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Neurophysiological Effects of Flickering Light in Patients with Perceived Electrical Hypersensitivity

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An increasing number of people in Sweden are claiming that they are hypersensitive to electricity. These patients suffer from skin as well as neurological symptoms when they are near computer monitors, fluorescent tubes, or other electrical appliances. Provocation studies with electromagnetic fields emitted from these appliances have, with only one exception, all been negative, indicating that there are other factors in the office environment that can effect the autonomic and/or central nervous system, resulting in the symptoms reported. Flickering light is one such factor and was therefore chosen as the exposure parameter in this study. Ten patients complaining of electrical hypersensitivity and the same number of healthy voluntary control subjects were exposed to amplitude-modulated light. The sensitivity of the brain to this type of visual stimulation was tested by means of objective electrophysiological methods such as electroretinography and visual evoked potential. A higher amplitude of brain cortical responses at all frequencies of stimulation was found when comparing patients with the control subjects, whereas no differences in retinal responses were revealed.

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In recent years, an increasing number of people have reported subjective symptoms and physiological reactions to various types of electrical equipment, including video display terminals (VDTs), fluorescent light, household appliances, televisions, etc. A few attempts have been made to describe this group of persons with so-called electrical hypersensitivity in the literature.[1-3] The most frequent complaints are headache, eye and general fatigue, and sensory symptoms such as itching, stinging, or burning sensations in facial skin. At first, the symptoms have an intermittent character and disappear after a short-term rest away from the source of the electricity, but for some patients the symptoms become more persistent. In severe cases, people can not use VDTs or artificial light at all. They usually associate their symptoms with electromagnetic (EM) fields emitted from these appliances. These emissions are, however, rather low, and during the last 5 years the electric and magnetic fields emitted from new VDTs sold in Sweden have decreased rapidly depending on recommendations from Swedish authorities and labor unions about a reduction in the strength of the emitted fields.[4,5] In spite of this, some patients still claim that even these new low-emission VDT monitors provoke symptoms.

Fluorescent lamps placed in the ceiling hardly give any contribution to the general magnetic field in a room. If the lamp is not properly grounded, it might be a dominating

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source of an electric field in the room; otherwise, the contribution to the electric field level is very small.[6] Indications that EM fields and/or fluorescent tubes might be of interest in this context was found in an epidemiological study performed in northern Sweden.[7] Case subjects were selected from a questionnaire study and were defined as working with VDTs for more than 1 hour per day and having sensory skin symptoms. Significantly more case than control subjects were found in offices with increased electric fields, and case subjects were more common than control subjects in offices with increased low-frequency magnetic fields from VDTs. Other factors, such as psychosocial factors and work organization, were also indicated as risk indicators.[8]

With one exception, all provocation studies to date with EM fields have been negative. Rea et al[1] examined 100 patients with claimed hypersensitivity and found that 16 of the subjects were able to react repeatedly to magnetic fields with frequencies from 0.1 Hz to 5 MHz. Others[9,10] could not find a significant correlation between exposure and subjective sensations and/or physiological reactions.

It seems likely that there are other factors in the office environment that can affect the autonomic and/or central nervous system, resulting in the experienced symptoms. Because both VDTs and fluorescent tubes generate amplitude-modulated light at frequencies of 50 to 100 Hz,[11] this might be a common symptom-provoking parameter in office environments. A recent study[12] on the effect of different types of fluorescent lights found a number of negative responses—subjective discomfort as well as electroencephalogram (EEG) changes—among test subjects when they were exposed to "daylight" lamps as compared with "warm-white" lamps. It is known that the daylight lamps used in the study had almost 100% amplitude-modulated light vs only 20% for the warm-white lamps.[11]

It is known that the nervous system is able to respond to amplitude-modulated light with frequencies above the critical flicker fusion frequency, ie, the frequency of the flickering light above which the light is perceived as continuous. The sensitivity of the retina and the brain to this kind of visual stimulation can be detected by means of conventional electrophysiological methods such as electroretinography (ERG), visual evoked potential (VEP),[13] and neuron activity.[14] In a normal situation, a linear relation in logarithmic coordinates between the amplitudes of ERG and VEP and the frequencies of the modulated light have been shown.[15,16] Brunett[17] reported that in people complaining of headaches, the VEP amplitude decreased less rapidly with increased stimulus frequency than it did in healthy subjects. In view of these results, it will be interesting to learn if persons with electrical hypersensitivity have an enhanced sensitivity to high-frequency amplitude-modulated light.

The study presented here (which constitutes the first part of a more extensive program) aims to answer the question of whether there are differences in neurophysiological responses to light stimulation of different frequencies and modulation depth between hypersensitive persons and healthy subjects.

Materials and Methods

Subjects

Ten patients (three men and seven women, 39 to 57 years of age; mean age, 47) participated in the study. Six patients were treated at the Department of Dermatology and four were treated at the Department of Occupational Health of Umeå University Hospital. The patients had neurological and/or skin symptoms that they claimed had resulted from VDT work, fluorescent light, or television exposure. The prevailing symptoms and the patients' experiences with appliances having provoked symptoms are presented in Table 1. The character of the symptoms varied widely among these patients. However, the group was selected according to the following criteria: (1) they usually had a combination of skin (mucous), eye, and neurological complaints, (2) they associated their complaints with EM fields emitted from the above-mentioned appliances, (3) clinical examinations did not show any psychiatric and/or somatic diseases severe enough to require medical treatment.

Facial skin symptoms (itching/stinging/tight or burning sensations) are the most common symptoms to appear first. Dryness, a gritty feeling, and (for patients 4, 7, and 8) foggy view were reported as eye symptoms. General neurological symptoms appeared as the first symptom only for patient 6, but as a part of the reported symptoms for all patients. The most common symptoms were headache, dizziness, fatigue, feeling "heavy-headed," and difficulty concentrating. Only patient 2 reported no such symptoms. Muscle weakness was reported as a provoked symptom for patient 6. Mucosal symptoms from throat and nose were reported from two patients.

The most common sources for the symptoms experienced were VDTs, fluorescent light, and nearby electric appliances. For one patient, heat was noted as a provoking source, and driving a car was noted as a provoking situation for another patient.

The duration of the disturbances varied from a couple of months to up to 8 years. The anamnesis showed that the patients had usually been in an intensive work period when the symptoms first appeared. Traditional skin therapy, antiallergic and sedative drugs, and cognitive psychotherapy have to some degree improved the conditions for some of the patients.

Ten subjects (four men, six women, 26 to 52 years of age; mean age, 37) without any problems associated with VDT work or light exposure served as the control group. Some of the patients, as well as the

TABLE 1
Age, Gender, Type/Site of Appeared Symptoms and Situations/Nearby Appliance When the Symptoms Appear, According to Each Patient's Own Experience

			Self-Reported Symptom Type			Situation/Appliance Provoking Symptoms*		
Patient	Gender	Age	First	Second	Third	First	Second	Third
1	F	44	Facial skin	Eyes	Neurological	VDTs	Fluor. tubes	Elec. appliance
2	M	50	Facial skin			VDTs	Fluor. tubes	Elec. appliance
3	F	49	Eyes	Facial skin	Neurological	Fluor. tubes	VDTs	Elec. appliance
4	F	57	Eyes	Neurological	Throat, nose	Fluor. tubes	Elec. appliance	VDTs
5	F	55	Facial skin	Neurological	Throat	Elec. appliance	Fluor. tubes	VDTs
6	F	44	Neurological	Facial skin	Muscle weakness	Fluor. tubes	VDTs	
7	F	39	Facial skin	Eyes	Neurological	Fluor. tubes	Driving a car	
8	M	40	Facial skin	Neurological	Eyes	VDTs	Fluor. tubes	Elec. appliance
9	M	46	Facial skin	Red skin on hands	Neurological	VDTs	Fluor. tubes	Heat
10	F	45	Facial skin	Neurological		VDTs	Fluor. tubes	

* VDT, visual display terminal; Fluor., fluorescent; Elec., electrical.

control subjects, also had minor vision dysfunction such as myopia (up to -4D) and astigmatism. Members of both the patient and volunteer groups were paid and informed in detail about the study's goal and procedures. The study was approved by the local ethics committee.

Procedure

The design of the investigation aimed to minimize the environmental and stress factors, and the total duration and complexity of examinations, which may lead to different emotional disturbances in hypersensitive patients.

The resting subject sat in an adjustable dentist's armchair in a special electrically shielded chamber with a background level of an extremely-low-frequency (ELF) magnetic field less than 20 nT (nanotesla) and a corresponding electric field less than 1 V/m (volt/meter). A white light-penetrable 0.45-m × 0.45-m screen with a centered black cross for eye fixation was placed in front of the subject at a distance of 1.0 to 1.2 m. All subjects were awake (no sedatives or mydriatics were given) and looked at the eye-fixation point throughout the stimulation period. One staff member (MS) sat with the patient in the test chamber during the entire test procedure, describing current steps of the investigation and asking about the appearance of negative sensations.

Before the light presentation, the subject's electrocardiogram results were recorded (interbeat intervals [IBI] were analyzed). The data-acquisition time span lasted 10 minutes, including 5 minutes of active communication with the staff member (MS), and a 5-minute relaxation period in darkened conditions.

Light presentation included two different kinds of stimulation: flash stimulation with a stroboscope discharge lamp, to examine the behavior of occipital VEPs and retinal ERGs at different frequencies, and a sector disc stimulation providing different depths of light modulation by means of a system of two slide projectors.

Because responses to high-frequency flickers were the focus of this study, steady-state protocol with unpatterned stimuli for VEP and EEG recording was used.[18,19] The stroboscope discharge lamp (Movistrob Model 350.10; BBE, Germany) was placed just behind the screen and its center matched the eye-fixation cross. The flash wave form was spiky, with a rise time of less than 1 μs and a fixed amplitude independent of the frequency. VEP and ERG were recorded at frequencies of 20, 25, 35, 45, 55, 65, and 75 flashes per second. The flash duration was approximately 10 μs.

The two-slide-projector system consisted of one projector with a lamp stabilizer providing direct-current light with an amplitude modulation of less than 1%, with a second slide projector emitting a beam of light (also direct-current) that was modulated by means of a rotating sector disc placed before the lens. Both projectors had independent transformers providing selective increase/decrease of their brightness, resulting in different degrees of light modulation on the screen. The peak value of the luminance of the screen was 500 cd/m^2. A schematic drawing of the projector system set-up is shown in Fig. 1.

The depth of the modulation was calculated as $(L_{max} - L_{min})/(L_{max} + L_{min})$, where L_{max} was the maximal value of luminance and L_{min} was the minimal value of luminance. We have used 100, 75, and 50% modulation at 45 Hz (Fig. 2). This frequency was chosen because of the distinctive responses it produced in all subjects, and because it is near the refresh-rate frequency of televisions and VDTs.

The wave form of the light emitted from the slide projectors and stroboscope was monitored with a photometer and the signal was displayed on a digital oscilloscope.

