

Hot Topics in DNA Repair No. 11

Are environmental electromagnetic fields genotoxic?

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Abstract

Long-term exposure to extremely-low-frequency¹ electromagnetic fields (ELF EMFs) greater than 0.4 μ T has been linked, by epidemiological studies, to a small elevated risk of childhood leukaemia. Laboratory-based experiments have been claimed to show that ELF EMFs induce a variety of biological responses, although these claims are controversial. Recent experiments by Ivancsits et al. [Mutat. Res. 519 (2002) 1; Int. Arch. Occup. Environ. Health 76 (2003) 431; Mech. Age. Dev. 124 (2003) 847; H.W. Rüdiger, S. Ivancsits, E. Diem, O. Jahn, Genotoxic effects of ELF-EMF on human cells in vitro, Bioelectromagnetics Society 25th Annual Meeting, Maui, USA, 2003] suggest that ELF EMFs are genotoxic, on the basis of observations that intermittent exposures induce single-strand breaks (SSB) and double-strand DNA breaks (DSB) in the DNA of cultured human fibroblasts. The implications of these findings are discussed.

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Electric power is an essential world commodity and is key to the growth of our technology-based society. Its benefits are overwhelming. However, there is some public concern that exposure to the extremely-low-frequency electromagnetic fields (ELF EMFs) associated with power distribution may increase the risk of cancer, especially childhood leukaemia.

Multiple epidemiological studies over 25 years have delivered mixed messages, but recent pooled (meta) analyses of several independent studies have indicated that long-term exposure to power-frequency magnetic fields averaging 0.4 μ T or more is linked to a doubling of the risk of childhood leukaemia [1,2]. However, identification of the link as causal is difficult to justify, for two main reasons.

Firstly, there is no established biophysical mechanism by which such weak magnetic fields could induce a biological response, although several possibilities have been suggested. One of the more plausible is based on the well-established perturbation of free-radical recombination by magnetic fields [3]. However, this perturbation is typically associated with relatively high field strengths (>1 mT) and it remains to be shown whether a particular biological circumstance exists that enables much weaker fields to promote a free-radical-mediated effect [4,5].

Secondly, laboratory-based experiments have failed to provide any convincing biological explanation for the epidemiological results. While there have been multiple in vivo and in vitro studies, using various animal and cellular

model systems – with a variety of read-outs, including mutation and gene activation – no robust and independently replicated biological response has been established for field strengths <100 μ T [6].

Nevertheless, the International Agency for Research on Cancer (IARC) has recently classified ELF EMFs as ‘possibly carcinogenic’ [7], a classification which necessarily implies that the epidemiological link may be causal and that, directly or indirectly, weak ELF magnetic fields may promote DNA damage; that is, they are genotoxic. It is therefore of some interest that experimental results apparently supporting this implication have recently emerged from one of the laboratories taking part in the collaborative EU-funded REFLEX project².

In particular, Ivancsits et al. [8–11] have reported experiments in which cultures of human diploid skin fibroblasts were exposed to 50-Hz magnetic fields (up to 2 mT) for up to 24 h and DNA damage was assessed using the comet assay, in both neutral and alkaline versions. There was a small but significant increase in DNA breakage in the cells exposed to fields as low as 35 μ T, as compared with sham-exposed cells. Intermittent exposure (5 min on, 10 min off) was effective, while continuous exposure was not. The response

¹ The extremely-low-frequency band is normally defined as 30–300 Hz.

² REFLEX (Risk Evaluation of Potential Environmental Hazards from Low Energy Electromagnetic Field Exposure using sensitive in vitro methods) a project funded by the European Union under the programme “Quality of Life and Management of Living Resources”, Key Action 4 “Environment and Health” QLK4-CT-1999-01574. The project has sponsored a variety of in vitro studies in a dozen European laboratories.

was dose-dependent up to 2 mT, and increased with time to a maximum after about 15 h of exposure. Similar effects were seen in rat granulosa cells, but there was no detectable EMF-induced damage in skeletal muscle cells or stimulated lymphocytes. In fibroblasts, however, exposure conditions producing maximum strand-break levels also induced a significant increase of micronuclei and chromosomal aberrations. The authors conclude that their data ‘strongly indicate a genotoxic potential of intermittent EMF’.

