Mr. Robert J. Pellatt
Commission Secretary
British Columbia Utilities Commission
Sixth Floor, 900 Howe Street, Box 250
Vancouver, BC, V6Z 2N3
Tel.: (604) 660 4700, Fax: (604) 660 1102
Email: Commission.Secretary@bcuc.com
Web site: http://www.bcuc.com

Via E-mail

Without Prejudice

February 19, 2007

Dear Mr. Pellatt,

Re: FortisBC Inc. Ellison Substation & Transmission Line Project No. 3698442 Certificate of Public
Convenience and Necessity Application (CPCN)
http://www.fortisbc.com/about/regulation.htm

1. Please accept this as my Evidence # 16 and Info Request # 15 into subject hearing for future
references and to be tested during the hearing process:

Robert J. Mairs et al., Microsatellite analysis for determination of the mutagenicity of
everestly lowfrequency electromagnetic fields and ionising radiation in vitro, Mutation
Research (2006), doi:10.1016/j.mrgentox.2006.08.005
source: http://www.buergerwelle.de/pdf/mairs_mutatres_microsatellites_elf_ir_2006.pdf

This is the first authorative study - only possible with new methodology - that shows how cancer
can be CAUSED by power-frequency electromagnetic fields (Extreme Low Frequency
Electromagnetic Fields, ELF-EMF). Please note that in this study, these fields are rotative, as
under power lines.
The methodology of this paper's study includes a more sensitive method of detecting DNA
damage involving the examination of microsatellite DNA sequences (microsatellite: see
The Scottish researchers Mairs et al. were not expecting this finding, having noted DNA damage effects at the 100 to 4,000mG (MRI, scans) "doses". They now plan to see what effects on DNA occur at exposures lower than 10mG.

Please note that the damage is significantly greater than that from exposure to gamma radiation, where low-level, or otherwise.

According to this study, power-frequency fields (ELF EMF) of 10 mG (milliGauss) magnetic field, after an equivalent of 1 night exposure, increases DNA damage 4-fold, affecting an estimated at least 35,000,000 Canadian and northern-state US persons who are exposed every night to such fields, mostly children.

Previous studies showed:

a) ELF EMF associated with various adverse biological effects,

b) ELF EMF can be co-carcinogen, and

c) once cancer is manifest, ELF EMF accelerate its promotion.

The study includes involvement of government health agency, academe and cancer institution stakeholder expert researchers.

Please also note that the study comes in the wake of WHO - International Agency for Research on Cancer evaluations of carcinogenic risk to humans, Volume 80 - and actually indicates power-frequency fields cause, rather co-cause cancer.

Homes typically are/can be exposed between 0.5 – 10 mG and above, also from external electropollution (i.e. power lines’ EMF, stray voltage).

2. May I cite here out of the paper’s Abstract:

"...we observed that exposure of UVW human glioma cells to ELF-EMF alone at a field strength of 1mT (50 Hz) for 12 h gave rise to 0.011 mutations/locus/cell. This was equivalent to a 3.75-fold increase in mutation induction compared with unexposed controls. Furthermore, ELF-EMF increased the mutagenic capacity of 0.3 and 3Gy -irradiation by factors of 2.6 and 2.75, respectively. These results suggest not only that ELF-EMF is mutagenic as a single agent but also that it can potentiate the mutagenicity of ionising radiation…"

3. The Commission is asked to please not base its decision making on industry consultant (Drs. Bailey and Erdreich from Exponent/New York, and former WHO now industry consultant Dr. Repacholi) who have been assisting in WHO’s and/or ICNIRP’s decision makings.

Furthermore the Commission is asked to have applied the precautionary principle, which is defined as follows at para. 7 of the Bergen Ministerial Declaration on Sustainable Development (1990), [2001] 2 S.C.R. 241 at para. 7:
In order to achieve sustainable development, policies must be based on the precautionary principle. Environmental measures must anticipate, prevent and attack the causes of environmental degradation. Where there are threats of serious or irreversible damage, lack of full scientific certainty should not be used as a reason for postponing measures to prevent environmental degradation.

