)			
		ELF-EMF-exposed	832	10.1 (4.3)	1660	0.31 (0.02)***	0.66 (0.06)***
MN	≤50 Hz	Control	780	9.6 (3.9)			
		ELF-EMF-exposed	784	10.1 (4.4)	1564	0.31 (0.02)***	0.64 (0.06)***
MN	≥60 Hz	Control	48	8.2 (2.6)			
		ELF-EMF-exposed	48	9.2 (3.3)	96	1.45 (0.36)**	1.06 (0.27)***
MN	0.0–0.5 mT	Control	187	10.7 (4.9)			
		ELF-EMF-exposed	187	10.4 (4.9)	374	0.07 (0.02)*	0.47 (0.13)***
MN	>0.5–1.0 mT	Control	392	5.8 (3.5)			
		ELF-EMF-exposed	396	7.7 (3.9)	788	0.90 (0.06)***	0.63 (0.09)***
MN	>1.0–5.0 mT	Control	137	5.5 (2.5)			
		ELF-EMF-exposed	137	8.8 (4.6)	274	3.71 (0.14)***	0.86 (0.16)***
MN	>5.0 mT	Control	85	5.1 (0.9)			
		ELF-EMF-exposed	85	8.0 (1.3)	170	2.47 (0.15)**	1.61 (0.26)***
MN	Occupational	Control	27	11.0 (7.0)			
		ELF-EMF-exposed	27	11.5 (7.1)	54	0.50 (1.92) ns	0.07 (0.28) ns

[a]E–C: Magnitude of weighted difference between ELF-EMF-exposed and control groups based on sample size and variance; [b]ES: Effect size; N: Sample size (i.e., number of experimental units); SD: Standard Deviation; SE: Standard Error; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; ns: Not significant. The indices reported in the historical database: MN – mean 9.0/1000 cells (SD = 8.0, $n = 8{,}667$).

exposure conditions (Hz and flux densities, $p < 0.001$), the only exception, again, was in cells from occupationally exposed individuals.

Sister chromatid exchanges

The results of the meta-analysis of the weighted mean E–C for SCE, presented in Table VI, indicated significant increase in mean ± SE values in ELF-EMF-exposed as compared with controls ($p < 0.05$); the exceptions were at >1.0–5.0 mT flux density and in cells from occupationally exposed individuals. The weighted mean E–C for SCE ranged between −0.06 and 1.42 (i.e., <2.0 SCE/cell in the ELF-EMF-exposed group). Also, see Table X for further results. The ES values for SCE were not significant at all exposure conditions ($p > 0.05$).

Epigenetic investigations

The pooled data for different exposure groups, E–C and ES, are presented in Table VII. The E–C data

Table VI. Meta-analysis of the pooled, E–C[a] and ES[b] data for sister chromatid exchanges/cell (SCE).

End Point	ELF-EMF Exposure	Group	Pooled data N	Pooled data Mean (SD)	E–C Total N	E–C Mean (SE)	ES Mean (SE)
SCE	Hz and Flux	Control	223	7.3 (1.6)			
		ELF-EMF-exposed	232	6.2 (1.5)	455	0.70 (0.03)***	0.10 (0.11) ns
SCE	≤50 Hz	Control	140	6.3 (1.7)			
		ELF-EMF-exposed	150	7.1 (1.6)	290	1.15 (0.04)***	0.03 (0.13) ns
SCE	≥60 Hz	Control	83	7.6 (1.4)			
		ELF-EMF-exposed	82	4.8 (1.2)	165	0.14 (0.05)**	0.24 (0.18) ns
SCE	0.0–0.5 mT	Control	70	10.0 (1.8)			
		ELF-EMF-exposed	69	7.7 (1.7)	139	0.14 (0.12)*	0.16 (0.20) ns
SCE	>0.5–1.0 mT	Control	34	4.4 (1.7)			
		ELF-EMF-exposed	34	4.6 (1.8)	68	0.16 (0.05)*	0.27 (0.31) ns
SCE	>1.0–5.0 mT	Control	58	4.7 (1.8)			
		ELF-EMF-exposed	60	5.0 (1.7)	118	−0.06 (0.13) ns	0.02 (0.22) ns
SCE	>5.0 mT	Control	13	6.1 (0.8)			
		ELF-EMF-exposed	14	7.2 (0.2)	27	1.42 (0.05)*	−0.13 (0.45) ns
SCE	Occupational	Control	48	6.9 (1.0)			
		ELF-EMF-exposed	55	6.4 (0.9)	103	0.04 (0.18) ns	0.07 (0.20) ns

[a]E–C: Magnitude of weighted difference between ELF-EMF-exposed and control groups based on sample size and variance; [b]ES: Effect size; N: Sample size (i.e., number of experimental units); SD: Standard Deviation; SE: Standard Error; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; ns: Not significant. The indices reported in the historical database: SCE – mean 7.6/cell (SD = 1.6, $n = 4{,}576$).