It is important to understand the theory behind the apparently simple technique used to measure damage, i.e. the comet assay. As originally devised by Östling and Johanson [12], the assay involved lysis and electrophoresis of cells at pH 9.5. The method of Singh et al. [13] employed a higher pH for electrophoresis – high enough to allow unwinding of the DNA. By analogy with various methods of measuring DNA single-strand breaks (SSB) that depend on strand separation of DNA in alkaline solution, Ivancsits et al., with many others, believe that while the alkaline comet assay detects SSB and double-strand breaks (DSB), the neutral assay will detect only DSB. Thus they conclude, from the similar levels of DNA breakage seen using the neutral and the alkaline versions of the comet assay, that the DNA breaks induced by EMF must be ‘mainly DSB’. If this were true, it would be a cause for serious concern, since DSB are less readily repaired and potentially more likely to kill the cell than are SSB.

However, Östling and Johanson [12] compare their method with the older ‘nucleoid sedimentation’ technique. Nucleoids are the protein- and membrane-depleted nuclear bodies left after lysis of cells with detergent and high salt. They comprise supercoiled loops of DNA linked to a residual nuclear matrix. Strand breaks (whether SSB or DSB) relax supercoiling and allow the DNA loops to be pulled into a ‘comet tail’ in the electric field. This explains the ability of their assay, at a pH too low to allow DNA strand separation, to detect breaks (mainly SSB) inflicted by low doses of ionising radiation; the simple neutral comet assay, like the alkaline version, does not discriminate between SSB and DSB. Thus it seems that, in the experiments of Ivancsits et al., virtually all of the damage ascribed to EMF may in fact be attributed to the less severe form of genetic damage, SSB, rather than DSB.

It is instructive to estimate how much damage (strand breaks) is actually present in cells treated with low EMF. The authors do not calibrate their assay (few comet users do), but since their conditions are similar to those generally used, it is probably safe to apply a published calibration curve [14], in which an increase of 20% in tail DNA corresponds to 1.5 Gy equivalents, or 0.5 breaks per 10^9 Da. Therefore, the increase of 2% reported here represents one break in 2×10^{10} Da, or a hundred or so strand breaks per cell. To put this in perspective, it is an order of magnitude less than the steady state level of 8-oxoguanine in cultured human cells as estimated using the comet as-

say with formamidopyrimidine DNA glycosylase (FPG) [15].

Several questions are posed by these results. Are the extra SSB the actual lesions produced by EMF, or are they intermediates in the repair of other lesions such as oxidised bases? (The extent of oxidative base damage could have been determined by including in the comet assay a digestion of nucleoid DNA with lesion-specific endonucleases, such as FPG [14].) There is no apparent explanation as to why intermittent, but not continuous exposures should induce DNA SSB. Also, while cell specificity is commonplace in biological responses, the fact that lymphocytes fail to respond is curious, especially because the epidemiological links with leukaemia suggest lymphocytes as the possible target cell for magnetic field exposure [1,2].

Finally, whereas in these experiments DNA damage was detected after intermittent exposure to a minimum field strength of 35 μ T, the average home has a much smaller average field strength, i.e. less than 1 μ T [16]. Therefore, even if DNA breaks were induced in the cell-culture system used, it is very difficult to interpret the results in terms of likely human hazard.

In spite of these qualifications, the experimental data are provocative and require independent verification. Meanwhile, the production of both SSB and DSB has also been reported by Lai and Singh [17] – in rat brain cells after 24-h continuous exposure to 10- μ T, 60-Hz fields. They used a more drastic neutral comet assay procedure which detects exclusively DSB; in this procedure, the nuclear matrix is degraded by RNase and proteinase digestion, so that the DNA is presumably no longer supercoiled.

The lack of independent replication has been a persistent feature of experimental studies looking for biological effects of weak ELF EMFs. It remains to be determined whether or not the present reports of DNA damage will be substantiated and whether it will be possible to draw any conclusions as to their possible relevance to a chain of events that might lead to leukaemia.

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