Wired vs. Wireless Internet in Schools

The Swiss Experience

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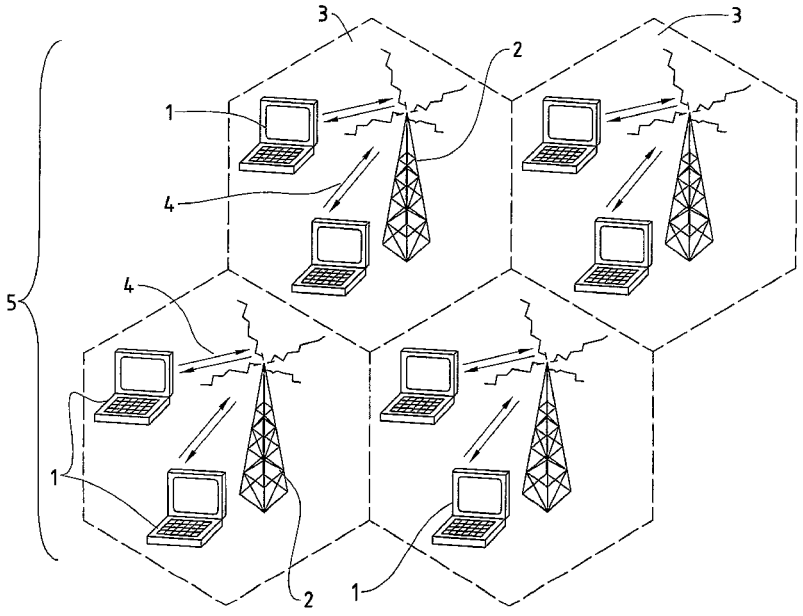

(57) Abstract: A method and system for reduction of electrosmog in wireless local networks, one or more mobile network units (1) communicating with a base station (2) of a wireless local network (5). After a predefinable time interval without connecting signal, the base station (2) changes over from the normal transmitting-receiving mode into a sleep mode, in which sleep mode no beacon signals and/or other radio frequency signals are transmitted from the base station (2). If a mobile network unit (1) requires a network connection, it transmits an alert signal, and, upon receiving the alert signal of the mobile network unit (1), the base station transmits beacon signals to the mobile network unit (1) and changes over into the normal transmitting-receiving mode.

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Reduction of Electrosmog in Wireless Local Networks

This invention relates to a method and system for reduction of electrosmog in wireless local area networks (WLAN), one or more mobile network units communicating with a base station by means of radio frequency signals in a wireless local area network, which base station amplifies the radio frequency signals of the mobile network unit and/or connects the wireless local area network to a wired fixed network by means of bridge functions. In particular, the invention relates to a method and system in which a WLAN comprises a plurality of access points with differing transmission cells.

The influence of electrosmog on the human body is a known problem. The health risk from mobile radio transmitters, handys and DECT telephones has been an explosive subject among the general public at least since the enormous breakthrough in mobile radio technology in the 1990s. To meet the concerns of science from the legislative side, the permissible limit values have thus been lowered several times, and technology has been increasingly focused on this problem. The risk of damage to health through electrosmog has also become better understood as a result of more recent and improved studies. When, for example, human blood cells are irradiated with electromagnetic fields, clear damage to hereditary material has been demonstrated and there have been indications of an increased cancer risk (Mashevich M., Folkman D., Kesar A., Barbul A., Korenstein R., Jerby E., Avivi L., Department of Human Genetics and Molecular Medicine, Tel-Aviv University, Tel-Aviv, Israel, "Exposure of human peripheral blood lymphocytes to electromagnetic fields associated with cellular phones leads to chromosomal instability," *Bioelectromagnetics,* 2003 Feb., 24(2): 82-90). In this study, for example, human peripheral lymphocytes were exposed to continuous electromagnetic fields of 830 MHz in order to examine whether this leads to losses or gains in chromosomes (aneuploidy). Bigger changes lead to instability of the genome (= the totality of all genes of a germinal cell) and thereby to cancer. The human peripheral blood lymphocytes (PBL) were irradiated at different average specific absorption rates (SAR) of 1.6 to 8.8 W/kg over a time period of 72 hours in an exposure system based on a parallel plate resonator in a temperature range of 34.5 to 37.5 °C. The average absorption rate (SAR) and

its distribution in the exposed tissue culture flask were determined by combining the measurement results with a numerical analysis based on a finite element simulation code. A linear increase in the chromosome No. 17 -- an aneuploidy (=numerical chromosome aberration) -- was observed as a function of the SAR, demonstrating that this radiation has a genotoxic effect. The SAR-dependent aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in the SAR dependent genetic toxicity. Control experiments (i.e. without any radio frequency radiation) carried out in the temperature range of 34.5 to 38.5 °C showed that elevated temperature is not associated with either the genetic or epigenetic alterations observed following RF radiation, these alterations being the increased levels of aneuploidy and the modification in replication of the centromeric DNA arrays. These findings indicate that the genotoxic effect of electromagnetic radiation is elicited via a non-thermal pathway. Moreover aneuploidy is to be considered as a known phenomenon in the increase of cancer risk.

Thus it has been possible to show that mobile radio radiation can cause damage to genetic material, in particular in human white blood cells, whereby both the DNA itself is damaged and the number of chromosomes changed. This mutation can consequently lead to increased cancer risk. In particular, it could also be shown that this destruction is not dependent upon temperature increases, i.e. is non-thermal. Based on the scientific studies in the field, and owing to increasing pressure from the public, especially in the industrialized countries, epidemiological studies have been systematized by the World Health Organization (WHO) in the last few years, such as e.g. the currently running WHO Interphone Project, in order to be able to assess more precisely the health risks from electrosmog and work out corresponding guidelines.

Local networks (LAN: Local Area Network) usually consist of so-called nodes which are connected via physical media, such as e.g. coaxial cable, twisted pair or optical fiber cable. These LANs are also referred to as wired LANs (wired fixed networks). In the last few years wireless LANs have

also become more and more popular (e.g. through developments such as the AirPort System of Apple Computer, Inc.). Wireless LANs -- also referred to as WLANs -- are especially suitable for integrating mobile units (nodes), such as e.g. laptops, notebooks, PDAs (Personal Digital Assistants) or mobile radio devices, in particular mobile radio telephones, with a corresponding interface, into a local computer network. The mobile nodes have an adaptor comprising a transceiver as well as a control card (such as e.g. infrared (IR) adaptor or a low frequency radio wave adaptor). The advantage of such mobile nodes is that they can be moved freely within the range of the wireless LANs. The mobile nodes communicate either directly with one another (peer-to-peer wireless LAN) or send their signal to a base station which amplifies the signal and/or passes it on. The base stations can likewise comprise bridge functions. Via such base stations with bridge functions, so-called access points (AP), the mobile nodes can access the wireless LAN on a wired LAN. Typical network functions of an access point comprise the transmission of messages of one mobile node to another, the sending of messages from the wired LAN to a mobile node and the transmission of messages of a mobile node to the wired LAN.

There exist many different access methods for WLAN in the state of the art which make it possible for a user of a mobile network device to access a wireless local network. One of these access methods, such as e.g. Carrier Sense Multiple Access/Collision Detection (CSMA/CD) or token passing have proved to be highly successful in their industrial application. Today the use of local or wide area networks usually does not have any clearly defined, predetermined characteristics anymore. With the growth of heterogeneous multimedia data exchange (e.g. video data streams, etc.) via WLANs, the Quality of Service (QoS) parameter for a particular type of data exchange (or application) has become more and more important. Such parameters comprise, for example, the highest possible bandwidth, lowest possible delay, etc. For such accesses, new access methods in the asynchronous or synchronous networks have been developed and can be found in the state of the art.

Together with the growth of the WLAN and the standardization of the access methods and the physical layer specifications for WLANS, such as e.g.

the 802.X physical layer protocols and non- 802.X protocols (e.g. ATM: Asynchronous Transfer Mode Protocol), the security needs of users and service providers of such networks have also become greater and greater. Unambiguous network recognition as well as user identification and/or authentication thereby complement one another. Within a WLAN, an AP transmits so-called Service Set IDentifier (SSID) when a mobile network unit tries to integrate itself in the wireless network. An SSID is an unambiguous identification, 32 characters long, which is assigned to the header of data messages sent over the network, and serves as a password for the mobile network units. The SSID differs from one WLAN to another. That means that all APs and mobile network units of a particular WLAN must use the same SSID. A network unit which cannot support the unambiguous SSID will not be granted any network access via a base station or respectively an AP. As mentioned, in the 802.X network technology, such as e.g. the 802.11 network technology, the network units normally communicate via an access point (AP). In the infrastructure mode, mobile network units can either communicate with one another or with network components of a wired network. An AP with bridge functions, which is connected to a wired network and one or more other access points, is referred to as the Basic Service Set (BSS). Designated as the Extended Service Set (ESS) are a plurality of BSS, which form in each case a sub-network. WLANs are usually operated in the infrastructure mode in order to provide access to other services, such as e.g. file server, printer services and/or the worldwide backbone network (Internet). In the 802.X technology, an SSID concerns in each case a Basic Service Set. Thus a mobile unit can only have network access to a BSS if it supports the corresponding SSID. SSIDs are sometimes referred to as network names since the SSIDs unambiguously designate or identify a network.

The physical range of an AP is called the Basic Service Area (BSA). If a mobile node is located within the BSA of an AP, it can communicate with this AP if the AP is likewise within the signal range (Dynamic Service Area (DSA)) of the mobile node. Mobile nodes typically have a signal strength of 100 mwatt to one watt. To connect the wireless LAN to the wired LAN, it is important for the AP to determine whether a particular message (information frame) on the network is intended for a node which lies within the wired LAN or within the

wireless LAN, and to pass on this information, if necessary, to the corresponding node. For this purpose APs have so-called bridge functions, e.g. corresponding to the standard IEEE Std 802.1D-1990 "Media Access Control Bridge" (31-74 ff). With such bridge functions, a new mobile node is registered in the wireless LAN, typically in a FDB (Filtering Database) of the AP in whose range the node is located. With each information frame on the LAN, the AP compares the destination address with the addresses (MAC addresses (Media Access Control Addresses)) which it has stored in the FDB, and sends, rejects or passes on the frame to the wired LAN or to the wireless LAN. The range of a wireless LAN is limited by factors such as e.g. wavelength of the signal, signal strength, impediments, etc. The radio frequency parameters cannot be selected freely, however. In most countries there are regulations, more or less strict, as mentioned further above, as concerns the low frequency transmission for wireless LANs (e.g. USA (FCC), Switzerland (BAKOM), etc.). This applies in particular to the USA, for example. In the USA the regulations are issued by the United States Federal Communications Commission (FCC) (D 15, Title 47, Code of Federal Regulations 1985). Three bandwidths are permitted: 902-928 MHz, 2400-2483.5 MHz and 5725-5850 MHz. Many applications today use the 900 MHz band. The quantity of data which can be transmitted over the 900 MHz band is limited, however, by the narrow frequency bandwidth in this band. Therefore more and more applications are using the frequency band around 2400 MHz. Future applications will presumably also use the band around 5800 MHz in order to meet the growing demand for high data throughput.