Table VII. Meta-analysis of the pooled, E–C[a] and ES[b] for DNA strand breaks evaluated as comet tail length in microns (SBM) and comet tail moment expressed as ratio (SBR), chromosomal aberrations/100 cells (CA), micronuclei/1000 cells (MN) and sister chromatid exchanges/cell (SCE) in epigenetic investigations.

End Point	ELF-EMF Exposure	Group	Pooled data N	Pooled data Mean (SD)	E–C Total N	E–C Mean (SE)	ES Mean (SE)
SBM	Hz and Flux	Control	104	0.8 (11.6)			
SBM	Hz and Flux	ELF-EMF alone	106	0.2 (17.7)	210	−0.07 (0.07) ns (a)	1.72 (0.20)*** (a)
SBM	Hz and Flux	Mutagen alone	96	12.9 (14.1)			
SBM	Hz and Flux	ELF-EMF + Mutagen	111	28.0 (18.7)	195	4.63 (0.76)*** (b)	0.31 (0.16)* (b)
SBR	Hz and Flux	Control	90	0.3 (0.6)			
SBR	Hz and Flux	ELF-EMF alone	90	0.2 (0.6)	180	−0.01 (0.01) ns (a)	0.17 (0.20) ns (a)
SBR	Hz and Flux	Mutagen alone	90	2.8 (1.0)			
SBR	Hz and Flux	ELF-EMF + Mutagen	90	1.8 (0.9)	180	0.27 (0.03)*** (b)	0.48 (0.21)* (b)
CA	Hz and Flux	Control	55	1.3 (2.9)			
CA	Hz and Flux	ELF-EMF alone	55	0.9 (2.9)	110	−0.09 (0.07) ns (a)	0.33 (0.25) ns (a)
CA	Hz and Flux	Mutagen alone	55	5.5 (8.1)			
CA	Hz and Flux	ELF-EMF + Mutagen	55	4.2 (9.0)	110	0.16 (0.11) ns (b)	0.61 (0.27)* (b)
MN	Hz and Flux	Control	360	5.4 (5.6)			
MN	Hz and Flux	ELF-EMF alone	360	11.5 (4.9)	720	4.50 (0.10)*** (a)	0.57 (0.10)*** (a)
MN	Hz and Flux	Mutagen alone	365	14.6 (12.6)			
MN	Hz and Flux	ELF-EMF + Mutagen	346	12.5 (13.9)	711	−0.22 (0.13) ns (b)	−0.01 (0.10) ns (b)
SCE	Hz and Flux	Control	36	3.7 (0.5)			
SCE	Hz and Flux	ELF-EMF alone	36	3.8 (0.6)	72	0.16 (0.08) ns (a)	0.25 (0.31) ns (a)
SCE	Hz and Flux	Mutagen alone	36	7.3 (1.4)			
SCE	Hz and Flux	ELF-EMF + Mutagen	36	11.1 (1.3)	72	1.46 (0.13)** (b)	0.59 (0.35)* (b)

[a]E–C: Magnitude of weighted difference between ELF-EMF-exposed and control groups based on sample size and variance; [b]ES: Effect size; N: Sample size (i.e., number of experimental units); SD: Standard Deviation; SE: Standard Error; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; ns: Not significant. (a): Difference between control and ELF-EMF exposure alone; (b): Difference between Mutagen treatment alone and ELF-EMF + Mutagen treatment.

for SBM, SBR, CA and SCE indicated no significance differences between ELF-EMF-exposure alone and control groups while the data for MN showed a significant increase in the former group as compared with the latter ($p < 0.001$). As expected, treatment with mutagen alone had significantly increased indices for all end-points ($p < 0.001$). A comparison between mutagen alone and ELF-EMF + mutagen treatment indicated significantly increased E–C for SBM, SBR, and SCE and non-significant effect for CA and MN; similar comparison of ES data indicated a significant effect for CA ($p < 0.01$).