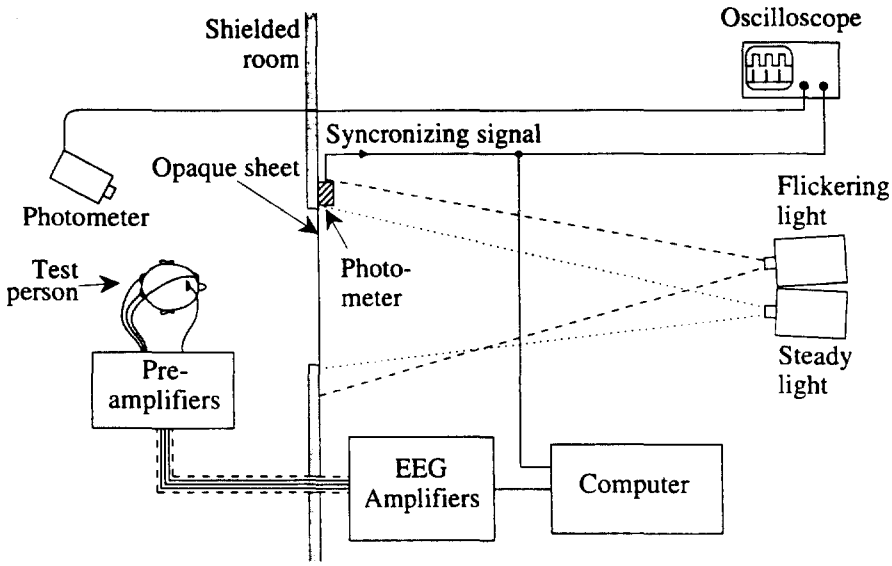

Fig. 1. A schematic drawing of the experimental set-up for the investigation of neurophysiological responses to different depths of light modulation. The test person sat in a electrically shielded chamber, looking at a white light-penetrable screen on which the light of interest was projected. Two slide projectors (one producing steady direct-current light, the other one emitting a beam of direct-current light modulated by means of a rotating sector disc placed before the lens) were placed outside the chamber. The synchronizing signal was picked up by a photometer placed within the area of the flickering light beam. The same set-up was used to perform flash light stimulation. In those tests, the stroboscope was placed just in front of the screen outside the chamber.

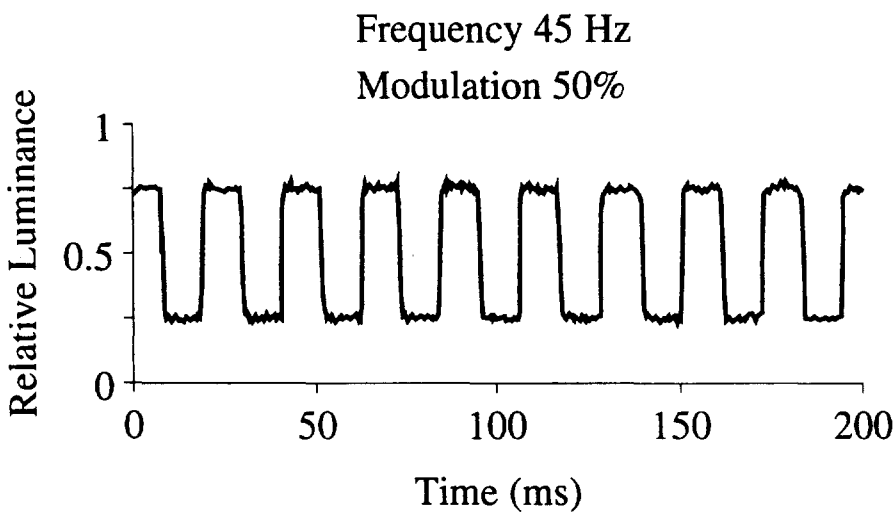

Fig. 2. Examples of the light presentations at 45 Hz when the modulation depth was 50%. The root-mean-square luminance value was kept constant for each of the modulation depths used.

The duration of light exposures at each frequency or depth of modulation varied from 1.5 to 2 minutes, depending on the minimal time needed for the data acquisition. There was a 2-minute pause between each set of light exposures to give the participants a rest period and time for retinal recovery. During these pauses, the subjects were allowed to close their eyes or look around, as well as talk with the personnel. The subjects were asked about their sensations and possible discomfort. None of the subjects complained about dry eyes, ocular discomfort, or headache as a result of the experimental procedure.

To ensure that the recorded signals were brain responses to the light stimulation, both the stroboscope and slide-projector stimulation were repeated under sham exposure conditions during which the light sources were covered with lightproof paper and the screen was constantly illuminated by stray light from an incandescent lamp.

Recording Arrangements

The VEP was recorded by Ag/AgCl disc electrodes filled with saline jelly and placed bilaterally 2 cm above the inion, 2.5 cm to the right and to the left from the midline, both of them referring to the central electrode placed between the Cz and Pz positions of the International 10–20 notation. The ERG was recorded by the same type of electrodes placed on the lateral margin of the left orbit referring to the forehead. A noncorneal recording of ERG using averaging techniques provides reliable and comparable data with the scleral lens electrode technique. The particular properties of these electrode methods (they are the least traumatizing, do not require use of an anesthetic, are more quickly applied, and eliminate the risk of corneal abrasion or conjunctival infection) were of primary importance in examinations of these hypersensitive persons.[20,21] Pupil dilation was not used.

The VEP and ERG samples were passed through an impedance matching circuit and amplified with 1 to 500 Hz bandpass. The VEP and ERG waves were digitized at 1000 samples/second with a 12-bit analog/digital converter and loaded into a personal computer for further processing. A train of pulses, synchronized with the stroboscope lamp or with the signals from the slide projector, was used as a trigger to average VEP and ERG responses. All epochs were stored on the hard disk for off-line analysis. Artifact epochs (eye blinks first) were automatically detected and excluded from further analysis. The number of single data acquisitions that were averaged varied between 200 and 1000 depending on the frequency of stimulation. The

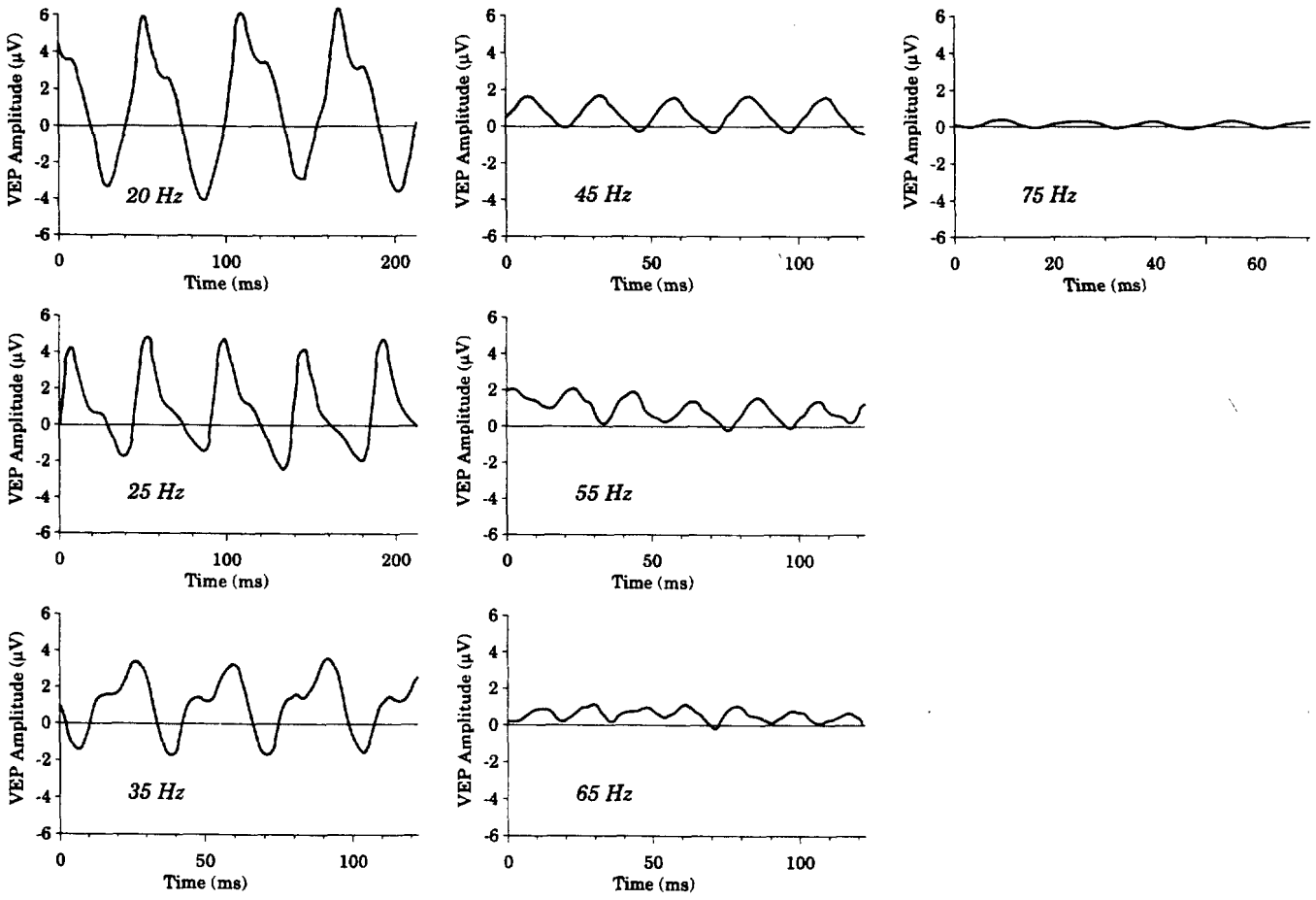

Fig. 3. Examples of occipital visual evoked potential responses for one subject at increasing stimulation frequencies from 20 to 75 Hz.

epoch of analysis included from 5 to 8 cycles of responses for each frequency of stimulation.

Statistical Analysis

The VEP responses to the amplitude-modulated light were, as expected, phase-locked to the periodic oscillations. Thus, the quantitative analysis of data was performed using the time-series analysis.[22] The occurrence and amplitude of synchronous oscillations in the VEP responses were approximated by a regression curve comprising the two first terms of the Fourier transform, using a least-square algorithm.[23]

The analysis of differences between samples (patients and the control subjects) was performed using a method of regression analysis. It was found that the VEP and ERG values (Y) fit an exponential decay model as a function of frequency:

$$Y = A + B(f - f_m) + e$$

where A and B are the regression coefficients, f is the frequency of stimulation, f_m is the mean value of the frequencies, and e is the normal stochastic error.

Equivalency of the slopes (B) and intercepts (A) for two samples was tested.[24] Statistics have Student's *t* distribution with 2(mn-2) or 2(mn-3) degrees of freedom, respectively; m is the number of subjects in each group, and n is the number of frequencies analyzed. Differences of $P < 0.05$ were considered as significant.

Results

As expected, the evoked responses had a nearly sinusoidal form, with the fundamental frequency equal to the stimulation frequency. Occipital VEPs of one subject during the stroboscope light stimulation at different frequencies are presented in Fig. 3. Data show the averaged VEP as a function of time. Note the typical decrement of the amplitude with increased stimulation frequency (Fig. 3). All subjects in both groups had discernible VEPs up to 55 Hz; many of them had VEPs up to 65 Hz, and three subjects (two in the patient group, and one from the control group) had VEPs up to 75 Hz.