Despite increasingly strict national guidelines with respect to legally specified limits, the impact of electrosmog in WLANs on the human body can be considerable. Moreover it is to be expected that this impact will continue to increase in the future for many people. Two factors in particular are playing a role in this: First, more and more applications require additional, usually higher-energy frequency bands in order to be able to meet the growing need with respect to transmission rate. Second, the need for WLAN expansion in the private sphere as well as in the public sphere, e.g. in airports, railway stations, trains, restaurants, exhibition halls, etc., has by far not yet reached its peak. With the state of the art as a basis, there has been a lot of effort put into providing evidence for the detrimental effects of electrosmog and setting

corresponding limits. Limits and guidelines alone will not suffice, however, to further contain the electrosmog in WLANs since the development in WLANs runs in exactly the opposite direction, as mentioned above. WLANs even represent zones in which people usually spend longer periods of time (place of work, Internet, network games, etc.) and are therefore to be considered as particularly problematic with respect to radiation impact. WLANs in the state of the art moreover send base stations, such as access points, so-called beacon signals periodically so that mobile units can recognize the network and authenticate themselves with an access point. These beacon signals comprise recognition signals, such as e.g. SSIDs and/or other radio frequency signals with control parameters. Even if no mobile units are located in the WLAN, the beacon signals continue to be transmitted periodically to the APs. This means that even when the WLAN is not being used at all, an underlying stress from electromagnetic radiation remains for persons in the Basic Service Area of an access point of the WLAN. For example, in the case of WLANs at places of employment, such as offices, etc., there exists therefore permanent stress from electrosmog from the WLAN on the employees of the company or organization. In the state of the art there exists only the possibility of further reducing the limits for electromagnetic radiation.

It is an object of this invention to propose a new method and system for reducing electrosmog in wireless local networks which do not have the drawbacks described above. In particular a solution should be proposed which can be managed without any disruptive software and/or hardware adaptations and is thus easily achievable for existing WLAN technologies.

These objects are achieved, according to the present invention, in particular through the elements of the independent claims. Further preferred embodiments follow moreover from the dependent claims and from the description.

In particular, these objects are achieved through the invention in that, for reducing electrosmog in wireless local area networks (WLANs), one or more mobile network units communicate with a base station in a wireless local network by means of radio frequency signals, which base station amplifies the

radio frequency signals of the mobile network unit and/or connects the wireless local area network to a wired fixed network by means of bridge functions, the base station changes over from the normal transmitting-receiving mode into a sleep mode after a predefinable time interval without connecting signal to a mobile network unit, in the sleep mode no recognition signals and/or other radio frequency signals being transmitted from the base station, the base station being ready to receive radio frequency signals, however, when needing a network connection, a mobile network unit transmits an alert signal to the base station, and upon receiving the alert signal of the mobile network unit, the base station transmits to the mobile network unit the recognition signals necessary for the connection and changes over into the normal transmitting and receiving mode. The invention as described above has the advantage that electrosmog in WLANs can be greatly reduced during times when there is no network activity. At the same time energy consumption is also reduced since in sleep mode no beacon signals or other radio frequency signals are transmitted from the base stations. The whole method and system is achievable in particular without any hardware changes of any kind in the mobile network unit being necessary on the user side, nor on the side of the base stations, and it is therefore simpler and less expensive to achieve compared with other solutions. This means that not only are the costs for new hardware saved, but also the costs for installing it. It must also be pointed out that in mobile network units weight and space considerations often play a role too. The present invention requires neither additional hardware space, nor does it result in increased weight of the mobile terminal (network unit). For company-internal WLANs, for example, it also further increases security, making it more difficult for the WLAN to be used by unauthorized persons e.g. outside of business hours since no periodic beacon signal is sent anymore by the base station or base stations if they are in sleep mode.

In an embodiment variant, when in need of a network connection, the mobile network unit transmits an alert signal only if it does not receive any recognition signal from a base station. This embodiment variant has the advantage, among other things, that no unnecessary alert signal has to be transmitted if the base station is already in normal transmitting-receiving mode.

This likewise results in a further reduction of electrosmog and at the same time energy saving in the mobile network units.

In another embodiment variant, only the base station in whose basic service area (BSA) the mobile network unit is located changes over into the normal transmitting and receiving mode, the other base stations of the wireless local network remaining in their previous operating mode. This embodiment variant has the advantage, among other things, that the electrosmog can be further reduced since for mobile units which are at times stationary, such as e.g. when working with a laptop at one's place of employment, only the needed base station goes back into the normal transmitting-receiving mode.

In still another embodiment variant, the base stations of the basic service areas (BSAs) bordering on the basic service area (BSA) of the base station in whose BSA the mobile network unit is located likewise change over automatically into the normal transmitting-receiving mode if they were previously in the sleep mode. This embodiment has, among other things, the same advantages as the preceding one, but during a shift of the mobile network unit from one BSA to the next, the base station of the bordering BSA is already in the normal transmitting and receiving mode.

In an embodiment variant, the base station of the wireless local network changes over from sleep mode into the normal transmitting-receiving mode only if a network-specific recognition signal of the alert signal corresponds to a stored recognition signal of the wireless local network. This embodiment has the advantage, among other things, that the user as well as the service provider of the WLAN is given additional security. Through the additional authentication by means of a network-specific recognition signal, an unauthorized person, such as someone outside the company in the case of company WLANs, cannot even activate the normal transmitting and receiving mode of the WLAN or respectively of the base station.

In an embodiment variant, at least parts of the network-specific recognition signal, such as e.g. supplementary information data, are definable for the wireless local network by a user of the mobile unit and/or by an operator.

This embodiment variant has, among other things, the same advantages as the preceding embodiment variant. The security can be further increased however through the addition of supplementary information data determinable by the user or operator. In an embodiment variant, these data can even be supplementary information data freely chosen by the user, whereby, as a borderline case, the supplementary information data could even be empty. As further embodiment variants, an unambiguous identification code of the user can be used as the supplementary information data. For example, this can be an IMSI (International Mobile Subscriber Identification) and/or a MSISDN (Mobile Subscriber ISDN) which is stored on a SIM (Subscriber Identification Module) card of the mobile network unit. This has the advantage, among other things, that a particular user can be identified by means of the MSISDN, and, if required, can be correspondingly authenticated, e.g. with a log-in password, etc., without the user having to be registered beforehand in the system, e.g. in a database. As an additional embodiment, it is even conceivable for the MSISDN of a mobile radio device of the user to be used as the MSISDN, for example, the mobile radio device being one from which an access request was previously sent to a central unit.

In a further embodiment variant, the alert signal is transmitted from the mobile unit in a network-independent way for each wireless local network. This embodiment variant has the advantage, among other things, that any mobile network unit can activate possibly available WLANs in a standard way, independently of a specific recognition signal, or at least can receive a beacon signal or similar signal of the network.

In another embodiment variant, the wireless local network is set up based on the 802.X network technology, the recognition signals containing the corresponding Service Set Identifiers (SSID). This embodiment variant has the advantage, among other things, that a standardized access method and standardized physical layer specifications with the 802.X layer protocols can be used for the WLANs. This allows a cost-effective implementation without it being necessary to depart from the standard methods. At the present time the standards of the Institute of Electrical and Electronics Engineers (IEEE) have taken hold worldwide in the WLAN area. Among the IEEE standards which

have gained acceptance are in particular the IEEE 802 standards for LAN (Local Area Network) technologies.

In another embodiment variant, the wireless local network is set up based on Bluetooth technology. Among other things, this embodiment variant has the same advantages as the preceding one. In particular, Bluetooth is supported by a wide range of well-known hardware and software producers, such as e.g. Ericsson, IBM, Intel, Nokia, Toshiba, etc., which are themselves members of the Bluetooth Special Interest Group, which defines the Bluetooth standard.

Embodiment variants of the present invention will be described in the following with reference to examples. The examples of the embodiments are illustrated by the following attached figures:

Figure 1 shows a block diagram illustrating schematically the architecture of an embodiment variant of a method and/or system according to the invention for reducing electrosmog in wireless local networks 5, one or more mobile network units 1 communicating by means of radio frequency signals 4 with a base station 2 of a wireless local network 5, which base station 2 amplifies the radio frequency signals 4 of the mobile network unit 1 and/or connects the wireless local network 5 to a wired fixed network by means of bridge functions.

Figure 2 shows a flow chart presenting schematically the architecture of a method and/or system in a wireless local network 5, whereby a beacon signal is constantly being transmitted from the base stations 2 in order to make a potential user aware of the availability of a WLAN 5.

Figure 3 shows a flow chart presenting schematically the architecture of a method and/or system according to the invention in a wireless local network 5, the WLAN 5 having two different operating modes, such as a normal transmitting - receiving mode and a sleep mode. The figure shows in particular the course of switchover from the sleep mode into the normal transmitting -

receiving mode when a mobile network unit 1 would like to use the wireless local network 5.

Figure 1 illustrates an architecture which can be used to achieve the invention. In this embodiment example, one or more mobile network units 1 communicate by means of radio frequency signals 4 with a base station 2, or respectively an access point, of a wireless local network 5. Wireless local networks 5 are also referred to as WLANs (Wireless Local Area Networks). A WLAN can be composed of one or more such base stations or respectively access points. The base station 2 amplifies the radio frequency signals 4 of the mobile network unit 1 and/or connects the wireless local network 5 by means of bridge functions to a wired fixed network. Base stations 5, or respectively access points, of a WLAN 5 can be connected e.g. via physical media such as, for instance, coaxial cable, twisted pair or fiber optic cable to assigned radius servers. The connection can comprise communication networks, such as, for example, mobile radio networks, such as a terrestrial mobile radio network, e.g. a GSM or UMTS network, or a satellite-based mobile radio network and/or one or more fixed networks, for instance the public switched telephone network (PSTN) and/or ISDN (Integrated Services Digital Network) or a suitable LAN (Local Area Network) or WAN (Wide Area Network). During log on of a mobile network unit 1 of a user in a WLAN 5, an identification code of the user is transmitted for authentication of the user together with supplementary information data, which can be determined by the user, via one of the APs 2 of the WLAN 5 to a central unit and/or radius server. The communication between the central unit and the access points 2 can take place e.g. via a TCP/IP interface and/or CORBA interface, an ATM module, a SMS and/or USSD gateway by means of special short messages, for example SMS (Short Message Services), USSD (Unstructured Supplementary Services Data) messages, or other techniques such as MExE (Mobile Execution Environment), via protocols such as GPRS (Generalized Packet Radio Service), WAP (Wireless Application Protocol) or another user information channel. The data transfer between the central unit and the access points 2 is initiated and carried out e.g. via transfer modules, implemented through software or hardware, of the central unit as well as of the access points. The mobile network units 1 or so-called mobile nodes can be e.g. laptops, notebooks, PDAs (Personal Digital

Assistants) or mobile radio devices, in particular mobile radio telephones. The mobile nodes are equipped through hardware and software with a corresponding interface in order to integrate them in a local wireless computer network (WLAN). They communicate by means of radio frequency signals with the access points 2 of the WLAN 5. The mobile nodes 1 can comprise e.g. an adaptor, which includes a transceiver as well as a control card (such as e.g. an infrared (IR) adaptor or a low frequency radio wave adaptor). The mobile nodes 1 are thereby able to move freely within the range of the wireless LAN 5. The access points 2 of the WLAN 5 can e.g. amplify the radio frequency signals of the mobile node 1 as well as comprise bridge functions which make it possible to access nodes 1 of a wired LAN from the wireless local network 5 and vice-versa. For transmission of the radio frequency signals, the access points 2 comprise at least one antenna. The antenna can be e.g. a dipole antenna, a loop radiator such as a folded dipole, a Marconi aerial or a ground plane antenna, a directional antenna such as e.g. a yagi aerial, a turnstile antenna or a parabolic aerial, an omnidirectional antenna or a fractal antenna system. The radio frequency signals lie typically in the frequency bands reserved for wireless LAN between 800 MHz and 6000 MHz, such as e.g. three frequency bands set by the United States Federal Communication Commission (FCC) in the USA: 902-928 MHz, 2400-2483.5 MHz and 5725-5850 MHz (D 15 of Title 47, Code of Federal Regulations). They can also be in the range of 400 MHz, for example, as is common e.g. with electronic, wireless garage openers, or at the WLL (Wireless Local Loop) frequencies auctioned a year ago in Germany and Switzerland, e.g. 26 GHz for wireless local loop methods. It is to be pointed out, however, that other frequencies are also possible, without affecting the scope of the invention. Thus, in principle, infrared signals can also be used for the invention such as e.g. IrDA, IR-LAN, etc. The bridge functions of the base station 2 can be achieved e.g. according to IEEE standard 802.1D-1990 "Media Access Control Bridges" pp. 31-47. In the WLAN network recognition and user identification and/or authentication complement one another. For network recognition, an AP periodically transmits so-called beacon signals within a WLAN, which signals comprise e.g. Service Set IDentifiers (SSID) and/or other control parameters for integrating a mobile network unit 1 into a wireless network. This applies in particular to the 802.X, such as e.g. the 802.11 network technologies, but also to Bluetooth and other network technologies. Beacon

signals are thus transmitted all the time to make potential users or respectively their mobile network units 1 aware of available WLANs 5. In the present invention, however, after a predefined time interval without a connection signal to a mobile network unit 1, the base station 2 switches over from normal transmitting and receiving mode to sleep mode. Understood by "normal transmitting and receiving mode" is the normal operating mode of the AP during which mobile network units 1 can access the APs or not.