Multiple regression analysis

The multiple regression analysis data for E–C and ES, and % contribution of Hz and flux density as well as their interaction on each end-point are presented in Table VIII. The details of the significant effects for the multiple regression analyses are given in Table IX.

The overall % contribution to the variability observed in E–C and ES for all end-points due to Hz groups and flux density groups were of smaller magnitude as compared with that obtained for goodness of fit data given in the last column of Table VIII. Nonetheless, some of them were significant ($p < 0.05$) and are explained in detail by the regression coefficients given in Table IX.

The variability in E–C for SBM, CA, MN and SCE due to Hz groups is not statistically significant ($p > 0.05$) while that for SBR is significant ($p < 0.001$). For all end-points, the variability in E–C due to flux density groups is statistically significant ($p < 0.01$). The interaction between Hz and flux density groups was also significant for all endpoints ($p < 0.01$) except for SCE which was not statistically significant. On the other hand, the variability in ES due to Hz and flux density groups are statistically significant for some endpoints, significant for Hz groups with SBR and CA ($p < 0.05$) and significant for flux density groups with SBM, CA, and MN ($p < 0.01$) . Similarly, the interaction between Hz and flux density groups was significant for only SBM and CA ($p < 0.05$). The detailed explanation for the coefficients which are significant in multiple regression analysis for the Hz and flux density groups and their interaction for E–C and ES values are presented in Table IX (the data in parenthesis describes the change due to Hz or flux density ± SE as well as their interaction).

Heterogeneity

The data for goodness of fit presented in the last column of Table VIII indicated that for each end-point (except SCE), random error accounted for a maximum variability (heterogeneity) observed in E–C values ($p < 0.01$). For ES values however, random error accounted for a significant amount of variability in SBM and CA ($p < 0.05$). When the goodness of fit showed a significant value ($p < 0.05$), the indication was that factors other than the ELF-EMF exposure characteristics are needed to explain more of the variability. The detailed explanation for these heterogeneity effects are described in Table X.

For SBM, the residuals for 18 out of 84 effects (21.4%) were outside the normal range, and this is due to the fact that 100% of these effects were from the studies that used longer electrophoresis duration. For SBR, the residuals for 95 out of 228 effects (41.7%) were outside the normal range, and 94.3% (90 of 95) of these effects were found to be from the studies that used the 'tail factor' method of assessment of DNA strand breaks. This leaves only five of 228 (2.2%) residuals outside of the normal range for SBR which is below the expected 5%. The effects for MN (1 out of 130, 0.8%) and SCE (0 out of 44, 0.0%) which are outside the normal range were found to be very few and the indices for these end-points were within the mean ± 2 SD observed in the historical database. Thus, the magnitude of heterogeneity for MN and SCE endpoints were small although they were not totally explained by Hz and/or flux density groups and their interaction alone.

The E–C multiple regression data obtained for CA indicated six of the 36 effects (16.7%) were larger than control mean + 2 SD (outside the expected normal range). These 16.7% effects were mainly due

Table VIII. Multiple regression analysis of the effects of ELF-EMF exposure characteristics on E–C[a] and ES[b] observed for each genotoxicity end-point. The data in the last column (goodness of fit) indicates unexplained variance. The multiple regression coefficients for significant effects ($p < 0.05$) are described in detail in Table IX.

End-point		Number of effects examined	Percentage contribution due to			
			Hz groups	Flux groups	Hz and Flux groups interaction	Regression 'goodness of fit'
SBM	E-C	84	0.11	21.26***	0.54***	78.09***
SBR	E-C	228	1.27***	0.00***	0.65***	98.09***
CA	E-C	36	0.27	8.28**	3.49***	87.96***
MN	E-C	130	0.19	6.66***	0.65**	92.51**
SCE	E-C	44	0.77	23.67***	1.55	74.01
SBM	ES	84	0.15	13.01***	2.25*	84.59*
SBR	ES	227	5.7***	0.23	0.72	93.35
CA	ES	36	3.34*	11.74**	8.91**	76.00**
MN	ES	130	0.29	4.52***	1.16	94.03
SCE	ES	42	13.50	17.99	14.43	54.08

[a]E–C: Magnitude of weighted difference between ELF-EMF-exposed and control groups based on sample size and variance; [b]ES: Effect size; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ns: Not significant.