Canada “advocated inclusion of the precautionary principle” during the Bergen Conference negotiations (D. VanderZwaag, CEPA Issue Elaboration Paper No. 18, CEPA and the Precautionary Principle/Approach (1995), at p. 8). The principle is codified in several items of domestic legislation: see for example the Oceans Act, S.C. 1996, c. 31, Preamble (para. 6); Canadian Environmental Protection Act, 1999, S.C. 1999, c. 33, s. 2(1)(a); Endangered Species Act, S.N.S. 1998, c. 11, ss. 2(1)(h) and 11(1).

4. **Info Request # 15 to FortisBC**

4.1 Does FortisBC and/or its experts agree with the content of Evidence # 16 Mairs et al. study. If not, where not and why not, please give detailed explanation so FortisBC arguments can be tested in the hearing.

4.2 Please state in detail how FortisBC is applying, and/or complying with, the precautionary principle - as indicated in section 3. above- with regards of subject project.

Respectfully submitted,

Hans Karow, CORE
Microsatellite analysis for determination of the mutagenicity of extremely low-frequency electromagnetic fields and ionising radiation in vitro

Robert J. Mairs a,b,*, Kate Hughes a, Sara Fitzsimmons a, Kevin M. Prise c, Anne Livingstone a, d, Lesley Wilson a, d, Nazia Baig a, Anne Marie Clark a, Alan Timpson a, Gaurang Patel c, M. Folkard c, Wilson J. Angerson e, Marie Boyd a

a Targeted Therapy Group, Division of Cancer Science and Molecular Pathology, Glasgow University, Cancer Research UK Beatson Laboratories, Glasgow G61 1BD, UK
b Department of Child Health, Yorkhill Hospital, Glasgow, UK
c Gray Cancer Institute, Mount Vernon Hospital, P.O. Box 100, Northwood HA6 2JR, UK
d Department of Clinical Physics, Western Infirmary, Glasgow G11 6NT, UK
e Department of Surgery, University of Glasgow, Royal Infirmary, Glasgow G31 2ER, UK

Received 28 March 2006; received in revised form 28 June 2006; accepted 1 August 2006

Abstract

Extremely low-frequency electromagnetic fields (ELF-EMF) have been reported to induce lesions in DNA and to enhance the mutagenicity of ionising radiation. However, the significance of these findings is uncertain because the determination of the carcinogenic potential of EMFs has largely been based on investigations of large chromosomal aberrations.

Using a more sensitive method of detecting DNA damage involving microsatellite sequences, we observed that exposure of UVW human glioma cells to ELF-EMF alone at a field strength of 1 mT (50 Hz) for 12 h gave rise to 0.011 mutations/locus/cell. This was equivalent to a 3.75-fold increase in mutation induction compared with unexposed controls. Furthermore, ELF-EMF increased the mutagenic capacity of 0.3 and 3 Gy H2O-radiation by factors of 2.6 and 2.75, respectively. These results suggest not only that ELF-EMF is mutagenic as a single agent but also that it can potentiate the mutagenicity of ionising radiation.

Treatment with 0.3 Gy induced more than 10 times more mutations per unit dose than irradiation with 3 Gy, indicating hyper-mutability at low dose.

© 2006 Elsevier B.V. All rights reserved.

Keywords: ELF-EMF; Gamma radiation; Microsatellite mutations

1. Introduction

Most people are exposed to extremely low-frequency electromagnetic fields (ELF-EMF) produced by power lines and electrical appliances. While some epidemiological studies have related exposure to ELF-EMF to an increased risk of certain types of adult and childhood cancer including leukaemia, cancer of the central ner-
vous system and lymphoma [1–4], others have failed to find such an association [5–8]. However, interpretation of these results is difficult because unreliable methods of assessment of exposure were employed and contact with other mutagens, such as cigarette smoke, was not taken into account.

In vitro studies with well-defined exposure conditions and end points may provide a more reliable means of estimating the possible carcinogenic potential of ELF-EMF than investigations based upon cancer risk estimates in human populations. Many such studies have been conducted, but contradictory results regarding the genotoxic potential of ELF-EMF and end points may provide a more reliable means of estimation that necessarily implies that weak ELF electromagnetic fields may promote DNA damage and hence may be genotoxic [19].

It has also been reported that EMF exposure alone is not genotoxic [11,12]. However, these methods cannot reveal molecular alterations, which may be subtle—perhaps involving modifications to one or a few nucleotide bases. Therefore, it is not surprising that the majority of such studies did not show ELF-EMF-related genotoxic effects. Nonetheless, genetic aberrations have been demonstrated in some studies [13–18] after exposure ELF-EMF according to various delivery schedules.