In a flow chart, Figure 2 illustrates how a mobile network unit 1 recognizes the WLAN and connects thereto before the user can authenticate himself e.g. with the central unit and/or radius server. As mentioned, the base station in normal transmitting and receiving mode transmits beacon signals periodically 11. Even when no mobile network units are located in the WLAN, the beacon signals continue to be periodically transmitted from the APs. The SSID can be an unambiguous identification symbol, 32 characters long, which is assigned to the header of data messages sent over the network and which serves as a password for the mobile network units. The SSID differs from one WLAN to another. That means that all APs and mobile network units of a particular WLAN must use the same SSID. A network unit which cannot support an unambiguous SSID will normally not be granted any network access via a base station or respectively an AP. In the secure access mode (802.X) of the APs, the SSID from base station 2 and mobile network unit 1 must agree. In the non-secure access mode, a mobile network unit 1 can log on with the configured SSID, a blank SSID, or with the SSID set on "any." The beacon signals can be transmitted encrypted or unencrypted. The 802.11 network standard uses for encryption purposes WEP (Wired Equivalent Privacy), for example. WEP operates in three modes: no encryption, 40-bit encryption and 128-bit encryption. The 802.11 standard encrypts only the data packets, however, and not the management packets. The SSID is part of the beacon and probe management signal and is not encrypted when WEP is activated. A mobile network unit 1 receives the beacon signal 13, and recognizes the WLAN 5 from the beacon. Default SSIDs of WLANs are e.g. “tsunami” - Cisco, “101” – 3Com, “RoamAbout Default Network Name” - Lucent/Cabletron, “Default SSID", “Compaq” - Compaq, “WLAN” – Addtron (a popular AP), “intel” - Intel, “linksys” – Linksys, “Wireless”. Thus if a mobile network unit 1 receives a

beacon signal 13, it logs on with the corresponding AP, and carries out the authentication 14 of the user, if necessary, e.g. with the central unit, before it has access to the WLAN 5. If the mobile node 1 does not receive any beacon signal, but nevertheless needs a WLAN connection, it continues to scan for beacon signals 15 until it has found an available WLAN. This applies to the normal transmitting and receiving mode. In the normal transmitting and receiving mode the AP automatically transmits a further beacon signal after a predefined time interval 12. In the case that a base station 2 switches over into sleep mode, no recognition signals and/or other radio frequency signals are transmitted anymore from the base station 2, i.e. also no beacon signals, but the base station 2 nevertheless remains ready to receive radio frequency signals 4 also in sleep mode.

Figure 3 illustrates the method according to the invention on the side of the AP 2 when the base station 2 is in sleep mode. If a mobile network unit 1 needs a network connection, it transmits an alert signal which is received by the base station 2. If, in the normal transmitting and receiving mode, the base station does not receive any connection signal from a mobile network unit 1, the AP 2 waits for a predefinable period of time 24, if thereafter it still does not receive any connection signal 25, the base station 2 switches over into sleep mode 26, and waits 27 for a connection signal from a mobile node 1. Upon receiving an alert signal from a mobile network unit 1, the base station 2 transmits 22 the recognition signals necessary for the connection and/or beacon signals to the mobile network unit 1 (e.g. beacon signal), and, as described under Figure 2, carries out the authentication of the user of the mobile network unit 1. All base stations 2 of a WLAN 5 can always switch together from sleep mode into the normal transmitting and receiving mode, or only those base stations 2 in whose basic service areas 3 the mobile network unit 1 is located, the other base stations 2 of the wireless local network 5 remaining in their previous operating mode. It can make sense in addition for the base stations 2 of basic service areas 3 bordering on the basic service areas 3 of the base station 2 in whose BSA the mobile node 1 is located to automatically switch over into the normal transmitting and receiving mode if they were previously in sleep mode. In an embodiment variant, the mobile network unit 1, when needing a network connection, can transmit an alert signal

only when no recognition signal is received from a base station 2, or automatically every time it needs a WLAN, for example. It is furthermore possible for the base station 2 of the wireless local network 5 to switch over from sleep mode into the normal transmitting-receiving mode only when a network-specific recognition of the alert signal corresponds with a stored recognition signal of the wireless local network 5. This results in additional protection against unauthorized use of the WLAN. The security of the WLAN 5 can be further increased in that at least parts of the network-specific recognition signal are definable for the wireless local network 5 by the user of the mobile unit 1 and/or by an operator. As a special embodiment variant, the MSISDN and/or IMSI of a mobile radio device of the user of the mobile network unit 1 can be used as the supplementary information data. Moreover this can be stored on a SIM (Subscriber Identification Module) card of the mobile network unit. For other embodiments it can be important, however, that the alert signal is transmitted from the mobile network unit 1 in a network-independent way. This could be advantageous in particular for WLANs in public buildings, airports, etc. It is important to point out that the method or respectively system according to the invention can be achieved without modification of existing hardware on the side of the base stations 1 and on the side of the mobile network units 1, requiring only modification of the corresponding software components. Of course it is also possible to achieve the method and system according to the invention through addition of corresponding hardware modules.

Claims

1. A method for reducing electrosmog in wireless local networks, one or more mobile network units (1) communicating with a base station (2) of a wireless local network (5) by means of radio frequency signals (4), which base station (2) amplifies the radio frequency signals (4) of the mobile network unit (1) and/or connects the wireless local network (5) to a wired fixed network by means of bridge functions, wherein

the base station (2) changes over from the normal transmitting-receiving mode into a sleep mode after a predefinable time interval without connecting signal to a mobile network unit (1), in the sleep mode no recognition signals and/or other radio frequency signals being transmitted from the base station (2), the base station being ready to receive radio frequency signals (4), however,

when needing a network connection, a mobile network unit (1) transmits an alert signal to the base station,

upon receiving the alert signal of the mobile network unit (1), the base station (2) transmits to the mobile network unit (1) the recognition signals necessary for the connection and changes over into transmitting and receiving mode.

2. The method according to claim 1, wherein, when in need of a network connection, the mobile network unit (1) transmits an alert signal only if it does not receive any recognition signal from a base station (2).

3. The method according to one of the claims 1 or 2, wherein only the base station in whose basic service area the mobile network unit (1) is located changes over into the normal transmitting and receiving mode, the other base stations (2) of the wireless local network (5) remaining in their previous operating mode.

4. The method according to claim 3, wherein the base stations (2) of the basic service areas (3) bordering on the basic service area (3) of the base station (2) in whose basic service area the mobile network unit (1) is located likewise change over automatically into the normal transmitting-receiving mode if they were previously in the sleep mode.

5. The method according to one of the claims 1 to 4, wherein the base station (2) of the wireless local network (5) changes over from sleep mode into the normal transmitting-receiving mode only if a network-specific recognition signal of the alert signal corresponds to a stored recognition signal of the wireless local network (5).

6. The method according to claim 5, wherein at least parts of the network-specific recognition signal are definable for the wireless local network (5) by a user of the mobile unit (1) and/or by an operator.

7. The method according to one of the claims 1 to 6, wherein the alert signal from the mobile network unit (1) is transmitted in a network independent way for every wireless local network (5).

8. The method according to one of the claims 1 to 7, wherein the wireless local network (5) is set up based on the 802.X network technology, the recognition signals containing the respective Service Set Identifier (SSID).

9. The method according to one of the claims 1 to 7, wherein the wireless local network (5) is set up based on Bluetooth technology.