Table IX. Detailed explanation for the E–C[a] and ES[b] coefficients which are significant in multiple regression analysis data in Table VIII. The data in parenthesis, [ ], describes the change due to Hz or flux (standard error).

Endpoint	Exposure effect on E–C or ES	E–C[a] or ES[b]	Explanation
Main effects			
MN	≤50 Hz	E–C	Effect of ≤50 Hz is larger than the effect of ≥60 Hz [0.91 (0.47), p 0.026].
SBM	≤50 Hz	E–C	Effect of ≤50 Hz is larger than the effect of ≥60 Hz [8.96 (5.48), p 0.050].
SBR	≤50 Hz	E–C	Effect of ≤50 Hz is larger than the effect of ≥60 Hz [2.70 (0.02), $p < 0.001$].
MN	0.0–0.5 mT	E–C	Effect of 0.0–0.05 mT is larger than >0.5–1.0 mT [2.37 (1.04), p 0.012].
SBM	0.0–0.5 mT	E–C	Effect of 0.0–0.05 mT is larger than >0.5–1.0 mT [2.37 (1.04), $p < 0.001$].
SBR	0.0–0.5 mT	E–C	Effect of 0.0–0.05 mT is smaller than >0.5–1.0 mT [2.37 (1.04), $p < 0.001$].
CA	>1.0–5.0 mT	E–C	Effect of >1.0–5.0 mT is larger than >0.5–1.0 mT [0.56 (0.09), $p < 0.001$].
MN	>1.0–5.0 mT	E–C	Effect of >1.0–5.0 mT is larger than >0.5–1.0 mT [4.34 (0.86), $p < 0.001$].
SCE	>1.0–5.0 mT	E–C	Effect of >1.0–5.0 mT is smaller than >0.5–1.0 mT [−0.30 (0.16), p 0.031].
MN	>5.0 mT	E–C	Effect of >5.0 mT larger than is larger than >0.5–1.0 mT [1.56 (0.16), $p < 0.001$].
SCE	>5.0 mT	E–C	Effect of >5.0 mT larger than is larger than >0.5–1.0 mT [1.54 (0.27), $p < 0.001$].
SBR	≤50 Hz	ES	Effect of ≤50 Hz is larger than the effect of ≥60 Hz [0.96 (0.15), $p < 0.001$].
CA	0.0–0.5 mT	ES	Effect of 0.0–0.5 mT is smaller than >0.5–1.0 mT [−0.72 (0.38), p 0.028].
SBM	0.0–0.5 mT	ES	Effect of 0.0–0.5 mT is larger than >0.5–1.0 mT [1.19 (0.19), $p < 0.001$].
CA	>1.0–5.0 mT	ES	Effect of >1.0–5.0 mT is larger than >0.5–1.0 mT [0.97 (0.45), p 0.015].
MN	>1.0–5.0 mT	ES	Effect of >1.0–5.0 mT is larger than >1.0–5.0 mT [1.52 (0.62), p 0.007].
MN	>5.0 mT	ES	Effect of >5.0 mT is larger than >1.0–5.0 mT[0.96 (0.27), $p < 0.001$].
CA	Occupationally exposed individuals	ES	Effect of occupational exposure is smaller than >1.0–5.0 mT [−0.64 (0.30), p 0.017].
MN	Occupationally exposed individuals	ES	Effect of occupational exposure is smaller than >0.5–1.0 mT [−0.58 (0.29), p 0.023].
Interaction of Hz and flux density.			
CA	50 Hz and >5.0 mT	E–C	Effect of >5.0 mT over >0.05–1.0 mT is larger in 50 Hz than in 60 Hz [6.40 (1.91), $p < 0.001$].
SBR	50 Hz and >5.0 mT	E–C	Effect of >5.0 mT over >0.5–1.0 mT is smaller in 50 Hz than in 60 Hz [−2.53 (0.92), p 0.003].
MN	50 Hz and 0.0–0.5 mT	E–C	Effect of 0.0–0.5 mT over >0.5–1.0 mT is smaller in 50 Hz than in 60 Hz [−3.21 (1.05), p 0.001].
SBM	50 Hz and 0.0–0.5 mT	E–C	Effect of 0.0–0.5 mT over >0.5–1.0 mT is smaller in 50 Hz than in 60 Hz [−19.01 (5.48), $p < 0.001$].
SBR	50 Hz and 0.0– 0.5 mT	E–C	Effect of 0.0–0.5 mT over >0.5–1.0 mT is larger in 50 Hz than in 60 Hz [1.60 (0.03), $p < 0.001$].
MN	50 Hz and >1.0–5.0 mT	E–C	Effect of >1.0–5.0 mT over >0.5–1.0 mT is smaller in 50 Hz than in 60 Hz [−1.57 (0.87), p 0.036].
SBR	50 Hz and >1.0–5.0 mT	E–C	Effect of >1.0–5.0 mT over >0.5–1.0 mT is smaller in 50 Hz than in 60 Hz [−1.57 (0.87), $p < 0.001$].
SBR	50 Hz and 0.0–0.5 mT	ES	Effect of 0.0–0.5 mT over >0.5–1.0 mT is smaller in 50 Hz than in 60 Hz [−1.96 ± 0.90, p 0.014].
CA	50 Hz and >1.0–5.0 mT	ES	Effect of >1.0–5.0 mT over >0.5–1.0 mT is smaller in 50 Hz than in 60 Hz [−1.57 ± 0.87, p 0.049].
MN	50 Hz and >1.0–5.0 mT	ES	Effect of >1.0–5.0 mT over >0.5–1.0 mT is smaller in 50 Hz than in 60 Hz [−1.57 ± 0.87, p 0.011].
SBM	50 Hz and >1.0–5.0 mT	ES	Effect of >1.0–5.0 mT over >0.5–1.0 mT is larger in 50 Hz than in 60 Hz [1.42 ± 0.87, p 0.052].

