TEST OF EFFECT OF ELECTROMAGNETIC FIELDS ON CELLS

Expt series	Preincubation time (h)	Exposure time (min)	EMF (μT r.m.s.)	*MYC*	*n*	*GAPDH*	*n*	β-actin mRNA	*n*
1	1	20	5.7	1.10 (0.89, 1.36)	5	–	–	1.15 (1.08, 1.22)	5
2	1	20	5.7	1.06 (0.94, 1.19)	5	-		1.06 (0.86, 1.30)	5
3	1	20	5.7	0.93 (0.86, 1.00)	12	0.95 (0.81, 1.12)	12	0.93 (0.81, 1.07)	7
4	1	60	5.7	0.93 (0.69, 1.25)	5	0.87 (0.77, 1.00)	5	0.85 (0.71, 1.03)	5
5	12	20	0.57	1.01 (0.92, 1.11)	5	0.92 (0.79, 1.08)	5	0.89 (0.72, 1.09)	5
6	12	20	5.7	0.98 (0.87, 1.12)	10	1.08 (0.95, 1.23)	10	1.11 (0.97, 1.26)	10
7	12	20	57	1.10 (0.98, 1.24)	5	0.99 (0.63, 1.57)	3	1.00 (0.90, 1.11)	7
8	12	20	0.1	1.04 (0.87, 1.25)	4	1.10 (0.98, 1.24)	4	-	
9	8 or 12	20	1.0	0.97 (0.88, 1.07)	9	1.03 (0.93, 1.13)	5	-	
10	8 or 12	20	10	1.05 (0.96, 1.15)	10	1.05 (0.93, 1.18)	7	-	
11	12	20	100	0.81 (0.56, 1.17)	4	0.97 (0.84, 1.12)	3	-	
3–11				0.98 (0.93, 1.03)	64	1.00 (0.94, 1.05)	54	0.98 (0.91, 1.05)	34

Experiments 1–7: Two sets of coils (applied a.c. field horizontal) constructed to the specifications of ref. 20 were located one on either side of a vertical mu-metal baffle within a mu-metal box in a water-jacketed incubator. Suspensions of HL60 cells (15 ml, 0.8–1.02106 ml[21] in RPMI 1640 medium containing 10% FCS) in T25 culture flasks were located in the centre of the coils. Experiments 8–11: six Helmholtz coil sets (applied a.c. field vertical), arranged with three in an equilateral triangle on either side of the vertical mu-metal baffle, were used. Cell suspensions (5 ml) as above were placed in the centre of the coils in 35-mm Petri dishes. Temperature (37±0.05 °C), CO_2 (5.0±0.05%) and magnetic field (residual geomagnetic field <1 μT, a.c. background <5 nT r.m.s.) inside the mu-metal box were logged every minute. No increase in background magnetic fields could be detected at the sham exposure coils at any of the field strengths used. The specific gene mRNAs from EMF-field-exposed (E) and control (C) cells were quantified from northern blots by phosphorimager and E/C ratios are given with 95% confidence limits. Expt 1: *MYC* and b-actin E/C ratios calculated without normalization for the amount of RNA in each sample; expt 2: the amounts of *MYC* and β-actin mRNA were normalized to the house-keeping gene, *GAPDH*; expts 3–11: ^{14}C rRNA labelling used for normalization; bottom row: means for experiments using ^{14}C rRNA normalization. To label cellular RNA with ^{14}C-uridine, cells were either incubated (0.5 μCi per 30 ml) throughout the 8- or 12-h preincubation in the field exposure incubator before field exposure, or were prelabelled for 16 h, concentrated by centrifugation and re-suspended in fresh complete culture medium with ^{14}C-uridine before placing in the field exposure incubator for 1 h preincubation before field exposure. A full technical account of these experiments will be described elsewhere.

SIR — Epidemiological studies have shown weak correlations between exposure to extremely low-frequency electromagnetic fields (EMFs) and cancers, particularly childhood leukaemias[11–13]. These observations prompted many *in vitro* cellular studies in which effects of EMFs were reported. However, no reported response has been widely and independently replicated, which is essential for critical evaluation. Key reports by Goodman and Henderson were the activation of proto-oncogenes in human leukaemic (HL60) and other cells[8,14–16], because of the essential role of proto-oncogenes in proliferation. EMFs increased the amounts of messenger RNA for *MYC* and β-actin in HL60 cells by 2– 4-fold after 20 min exposure to a 60-Hz horizontal sinusoidal field (5.7 mT r.m.s.)[8,14], although smaller effects for the same genes (mean 1.3-fold) were reported subsequently[15,16]. We have attempted a systematic replication of this effect.