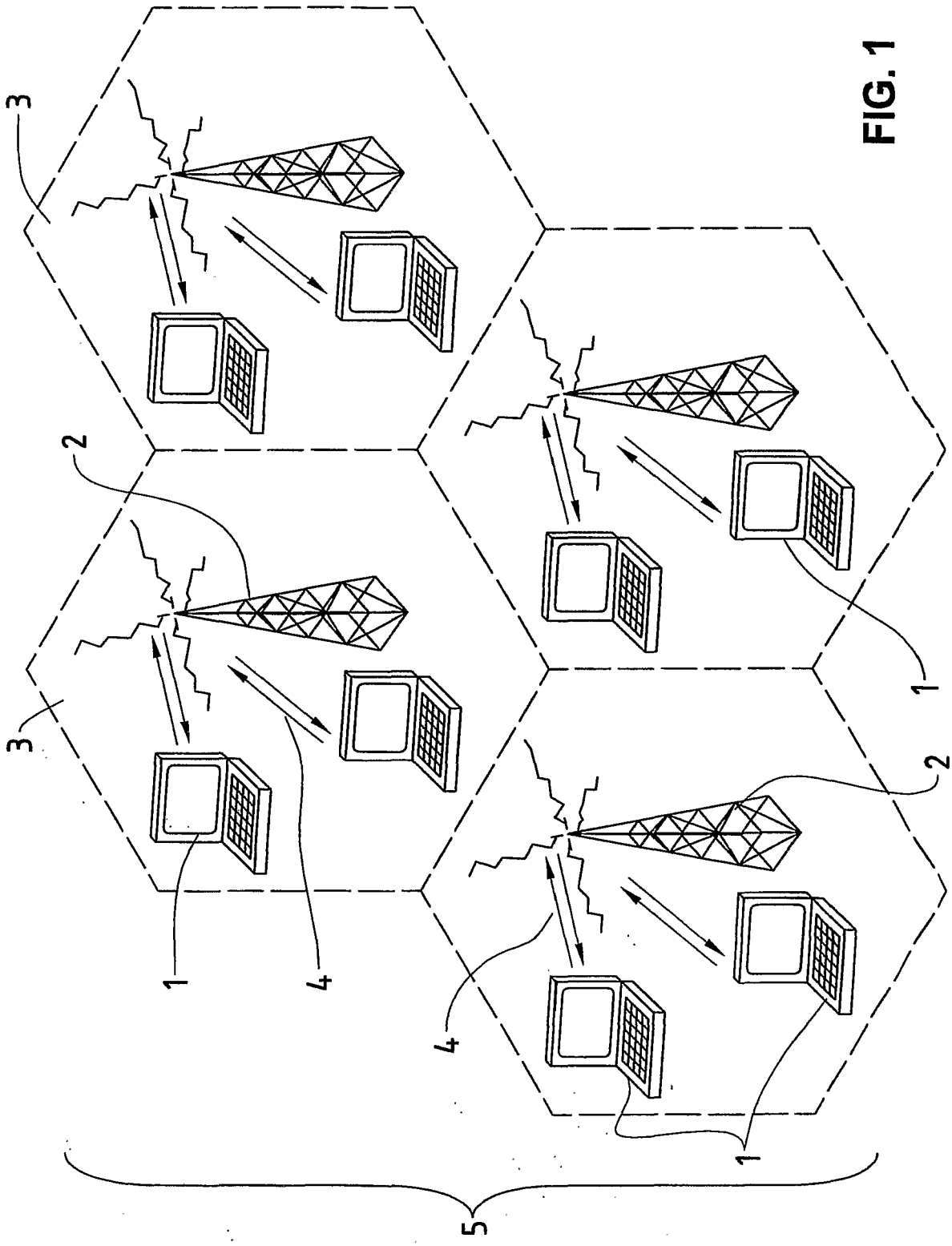

FIG. 1

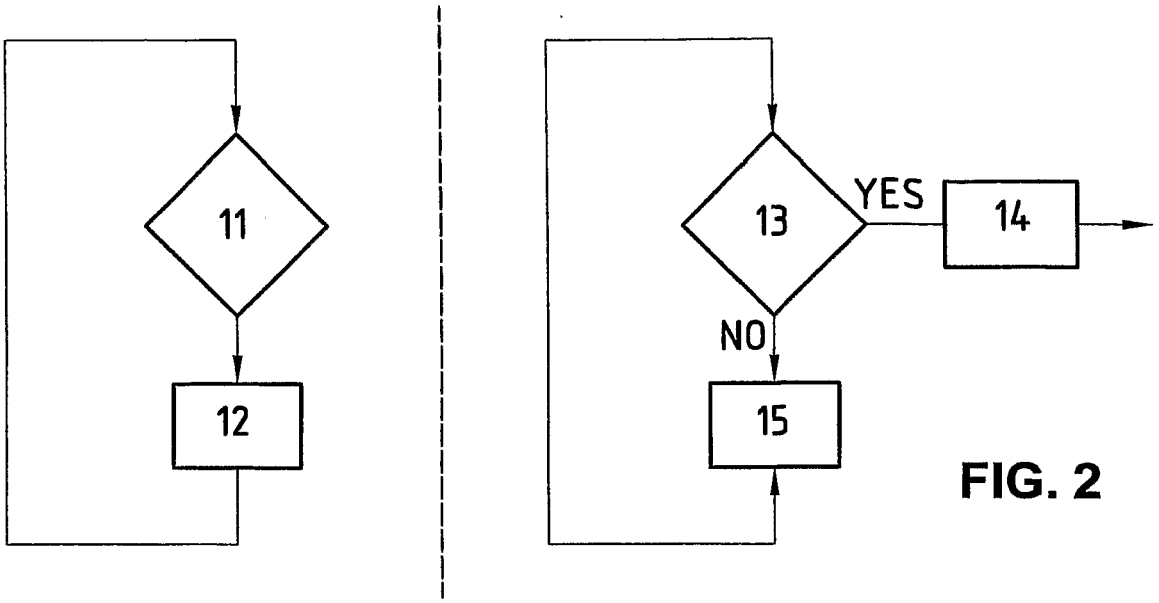

FIG. 2

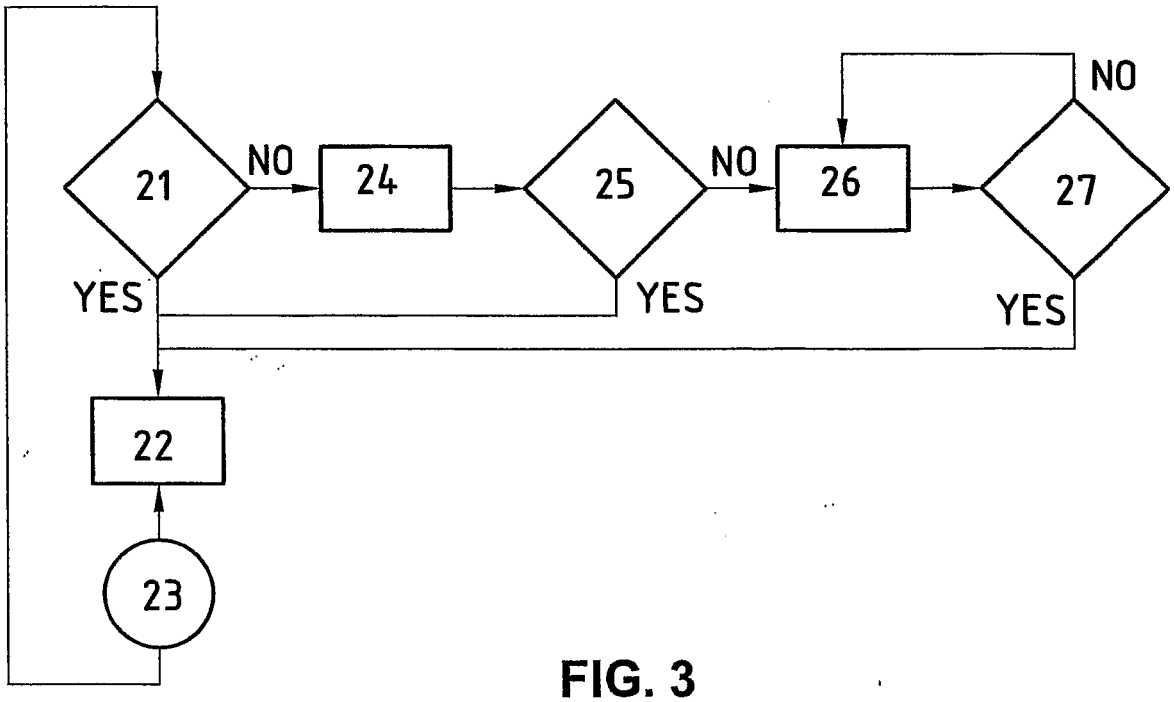

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CH 03/00138

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 H04Q7/32 H04Q7/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 H04Q H04B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 884 196 A (LEKVEN ERIC J ET AL) 16 March 1999 (1999-03-16) abstract figure 2 column 6, line 11 - line 31	1-9
A	WO 02 093778 A (QUALCOMM INC) 21 November 2002 (2002-11-21) abstract paragraph '0009! - paragraph '0010! claim 1	1-9
A	US 6 339 694 B1 (NUCKOLS JEFFREY R ET AL) 15 January 2002 (2002-01-15) column 3, line 43 - line 60 abstract	1-9

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 2003	22/10/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Dionisi, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CH 03/00138

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02 07464 A (ERICSSON TELEFON AB L M) 24 January 2002 (2002-01-24) page 2, line 5 - line 23 page 15, line 11 - line 15	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CH 03/00138

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5884196 A	16-03-1999	AU 717244 B2	23-03-2000
		AU 3569397 A	05-01-1998
		BR 9709555 A	11-01-2000
		CN 1228230 A	08-09-1999
		EP 0903047 A2	24-03-1999
		JP 2000515334 T	14-11-2000
		KR 2000016550 A	25-03-2000
		WO 9747149 A2	11-12-1997
WO 02093778 A	21-11-2002	US 2002177461 A1	28-11-2002
		US 2002173325 A1	21-11-2002
		US 2002173326 A1	21-11-2002
		US 2002172165 A1	21-11-2002
		WO 02093788 A1	21-11-2002
		WO 02098015 A1	05-12-2002
		WO 02093953 A1	21-11-2002
		WO 02093954 A1	21-11-2002
		WO 02093948 A1	21-11-2002
		WO 02093812 A2	21-11-2002
		WO 02093778 A1	21-11-2002
		US 2003008657 A1	09-01-2003
		US 2002173327 A1	21-11-2002
		US 2002172169 A1	21-11-2002
US 6339694 B1	15-01-2002	NONE	
WO 0207464 A	24-01-2002	US 6584330 B1	24-06-2003
		AU 7121601 A	30-01-2002
		WO 0207464 A1	24-01-2002

Box No. VIII (iv) DECLARATION: INVENTORSHIP (only for the purposes of the designation of the United States of America)

The declaration must conform to the following standardized wording provided for in Section 214; see Notes to Boxes Nos. VIII, VIII (i) to (v) (in general) and the specific Notes to Box No. VIII (iv). If this Box is not used, this sheet should not be included in the request.

Declaration of inventorship (Rules 4.17(iv) and 51*bis*.1(a)(iv)) for the purposes of the designation of the United States of America:

I hereby declare that I believe I am the original, first and sole (if only one inventor is listed below) or joint (if more than one inventor is listed below) inventor of the subject matter which is claimed and for which a patent is sought.

This declaration is directed to the international application of which it forms a part (if filing declaration with application).

This declaration is directed to international application No. PCT/..... (if furnishing declaration pursuant to Rule 26*ter*).

I hereby declare that my residence, mailing address, and citizenship are as stated next to my name.

I hereby state that I have reviewed and understand the contents of the above-identified international application, including the claims of said application. I have identified in the request of said application, in compliance with PCT Rule 4.10, any claim to foreign priority, and I have identified below, under the heading "Prior Applications," by application number, country or Member of the World Trade Organization, day, month and year of filing, any application for a patent or inventor's certificate filed in a country other than the United States of America, including any PCT international application designating at least one country other than the United States of America, having a filing date before that of the application on which foreign priority is claimed.

Prior Applications: .....

.....

I hereby acknowledge the duty to disclose information that is known by me to be material to patentability as defined by 37 C.F.R. § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the PCT international filing date of the continuation-in-part application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name: FERRAN MORENO BLANCA

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Mailing Address: OSTERMUNDIGENSTRASSE 93 , 3050 BERNE (Switzerland)

.....

Citizenship: SPANISH

Inventor's Signature: MBB
(if not contained in the request, or if declaration is corrected or added under Rule 26*ter* after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: 25.02.2003
(of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26*ter* after the filing of the international application)

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Citizenship: Swiss

Inventor's Signature: MBischoff
(if not contained in the request, or if declaration is corrected or added under Rule 26*ter* after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: 25.02.2003
(of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26*ter* after the filing of the international application)

☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (iv)".

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 154274.1/LE/mb	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/CH 03/00138	International filing date *(day/month/year)* 24.02.2003	Priority date *(day/month/year)* 24.02.2003
International Patent Classification (IPC) or both national classification and IPC H04Q7/32		
Applicant SWISSCOM AG et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07.09.2004	09.05.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Schweitzer, J-C Telephone No. +49 89 2399-8963

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-15 as originally filed

Claims, Numbers

1-9 as originally filed

Drawings, Sheets

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-9
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-9
	No:	Claims	

2. Citations and explanations

   see separate sheet

Concerning section V.2 (reasoned statement under Article 35(2) PCT)

Claim 1 relates to a method for reducing electrosmog in a wireless local network by putting a base station into sleep under certain conditions.

The nearest prior is given by the cited document **WO-A-02/07464 (Ericsson),** hereinafter referred to as document **D1**, which discloses a method for reducing energy consumption (and, thus, implicitly electrosmog) in a base station (node) of a wireless local network, by turning off or putting into sleep some of the node's equipments or traffic carriers during periods of low traffic.