[a]E-C: Magnitude of weighted difference between ELF-EMF-exposed and control groups based on sample size and variance; [b]ES: Effect size; SBM: DNA strand breaks evaluated as comet tail length in microns; SBR: DNA strand breaks evaluated as comet tail moment expressed as ratio; CA: Chromosomal aberrations/100 cells; MN: Micronuclei/1000 cells; SCE: Sister chromatid exchanges/cell.

to two effects which were explained in Table IX: (i) When the cells were exposed to >1.0–5.0 mT flux density (0.56 ± 0.09 in main effects) and (ii) when there was an interaction effect of ≥50 Hz exposure at >5.0 mT (6.4 ± 1.91). Thus, exposure of the cells to >1.0–5.0 mT flux density (2 of 10 or 20% of the effects) and ≥50 Hz at >5.0 mT flux density (1 of 3 or 33% of the effects) resulted in an abnormally high incidence of CA. The remaining three large effects, when averaged in with 10 other effects in the same category resulted in a non-significant and small multiple regression effect for E-C. These three large effects could not be explained by Hz and flux density.

Publication bias

The publication bias was graphically presented in Figure 1. Although there were a total of 87 publications, some investigators have examined one

Table X. Heterogeneity in ELF-EMF exposure characteristics on the effects observed in the end-points investigated.

End-point		Number of E–C effects examined (N)	ELF-EMF publications		
			Sample size Controls	Controls (C)	ELF-exposed (E)
SBM	Mean		545	4.76	2.83
	SD			5.78	10.91
	Upper limit*			11.56	
	E–C Range**	84		−1.2 to 105.0 (18 of 84 = 21.4%)	
				100% (of 21.4%) studies used longer electrophoresis duration	
SBR	Mean		1575	3.80	5.39
	SD			0.82	3.43
	Upper limit*			1.64	
	E–C Range**	228		−5.3 to 12.5 (95 of 228 = 41.7%)	
				−5.3 to 12.5 (4 of 228 = 1.8% below the normal)	
				94.3% (of 41.7%) studies used tail factor method	
CA/100 cells	Mean		262	3.43	4.66
	SD			0.85	1.71
	Upper limit*			1.70	
	E–C Range**	36		−1.5 to 6.7 (6 of 36 = 16.7%)	
	Historical Controls:				
	Mean (SD)			1.5 (3.7)	
	Upper limit*			7.40	
	E–C Range**			−1.5 to 6.7 (0 of 36 = 0.0%)	
MN/1000 cells					
	Mean		828	9.55	10.07
	SD			3.82	4.31
	Upper limit*			7.64	
	E–C Range**	130		−6.0 to 7.9 (1 of 130 = 0.8%)	
	Historical Controls:				
	Mean (SD)			9.00 (8.00)	
	Upper limit*			16.00	
	E–C Range**			−6.0 to 7.9 (0 of 130 = 0.0%)	
SCE/cell					
	Mean		223	7.33	6.15
	SD			1.59	1.49
	Upper limit*			2.18	
	E–C Range**	44		−1.3 to 1.6 (0 of 44 = 0.0%)	
	Historical Controls:				
	Mean (SD)			7.60 (1.60)	
	Upper limit*			3.20	
	E–C Range**			−1.3 to 1.6 (0 of 44 = 0.0%)	