Mean values of VEP and ERG amplitudes (peak to peak) at the investigated frequencies and standard errors of the mean for patients and control subjects are presented in Fig. 4. The results show an exponential amplitude-frequency dependence. The data obtained in the control group were close to those published by Van der Tweel et al[25] and Brundrett[17] for their subjects without visual abnormalities.

The dynamics of the VEP amplitude showed a statistically significant difference in the amplitudes (ie, intercepts) of the curves between pa-

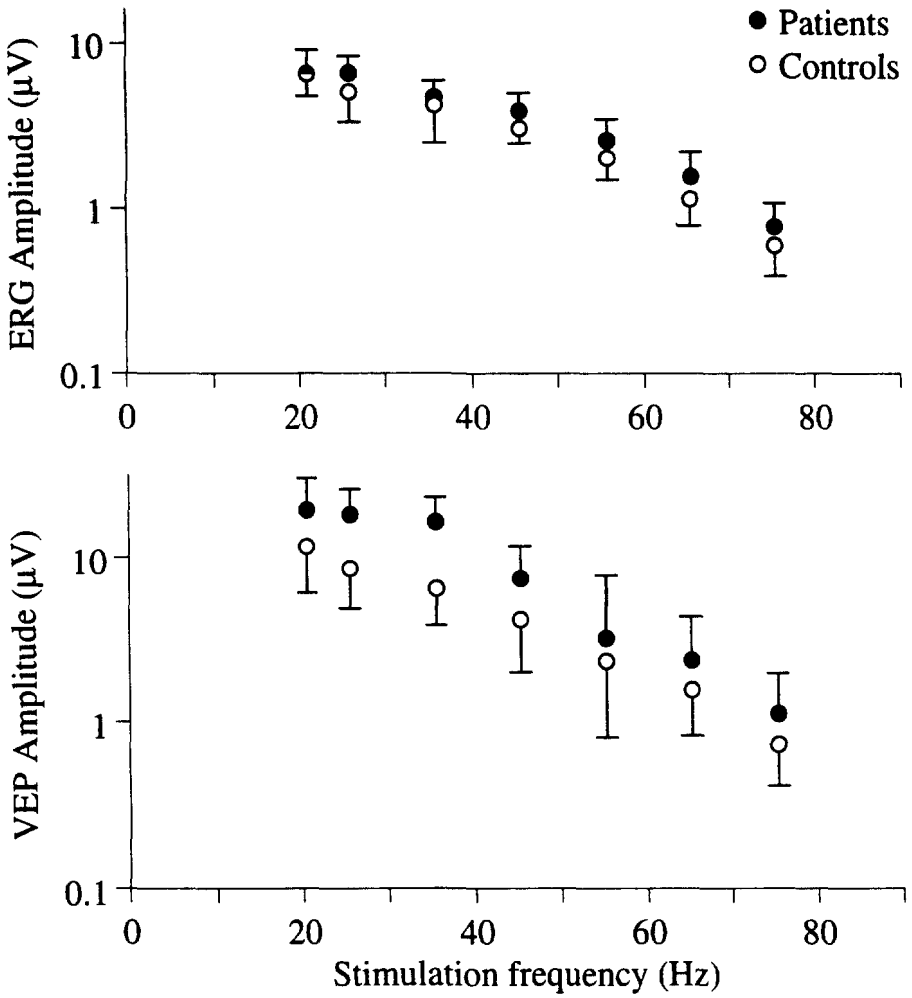

Fig. 4. The mean values of the electroretinography (ERG) and visual evoked potential (VEP) amplitude responses, in logarithmic scale, at different stroboscope flash frequencies for the patients and the controls. The lines indicate the standard errors.

tients (p) and control (c) subject groups ($A_p = 1.02$; $A_c = 0.81$; $P < 0.05$). There was no significant difference between groups in the slope of the VEP curves ($B_p = 0.025$; $B_c = 0.022$), and no difference was seen between the curves in the ERG recordings.

The experiments with different modulation depths at 45-Hz stimulation showed some differences between patients and control subjects at 100% and 50% modulation depths. The VEP amplitudes were higher among patients ($P < 0.05$) (Fig. 5). The mean values of the ERG for the patients were also higher compared with those of the control subjects, but the difference was not statistically significant.

The mean values of the IBI (Table 2) significantly increased ($P < 0.05$) during the relaxation period in patients, whereas it did not change in control subjects. It should be noted that background values of IBI among the patients were significantly lower compared with those of control subjects. The average data in the control group showed no changes in IBI during the relaxation period.

Discussion

In this study, physiological measurements of reactivity to high-frequency flickering light were applied, because this environmental factor presumably has a significance in the genesis of symptoms of electrical hypersensitivity.

The significant difference between patients and control subjects was revealed as a shift of the curves approximating VEP amplitude-frequency dependence. The difference indicates that the amplitude of occipital reactions to light stimulation is distinctively higher among patients, and this might be considered as an objective sign of hypersensitivity to amplitude-modulated light stimulation.

It is necessary to interpret this difference in VEP amplitude between patients and control subjects with caution. Interindividual variability and age difference may influence this variable. From the difference between the mean age in the control and patient groups (37 vs 47, respectively), one may expect that control subjects have responses with higher amplitude because sensitivity to high-frequency flicker decreases with age.[17,19] The data obtained in the study presented here show the opposite situation: higher amplitude was obtained in the patient group.

At the same time, the slope of the curves showed a nonsignificant difference. Brundrett[17] investigated VEP in five persons with complaints of headache or eyestrain associated with work in rooms with fluorescent light. Sufferers tended to have a slower decline in evoked responses with frequency increase. In physiological terms, this means that the most prominent differences of brain reactivity are displayed at higher frequencies of light stimulation. In the study presented here, we found a constant shift toward hyperreactivity at all observed frequencies.

One may assume that the hypersensitivity to flicker displayed in VEP correlates with changes of subjective perception (for instance, with Critical Fusion Frequency [CFF]) because previous studies[26] have shown close correlation between the VEP dynamics and CFF. In the study presented here, the general duration of light exposures was limited by objective physiological measurements and did not include CFF in-

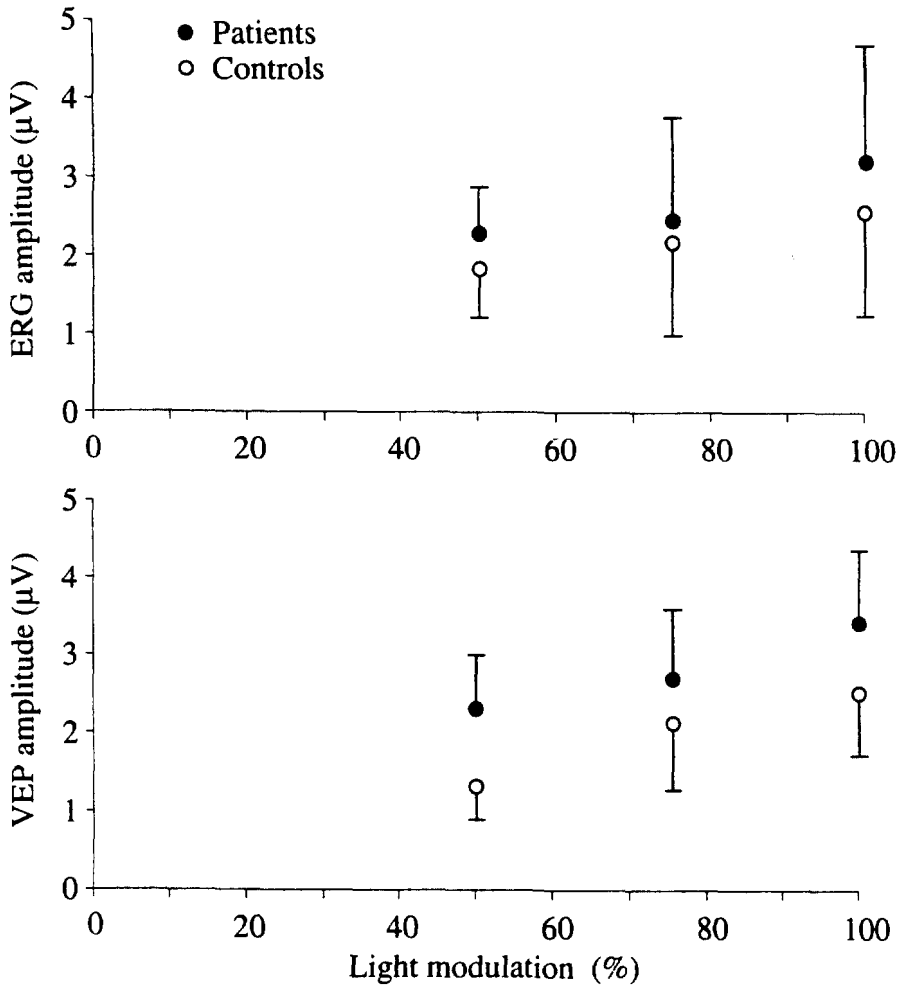

Fig. 5. The mean values of the ERG and VEP amplitude responses, in logarithmic scale, at different depths of the modulated light. The stimulation frequency was 45 Hz. The lines indicate the standard errors.

TABLE 2
Heart Interbeat Intervals (in seconds) During Active Communication and Relaxed Conditions*

	Beat Interval(s)	
Period	Control Group	Patient Group
Active	0.84 ± 0.10	0.72 ± 0.09
Related	0.85 ± 0.09	0.75 ± 0.08

* Data acquisition time span was 5 minutes.

vestigation because an overload of the patients must be excluded. This will, however, be included in future studies.

Another consistent line of further experiments might be focused on the functioning of magnocellular division of the visual system. Because the difference of brain responses was found at high-frequency flicker, it may be assumed that those pathways are basically affected in these patients. It is known that magnocellular pathways provide reactivity to high-frequency stimulation, including both temporal and spatial ones (as well as low-contrast changes). Further experiments with special methods of visual stimulation would elucidate the possible functional peculiarities of these pathways on such patients.

In contrast with the VEP data, significant differences in ERG were not found either in the slopes or the shift of curves. Moreover, discernible ERG at frequencies of 55 to 75 Hz were seldom recorded, whereas some studies have shown that ERG can be recorded at 120 Hz and higher.[16] This difference is likely a result of the use of noncorneal (skin) electrodes in the study presented here, whereas the researchers cited in reference 16 used scleral-contact electrodes.

Although noncorneal ERG responses decline with frequency more rapidly compared with corneal electrodes, this tendency was the same for patients and the control subjects. Furthermore, no differences were observed at 20 to 45 Hz where ERG were distinctly recorded in both groups.

The significant differences in VEP between the patients and the control subjects and the similarity of ERG points to a specific hypersynchronization of cortical responses to high-frequency light stimulation on patients complaining of hypersensitivity, which does not correlate with changes in peripheral, retinal reactivity.