Vijayalaxmi and Obe [10] examined all reports between 1990 and 2003 of ELF-EMF-induced genotoxicity and concluded that 46% of studies did not identify increased genetic damage; 22% of such investigations did indicate a genotoxic effect of EMF exposure and 32% of the studies were inconclusive. The International Agency for Research on Cancer (IARC) has recently classified ELF-EMF as possibly carcinogenic—a categorization that necessarily implies that weak ELF electromagnetic fields may promote DNA damage and hence may be genotoxic [19].

We propose that part of the reason for the controversy associated with the mutagenicity of ELF-EMF is that the techniques used, while capable of identifying extensive and severe cellular DNA damage, are not sensitive enough to detect small-scale but potentially harmful genetic damage. Therefore, we have developed a more sensitive method of detecting DNA damage involving examination of microsatellite DNA sequences.

Microsatellites are non-coding DNA sequences distributed throughout the genome. They comprise variable numbers of short repeats of one to five base pair units. The role of microsatellites is unclear. While they may exert subtle influences on the regulation of gene expression, most human microsatellites probably have no biological role [reviewed in 25,26]. Higher spontaneous mutation frequencies in normal tissues have been reported in microsatellite sequences [27] and mutations occur 100 times more often in microsatellites than in coding genes [28]. Several studies have shown that such repeat sequences are hypermutable compared with the HPRT locus [29] and are hypermutable by radiation in somatic [30,31] and germ-line cells [32–36].

We have found the utilisation of microsatellite sequences for determination of radiation-induced mutations to be at least 1000 times more sensitive than analysis of coding genes such as the HPRT gene—the current gold standard method for detection of DNA mutation [31]. We now report on the refinement of this methodology using an automated, non-radioactive and high-throughput system for detecting and analysing DNA fragments after exposure of cultured cells to EMF, ionising radiation or a combination of the two.

2. Materials and methods

2.1. Cells and y-irradiation procedure

Analyses were conducted using the glioma cell line UVW (passage 7), which has been established and characterised in our laboratory in terms of growth properties and sensitivity to low-dose y-irradiation [31]. The doubling time of this cell line is approximately 18 h. The cells were cultured in Eagle’s minimum essential medium supplemented with 10% foetal bovine serum and 2 mM glutamine, at 37 °C in an atmosphere of 5% CO2. Media and supplements were obtained from Invitrogen, UK. Plastic-ware was obtained from McQuilken, UK.

Cells were seeded into T25 flasks and grown until they were 70% confluent (in exponential growth phase). They were treated with 0, 0.3 or 3 Gy using a 60Co source at a dose-rate of 0.3 Gy/min, and transferred immediately to an EMF generator.
The EMF irradiator comprised two identical solenoids, each 300 mm long by 120 mm diameter. Each coil was constructed from 400 turns of 1.5-mm diameter copper wire, with an additional 52 turns of wire at either end that could be energised independently from the main coil. These ‘shim’ coils were used to improve the field uniformity within the sample region, and it was possible to achieve a measured variation in field strength of less than 1% within a 200 mm by 100 mm diameter cylindrical volume. Each coil was housed within its own double-skinned mu-metal enclosure, supported horizontally in a temperature-controlled Hereaus CO₂ incubator [37]. The field attenuation between coils was >2000-fold. Additional temperature stabilisation was provided by pumping water from a reservoir (within the incubator) through tubing wound around each coil. Six thermocouples sited within each solenoid, and within the incubator were used to monitor the temperature. The coils were independently energised, using two 50-Hz ac power-supplies. These were computer-controlled and a versatile computer package was developed (using ‘Labview’) to control and log each experiment. The software allowed 50-Hz ac fields to be generated in either one or both of the coils.

Six T25 flasks were placed inside each coil. One coil was energised and the other used as a sham control under blinded conditions with both coils maintained at 36.5°C. Cells in the exposed coil were subjected to a field strength of 1 mT (50 Hz) for 12 h. Field strength and temperature in both coils were monitored throughout the procedure by computer. The following treatments were applied: 0 Gy external beam irradiation; ±1 mT (50 Hz) EMF irradiation; 0.3 Gy external beam irradiation ±1 mT (50 Hz) EMF irradiation; and 3 Gy external beam irradiation ±1 mT (50 Hz) EMF irradiation. Subsequent manipulations were conducted by technical staff who were blinded to the irradiation treatments.