N: Number of E–C values examined in the multiple regression; *Upper limit is 2 × SD above control mean, i.e., 97.5 percentile; **E–C range is the minimum and maximum for all E–C residual values (i.e., deviations from predictions) used in the multiple regression; SBM: DNA strand breaks evaluated as comet tail length in microns; SBR: DNA strand breaks evaluated as comet tail moment expressed as ratio; CA: Chromosomal aberrations/100 cells; MN: Micronuclei/1000 cells. SCE: Sister chromatid exchanges/cell.

or more end-points in several different ELF-EMF exposure conditions. Consequently, the X-axis and Y-axis in Figure 1 has several data points each representing the ES value of one end-point indicating 'near zero' or 'no' effect in one ELF-EMF exposure condition, respectively. The overall data did not appear as 'pyramid' with the mean ES approximately at the center with 'negative' and 'positive' publications (absence and presence of significant differences between ELF-EMF-exposed and controls). In contrast, the data were largely 'skewed' indicating the existence of a significant publication bias ($p < 0.001$). The 'skew' was due to large SE in studies with large ES values despite a small mean E–C difference. Finally, since the meta-analysis strongly suggested the presence of publication bias, the conclusions should be regarded as 'tentative'. For example, there was only one instance in which the cells which were exposed to $\leq$50 Hz at $>$5 mT exhibited the largest increase in CA; these observations must be replicated. The practical reality is that the data from diverse investigations were used for meta-analysis and this needs to be accepted while drawing final conclusions.

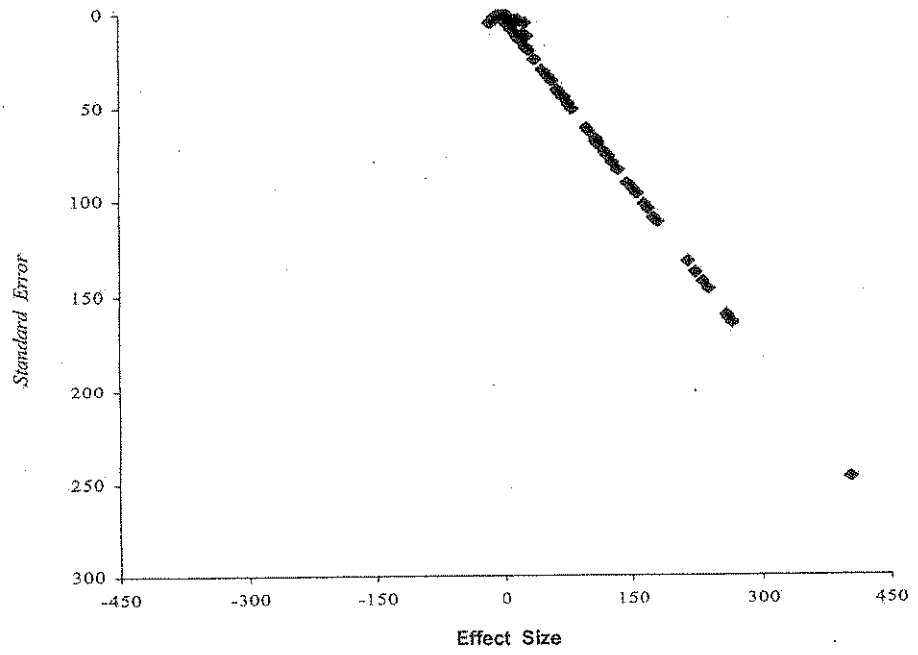

Figure 1. Each data-point in the figure represents one effect from a group of ELF-EMF-exposed and control conditions, in one publication, for one genotoxicity end-point. There were a total of 522 data-points (96 data-points <0 value, 49 data-points with 0 value and 377 data-points >0 value). The effect size and the standard error are represented on X-axis and Y-axis, respectively. The 'skewed' publication bias is due the investigations reporting 'positive' results (significant difference between ELF-EMF-exposed and control groups of cells) with large standard error. Detailed explanation is given in the text.