It is not possible to draw any conclusions from this study with regard to possible explanations of the subjective symptoms reported or its connection to electromagnetic fields. However, this study indicates that patients with perceived electrical hypersensitivity are hyperreactive in their nervous system. This is in agreement with the result of earlier studies.[1,3,27]

The following hypothesis is, however, advanced as a basis for further examination: One may speculate that a stable enhancement of cortical synchronization to flicker in the projection areas of the brain might lead to generalization of this process on extended structures and areas involving the autonomic nervous system and neurochemical balance. In this case, the cortical hypersynchronization resulting from exposure to flickering light might be considered as a trigger for further neurochemical disturbances, leading to typical symptoms of hypersensitivity. Such a mechanism is only presumed now, and special experimental proofs are needed to confirm it.

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Lymphomas in Eμ-*Pim1* Transgenic Mice Exposed to Pulsed 900 MHz Electromagnetic Fields

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Repacholi, M. H., Basten, A., Gebski, V., Noonan, D., Finnie, J. and Harris, A. W. Lymphomas in Eμ-*Pim1* Transgenic Mice Exposed to Pulsed 900 MHz Electromagnetic Fields. *Radiat. Res.* 147, 631–640 (1997).

Whether radiofrequency (RF) fields are carcinogenic is controversial; epidemiological data have been inconclusive and animal tests limited. The aim of the present study was to determine whether long-term exposure to pulse-modulated RF fields similar to those used in digital mobile telecommunications would increase the incidence of lymphoma in Eμ-*Pim1* transgenic mice, which are moderately predisposed to develop lymphoma spontaneously. One hundred female Eμ-*Pim1* mice were sham-exposed and 101 were exposed for two 30-min periods per day for up to 18 months to plane-wave fields of 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms. Incident power densities were 2.6–13 W/m^2 and specific absorption rates were 0.008–4.2 W/kg, averaging 0.13–1.4 W/kg. Lymphoma risk was found to be significantly higher in the exposed mice than in the controls (OR = 2.4, P = 0.006, 95% CI = 1.3–4.5). Follicular lymphomas were the major contributor to the increased tumor incidence. Thus long-term intermittent exposure to RF fields can enhance the probability that mice carrying a lymphomagenic oncogene will develop lymphomas. We suggest that such genetically cancer-prone mice provide an experimental system for more detailed assessment of dose–response relationships for risk of cancer after RF-field exposure. © 1997 by Radiation Research Society

INTRODUCTION

Concern has been expressed for a number of years that exposure to radiofrequency (RF) fields emanating from telecommunications devices, heating equipment and radar and television transmitters may increase the incidence of cancer in humans. Epidemiological studies have not indicated an increased cancer risk, but the methodology and exposure assessments are generally considered to have been suboptimal (*1–3*).

The mechanisms presently known by which normal cells are transformed into neoplastic cells involve alterations to the structure of somatic cell DNA such as point mutations, translocations, deletions, amplifications and retroviral provirus insertions (*4, 5*). Experiments reviewed for the World Health Organization (*2*) and for the National Radiological Protection Board of the UK (*1*) did not demonstrate convincingly any direct damage to DNA after acute or chronic exposure of biological systems to RF fields. In particular, when temperatures were maintained within normal physiological limits, no evidence for induction of DNA breaks or chromosome aberrations was found. On the other hand, two recent studies have suggested that RF fields can affect DNA. In the first, Sarkar *et al.* (*6*) found evidence of an alteration in the length of a DNA microsatellite sequence in brain and testis cells of mice exposed to 2.45 GHz fields at a specific power absorption rate (SAR) of 1.2 W/kg for 2 h/day for up to 200 days. In the second, Lai and Singh (*7*) reported the occurrence of single-strand breaks in rat brain DNA shortly after the animals had been exposed for 2 h to pulsed or continuous-wave 2.45 GHz fields with SARs of 0.6 or 1.2 W/kg. Until these results and their interpretation are confirmed, doubt will remain as to whether RF fields can induce any of the types of genetic change in cells that lead to malignancy.

A number of studies in experimental animals have sought to determine directly whether RF fields can affect the development of cancer. Szmigielski *et al.* (*8*) and Szudzinski *et al.* (*9*) reported that chronic exposure of mice to RF fields (2.45 GHz, SAR 2–8 W/kg, 2 h/day, 5–6 days per week for up to 12 months) accelerated the development of metastatic colonies from transplanted sarcoma cells and increased the incidence of primary mammary tumors in predisposed animals and of skin tumors induced with 3,4-benzopyrene. Further work by this group (*10*) found that similar exposures increased the number of hepatomas, sarcomas and skin tumors in mice treated with chemical carcinogens. On the other hand, Wu *et al.* (*11*) were unable to demonstrate significant enhancement of colon carcinogenesis by

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dimethylhydrazine in mice chronically exposed to 2.45 GHz fields, and two other studies of transplanted melanoma (*12*) and brain tumors (*13*) in mice likewise failed to find significant effects of 2.45 GHz or 915 MHz fields, respectively. Furthermore, a large study of rats exposed for 21.5 h/day for 2 years to 2.45 GHz fields pulsed at 800 Hz and producing SARs of 0.15–0.4 W/kg did not show any alterations in over 150 parameters of health and longevity (*14*). No single type of tumor was increased in frequency to a statistically significant extent in the exposed animals. The overall incidence of malignancies was raised significantly, but the authors of the study (*14*) questioned the biological significance of this finding because the higher incidence levels of specific malignancies were similar to those reported previously for unexposed rats of the strain used.

The overall conclusion from the studies published so far is that uncertainty persists as to whether exposure to RF fields can influence the process of carcinogenesis. One way of attempting to resolve this issue is to perform further tests under carefully controlled conditions using large numbers of animals with a genetic predisposition to develop tumors, the incidence of which is greatly increased by weakly carcinogenic influences. Transgenic mice expressing an activated *Pim1* oncogene in their lymphoid cells seemed to fulfill these criteria for malignant lymphoma (*15, 16*). We therefore performed a study designed to test whether long-term exposure of Eμ-*Pim1* mice to pulse-modulated 900 MHz fields can increase the incidence of lymphoma. The pulse modulation and the frequency were selected to correspond to those of the recently introduced digital system of cellular mobile telecommunications. This paper describes the experimental system and the results, which show a moderate but statistically significant increase in lymphomas in the exposed animals.

MATERIALS AND METHODS

Mice

The characteristics of the ppG64 strain of Eμ-*Pim1* transgenic mice have been described (*15–17*). Virgin, hemizygous-transgenic females and nontransgenic C57BL/6NTac females were purchased from GenPharm International (Mountain View, CA). From an original mixed genetic background derived from two mouse strains (C57BL/LiA and CBA), the transgenic mice used here were the product of the fourth successive backcross with the inbred wild-type C57BL/6NTac strain and were therefore expected to be >90% C57BL in genetic composition. The specific-pathogen-free (SPF) animals were air-freighted to Australia at 4–6 weeks of age, transferred to an SPF facility, ear-clipped for identification and distributed randomly into two groups. After 10 days' conditioning to their new environment and diet, they were entered into the study. The animal experimentation was approved by the Animal Experimentation Ethics Committee of the Institute of Medical and Veterinary Science, Adelaide, South Australia, and conducted in accordance with its guidelines.

Study Design

The strain of *Pim1* transgenic mice used here has been reported to develop lymphoma to an incidence of 5–10% in the first 10 months of life (*15, 17*). Information provided by the commercial supplier of these mice indicated that by 18 months the incidence of lymphoid tumors reaches about 15%, a level that is well suited as a baseline against which to detect moderately carcinogenic influences. Statistical calculations showed that the use of approximately 100 animals per exposure group in an 18-month study would allow the detection of as little as a doubling of lymphoma incidence with 95% confidence. The study was designed as a blinded trial. The mice and the samples taken from them for pathological analysis were coded to ensure they would be assessed without knowledge of their derivation with respect to RF-field exposure. The code was broken only after statistical analysis of the results had been completed.

Animal Husbandry

The animals were maintained in a disinfected facility kept at positive pressure by a supply of filtered air at the rate of 15–20 room volumes per hour. Animal care staff entered through an air-lock and exchanged their clothing for sterile overalls, gloves, masks, hats and boots. Air temperature was maintained at 22 ± 2°C. The lights were on from 0600 h to 1800 h each day.

From the initiation of the study, the mice were housed in groups of five in 180 × 150 × 300-mm filter-top transparent polycarbonate cages (Tecniplast, Buguggiate, Italy) in which the steel-grille lid had been replaced by a perforated glass lid, the food pellets were placed on the floor, and the glass water bottle was end-mounted distal to the ground plane of the RF-field source to minimize perturbations to the RF field. The sawdust bedding, food pellets (Joint Stock Ration II from Milling Industries Stockfeeds, Murray Bridge, South Australia), water (acidified with 4 m*M* HCl) and equipment were sterilized before transfer into the facility. Twice weekly, the cages were cleaned and fresh food pellets and water were provided. The mice were weighed weekly and the data recorded on a computer system that would sound an alert if an individual weight differed from the previous value by more than 10%. To ensure equal average exposures to the RF fields, cages were moved clockwise to the next position after cleaning. All mice were inspected closely during the weekly weighing. They were also observed daily and disturbed to check their mobility. When any showed dyspnea, reduced mobility, weight loss, a local swelling or any other clinical abnormality, they were designated for closer monitoring and submitted for pathological assessment when the abnormality was judged to be life-threatening or causing significant distress.

Pathology

Animals were normally submitted live for pathological assessment and killed by anesthetic overdose. Any mouse found dead in the cage was placed on ice or refrigerated at 4°C and subsequently submitted on ice to the pathology laboratory. A full necropsy was performed. Samples of thymus, lymph nodes (if enlarged), spleen, liver, lung, kidney, adrenal, large and small bowel, urogenital system, eyes, brain and any tissue appearing abnormal at autopsy were immersion-fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 3 μm and stained with hematoxylin and eosin. Histological assessment of lymphomas and any other pathology was then performed. Lymphomas were diagnosed and classified predominantly on morphological criteria (e.g. see ref. *18*). Any mice that were clinically healthy after 18 months of exposure or sham exposure were counted as survivors and discarded without further investigation.

Representative cases of lymphoma were immunophenotyped with the aim of determining their cell lineage of origin. Samples of enlarged lymphoid organs were dispersed mechanically into single-cell suspensions in RPMI-1640 culture medium (Commonwealth Serum Laboratories, Parkville, Victoria, Australia) containing 10% dimethyl sulfoxide and slow-frozen in 1-ml cryotubes (Nunc, Denmark) for storage in liquid nitrogen. Accumulated batches of these frozen cells were subsequently thawed and tested for the presence of lymphoid tumor cells expressing T- or B-lineage cell surface markers by standard methods of immunofluorescence staining. The reagents used were fluorochrome-conjugated antibodies against CD45R(B220) (clone RA3-6B2 from Caltag, South San Francisco, CA), immunoglobulin (sheep anti-mouse immunoglobulin from Silenus, Hawthorn, Victoria, Australia), Thy1, CD4 and CD8

(clones 30-H12, GK1.5 and 53-6.7, respectively, from Becton Dickinson, San Jose, CA). Staining was assessed by fluorescence microscopy.