2.2. Post-irradiation procedure

Immediately after irradiation, the cell density was adjusted to enable the deposition, on average, of one cell per well of 96-well culture dishes. This procedure was adopted to ensure that cells were separated as soon as possible after treatment, before they started to divide, so that each clone represented one irradiated cell. Microscopic examination was performed after 3–5 days. Wells containing more than one colony (hence derived from more than one cell) were discarded.

The cells were incubated at 37 °C and 5% CO₂ for approximately 2 weeks. Each colony was transferred to a single well of a six-well plate and again cultivated for 2 weeks. Once confluent, these cells were transferred to T25 flasks and grown for approximately 10 days, yielding enough cells to produce sufficient DNA for mutation analysis. DNA was then extracted from each colony of cells [38], yielding a DNA sample derived from a single irradiated cell. One hundred clones from each treatment group were analysed for the presence of mutations.

2.3. Fluorescent PCR

PCR reactions were carried out in a final volume of 12.5 µl consisting of 10 ng DNA, 0.3 µM forward primer, 0.3 µM reverse primer, 2.5 mM MgCl₂, 200 µM dNTPs (Larova Biochemie GmbH, Germany), 1.25 µl NH₄-based reaction buffer (BioTaq, Bioline, London, UK) and 2 U of Taq polymerase (BioTaq, Bioline, London, UK). The forward primer was conjugated at its 5’ end to one of three fluorescent dyes, enabling automated fragment analysis, and purified by HPLC (Proligo, Paris). Sequences were amplified in 30 cycles at 94 °C for 30 s, at 60 °C for 30 s, at 72 °C for 30 s and at 72 °C for 20 min using a DNA Engine Peltier Thermal Cycler (GRI, Essex, UK). Thirteen different microsatellite loci were analysed. These are distributed randomly throughout the genome. The loci analysed and the corresponding primers are shown in Table 1.

2.4. Analysis of fluorescent DNA fragments

PCR reactions incorporating all three dyes were analysed simultaneously by adding 0.5 µl of each PCR product to the same well of a 96-well plate with 0.2 µl DNA size standards.
Previously reported a microsatellite mutation rate, follow- ing capillary gel electrophoresis, we observed 3.0 × 10⁻³ mutations/locus/Gy/cell after a 3-Gy treatment, suggesting that the automated method enabled the detection of 3.4 times more mutations than the manual method. This apparent heightened sensitivity may be due to our ability to score with confidence AI mutations revealed by fluorescent fragment analysis. Such genetic aberrations could not be identified unambiguously in autoradiograms of electrophoresed microsatellite sequences.

In the absence of ionising radiation, cells exposed to 1 mT ELF-EMF alone experienced a greater rate of mutation induction, by a factor of 3.75 (P = 0.019), than cells that were sham-exposed. This was unexpected because the majority of previous observations of genotoxicity by ELF-EMF occurred when ELF-EMF was applied either at much higher doses (10–400 mT) or in tandem with other genotoxic or mutagenic agents such as γ-irradiation [10]. This also suggests that this phenomenon may not have been revealed previously utilising less sensitive methodology.

A second aspect of the study was the examination of the effect of the combination of ELF-EMF with the well-characterised mutagen γ-radiation. This allowed comparison with previous findings suggesting that EMF alone is not genotoxic but acts as a promoter of DNA damage by ionising radiation [42–44]. Our results indicate that ELF-EMF increased the mutagenic capacity of 0.3 and 3 Gy γ-irradiation by factors of 2.62 (P = 0.002) and 2.75 (P = 0.002), respectively (Table 2). Therefore, these results suggest not only that EMF may increase the DNA damage induced by ionising radiation but also that EMF is mutagenic as a single agent.

An important finding was that, in the absence of EMF, 0.3 Gy was 11.0 times more efficient than 3 Gy, per unit dose, in inducing mutations. When applied in combination with 1 mT EMF, 0.3 Gy was 10.2 times more mutagenic than 3 Gy. We have previously reported that UVW cells, in common with most tumour-derived cell lines, showed evidence of increased sensitivity (measured as clonogenic survival) per unit dose to radiation in the range 5–50 cGy [45]. This feature, known as low-dose hyper-radiosensitivity (HRS) [46], may be exploited to enhance the efficacy of radiotherapy of resistant tumours by ultra-fractionation—the administration of multiple fractions of <0.5 Gy [47]. However, it is not known whether HRS could also increase the severity of adverse effects on normal tissues exposed to low-dose radiation.