In accordance with the invention, by contrast, when the base station has not received any connection signal from a mobile unit for a predetermined time period, it switches into a sleep mode wherein no recognition (beacon) signal is transmitted. Thus, instead of periodically sending such recognition signals, which are normally required by the mobile units to "recognize" the wireless network and authenticate themselves with the base station, the invention proposes to stop, during the sleep mode, the transmission of such recognition signals, while still allowing the reception of radio signals. A mobile unit needing a connection transmits an alert signal to the base station, which then changes over to the normal transmitting/receiving mode.

This claimed concept of underlying the invention permitting to reduce electrosmog in the vicinity of base stations is neither taught, nor rendered obvious, alone or in combination, by the prior art documents cited in the International Search Report.

The above-cited **D1** merely suggests the idea of turning off traffic carriers or specific circuits in the base station, but does not mention stopping the sending of beacons signals. The remaining cited documents concern remote units (mobile stations) having a sleep/dormant mode, rather than base stations or access points, and thus provide no incentive for the skilled person to arrive at the present invention.

Claim 1 is therefore novel and considered to involve the required inventive step, Articles 33(2) and (3) PCT. The subject-matter of claim 1 is also industrially applicable.

Dependent **claims 2 to 9** relate to further implementing details of the method defined by claim 1 to which they refer and are thus equally novel, inventive and industrially applicable.

Additional remarks concerning the clarity of the claims.

Claim 1 states, at lines 4 to 6, that the *"base station amplifies the radio frequency signals to the mobile network unit and/or connects the wireless local network to wired fixed network..."*. The term "and/or" is however misleading, as it is actually clear that the base station -inter alia- has to perform both tasks/functions, that is amplifying radio signals to the mobile units (stations) and connecting the wireless and the fixed networks. The expression "and/or" should thus correctly read "and".

Moreover, in claim 1, it should be made clear that the expression "without connecting signals" actually means "without receiving any connection signal", as it is clear from the description, see e.g. at page 14, line 17.

Remarks concerning the form and contents of the application:

The independent claim is not drafted in the proper two-part "characterised" form recommended by Rule 6.3.(b),(I),(ii) PCT, having a preamble that correctly reflects the nearest prior art represented by the above noted **D1**.

In order to meet the requirements of Rule 5.1.(a),(ii) PCT, the prior art document **D1** noted above should be acknowledged by reference and briefly discussed in the introductory part of the description.

REDUCTION OF ELECTROSMOG IN WIRELESS LOCAL NETWORKS

Legal status of WO2004075583 (A1) 2004-09-02:

WO F	**0300138 W** (Patent of invention)
PRS Date :	2004/09/02
PRS Code :	AK
Code Expl.:	+ DESIGNATED STATES
KD OF CORRESP. PAT. :	A1
DESIGNATED COUNTR. :	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
PRS Date :	2004/09/02
PRS Code :	AL
Code Expl.:	+ DESIGNATED COUNTRIES FOR REGIONAL PATENTS
KD OF CORRESP. PAT. :	A1
DESIGNATED COUNTR. :	GH GM KE LS MW MZ SD SL SZ TZ UG ZM ZW AM AZ BY KG KZ MD RU TJ TM AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT SE SI SK TR BF BJ CF CG CI CM GA GN GQ GW ML MR NE SN TD TG
PRS Date :	2004/10/27
PRS Code :	121
Code Expl.:	EP: THE EPO HAS BEEN INFORMED BY WIPO THAT EP WAS DESIGNATED IN THIS APPLICATION
PRS Date :	2004/11/11
PRS Code :	DFPE
Code Expl.:	REQUEST FOR PRELIMINARY EXAMINATION FILED PRIOR TO EXPIRATION OF 19TH MONTH FROM PRIORITY DATE (PCT APPLICATION FILED BEFORE 20040101)
PRS Date :	2005/08/24
PRS Code :	WWE
Code Expl.:	+ WIPO INFORMATION: ENTRY INTO NATIONAL PHASE
CC OF CORRESP. PAT. :	EP
CORRESP. PATENT D. :	2003815938
PRS Date :	2005/11/23
PRS Code :	WWP
Code Expl.:	+ WIPO INFORMATION: PUBLISHED IN NATIONAL OFFICE
CC OF CORRESP. PAT. :	EP
CORRESP. PATENT D. :	2003815938
PRS Date :	2007/01/26
PRS Code :	WWW
Code Expl.:	- WIPO INFORMATION: WITHDRAWN IN NATIONAL OFFICE
CC OF CORRESP. PAT. :	JP
PRS Date :	2007/01/26
PRS Code :	NENP JP
Code Expl.:	NON-ENTRY INTO THE NATIONAL PHASE IN:

Bioelectromagnetics 24:82–90 (2003)

Exposure of Human Peripheral Blood Lymphocytes to Electromagnetic Fields Associated With Cellular Phones Leads to Chromosomal Instability

Maya Mashevich,[1,3] Dan Folkman,[2] Amit Kesar,[2] Alexander Barbul,[3] Rafi Korenstein,[3]* Eli Jerby,[2] and Lydia Avivi[1]

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[2]Department of Electrical Engineering-Physical Electronics, Tel-Aviv University, Tel-Aviv, Israel
[3]Department of Physiology and Pharmacology, Tel-Aviv University, Tel-Aviv, Israel

Whether exposure to radiation emitted from cellular phones poses a health hazard is at the focus of current debate. We have examined whether in vitro exposure of human peripheral blood lymphocytes (PBL) to continuous 830 MHz electromagnetic fields causes losses and gains of chromosomes (aneuploidy), a major ''somatic mutation'' leading to genomic instability and thereby to cancer. PBL were irradiated at different average absorption rates (SAR) in the range of 1.6–8.8 W/kg for 72 hr in an exposure system based on a parallel plate resonator at temperatures ranging from 34.5–37.5 °C. The averaged SAR and its distribution in the exposed tissue culture flask were determined by combining measurements and numerical analysis based on a finite element simulation code. A linear increase in chromosome 17 aneuploidy was observed as a function of the SAR value, demonstrating that this radiation has a genotoxic effect. The SAR dependent aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in the SAR dependent genetic toxicity. Control experiments (i.e., without any RF radiation) carried out in the temperature range of 34.5–38.5 °C showed that elevated temperature is not associated with either the genetic or epigenetic alterations observed following RF radiation—the increased levels of aneuploidy and the modification in replication of the centromeric DNA arrays. These findings indicate that the genotoxic effect of the electromagnetic radiation is elicited via a non-thermal pathway. Moreover, the fact that aneuploidy is a phenomenon known to increase the risk for cancer, should be taken into consideration in future evaluation of exposure guidelines. Bioelectromagnetics 24:82–90, 2003.

Key words: continuous RF fields; nonthermal effects; aneuploidy; centromeric DNA replication; carcinogenesis

INTRODUCTION

The exponential increase in the use of cellular mobile communication over the last few years leads millions in the world's population to be exposed to radiofrequency (RF) electromagnetic radiation. This increased environmental exposure of humans to RF radiation raises questions regarding the biological and health consequences of this exposure, especially its long term effects. In particular, the connection between cancer hazard and exposure to RF radiation is continuously debated [reviewed in Szmigielski, 1996; Rothman et al., 1996; Valberg, 1997; Moulder et al., 1999].

The conflicting evidence arises from difficulties in differentiating between the exposed and unexposed individuals while conducting epidemiological studies relying on small cohorts, as well as from the insufficient elapsed time for the cancer to appear in populations. Thus, a large epidemiological study, which followed cancer morbidity in the whole population of military

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career personnel in Poland during a 15 year period (1971–1985), reported that leukemia and brain tumors are significantly higher in RF exposed personnel compared with unexposed ones [Szmigielski, 1996]. This study was supported by another, conducted on laboratory animals, which presented evidence that exposure to RF radiation leads to an increased risk for lymphoma [Repacholi et al., 1997]. However, there are studies arguing that the increased cancer risk reported in connection with exposure is due to various experimental errors and is not the result of the RF radiation per se [reviewed in Valberg, 1997]. Thus, the question whether exposure to RF radiation leads to cancer, although of grave concern for the general public, still remains a puzzling issue, which is awaiting the arrival of more modern tools to be resolved.

Exploring the genetic effects of RF radiation was previously undertaken using classical and modern genetic tools assessing gene mutation, chromosomal alterations in somatic cells, DNA repair processes, and cell transformation assays [for review see Verschaeve and Maes, 1998; Brusick et al., 1998]. However, many of these studies assayed genetic parameters such as sister chromatid exchange and chromosomal aberrations that were previously shown to be typically associated with ionizing radiation.

One of the most relevant genetic changes associated with the cancerous process is alteration in chromosome complement [aneuploidy; Duesberg et al., 2000].

There is an increasing evidence that in preneoplastic cells, the genetic material undergoes continuous change, resulting in the accumulation of multiple mutations and alterations in the genomic DNA content [Loeb, 1991; Jackson and Loeb, 1998; Duesberg et al., 2000; Loeb and Loeb, 2000]. Among modified genes are those that function in guaranteeing the stability of the genome. Loss in this ability results in a ''mutator phenotype.'' Evidence for a mutator phenotype is the frequent occurrence of aneuploidy displayed in most of human cancers [Lengauer et al., 1998; Pihan and Doxsey, 1999; Duesberg and Rasnick, 2000; Li et al., 2000; Bialy, 2001]. New insights into the analysis of the neoplastic process suggest that aneuploidy is neither the result of gene mutation nor the outcome of some other genomic modification, but constitutes ''the somatic mutation [itself] that makes cancer'' [reviewed in Duesberg and Rasnick, 2000]. Thus, aneuploidy appears to be the cause and not the result of carcinogenesis.

Aneuploidy results either from chromosome malsegregation or from chromosome fragmentation. It at once alters the dosage of a large number of genes, giving rise to multiple changes in gene expression of both genes whose dosage was altered as well as those that remained in two copies, but whose function depends on two intact copies of other genes. The gross alteration in gene expression accompanying an unbalanced chromosome complement frequently leads to malfunctioning of the chromosome segregating apparatus, making aneuploidy an autocatalytic process, which may continuously destabilize the genome and thus facilitate embarking on the road to cancer. Consequently, aneuploidy accompanied by an abnormal behavior of the chromosome segregating apparatus and modification in gene expression is a hallmark of the preneoplastic phenotype, and as such provides a reliable genetic marker for the identification of agents possessing carcinogenic activity.

Recent studies reveal that there is a correlation between aneuploidy and loss of synchrony in replication timing of homologous DNA counterparts lacking transcriptional ability, such as highly repetitive DNA arrays (satellite DNA) [Litmanovitch et al., 1998]. These arrays, which in man are associated with chromosome mover components (centromeres), replicate synchronously in euploid cells [chromosomally balanced cells; Litmanovitch et al., 1998]. However, an increase in aneuploidy is accompanied by elevation of asynchrony in replication timing of homologous satellite DNA arrays. Thus, asynchronous replication of homologous counterparts of satellite DNA arrays was observed in ovarian cancer tumors [Litmanovitch, 1996], in lymphocytes of patients suffering from familial ovarian cancer [Litmanovitch, 1996; Litmanovitch et al., 1998], and in blood malignancies [Korenstein-Ilan, 2000]. It is not yet clear whether aneuploidy is the cause or the effect of the loss of synchrony in replication timing of homologous centromeres. Whatever the mechanism, a continuous damage in replication timing of centromeres affects the whole mitotic machinery, which is dependent on temporal control of successive events [reviewed in Litmanovitch et al., 1998].