Monitoring of SPF Status

Wild-type female C57BL/6NTac mice (from GenPharm) were used as sentinels distributed randomly among the Eμ-*Pim1* animals in the exposed and sham-exposed groups. Each month, one sentinel from each group was sent to the pathogen testing service of the Central Veterinary Laboratory (Adelaide, South Australia). The mice were examined there for a broad range of pathogenic viruses, chlamydia, mycoplasma, bacteria and parasites by serological assays, culture tests, gross autopsy examination, direct microscopy and histology. Apart from occasional detection of a questionably pathogenic protozoan (trichomonad), the mice remained free of known infectious disease organisms through the study period.

The Exposure Facility

Exposed and sham-exposed mice were housed in separate, adjacent rooms. The exposure room was 2.6 m long, 2.2 m wide and 2.45 m high, the other room 2.6 × 1.8 × 2.45 m. The rooms were lined individually with overlapping sheets of 1-mm-thick aluminum, which gave a shielding effectiveness of 40 dB at 900 MHz. Air-conditioning ducts were screened, and the doorway was fitted with metal fingers to achieve a conductive seal with the aluminum sheet covering the door. Each room was designed to contain a vertical ground plane, 2.5 m wide and 2.2 m high, running parallel to the 2.6-m-long wall, with a one-quarter-wave monopole antenna located at the center of the ground plane. Twenty lucite stands (150 × 300 mm) for mouse cages were mounted perpendicular to the ground plane in a circular array with the center of each stand 0.65 m from the antenna. The far field of the quarter-wave antenna, acting on the ground plane as a half-wave antenna, was located beyond a distance of $2D^2/\lambda$ = 165 mm. All exposures of the mice therefore occurred in the far field.

The monopole antenna was fed by a 900 MHz 70-W amplifier to produce an RF field that was modulated at a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms. The duty cycle of the transmitter was 0.13, giving an average power output of 9.1 W. The amplifier was under computer control and the power output was monitored while the antenna was energized. Animals were exposed daily for 30 min preceding lights on at 0600 h and 30 min before lights off, 12 h later, in the evening, when the mice were expected to be in their most active state. The sham-exposure room was set up identically so that the animal care staff could not discriminate between the two groups of mice, but the antenna in that room was not energized.

RF-Field Dosimetry

The RF fields were measured with a broadband meter (model 8616, Narda Microwave Corp., Hauppauge, NY) and an isotropic electric field probe (Narda 8662B), the calibration of which was verified at the Australian Radiation Laboratory before and after use. Measurement of the RF power levels at each of the 20 mouse cage positions were made while the other 19 cages were present with their complement of five mice, food and full water bottle. The root mean square RF power density (corrected for the probe calibration factor) was measured at 10 mm from the ground plane and at the distal end of each mouse cage stand (300 mm). The values at these various positions ranged from 2.6 to 13 W/m^2. Numerous measurements of the field distribution inside the room were conducted to assess the interference patterns produced by reflections from the aluminum walls. While significant variations could be detected in the room, the variation in the vicinity of the animal cages was within the range of values given above.

The SAR evaluation for a single mouse was determined experimentally because there was a substantial range of body weights and fat content among the mice used in the present study that did not fit the standard mouse models of the Dosimetry Handbook (*19*). The accuracy of these measurements was estimated at ±1.6 dB (*20*). Measurements of the electric fields induced by RF fields were made in three phantoms representing small, medium and large mice. Two tissue-equivalent gels were used in constructing the phantoms using the following complex dielectric constants as a guide:

Average human tissue at 900 MHz: $\varepsilon_r' = 34.3$, $\varepsilon_r'' = 21.3$ (from ref. *19*).
Fat at 900 MHz and 37°C: $\varepsilon_r' = 9.94$,
$\varepsilon_r'' = 3.46$ (from C. Gabriel, unpublished data).

The gels were contained within thin plastic shells of dimensions determined from outline tracings of mice weighing 26, 34 and 64 g. The two larger body shells (excluding the head) were lined with fat-equivalent gel to account for approximately 30% of the total body mass, and the remaining space was filled with human tissue-equivalent gel. The small mouse phantom contained no "fat."

A miniature isotropic E-field probe with 1.5-mm dipoles (Narda 8021) was inserted into the phantoms to measure the internal electric fields in V^2/m^2. Linearity and isotropicity of the probe response at 900 MHz were verified. Using the procedure of Hill (*21*), the enhancement factor for responses in gels relative to those in air was determined to be 2.42. All measurements were conducted in a shielded semi-anechoic room. A coaxial-to-waveguide adapter was used to generate a continuous-wave exposure field at 900 MHz. The waveguide flange was WG-4 with internal dimensions of 124 × 248 mm. The mouse phantoms were placed on the bore-sight of the aperture at a distance of 0.7 m, which was in the far field by the $2D^2/\lambda$ criterion, and the phantom and the adapter were oriented to produce E, H or K polarization relative to the long axis of the phantom. The incident power flux density ($S = E^2/377$ W/m^2) was measured with the Narda probe at the position occupied by the phantom.

Midline measurements were made at 0.25, 0.5 and 0.75 along the length of the phantom by inserting the Narda probe through predrilled holes along the top of the shell. The SAR was calculated for each point, using $SAR = \sigma E^2/\rho$, where $\sigma = 1.066$ *S*/m and $\rho = 1000$ kg/m^3. These measurements were averaged to arrive at the whole-body average SAR for the phantom and then divided by the measured power flux density.

Empirical calculations of the SAR values using spheroidal models for various weight groups of five mice were derived from the Radiofrequency Dosimetry Handbook (*19*). Estimates of the equivalent whole-body SAR values, at 900 MHz for E-polarization and for five mice at variable orientation in a close-packed group, are given in Table II.

Statistical Methods

Evaluation of end points such as lymphoma occurrence and time to occurrence in the mice was performed using logistic regression (which allows adjustment for related factors such as age and weight of the animals) and survival analysis. If exposure is the only variable used in a logistic regression model, the results are analogous to those of a χ^2 test for a 2 × 2 table. Cause-specific incidence of disease was analyzed using a competing risks model which accounts for mice dying of causes other than lymphoma (renal disease, etc.). The incidence of specific disease such as lymphoblastic lymphoma can then easily be adjusted for mice developing non-lymphoblastic lymphoma. The method of Pepe (*22*) allowed for such comparisons without requiring the competing causes of death to be independent. Comparisons of disease occurrence were performed using the conditional binomial exact test (*23*), which, while being analogous to the standard χ^2 test for large samples, is more powerful in analyzing 2 × 2 tables when frequencies are low.

RESULTS

Dosimetry of RF Energy Absorption by Mice

The SAR values measured for an individual mouse ranged from 0.0078 to 4.2 W/kg. The lower value was the product of the measured H-polarization SAR of 0.003 (W/kg)/(W/m^2) for a small mouse (Table I), and the minimum power density exposure of 2.6 W/m^2. The upper value

TABLE I
Average Whole-Body SAR per Incident Power Flux Density for Each Polarization (E, H and K) for Small, Medium and Large Mouse Phantoms

Mouse phantom	SAR [(W/kg)/(W/m^2)] E	H	K
Large	0.31	0.011	0.056
Medium	0.32	0.009	0.037
Small	0.24	0.003	0.029

Note. Small, medium and large phantoms represented mice of 26, 34 and 62 g, respectively.

applies to the E-polarization SAR of 0.32 (W/kg)/(W/m^2) for a medium-size mouse during its exposure to the maximum power density of 13 W/m^2. Our estimate for the range of SAR values applying to animals in groups of five comes from adjusting the values shown in Table II according to the measured maximum and minimum power densities. This yielded an SAR range of 0.13–1.4 W/kg.

Mouse Body Weight

As the experiment progressed, the mice showed a tendency to obesity. Allowance for this was made in the estimation of SAR values. While the body weight of 1-year-old virgin females of common inbred strains such as C57BL/6J is 20–25 g in our experience (see also, e.g., ref. *24*), the *Pim1* mice at 1 year averaged 36.3 ± 7.6 g (n = 69) in the exposed group and 35.7 ± 6.2 g (n = 82) in the sham-exposed group. The mean for 95 non-transgenic control mice of the same age was 39.1 ± 6.5 g. Hence the accumulation of weight was not affected by RF-field exposure and was not caused by the transgene, but was a characteristic of the C57BL/6NTac mouse strain that provided the genetic background for the *Pim1* transgene.

Diseases Found

Over the 18-month course of this exposure study, the mice developed several abnormalities at varying frequency. Some of these had not been reported previously in *Pim1* mice. The numbers of animals from the exposed and sham-exposed groups found in the major diagnostic categories are shown in Table III.

TABLE II
Values of Whole-Body SAR for Exposure to an Average Power Density of 10 W/m^2, for Groups of Five Mice, as Determined from Durney *et al.* (*19*)

Total body mass (g)	SAR (W/kg)
5 × 26	1.09
5 × 32	0.92
5 × 62	0.49

Renal disease. A lethal renal disease occurred. It first appeared in a few terminally ill animals at 5–8 months of age, reaching a cumulative incidence in both the RF-exposed and the sham-exposed groups of about 10% at 19 months of age, when the experiment was completed. It was the sole cause of terminal illness in 7–8% of the animals. At autopsy, these mice often showed anasarca, the subcutaneous connective tissues having a gelatinous and shiny appearance. Both kidneys were pale and enlarged. In histological sections of these kidneys, most, if not all, glomeruli were abnormal. The most striking change was ballooning of the glomerular capillaries, which were filled with amorphous eosinophilic material (Fig. 1). This disease was also detected histologically in variably milder form in a number of the animals that were killed with other predominant diseases. The substantial incidence of renal pathology seemed to be a product of transgene action, since we saw only a single case in a group of 197 non-transgenic female C57BL/6NTac mice housed in the same SPF facility for 19 months (our unpublished observations).

Lymphoblastic lymphoma. The predominant malignant disease found in the transgenic mice up to about 10 months of age was thymic lymphoblastic lymphoma, as expected from previous studies of mice expressing the *Pim1* proto-oncogene (*15, 16*). Cells obtained from several representative tumors tested for surface markers by immunofluorescence stained strongly for the T-cell markers Thy1, CD8 and/or CD4. Mice developing this tumor were recognizable only at a late stage of their disease when they suffered respiratory distress. The terminal stage developed too rapidly for the tumor to be detected by the weekly weighing regimen. As a result, the first three cases were mice found dead in the cage, although a diagnosis was still made from the histological appearance of the tissues. Subsequently, the mice were examined more frequently to identify cases

TABLE III
Cases of Lymphoma and Other Diseases among Eμ-*Pim1* Mice Exposed or Sham-Exposed to 900 MHz Fields

Group	n	Lymphoma: Lymphoblastic	Lymphoma: Nonlymphoblastic	Lymphoma: Total	Renal disease[a]: Alone	Renal disease[a]: Total	Other disease[b]	Undiagnosable[c]
Control	100	3	19	22	7	11	8	7
Exposed	101	6	37	43	8	10	12	7

[a]Terminal glomerulopathy; some of these mice also had lymphoma.
[b]Other deaths due to miscellaneous causes, including dehydration, injuries, hepatoma and amyloidosis.
[c]Mice found dead, with tissues too autolyzed for pathological evaluation.