Comparison of meta-analysis data with those in historical database for CA, MN and SCE

Several hundred reports were the published literature (some were mentioned before) in which the incidence of CA, MN and SCE in normal normal/spontaneous cells were reported and the indices were used as biomarkers to predict the carcinogenic potential of exposure to genotoxic agents present in our environment. The mean incidence (and the range of values) for CA, MN and SCE reported for freshly collected peripheral blood lymphocytes from normal individuals in the historical database with large sample size are as follows: CA – 1.5/100 cells (SD 3.7; $n = 15594$); MN – 9.0/1000 cells (SD 8.0; $n = 8667$); SCE – 7.6/cell (SD 1.6; $n = 4576$). The maximum indices obtained in ELF-EMF-exposed and control groups in the meta-analysis were similar to the above indices in the historical database (with very few exceptions).

Cytogenetic end-points as biomarkers for cancer risk assessment

When the cells are exposed to genotoxic agents, the primary lesions that are induced in the DNA are base damage, single strand breaks, double strand breaks, etc. Cells also have inherent mechanism(s) to repair some or all of these lesions. The un-repaired and/or mis-repaired lesions lead to the formation of CA, MN and SCE (see below). The original technique for the evaluation of DNA strand breaks in single cells included embedding of cells in agarose, lysis and electrophoresis; cells which were exposed to gamma rays displayed a significant increase in the length of DNA migration as compared with those that were not irradiated (Ostling and Johanson 1984). Researchers in various laboratories have made several modifications to the laboratory protocol used for the comet assay (Singh et al. 1988, 1994, Olive et al. 1990, Vijayalaxmi et al. 1992) and reported the data in freshly collected and/or cultured rodent and human cells exposed in vitro and in vivo the ELF-EMF at various frequencies and flux densities. Ivancsits et al. (2002, 2003a, 2003b, 2005) exposed unsynchronized, continuously growing cultured rodent and human cells to 50 Hz ELF-EMF at 0.02–1.0 mT. The exposure period ranged from 1–24 h after which the cells were used in the comet assay. The comets were classified 'visually' into A, B, C, D and E categories which were then subjected to approximate transformation factors (weighted as A × 2.5, B × 12.5, C × 30.0, D × 67.5 and E × 97.5) to derive an 'objective' 'tail factor' (Diem et al. 2002). Vijayalaxmi et al. (2006) expressed concern over the presence and contribution of 'confounding' cells (in S-phase of the cell cycle and

apoptosis) to the tail factor. It is imperative for future researchers to include cell cycle analysis to determine the numbers of cells in S-phase as well as enumeration of apoptotic cells when the comet assay is applied to unsynchronized, continuously growing cells. So far, there has been no information on human health hazard risk evaluation using the data reported from DNA strand breaks (using the comet assay), although the assay has been increasingly used in recent years. It is worth mentioning the observations of the comet assay that was conducted in regular genetic toxicology investigations in which cells from eight different organs of mice treated with 208 chemicals (groups 1, 2A, 2B, 3 and 4) selected from the carcinogenicity database of the International Agency for Research on Cancer and from the U. S. National Toxicology Program were examined. The results were compared with those obtained from other genotoxicity end-points used for the assessment of genetic damage, namely, Ames test in bacterial tester strains, CA, MN and unscheduled DNA synthesis. The overall conclusion was that no single test was capable of detecting all relevant genotoxic/carcinogenic agents and the recommendation was to conduct a battery of in vitro and in vivo tests for genotoxicity (Sasaki et al. 2000).

The incidence of CA had been investigated for several decades to monitor whole-body and environmental exposures to genotoxic carcinogens. At the molecular level, the spontaneous and/or induced strand breaks in the DNA that remain un-repaired and/or that undergo aberrant repair lead to the formation of CA. The analysis CA is more refined in recent years so that the cells in their first mitotic metaphase only were examined since unstable aberrations such as dicentric and ring chromosomes are eliminated in successive cell divisions. At least 100 metaphases per sample were recommended for detailed CA evaluations. However, not all researchers in ELF-EMF field appeared to have followed the classification and recommendation. CA, as a biomarker to predict carcinogenic risk in humans, has been systematically examined by several researchers. The data from several studies have indicated that the aberration frequencies were increased even prior to the clinical manifestation of disease. Despite the fact that CA analysis is time-consuming, it is the most reliable biomarker to predict increased cancer risk in humans (Tucker et al. 1997, Hagmar et al. 2004, Bonassi et al. 2005, Norppa et al. 2006).