The present study employs aneuploidy and replication assays, based on interphase fluorescence in situ hybridization (FISH), to address the question whether in vitro exposure of human peripheral blood cells to mobile telephone frequencies (continuous 830 MHz) leads to genetic effects that are associated with increased risk for cancer. The FISH replication assay was found to reliably detect replication timing [Mukherjee et al., 1992; Selig et al., 1992; Bickmore and Carothers, 1995; Boggs and Chinault, 1997; Haaf, 1997; Simon et al., 1999], while avoiding the labor intensive classical methodologies. These include thymidine and BudR incorporation [Miller et al., 1973], which themselves influence the course of replication,

followed by Southern blotting [Braunstein et al., 1982] or cell sorting [Hansen et al., 1993]. They necessitate large cell populations and are not sufficiently reproducible [Mukherjee et al., 1992; Simon et al., 1999]. Also, using the FISH replication assay we can simultaneously assess the frequency of losses and gains of the locus identified by the probe used [reviewed in Litmanovitch et al., 1998 and references therein].

MATERIALS AND METHODS

Samples

Peripheral blood samples were obtained with informed consent from ten healthy male volunteers. The blood samples of the first group of five donors were employed for exposure to the continuous wave (CW) 830 MHz RF radiation. Each volunteer donated several samples at different times, each used for a different level of exposure to RF. Blood samples from a second group of five donors were exposed to different temperatures in the range of 34.5–41.0 °C.

Culture Preparation

Heparinized blood was collected from each donor. Three milliliter of peripheral blood cells were added to 62 ml of culture medium (F10) supplemented with 20% fetal calf serum, 3% phytohemagglutinin, 0.2% heparin, and 1% antibiotics (a standard solution of penicillin and streptomycin), in 25 cm^2 culture flasks (TPP, Switzerland). This medium is used in the preparation of phytohemagglutinin stimulated lymphocytes for routine karyotyping analysis [Rooney and Czepulkowsli, 1992].

The samples exposed to RF radiation were inserted into the exposure system, which was later placed in the incubator. The samples were continuously exposed to CW 830 MHz radiation for the duration of the culturing period of 72 hr. The unexposed (sham exposed) sample was placed in the same incubator, at a different height level, with a metal plate between the two samples. The sham exposed samples were also grown uninterrupted for the same period of time. An additional control flask was grown in a different incubator which was set to 37 °C. Following culturing, the samples were harvested for cytogenetic analysis [Rooney and Czepulkowsli, 1992]. No significant differences were found between the two types of control.

RF Exposure Setup

Figure 1 shows a schematic block diagram of the RF exposure setup. It consists of an exposure cell, an RF generator, and diagnostic means to measure the RF power and the culture temperature. The exposure cell

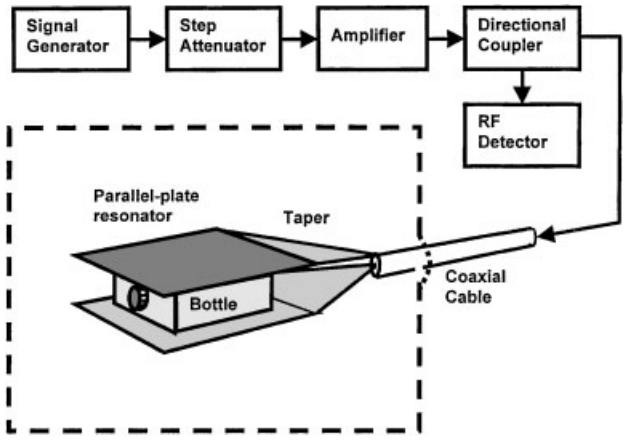

Fig. 1. Schematic block diagram of the RF exposure system.

utilized a standard tissue culture plastic bottle (TPP, 25 cm^2) in which the blood cells were cultured. The bottle was situated within a parallel plate RF resonator fed by a coaxial cable through a tapered transition section. The parallel plate resonator dimensions are 6 cm length, 5 cm width, and 2.4 cm height (i.e., spacing between the plates). The entire exposure cell was installed within an incubator to keep it at the appropriate temperature.

The RF generator unit consists of a CW generator, a digital step attenuator, and an RF amplifier (Mini-Circuits' ZOS-1025, ZFAT 1-2-4, and ZHL-2-8, respectively). The output RF power feeding the exposure cell is coupled by a directional coupler (ZEDC-10-2B) to an RF detector (ACSP-2517NC3). The input RF power to the exposure cell is 1 W or less.

Numerical Dosimetry

The distribution of the electromagnetic radiation inside the exposure cell (including the incubator's wall effect) was computed by an Ansoft HFSS simulation code. The mesh resolution was chosen as ~1/6 of the wavelength in each medium. At 850 MHz, the free space wavelength is ~35 cm, whereas the wavelength in the culture liquid is ~4.1 cm. The relative dielectric constant of the culture medium was found experimentally to be $\varepsilon_T \cong 73–j34$ at 811 MHz and 37 °C.

The HFSS results show that $^2/_3$ of the incident RF power is absorbed by the blood culture, whereas the remainder is dissipated by reflection to the input port and by radiation to the incubator. Figure 2 demonstrates the absorbed power distribution profile at the bottom of the bottle by a 3D contour plot. The accuracy of the HFSS simulation was estimated to be ± 5%. The slight asymmetry in the RF distribution profile is caused by the uneven reflection from the incubator walls (included in the simulation). For a 1 W input power computation,

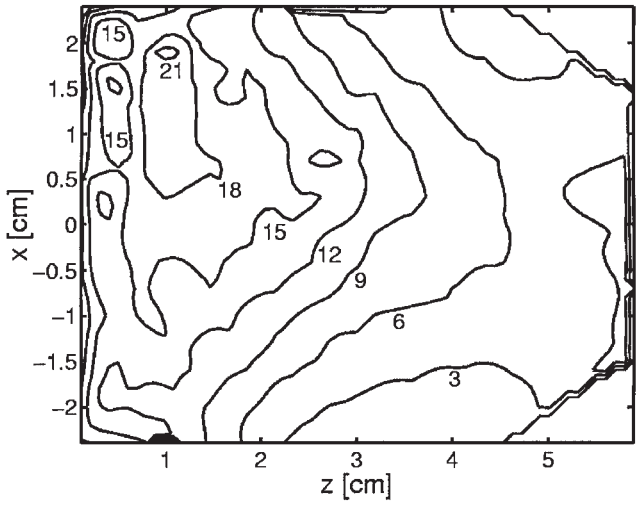

Fig. 2. Contour map of the absorbed power in W/kg at the bottom of the bottle for 1 W input power, based on numerical simulation of the exposed bottle in the incubator. The z-axis represents the longitudinal dimension of the bottle and the x-axis represents the perpendicular dimension.

the maximum power density absorbed in the culture is 24 W/kg, whereas the average power density is 9.4 W/kg. These results also indicate the maximum and average specific absorption rate (SAR) levels, respectively. The SAR levels corresponding to the lower input RF powers applied in the exposure experiments are computed accordingly.

Temperature Measurements of the Samples

The SAR distribution in the medium was inhomogeneous in the culture flask. It possessed a maximum at the flask's area nearest to the coaxial feeding and a minimum at the area furthest from it. Therefore, we made an attempt to measure on line, under steady state conditions of RF irradiation, temperature difference at various points in the flask. It should be taken into consideration that the heat transfer in the medium across the SAR gradient is much faster than the heat transfer to the surrounding environment. Thus, the temperature difference across this SAR gradient (SAR_{diff}) should be proportional to the SAR_{diff}/c_w, where c_w is the specific heat capacity of the aqueous medium. A constant reading was attained (thermal steady state conditions) 4 hr after initiation of exposure to the RF radiation.

For the measurement of temperature difference in the medium, during irradiation, before and after establishment of steady state conditions, we employed an array of four thermistors made adequate for temperature measurements in RF radiation environments by using both RF radiation shielding and high resistance cables. The four probes were inserted into the medium through the flask's cap. Two of the probes were positioned in the region of high SAR, whereas the two other probes were positioned in the area of low SAR. The maximal temperature difference between the probes at the two SAR regions did not exceed 1 °C.

In a different set of temperature measurements we determined, by a calibrated thermocouple, the average temperature of the medium in the bottle, within 4–8 s after the termination of the exposure to RF. During the exposure to RF radiation, the thermocouple (accuracy of ± 0.5 C°) was inserted in the medium at the cap area, which was unexposed to the RF radiation. Immediately after the termination of the exposure, the medium was quickly mixed and the thermocouple was pushed into it. The dependence of temperature rise, relative to the unexposed control, as a function of SAR level is shown in Figure 3 (the data points fitted to a linear function yielded $R^2 = 0.55$). The rise in the temperature can be attributed to the inefficient heat exchange between the exposed sample and the incubator environment in which it was positioned.

In order to prevent the average temperature elevation from exceeding 38.0 °C, the incubator was set to lower temperatures to compensate for the heating, especially when exposing the cells to the high SAR levels. For example, at the highest average SAR of 8.8 W/kg, the temperature of the incubator was set to 33.5 °C (Fig. 3).

Fluorescence In Situ Hybridization (FISH)

We used directly labeled commercial probe (Vysis Inc., Downers Grove, IL) for the centromeric repetitive (α-satellite) DNA arrays of chromosome 17 (CEN17). In situ hybridization, post washing, and detection were performed in accordance with the protocol of Insitus Biotechnologies (Albuquerque, NM),

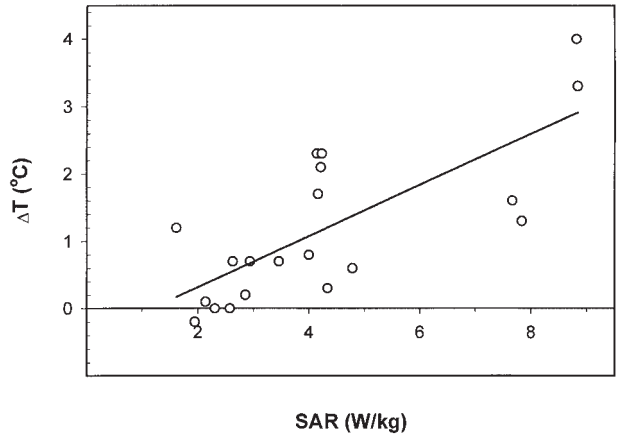

Fig. 3. Dependence of temperature rise, relative to the unexposed control, on specific absorption rate (SAR) levels.

with slight modifications. Five microliter of the probe solution were placed on the targeted area of the sample slides, covered with a 12 mm round silanized coverslip (Insitus Biotechnologies), and then sealed with rubber cement. The slides were placed into a microheating system (True Temp; Robbins Scientific Corp., Sunnydale, CA) at 76 °C and denatured for 6 min at that temperature. Then, the True Temp was turned off, and the slides were allowed to hybridize overnight in the instrument.

Post hybridization wash for probe CEN17 was carried out by immersing the slides in 0.4 × SSC/0.3% NP40 (1 × SSC = 150 mM NaCl, 15 mM sodium citrate) for 2 min followed by 1 min in 2 × SSC/0.1% NP40, both at 76 °C. After draining off excess liquid and brief drying, the slides were treated with 15 µl/test of a solution of antifade containing 6-diamidino-2-phenylindole (DAPI) as counterstain at 3 µg/ml (Vectashield, Vector Labs). Slides were covered with glass coverslips (22 × 60 mm) and stored at −20 °C until analysis (between 1 hr and 2 days).