FIG. 1. Histological appearance of the distinctive renal disease in Eμ-*Pim1* mice. The photomicrograph of a hematoxylin and eosin-stained section of a kidney from such a mouse killed when terminally ill shows dilated glomerular capillaries (some examples marked with arrows) filled with amorphous eosinophilic material (scale bar, 100 μm).

before death. In this disease, masses of uniform lymphoblasts replaced most of the normal lymphocytes in the thymus and formed major deposits in the spleen, lymph nodes, lungs, liver, kidneys and bone marrow. An example is shown in Fig. 2. Of the 201 transgenic animals in this study, 9 were diagnosed with lymphoblastic lymphoma (3 in the control group and 6 in the exposed group). Only one of these occurred beyond 1 year of age.

Non-lymphoblastic lymphoma. From 10 months of age onward, some of the mice started to become ill with lymphomas that were different from the lymphoblastic tumors found in the younger animals. The new cases continued to appear through to the end of the experiment, at which time they had reached a total of 56, with 19 in the control group and 37 in the exposed group. Attempts to immunophenotype such tumors using cell suspensions gave inconclusive results, possibly because the tumor cells did not survive the dispersion and freeze–thawing procedure. These mice did not present with dyspnea and a large thymus, but commonly with readily palpable splenomegaly, or with swelling in the ventral neck region due to enlargement of the cervical lymph nodes. Histologically, none of these was lymphoblastic. Most showed follicular lymphoma in the spleen (Fig. 3), some lymph nodes, the lungs and, to varying extent, the liver. A number had histiocytic morphology, some with giant multinucleate cells scattered among the histiocytic sarcoma cells. Of the four remaining cases, two had diffuse large-cell lymphoma and two had small-cell lymphoma. Eight representative cases of follicular lymphoma and two of histiocytic sarcoma were assessed independently by Dr. T. N. Fredrickson (Registry of Experimental Cancers, National Cancer Institute, Bethesda, MD) and confirmed as lymphomas of probable follicular center B-cell origin and of histiocytic sarcoma, respectively.

Miscellaneous diseases and deaths. The SPF status of the facility was maintained throughout the study, so there were no outbreaks of infectious disease. A total of 20 mice killed with abnormal clinical signs were found to have no histological evidence of lymphoma. Two had hepatoma, 1 had amyloidosis, 2 had signs of central nervous system disorder, 2 appeared dehydrated and 7 had wounds or signs of local infection secondary to trauma. In the remaining 6, no cause of illness could be discerned. In 14 additional cases, no specific diagnosis could be made because the animals had been found dead in the cage and their tissues were too autolyzed for histopathological assessment. The miscellaneous and undiagnosable cases occurred in approximately equal numbers in the exposed and sham-exposed groups (Table III).

Statistical Analysis

The increase in the proportion of mice contracting a lymphoma of any type in the RF-field-exposed group from 22% to 43% was found to be significant ($P < 0.001$) by the conditional binomial exact test. A multivariate analysis using logistic regression was also performed to test the significance of this difference after adjusting for any differences in age and body weight. In this analysis, an additional adjustment was made for mice dying from causes other than lymphoma. Taking into account all competing risks to survival, the total lymphoma incidence in the exposed group was found to be over twice that found in the controls. The odds ratio was 2.42 at $P = 0.006$ with a 95% confidence interval of 1.3–4.5.

The crude proportions of mice contracting thymic lymphoblastic lymphoma in the control and exposed groups were 3 and 6%, respectively. Because the number of cases was small, this difference was not significant by the conditional binomial exact test ($P = 0.38$). Multivariate logistic regression analysis, and competing risks analysis adjusting for all other causes of death yielded a nonsignificant difference for lymphoblastic tumors between the exposure groups ($P = 0.95$ and $P = 0.33$, respectively).

The crude cumulative incidence of non-lymphoblastic lymphomas was 19% in the control mice and 37% in the exposed animals. This was significant by the binomial exact test at a confidence level of 99.8%. When adjusted for age and weight, the excess incidence in the group exposed to RF fields was found to be 2.7-fold with a 95% confidence interval of 1.4–5.4 ($P = 0.002$). After adjustment for competing risks, the difference in the time to appearance of non-lymphoblastic lymphoma was also highly significantly different. The increase in the probability of lymphoma with age is shown in Fig. 4. The probability that the faster rate of appearance of these tumors in the exposed mice was due to chance was calculated to be 0.014.

DISCUSSION

In the present study we sought to determine whether oncogene-transgenic mice could be used to detect a carcinogenic effect of exposure to RF fields. Mice of the Eμ-*Pim1* transgenic strain employed here express the *Pim1*

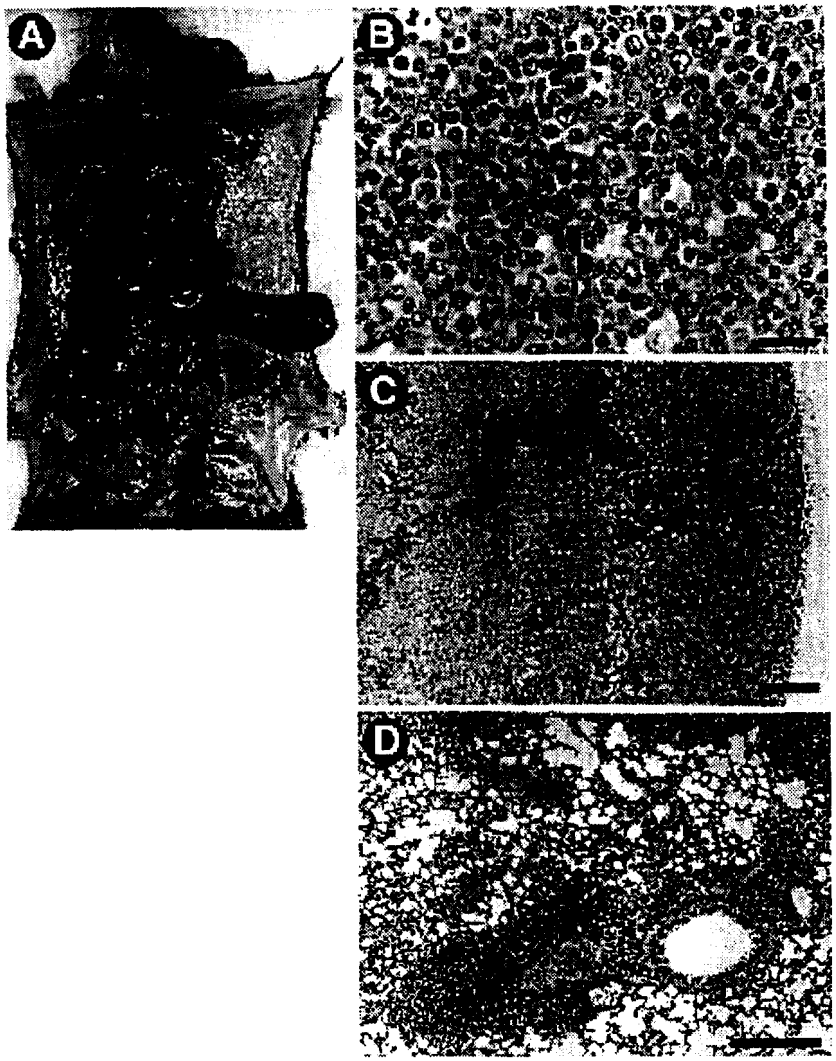

FIG. 2. A typical case of advanced lymphoblastic lymphoma of thymic origin in an Eμ-*Pim1* mouse. The panels show (A) a postmortem dissection exposing greatly enlarged thymus, spleen and lymph nodes, and hematoxylin and eosin-stained sections revealing (B) a mass of lymphoblasts, with frequent mitotic figures and some pycnotic tumor cells, filling the enlarged thymus (scale bar, 20 μm), (C) extensive infiltration by lymphoblasts (darkly stained regions) of the cortex of the kidney (scale bar, 400 μm), and (D) periarterial tumor nodules and diffuse infiltration of alveolar septa by lymphoblasts in the lung (scale bar, 200 μm).

oncogene in their lymphoid cells and have a modest propensity to contract malignant lymphoma spontaneously. Previous reports had indicated that they are specifically predisposed to develop thymic T-cell lymphoblastic lymphoma (*15, 17*), although one case of follicular lymphoma was also recorded (*15*). However, those reports did not document the fate of the mice that survived beyond about 9 months of age. In the present study, lymphoblastic lymphoma occurred in 3–6% of the mice, but we also found that about 10% of the animals developed a terminal renal disease from 6 months of age onward, and 20–40% developed non-lymphoblastic lymphomas after 10 months and up to 19 months, when the study was terminated. The predominant tumor type in this category was follicular lymphoma, amounting to about 80% of the non-lymphoblastic lymphoma cases. Follicular lymphoma is a neoplasm derived from the germinal center B lymphocytes of lymphoid tissue and is a common lymphoid malignancy in humans (*25, 26*). Of the remaining non-lymphoblastic tumors in the *Pim1* mice, all but two (which were hepatomas) were found predominantly in lymphoid tissues and were therefore counted as lymphomas. Some of them were diagnosed histologically as histiocytic sarcoma. They were likely to be of either B-cell or macrophage origin.

The incidence of lymphoma was higher in the RF-field-exposed *Pim1* mice than in the sham-exposed animals. For lymphoblastic lymphomas, the 2-fold increase in frequency was not statistically significant because the number of cases of that type of lymphoma was small. On the other hand, the increased incidence of all types of lymphoma and of non-lymphoblastic lymphoma was highly significant. With a lymphoma incidence of about 20% in the sham-exposed animals, groups of 100 mice were sufficient to obtain statistical significance from a 2-fold or greater increase in lym-

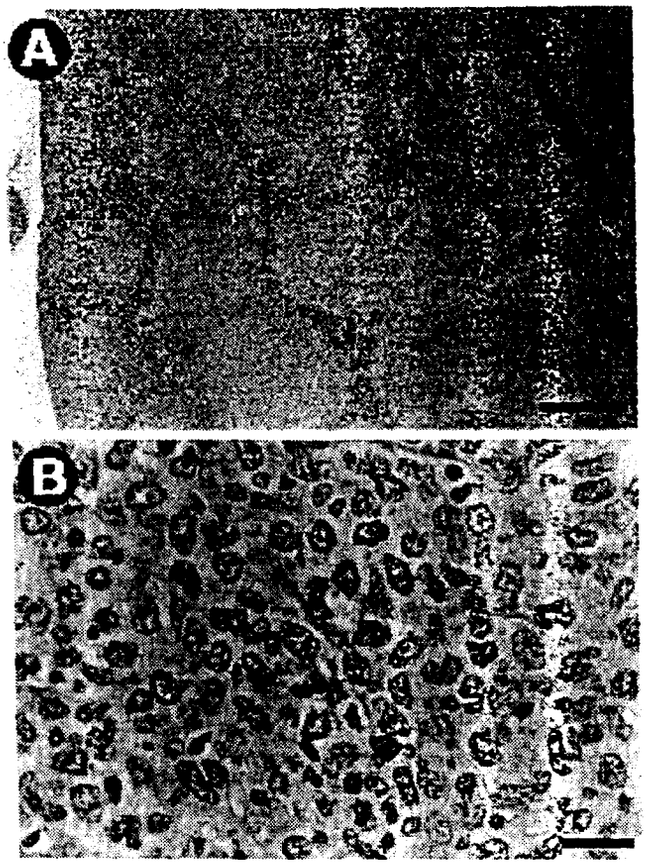

FIG. 3. A representative case of follicular lymphoma in an Eμ-*Pim1* mouse. Photomicrographs of hematoxylin and eosin-stained tissue sections show (A) a low-power view of the spleen in which numerous large tumor nodules have replaced the normal small islands of white pulp (scale bar, 500 μm), and (B) a high-power view of the tumor cells (scale bar, 20 μm).

phomas despite the competing risks of renal failure and incidental abnormalities, which were not altered by RF-field exposure. In the event, we found that RF-field exposure was associated with an overall increase of 2.4-fold in the risk of developing lymphoma. The statistical probability that the apparent increase was due to chance was calculated to be less than 1%.