HRS may be a manifestation of incomplete activation of repair mechanisms in response to low radiation dosage [48]. Failure to restore damaged DNA, associated with inefficiency of cell kill at low dose, suggests that a consequence could be increased frequency of mutations. If our finding that 0.3 Gy treatment induced more mutations per unit dose than 3 Gy, were to be corroborated in other models that also display low-dose HRS, the use
Fig. 1. Microsatellite mutation detection by electrophoresis of fluorescently labelled PCR products. The chromatograms depict abundance of product (fluorescence intensity) against size (nucleotides) of alleles at loci D18S61 (a), D5S2088 (b) and D7S500 (c) for four of the 100 clones examined per locus. (a) Loss of heterozygosity: at this locus, clones 1, 2, and 4 are heterozygous. Clone 3 exhibits loss of one allele. (b) Change in allele size: at this locus, clones 1, 2, and 4 are homozygous. Clone 3 has acquired a larger allele, in addition to the normal allele. (c) Allelic imbalance: in clone 3, some of the cells in the population have lost one allele. This is manifest by the reduced height of one allelic peak.
of ultra-fractionated radiotherapy, designed to exploit HRS at sub-Gy doses of radiation, would be contraindicated. The increased frequency of mutations produced by combining EMF with γ-radiation treatment suggests that EMF exposure may inhibit the repair of DNA lesions. We have demonstrated in this and previous studies, that microsatellite loci are suitable for use as reporter sequences for DNA-damage induction. Instability of microsatellite and minisatellite sequences are associated with at least 35 human diseases, including tumour-associated microsatellite instability and neurological disorders including fragile X syndrome, myotonic dystrophy and Huntington’s disease [49]. Whether the changes in repetitive sequences of DNA demonstrated in this

Table 2
Frequency of mutations at 1300 microsatellite loci (13 loci/cell × 100 cells) in each of six treatment groups exposed to gamma and/or EMF radiation

<table>
<thead>
<tr>
<th>Gamma</th>
<th>EMF</th>
<th>No. of mutations</th>
<th>Mutation ratea (/locus/cell × 10⁻³)</th>
<th>Ratio (EMF exposed/not exposed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AL</td>
<td>LOH</td>
<td>Δ size</td>
</tr>
<tr>
<td>0 Gy</td>
<td>0 mT</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1 mT</td>
<td>7</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>0.3 Gy</td>
<td>0 mT</td>
<td>6</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>1 mT</td>
<td>24</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>3 Gy</td>
<td>0 mT</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1 mT</td>
<td>18</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

AI: allelic imbalance; LOH: loss of heterozygosity; Δ size: shorter or longer alleles, suggestive of alteration of repeat number.

a 95% confidence intervals are shown in parentheses.
study reflect changes in coding sequences and whether there is a physiological consequence of these alterations (e.g. cancer induction or promotion) are topics of intense debate, which require further study [26].

4. Conclusions

The treatment of cells with ELF-EMF of field strength 1 mT, in the absence of ionising radiation, induced more mutations than were observed in untreated controls. It is necessary to determine the mutation rate in response to lower magnetic flux density (at the μT level) to assess the significance of the ELF-EMF exposure that is of concern to most of the population.

Exposure of cells to both γ-radiation and ELF-EMF radiation resulted in an increased mutation frequency compared with the effect of ionising radiation alone. These results suggest not only that ELF-EMF is mutagenic as a single agent but also that it can potentiate the mutagenicity of ionising radiation.

Finally, the observation of higher mutation rate per unit dose at 0.3 Gy compared to 3 Gy indicates that further study of the potential adverse effects of low-dose ionising radiation is warranted.

Acknowledgements

This work was supported by the Department of Health, the Clerk Maxwell Cancer Research Fund and North Glasgow University Hospitals. We are grateful for the technical assistance of Stuart Gilchrist, Gray Cancer Institute, Northwood, London.

References


Please cite this article as: Robert J. Mairs et al., Microsatellite analysis for determination of the mutagenicity of extremely low-frequency electromagnetic fields and ionising radiation in vitro, Mutation Research (2006), doi:10.1016/j.mrgentox.2006.08.005