Cytogenetic Evaluation

For the analysis of aneuploidy, 200 cells were blindly scored from each sample. In each scored cell the copy number (number of FISH signals) of CEN17 was determined.

For the analysis of replication timing, 100 cells, each containing two hybridization signals, were scored. Accordingly, an α-satellite array in the course of replication reveals two differently shaped configurations depending on its replication status [Selig et al., 1992]. The cells were divided into two categories: a single dot and a large beaded signal (singlet; S) representing an unreplicated sequence and a doubled dot and elongated rod like beaded signal (doublet; D), indicating that the sequence has already replicated. Thus, in a population of replicating cells, out of the total population of cells with two hybridization signals, the frequency (%) of cells containing two dissimilar (asynchronous) signals (%SD), represents the level of asynchrony in replication.

Statistical Analysis

Statistical significance was analyzed by two tailed Student's *t*-test.

RESULTS

Effect of Exposure on Loss and Gain of Chromosome 17

The change in the aneuploidy of chromosome 17 following exposure to different levels of SAR in the range of 1.6–8.8 W/kg is given in Figure 4a. An increase in the aneuploidy level is observed upon elevation of the SAR level. The dependence of aneuploidy of chromosome 17 on the SAR level was fitted to a polynomial of the second order ($R^2 = 0.35$).

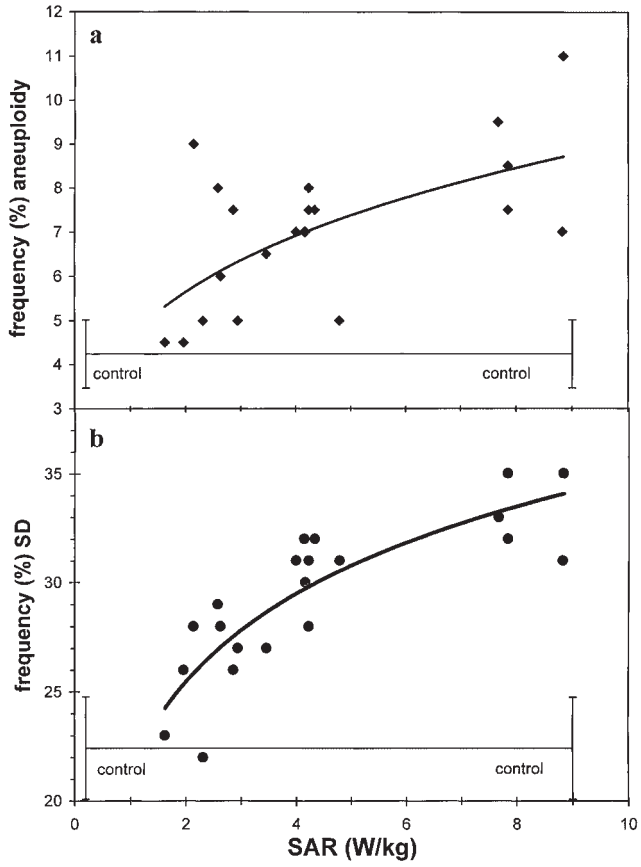

Fig. 4. Dependence on exposure to RF of human peripheral blood cells in the specific absorption rate (SAR) range of 1.6–8.8 W/kg of chromosome 17 aneuploidy (**a**) and frequency of asynchronous replication of α-satellite DNA of chromosome 17 (%SD) (**b**). Each point represents a single experiment carried out at the appropriate average SAR per flask.

In order to compare aneuploidy changes at the different levels of exposure, the samples were subdivided into four groups according to their average SAR level of exposure. Since the exposure was not evenly distributed over the whole range, the samples were grouped and subdivided into four levels of exposure: 1.6–2.3 (2.0 ± 0.3 W/kg; mean ± standard deviation; n = 4), 2.6–3.5 (2.9 ± 0.3 W/kg; n = 5), 4.0–4.8 (4.3 ± 0.2 W/kg; n = 7), and 7.8–8.8 W/kg (8.2 ± 0.6 W/kg; n = 5) which were represented as 1st to 4th levels, respectively. The dependence of aneuploidy of chromosome 17 on the four exposure levels is shown in Figure 5a. No significant increase in aneuploidy was observed following exposure to the 1st level of exposure as compared to control ($P > 0.4$). However, exposure to

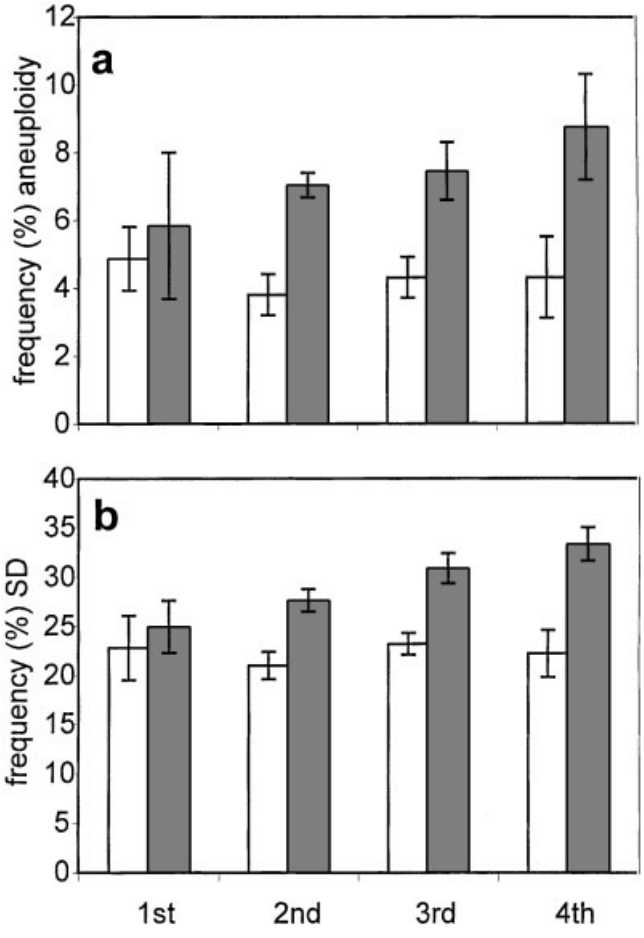

Fig. 5. Mean and standard deviation of the level of aneuploidy of chromosome 17 (**a**) and asynchronous replication of α-satellite DNA of chromosome 17 (%SD) (**b**) in human peripheral blood cells following exposure to four different levels of average specific absorption rate (SAR) of 2.0 ± 0.3 ($n = 4$), 2.9 ± 0.3 ($n = 5$), 4.3 ± 0.2 ($n = 7$), and 8.2 ± 0.6 ($n = 5$) W/kg (1st to 4th levels, respectively). Empty columns, controls; full columns, exposed samples.

the 2nd level resulted in a 65% increase of aneuploidy as compared to control ($P < 0.004$). Exposure to the 3rd level yielded an increase of 70% ($P < 0.002$), whereas exposure to the 4th level led to a 100% increase in the level of aneuploidy ($P < 0.00002$).

Effect of Exposure on the Temporal Order of Replication of the Centromere of Chromosome 17

The frequency of cells showing asynchronous replication (%SD) of CENT17 following exposure to RF increased in a similar manner as that of the aneuploidy frequency (compare Fig. 4a and 4b). The dependence of %SD on the SAR level was fitted to a logarithmic function ($R^2 = 0.55$).

The frequency of SD following exposure to the 1st SAR level was not significantly different from control ($P > 0.3$). However, exposure to the 2nd level of SAR brought about a 29% increase in the level of SD ($P < 0.00005$). The exposure to the 3rd level resulted in a 32% increase relative to control ($P < 0.00001$). The largest effect on SD was obtained following exposure to the 4th level of SAR, where an increase of 49% ($P < 0.00004$) was attained.

Effect of Temperature on the Aneuploidy and the Temporal Order of Replication of the Centromere of Chromosome 17

Since exposure to increasing SAR levels is accompanied by elevation in the sample temperature (Fig. 3), we determined the effect of this increase on both aneuploidy and the level of replication asynchrony of CENT17 in the absence of RF radiation. The PBL samples were exposed to different temperatures in the range of 34.5–41.0 °C for 72 hr. Both the aneuploidy level and the level of temporal replication were found to be unaffected by the temperature variation in the range of 34.5 to 38.5 °C ($4.4 \pm 0.8\%$ and $21.5 \pm 2.4\%$, respectively; Fig. 6). However, elevation of the temperature to 40 °C led to an increased level of $7.8 \pm 0.3\%$ in aneuploidy ($P < 10^{-11}$), while further increase to 41 °C had no additional effect ($8.1 \pm 0.7\%$). Interestingly, there were no differences in the frequency of asynchronous replication in any of the temperatures in the range tested.

DISCUSSION

The exposure of PBL to CW 830 MHz radiation of increasing average SAR levels in the range of 1.6–8.8 W/kg resulted in an increase in the losses and gains of chromosome 17 (Figs. 4a and 5a). The averaged SAR and its distribution in the exposed tissue culture flask were determined by combining measurements and numerical analysis based on finite elements. The analysis shows a 2.5 fold ratio between the maximal and the average SAR level in the exposed cells. Thus, the inhomogeneous exposure does not allow for the determination of the exact SAR threshold leading to aneuploidy, but rather confines it to an average SAR value of 2.9 ± 0.35 W/kg. This SAR range is higher than the restricted ICNIRP guideline of occupational whole body average exposure of 0.4 W/kg but lower than the localized exposure limit for the head and trunk of 10 W/kg [ICNIRP guidelines, 1998].

Exposure guideline limits set for RF radiation, assume that biological effects solely result from tissue heating [ICNIRP guidelines, 1998]. The possibility of RF induced effects via nonthermal pathways is a highly

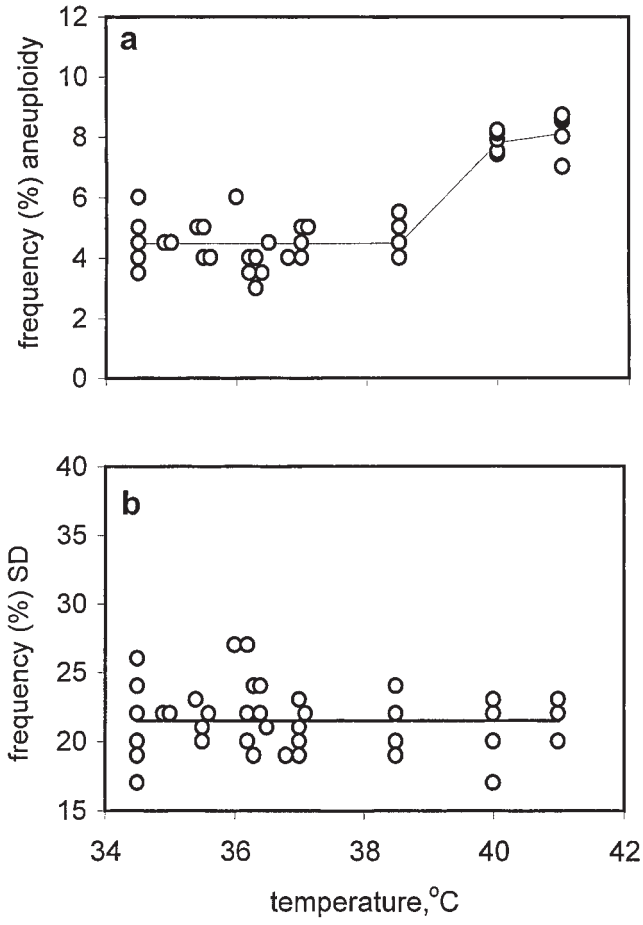

Fig. 6. The level of aneuploidy of chromosome 17 (**a**) and asynchronous replication of α-satellite DNA of chromosome 17 (%SD) (**b**) in peripheral blood cells following culturing at different temperatures in the range 34.5–41.0 °C.

controversial and debated question. In order to determine whether the observed increase of aneuploidy originates from sample heating, we studied the sole effect of temperature on the level of aneuploidy of chromosome 17. No effect of temperature elevation in the range of 34.5–38.5 °C on the level of aneuploidy was observed. However, at higher temperatures of 40–41.0 °C a significant increase (about 80%) in the aneuploidy level was observed. This finding is in agreement with a previous report [Prabhakara and Murthy, 1995] that demonstrated, based on metaphase analysis, an increase in aneuploidy from a control level of 3.6% to the level of 12.7% in human lymphocytes exposed to temperature of 43 °C.