By what mechanism can RF fields perturb biological systems? Unlike ionizing radiation or ultraviolet light, the photon energy of RF fields is much too low to break chemical bonds directly. However, RF fields induce electric fields that result in the flow of ions and rotation of asymmetric charged molecules (dipoles). This increase in linear and rotational energy is rapidly dissipated by molecular collisions, which generate heat. The field-induced molecular rotation is known as dielectric dispersion and is maximal for a given dipole at a characteristic relaxation frequency. At 900 MHz, the dominant relaxation phenomenon (in which there is a rapid change in the dielectric constant and conductivity of the absorbing tissue) is the δ-dispersion, which results from the relaxation of bound water, amino acids and charged side chains in proteins (*2*). The δ-dispersion and, to a lesser extent, the other relaxation phenomena are responsible for the eventual heating of tissue after absorption of RF energy. Under the conditions used in the present study, the thermal load induced in an exposed mouse would be small relative to the heat generated by normal metabolic activity. Only the SAR values at the upper end of the range measured here would add significantly to the resting metabolic rate in the mouse of 7–15 W/kg (*27*). Some investigators suggested earlier that resonant excitation of particular molecules such as DNA may lead to specific biological effects independent of heating (*28*), but subsequent tests of whether resonant absorption occurs in DNA gave negative results (*29, 30*). Others have postulated that an effect on the molecular interactions responsible for transducing mitogenic signals from the cell surface may enable RF fields to influence cellular processes leading to malignancy (*31, 32*), but the evidence for such a mechanism is not compelling.

A number of previous efforts to discern effects of RF exposure on lymphoid cells *in vitro* have been documented. An early report of an RF-field-induced increase in lymphoblastic transformation (*33*) was not confirmed by subsequent studies (*34–36*). Some evidence that RF fields can induce an alteration in antibody binding to mouse B-cell surface immunoglobulin (*37*) and inhibition of T-cell cytotoxic activity (*38*) has been described, but this has not been confirmed or extended using the more meaningful and sophisticated assays available today. In other reports, tests for effects of pulse-modulated RF fields on the capping of mouse B-cell surface immunoglobulin (*39*) or on DNA or protein synthesis in mitogen-activated lymphocytes *in vitro* (*40*) have yielded negative results. Thus the limited literature available on the subject does not seem to offer a mechanism by which RF-field exposure, either directly, or indirectly through effects on immune competence, could increase the incidence of lymphoid malignancy.

The activated *Pim1* oncogene in the Eμ-*Pim1* mouse does not act alone to transform lymphocytes to the malignant state. The lymphomas arise in a stochastic fashion as they do in other strains of oncogene-transgenic mice (*41*), and the current view is that acquisition of malignancy requires multiple somatic mutations which activate cooperating sets of oncogenes and genes that prolong cell survival, as well as inactivating tumor suppressor genes (see reviews in refs. *42* and *43*). In the case of the *Pim1* mouse the lymphocytes start their existence one step toward malignancy but must undergo mutation in endogenous genes before one of the cells can initiate a lymphoma. Lymphomas accelerated by chemical carcinogens in *Pim1* mice were found by Breuer *et al.* (*16*) to over-express the *Myc* gene, which has proliferation-promoting activity. There is no convincing evidence that RF fields can induce mutation or activate genes directly, but if such fields can cause an increase in gene expression, perhaps as a result of transient low-level warming of exposed tissues, then they might increase the likelihood of spontaneous mutation in the precancerous *Pim1*-expressing lymphocytes by stimulating cell proliferation.

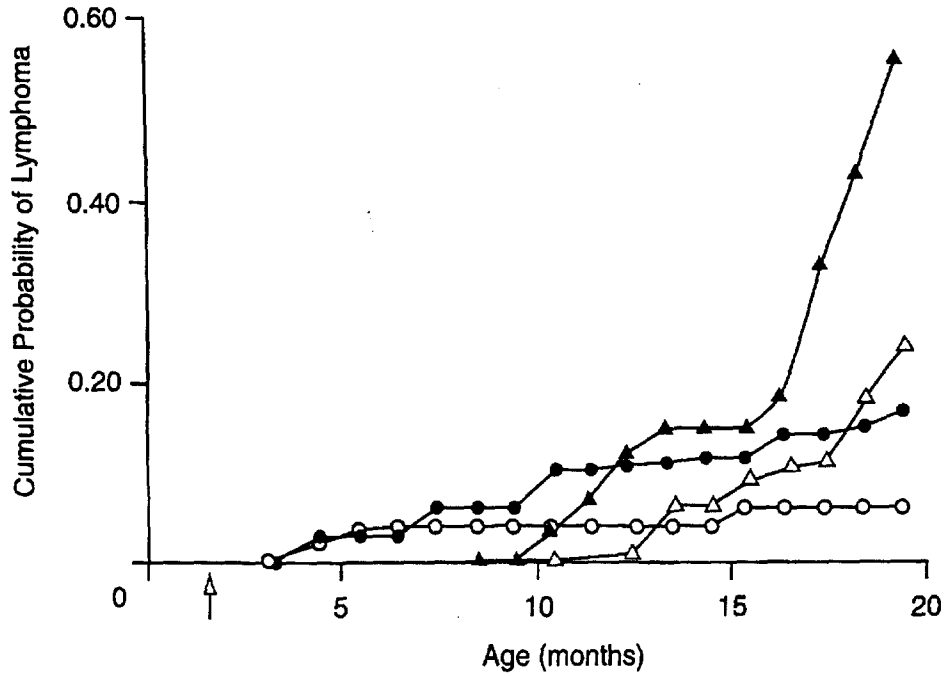

FIG. 4. Cumulative probability of development of lymphoma with age in Eμ-*Pim1* mice. (●, ○) Lymphoblastic lymphoma and (▲, △) non-lymphoblastic lymphoma in RF-field-exposed (●, ▲) and sham-exposed (○, △) animals. The cumulative probability values were calculated by adjusting the crude incidence of lymphoma for losses of mice to other causes such as other tumors, renal disease, incidental injuries and undiagnosed terminal illness (see Table III).

Stimulated cell proliferation after tissue damage has been proposed by Ames *et al.* (*44*, *45*) to account for the tumorigenic effects of high doses of non-mutagenic chemicals in tests of carcinogens in rodents. By analogy, a small enhancement of proliferation on a daily basis by RF-field exposure might suffice to increase the rate of initiation of lymphoma by the factor observed here in the *Pim1* mice.

While the increase in the incidence of lymphoma found here was highly significant statistically, and the exposure conditions were designed to mimic the fields generated by a digital mobile telephone, the implications of the study for the risk of carcinogenesis in humans are unclear. It is difficult to extrapolate directly from mice to humans due to differences in their absorption of energy from RF fields. The mice were exposed approximately 0.65 m from the radiating antenna, i.e. in its far field, where the magnetic and electric field vectors are orthogonal. By contrast, the head of a human using a cellular telephone is in the near field, where the magnetic and electric field strengths do not have a constant relationship. Further, 900 MHz RF energy is absorbed almost uniformly throughout the mouse, whereas in humans it is absorbed in a non-uniform manner in the skin and underlying muscle, and the eye, with little penetration to deeper tissues (*46*, *47*). The RF energy absorbed by the *Pim1* mice during their exposure ranged from 0.008 W/kg up to 4.2 W/kg. This estimate took into account their varying orientation to the incident RF field and the varying incident power density as they moved around the cage, their change in body mass with age and their tendency to rest as a close-packed group. Since the variation is so wide, it is not possible to determine what SAR or SAR range was responsible for causing the increased incidence of lymphoma. However, on the basis of studies reported previously, one would expect that the higher SARs would have done so. It seems important in light of the present results to determine the relationship between exposure dose and lymphoma incidence. One way to reduce the uncertainty of SAR values would be to restrict the movement of the mice during their exposure, such as by placing them in a tube having a fixed orientation to the field. For 30-min exposure periods this would be a feasible option for use in future studies.

There is a need to replicate and extend this study to test whether the tumor-prone transgenic mouse is a reproducible system for assaying biological effects of RF fields. The *Pim1* mouse model used here is somewhat complicated by its propensity to develop at least two types of lymphoid tumor and an unusual renal disease. Other mice carrying an activated oncogene or an inactivated tumor suppressor gene have the potential to be useful in testing whether the provocative findings described here have some more general validity. Transgenic mice bearing an activated *Abl* (*48*) or cyclin D1 (*49*) oncogene, or mice with a deleted *Rb* (*50*) or *p53* tumor suppressor gene (*51–53*), for example, develop various tumors and could be candidates for such testing.

The *Pim1* mouse would be expected to respond to carcinogenic agents with an increase in lymphomas because it expresses an activated oncogene selectively in its lymphoid cells. Hence we would not interpret the results as indicating

that RF-field exposure would be specifically lymphomagenic in normal animals. Other types of cancer might be induced either more or less easily in other tumor-prone animals. No humans are presently known to carry an activated *Pim1* gene, but some individuals inherit mutations in other genes, such as *p53* in the Li-Fraumeni syndrome (*54*), that predispose them to develop cancer, and these individuals may comprise a subpopulation at special risk from agents that would pose an otherwise insignificant risk of cancer. That is not to imply that any humans at all are necessarily at increased risk of cancer as a consequence of exposure to RF fields. No single experiment on animals can allow such a conclusion. Rather, we believe the study reported here indicates a need for further research. Tumorigenesis in genetically predisposed mice may provide a useful assay for interactions between RF fields and biological systems. With the current rapid expansion in the use of RF fields for telecommunications, a reliable assay is required to enable a better assessment of the limits to safe levels of human exposure.

ACKNOWLEDGMENTS

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Senator Lyn Allison

Australian Democrats

Democrat Spokesperson on Telecommunications

7th May, 1997 *MEDIA RELEASE* 97/299

Government refuses to review mobile phone safety standard as study reveals "hot spots" in users' heads

The Federal Government today refused to review the safety standard for mobile phones.