The existence of MN as a separate entity in a cell, apart from the main nucleus, was known for decades and the indices were widely used to monitor occupational and environmental exposure to genotoxic agents. MN may contain portions of broken chromosomes (clastogenic effect) or whole chromosomes which were not incorporated into daughter cells during cell division due to spindle disruption (aneugenic effect). Preliminary evidence has been presented that an increased incidence of MN predicts enhanced risk of cancer in humans (Bonassi et al. 2007). An important suggestion has been made to use fluorescence in situ hybridization techniques to recognize the presence or absence of centromere in order to explain the clastogenic or aneugenic effect of the test agent, respectively. However, the presence of broken chromosomal fragments with intact centromere which gives the appearance of whole chromosome in MN can not be ruled out.

SCE are cytological manifestation of consequences of errors in DNA replication resulting in interchanges between the two chromatids of the same chromosome at apparently homologous loci, possibly at the replication fork itself (Painter 1980). Although evaluation of SCE is generally considered a more sensitive indicator of exposure to genotoxic agents, their indices did not appear to have a predictive value for human health risk assessment. Nonetheless, SCE would remain a valuable end-point among the short-term assay systems because of the sensitivity and less effort is needed for their analysis, especially when the exposed cells/subjects and their matched controls were sampled simultaneously and scorer bias was eliminated.

The epigenetic investigations are important since in real life, people are exposed to a variety of environmental insults simultaneously and/or sequentially. Theoretically it may well be that ELF-EMF exposure *per se* is not genotoxic, but such exposure could enhance the cytogenetic damage induced by other chemical and/or physical genotoxic agents (i.e., the influence of ELF-EMF exposure could be epigenetic or non-genotoxic). Hence, this important issue was addressed in a total of 33 ELF-EMF investigations.

Considering the above discussion, it is note worthy that the genotoxicity indices for SBM, SBR, CA, MN and SCE obtained in the meta-analysis for ELF-EMF-exposed and control groups were similar to the 'spontaneous' indices reported in the historical database.

Perspectives from meta-analysis and conclusions

Cytogenetic investigations are important since most genotoxic agents are also carcinogens. Besides, the epigenetic effect of some non-genotoxic agents can contribute to the development of cancer by enhancing the damage induced by known genotoxic agents. It is clear from the above discussion that no single genotoxic end-point, by itself, is capable of providing a precise estimate of the genotoxic potential and the consequent cancer risk from

occupational and environmental agents (Sasaki et al. 2000). Perhaps, the evaluation of CA is the best among the end-points available for such purpose. In order to protect the general public and occupationally involved individuals, several national and international organizations have suggested guidelines for limiting exposure to ELF-EMF. For both 50 Hz and 60 Hz, the guidelines for the general public are 5 kV/m and 100 μT for continuous exposure and 10 kV/m and 1000 μT for short-term exposure; the guidelines for occupational exposures are 10 kV/m and 500 μT for continuous exposure and 30 kV/m and 5000 μT for short-term exposures (ICNIRP 1998). Also, the guidelines for residential and occupational exposures to 60 Hz are 10 kV/m and 1330 μT, and the standards for 50 Hz are 12 kV/m and 1600 μT (NRPB 2001). These standards are based on keeping the electric currents induced by power frequency fields to <10 mA/m^2. Above these levels, evidence was presented for a direct stimulation of neuronal and cardiac tissue. When the investigations were conducted under these recommended safety guidelines, the overall genotoxicity indices obtained in the meta-analysis were similar, in ELF-EMF-exposed and controls, to those reported in historical database. It must be pointed out that the meta-analysis focused on only three specific ELF-EMF exposure characteristics, and the multiple regression analysis and goodness of fit did indicate that factors other than the three ELF-EMF exposure characteristics are needed to explain more of the variability reported in the investigations. Since no single genotoxic end-point, by itself, is capable of determining the genotoxic potential and the consequent cancer risk from occupational and environmental agents (Sasaki et al. 2000, Bonassi et al. 2005), it is relevant to include more than one genotoxicity end-point for DNA damage assessment in future ELF-EMF research investigations.

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