HL60 cells were exposed to EMFs (expt 1 in table above) under very similar conditions to those described[8,14], except that coil sets for exposed and control cells were switched randomly between experiments so that the experiments were performed blind, and mRNAs were quantified from northern blots rather than dot blots. The ratio of *MYC* mRNA from exposed and control cells was 1.10, with large confidence limits of 0.89 and 1.36 due to the absence of normalization by direct measurement of the amounts of RNA in each sample.

In further experiments, we observed no EMF effect when *MYC* and β-actin mRNAs were normalized to the house-keeping gene, *GAPDH* (expt 2 in table), or by labelling the RNA with ^{14}C-uridine (expts 3–11), although the confidence limits were substantially reduced. In all experiments, TPA (phorbol 12-myristate 13-acetate) was used as a positive control for activation of the *MYC* gene. The ratio of *MYC* mRNA in TPA-treated cells to control cells was 2.18 (2.01, 2.36; $n=9$) and therefore field effects of the magnitude reported should be readily detectable.

Unsatisfactory features of previous procedures[8,14] are that the cells were equilibrated for only 1 h before the field was applied and after exposure were placed on ice for 10 min at ambient EMFs before centrifugation and RNA preparation. We therefore performed experiments in which cells were maintained in the exposure incubator for 12 h before the field was applied and RNA was prepared immediately after removing the cells from the incubator. No effect on *MYC* or β-actin expression was observed (expts 5–7 in table). Experiments in which the cells were lysed *in situ* before they were removed from the incubator showed no effect on *MYC* expression (expts 8–11). Summation of the data for experiments 3–11 gave a mean ratio for *MYC* mRNA of 0.98 (0.93, 1.03).

It is very unlikely that a *MYC* gene response depends on the subculture of HL60 cells used, as Goodman and Henderson have reported responses to EMFs in seven different types of cells[8,14–19], with no reports of cell types insensitive to EMFs. It is therefore likely that either there is an elusive requirement for an EMF gene response yet to be defined or that there was a systematic error in the experiments showing a positive response. The data do not eliminate the possibility of very small EMF effects on gene expression in HL60 cells below the levels defined here.

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1. Theriault, G. *et al. Am. J. Epidem.* **139,** 550–572 (1994).
2. Tynes, T., Jynge, H. & Vistnes, A.I. *Am. J. Epidem.* **139,** 645–653 (1994).
3. Guenel, P., Raskmark, P., Andersen, J.B. & Lynge, E. *Br. J. ind. Med.* **50,** 758–764 (1993).
4. Floderus, B. *et al. Cancer Causes Control* **4,** 465–476 (1993).
5. Kobayashi, A.K., Kirschvink, J.L. & Nesson, M.H. *Nature* **374,** 123 (1995).
6. Goodman, R., Bumann, J., Wei, L.-X. & Shirley-Henderson, A. *Electro- Magnetobiol.* **11,** 19–28 (1992).
7. Wei, L.-W., Goodman, R. & Henderson, A. *Bioelectromagnetics* **11,** 269–272 (1990).
8. Goodman, R. & Shirley-Henderson, A. *Bioelectrochem. Bioenerget.* **25,** 335–355 (1991).
9. Goodman, R. & Shirley-Henderson, A. *Cancer Cells* **2,** 355–359 (1990).
10. Goodman, R., Wei, L.-X., Xu, J.-C. & Henderson, A. *Biochim. biophys. Acta* **1009,** 216–220 (1989).
11. Wertheimer, N. & Leeper, E. *Am. J. Epidem.* **109,** 273–384 (1979).
12. Feychting, M. & Ahlbom, *A. Am. J. Epidem.* **138,** 467–481 (1993).
13. NRPB *Documents of the NRPB* No. 3, 1–138 (1992).
14. Goodman, R., Wei, L.X., Bumann, J. & Shirley, H.A. *Bioelectrochem. Bioenerget.* **29,** 185–192 (1992).
15. Broude, N., Karabakhtsian, R., Shalts, N., Goodman, R. & Henderson, A.S. *Bioelectrochem. Bioenerget.* **33,** 19–23 (1994).
16. Karbakhtsian, R. *et al. FEBS Lett.* **349,** 1–6 (1994).
17. Weisbrot, D.R., Khorkova, O., Lin, H., Henderson, A.S. & Goodman, R. *Bioelectrochem. Bioenerget.* **31,** 167–177 (1993).
18. Lin, H., Goodman, R. & Shirley-Henderson, A. *J. cell. Biochem.* **54,** 281–288 (1994).
19. Gold, S., Goodman, R. & Shirley-Henderson, A. *Bioelectromagnetics* **15,** 329–336 (1994).
20. Goodman, R. *et al. Bioelectrochem. Bioenerget.* **33,** 115–120 (1994).