Ironically, the Government's position was announced on the same day a medical study reported "hot spots" in the head, dizziness, nausea, and blurred vision by frequent mobile phone users.

Responding to a question from the Democrat Spokesperson on Telecommunications, Senator Lyn Allison, the Minister for Communications, Senator Richard Alston repeated his view that "there is no substantiated evidence of adverse health and safety effects from radio frequency emissions at typical levels."

Senator Allison said the Government's position was getting harder to defend.

She said: "Not so long ago, we learned that microwave emissions can break strands of DNA.

"Last week, we learned that mice exposed to mobile phone emissions are twice as likely to develop cancer.

"Today, an occupational medical expert reports consistent symptoms in an unrelated group of mobile phone users.

"The Government is sounding increasingly like an apologist for a business resembling the tobacco industry of the fifties," Senator Allison said.

The Democrats are calling for:

- **a review of the Australian mobile phone safety standard;**
- **a review of advertising guidelines for mobile phones**, in particular, the targeting of very young people;
- **a transfer of the responsibility** of mobile phone policy from the **Department of Communications** (DOCA) to the **Health Department**; and
- a requirement for mobile phone companies and medical practitioners to **record health complaints relating to** mobile phone use in particular and exposure to other EMR producing telecommunications equipment.

For further information, contact Matthew Townsend on (06) 277 3076, 0411 22 02 77 or Senator Allison direct on mobile (015) 691 512 or pager (03) 9483 7892.

— Australia —

Mobile users report aches

By SASHA BASKETT

A STUDY of mobile phone users by a Melbourne doctor has found users can suffer from a range of headache-type symptoms.

Former chief medical officer with Telstra Dr Bruce Hocking has just completed a survey of 40 mobile phone users from all over Australia who had reported headaches.

In what he believes is the first survey worldwide of this kind, occupational medicine consultant Dr Hocking said he had sound evidence that they were affected by radio frequency radiation from mobile phones.

"I have also found some symptoms that are suggestive or indicative that they may also affect the skull," he said.

"There is also some evidence from respondents of speckling in their vision, dizziness and migraines."

Dr Hocking said the respondents had a range of jobs and were not all heavy mobile phone users.

"They did not get these symptoms when using a normal telephone," he said.

A report in London's *Sunday Times* this week said mobile phones emitted up to 70 per cent of their radiation into the user's head and created "hot spots" in the brain.

Stuart Roache, managing director of Mobiletronics, dismissed the claims, saying: "Do you think people would still buy them and they would still be made if they were that dangerous?"

Herald Sun technology writer Peter Familari said: "It is drawing a long bow to suggest that radiation would affect the average mobile phone user in the natural course of their job.

"The normal family television would emit more radiation than a digital or analog mobile phone and you would have to stand with your face to the screen for a very long time to be affected."

Mobile phone user Kevin Ross has fears about radiation and says there needs to be a lot more research. "It could just be another scare, but it's possible it's true."

Herald Sun, Tuesday, April 16, 1996

Phones linked to asthma

RADIATION from mobile phones may make asthmatics more susceptible to attacks, research shows.

Tests by a researcher at St Vincent's Hospital in Sydney showed that when cells which produced allergic reactions were subjected to radiation from analogue mobile phones, they reacted similarly to being exposed to pollen.

Hospital immunology scientist Dr Peter French, bathed mast cells (which contain histamines that react when irritated) with the analogue frequency of 835 megahertz.

The histamines in the mast cells tended to be more sensitive to an allergic reaction, such as shortness of breath and runny noses.

"We found that after exposure to the frequency, the cells exhibited a similar rection to what they do after exposure to pollen," Dr French said.

Dr French plans to discuss his findings with Telecom.

WHIN DOUBLE ISSUE!
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ELECTROMAGNETIC RADIATION

Mobile phone can bake your brains warn experts

MICK KING'S PHONE keeps ringing, as British Telecom engineers look totheir union rep for guidance on the hazards of their company-issue mobile phones.

Mick says one BT maintenance engineer, who used a mobile for one to two hours a day, has already turned his in and is glad to to see the back of it. "He'd been suffering from quite severe headaches and fuzziness. When he gave up using the mobile the symptoms went away."

In his role as Communication Workers Union (CWU) safety officer for Thames Central Branch, Mick is still fielding a couple of inquiries a week, two months after Watchdog HealthCheck, a prime time UK magazine programme, first sparked controversy about mobile phone safety.

The programme assembled evidence from six eminent scientists in the US, Australia and Sweden. They warned that radiation emitted from the radio transmitter in mobile phones may be linked to diseases such as asthma, Alzheimer's, cancer and eye and neck problems. Two of the scientists had stopped using the phones altogether.

Immunologist Dr Peter French, president of the Australia and New Zealand Cell Biology Society, told the programme: "I have a mobile phone but now I only use it when absolutely essential. I switch ears if the call goes on longer than a minute or two."

Dr Bruce Hocking, a former medical director of the state-owned Australian telephone company Telstra, has investigated claims made by customers and staff at the company that mobile phones were making them ill. He has logged 40 cases of users who reported severe headaches and muscle pain in the neck and upper arm.

And University of Washington experts Dr Henry Lai and Dr Narenda Singh warned that exposure can damage the body's genetic building block, DNA. They say "hot spots" might develop inside the brain, causing damage which might lead to Alzheimer's disease or cancer.

BBC's HealthCheck, seeing the calls pour in - over 1,000 in total - returned to the story in three separate June episodes.

One UK employer, Northumberland County Council, is monitoring the health of an employee who reported dizziness and nausea after using a hand-held mobile phone in a car. The symptoms stopped when the man switched to a hands-free phone with an external aerial.

And a former mobile phone industry employee whose monthly mobile bill regularly ran to £700 - he spent four to five hours each day on the phone in his car - is now taking legal advice after developing a rare neurological condition which stopped him working for two years and which eventually required surgery.

In-car users are alleged to be at a higher risk because of a "resonance" effect as microwaves bounce around inside of the steel body of the vehicle, creating a low-grade microwave cooker on wheels. Volkswagen now includes a mobile phones health warning in its handbook for new car owners.

Some UK employers now advise employees to use normal phones where there is a choice, to keep mobile calls short and to never use hand-held phones in cars. This shouldn't be too much to ask - it is illegal to use a mobile in motion. At least one person in the UK has been killed by a driver losing control of the car when answering the telephone.

While mobile phone users ponder the conflicting claims of health problems linked to the use of hand-held sets, the industry may have more pressing concerns.

Three years ago share prices of some mobile phone manufacturers tumbled after a US man announced on Larry King Live, a top-rating TV talk show, that he intended to sue NEC Corp. after the death of his wife - a frequent cellphone user - from brain cancer. Several other personal injury cases are being pursued in the US.

There are commonsense precautions that can reduce whatever risks there might be.

Use an ordinary phone when you can. Minimise the time spent on the mobile and never use it in the car. And one company, Microshield, has produced a guard costing under £50 which it claims can reduce by 90 per cent the microwave emissions.

In the meantime, CWU's Mick King is concerned that the union members he represents are still required to use mobile phones at work. "People within BT - jointers, faultsmen, for example - use mobile phones for a lot longer than average. They have their head stuck to the phone for up to two hours a day.

"Our members are concerned, they are worried. They don't feel safe with them."

Based on a article from CWU Voice CWU, UK, August 1996

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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November 25, 1996

Honorable Phil Gramm
United States Senator
2323 Bryan Street, #1500
Dallas, TX 75201

Dear Senator Gramm:

Thank you for your letter of November 5, 1996, in behalf of your constituents, Mr. [redacted] and [redacted] of [redacted], Texas. Mr. and Ms. [redacted] believe that members of their family and others are suffering from various symptoms caused by exposure to radiation from cellular telephone base stations which utilize the new digital technology for signal modulation.

I have enclosed a fact sheet with more information on this issue. To summarize, there is insufficient information available at present to enable the Environmental Protection Agency to advise you on any electrosensitive effects which may be associated with exposure to radiation from digital cellular base stations. At least one study is underway (in Sweden), and others are likely to follow, particularly if complaints of electrosensitivity continue to increase. Cellular telephone base stations emit radiation levels which fall below the guidelines recently promulgated by the Federal Communications Commission.

I hope you find this information helpful in responding to your constituents. If I can be of further assistance, please contact me.

Sincerely yours,

//// signed by "Carroll" for
the Regional Administrator ////

Jane N. Saginaw
Regional Administrator

Enclosure

Fact Sheet
November 1996

Electrosensitivity and Digital Cellular Base Stations

The Environmental Protection Agency (EPA) has for many years received similar complaints from relatively few individuals living in the general vicinity of air traffic control radar transmitters, which are pulsed systems similar in many respects to digital cellular telephone systems. Clicking, buzzing, hissing and knocking sounds are known effects in some individuals exposed to high intensity radar signals. However, environmental exposure to pulsed radiation from cellular telephone base stations is at a very much lower intensity than that of radar signals known to stimulate the impression of audible noises in humans.

With the advent of digital cellular telephone and paging systems, the number of complaints similar to those of [redacted] has increased significantly, both in the United States and world-wide. Symptoms attributed to radio frequency exposure such as nausea, headaches, dizziness, pain in the eyes, ringing of ears, screeching and sizzling sounds, and irregular heartbeat are described collectively by the term, "electrosensitivity." These symptoms are very difficult to quantify in research studies, so little information is available on electrosensitivity to radiofrequency radiation. To our knowledge, the only research program underway at present to address electrosensitivity has just begun in Sweden. Its purpose is to determine whether or not reports of electrosensitivity to radiation from digital cellular telephone and paging systems reflect a real physiological problem. Research programs sponsored by the cellular telephone industry are currently underway, but these programs primarily focus on cancer.

The World Health Organization (WHO) is in the initial stages of planning a research program to investigate health effects of exposures to low levels of radiofrequency fields. Environmental Protection Agency (EPA) staff recently received a draft report from WHO that will serve as a basis for discussion to identify the gaps in knowledge, so that research can be targeted to better assess health risks from exposure to low levels of radiofrequency radiation. EPA staff are in the process of preparing comments which will identify biological effects of pulsed radiofrequency radiation as a significant gap in knowledge which needs to be studied.

In August 1996, the Federal Communications Commission (FCC) adopted exposure guidelines recommended by the National Council on Radiation Protection and Measurements (NCRP). The NCRP is a congressionally-chartered organization of radiation experts that collect, analyze, develop, and disseminate in the public interest information and recommendations about protection against

radiation. The EPA supported the adoption of the NCRP guidelines by the FCC. The new FCC guidelines apply to all radio frequency sources which the FCC regulates, including the new digitally modulated systems. Cellular telephone base stations are known to be low power radiofrequency radiation sources, and the radiation levels in areas accessible to the public fall below the FCC guidelines.

"ELECTROSENSITIVITY", "ELECTROSUPERSENSITIVITY" AND "SCREEN DERMATITIS": PRELIMINARY OBSERVATIONS FROM ON-GOING STUDIES IN THE HUMAN SKIN

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