However, since in our RF exposure experiments the temperature did not exceed, even locally, 38.0 °C, it must be concluded that the increase in aneuploidy observed by us is attributable to a nonthermal effect of the RF radiation. The nonthermal origin of our observed genetic effects is further supported by the finding that exposure of the PBL to RF radiation resulted in a SAR dependent increase in the level of the asynchronous replication of the homologous centromeres of chromosome 17, though the level of asynchrony was found to be independent of temperature elevation in the range of 34.5–41.0 °C.

The possibility that biological effects following exposure to the microwave region of the electromagnetic spectrum under very low exposure intensity, are elicited via a nonthermal mechanism has been debated in the literature [Foster, 2000]. However, a recent report shows that prolonged exposure to extremely low intensity microwave fields (SAR of 0.001 W/kg) can induce heat shock proteins (HSP) in the soil nematode *Caenorhabditis elegans* via a nonthermal mechanism [de Pomerai et al., 2000a,b].

The mechanism by which the RF radiation induces loss and gain of chromosomes, probably does not involve a direct effect of the radiation on structural alteration of DNA due to the high energetic expenditure of bond breakage. However, it may affect the function of proteins that regulate the pathway of chromosome segregation on the one hand and DNA replication on the other. That heat shock proteins (HSP) are induced via a nonthermal pathway following exposure to both ELF [Lin et al., 1997; Lin et al., 1998; Tsurita et al., 1999] and microwave [de Pomerai et al., 2000a,b] is in accordance with the notion that HSP are nonspecific stress proteins which are induced by many kinds of environmental changes and act as molecular chaperones which are involved in the defense mechanism against proteitoxic stresses such as heat and chemicals [Ohtsuka and Hata, 2000]. Indeed there are reports suggesting that nonthermal exposure to microwaves affects protein structural rearrangements [Porcelli et al., 1997; La Cara et al., 1999; Bohr and Bohr, 2000a,b], which in a cell can be repaired by HSP.

The possible significance of our study emerges when comparing the RF induced changes in the frequency of aneuploidy in our in vitro exposed PBL with those observed in PBL of patients suffering from hematological malignancies. The reported level of aneuploidy of chromosome 17 of patients suffering from hematological malignancies [Korenstein-Ilan, 2000] is twice the value obtained at the highest SAR level of our exposure ($16.0 \pm 5.0\%$ and $8.7 \pm 1.6\%$, respectively). It should be stated that the aneuploidy observed in cancer arises from a continuous defect in the segregating apparatus and thus is not chromosome specific. Therefore, it might be speculated that the RF induced alteration of aneuploidy is likewise nonchromosome specific and not restricted to chromosome 17.

If one accepts the notion that aneuploidy is an autocatalytic process leading to the transition from a pre-neoplastic phenotype into a neoplastic one [Duesberg and Rasnick, 2000], then the elevation of aneuploidy to a level that is on one hand twice than the control and on the other hand half the value typical of hematological malignancies, may reflect a situation which constitutes a risk for cancer. This suggestion is further supported by a recent epidemiological study [Lalic et al., 2001], where the genotoxic effects of occupational exposure to ionizing and nonionizing radiation were investigated in 25 physicians and nurses working in hospitals and in 20 individuals working at radio relay stations. Chromosomal aberration analysis of peripheral blood lymphocytes showed that the number of chromosome aberrations in people exposed to ionizing and radio frequency radiation were almost equally increased compared to those of unexposed subjects.

The RF induction of improper chromosome segregation was accompanied by increase in the abnormal (asynchronous) replication of the homologous centromeric arrays. Though the level of asynchronous replication timing of CENT17 following exposure to the highest SAR was 1.5 fold higher than in controls, it had a close value to the one observed in patients suffering from hematological malignancies [Korenstein-Ilan, 2000] ($33.2 \pm 1.9\%$ and $37.5 \pm 4.4\%$, respectively). This again suggests that the RF induced elevation in the asynchronous replication pattern of the centromeric DNA arrays can be considered to be associated with an epigenotype of cancer.

CONCLUSIONS

Our results indicate that human cells exposed to RF radiation acquire premalignant genotypes associated with elevated levels of aneuploidy and abnormalities in replication mode as expressed in asynchrony in the replication timing of homologous chromosomal regions associated with chromosome segregation. These findings support the view that exposure to RF radiation of average SAR values of 2.6–8.8 W/kg may lead, through a nonthermal pathway, to a carcinogenic activity. Our study does not elucidate the specific primary mechanism by which radiation interacts with the cell and alters its genetic material. However, it does demonstrate that exposure to RF radiation results in a gross somatic mutation leading to major modulation in gene expression which may be amplified by epigenetic mechanism of gene expression reflected by the asynchrony in the replication timing of the homologous DNA loci. Our work shows that aneuploidy was generated by the exposure to the electromagnetic radiation. As, according to the aneuploidy theory, random chromosome number mutations evolving autocatalytically from the status of aneuploidy open the road to cancer, our results suggest the exposure to RF radiation at a SAR value of 2.9 W/Kg and above pose a risk for cancer.

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NONSTOP PULSED 2.4 GHZ RADIATION INSIDE US HOMES

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Abstract
The use of DECT (Digital Enhanced Cordless Telecommunication) cordless phones has been a major health and environmental concern in Europe and especially in Germany for years. The biological concern arose from studies on HF (high frequency) sources such as GSM cellular phones and towers. Digital cordless phones are also available in the USA – marketed as 2.4 GHz digital technology. A digital cordless phone was placed in a representative private home in California and HF measurements were conducted at different locations inside, using frequency selective spectrum analysis to obtain the cordless phone power densities. The results showed that the radiation patterns and levels emitted by the small cordless phone base station are almost identical to the DECT technology – also digitally pulsed and permanent microwave radiation. The power density values presented for each room inside the home can be compared to average DECT cordless phone radiation exposures found in German homes. The maximum power density was found to be over 500,000 µW/m^2 at a normally encountered distance (about 1 - 2 feet) if the base station is placed on an office desk or bedside table. The radiation peak values in the same room are higher than those encountered in proximity to cellular base stations located near residential buildings.

Introduction

DECT cordless phones usage has been a major health and environmental concern in Europe and especially in Germany for years. Now multiple handsets cordless phones are also available in the USA – extolled as 2.4 GHz digital technology with multiple handsets following the DFHSS (Digital Frequency Hopping Spread Spectrum) standard which is almost identical to DECT. The biological concern arose from studies on high frequency (HF) sources such as cellular phones and cellular phone base stations with GSM technology. The digital pulsed pattern of GSM and DECT radiation has come under suspicion to cause e.g. brain cancer, lymphoma, and changes in the brain blood barrier. The problem with the cordless DECT phones is, that the base station permanently emits full power pulsed microwave radiation, whether the phone is used or not. This creates constant exposure to high levels of the most critical type of HF radiation known throughout the entire home or office. The DECT technology is a European standard for cordless phones in the range of microwave radiation (1.8 to 1.9 Gigahertz, GHz). Identical permanently emitting portable phones with the special option of multiple handsets are available in the US and therefore the exposure issues are relevant for US population. In the US, the cordless phone manufacturers established the 2.4 GHz digital pulsed technology in the range of 2.4 – 2.5 GHz. Cordless phones such as e.g. the GIGASET (same name as a DECT cordless phone series by the same manufacturer in Germany) are available in USA.

Methods

A GIGASET cordless phone model was selected as a representative DFHSS 2.4 GHz phone for typical home and office usage. The base station was purchased in the US and placed on a wooden office desk in a representative 3 bedroom residential building in California. In the first test set, the power density in the room was measured prior to the activation of the base station to obtain background levels at the test site. Power density measurements were performed at different distances and directions from the phone (see table 1 and 2) with an Advantest R4131C spectrum analyzer (Rohde & Schwarz) and a calibrated periodic logarithmic log.per. antenna UKLP 9140-A (Schwarzbeck). The power density measurements were conducted under real-life conditions and only peak values (pulse maximum) were measured. All measurements were conducted following VDB guideline (VDB 2002) and the Swiss BUWAL guideline (BUWAL 2002). The power density levels are given in µW/m^2 (microwatt per square meter). 1 µW/m^2 equals 0.1 nW/cm^2 (nanowatt per square centimeter). The background level was <0.3 µW/m^2 (-58 dBm) in the range of all wireless, analogue or digital cordless, and cellular phone applications (0.3 to 3.5 GHz). See figure 1.

Results

High frequency measurements were conducted and showed that the radiation patterns and levels emitted by GIGASET 2.4 GHz cordless phone base station are identical to the DECT technology – also digitally pulsed with permanent microwave radiation. For comparison, the radiation levels from the GIGASET located in the same room are even higher than encountered in proximity (50 to 100 feet) to most cellular base station located on pole mount positions or on top of office buildings. However, in this case the source of the radiation is a desktop personal cordless phone.

Figure 1: Spectrum analysis (no 2.4 GHz)

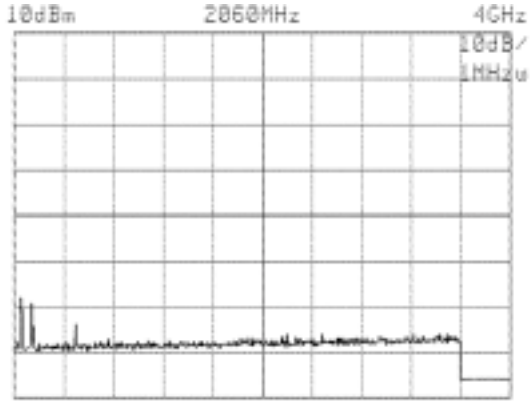
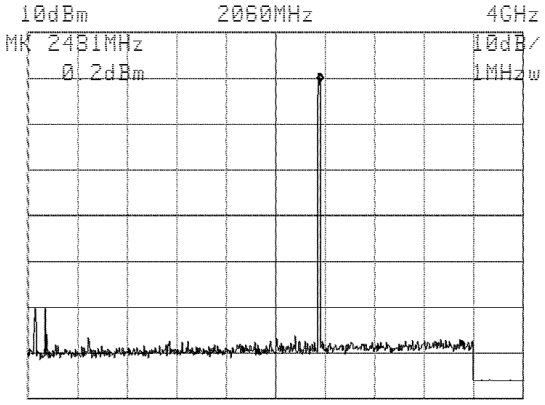


Figure 2: Spectrum analysis (with 2.4 GHz)



After the phone was plugged in, the radiation level rose to 673,000 µW/m^2 (+0.2 dBm) in a normally encountered distance (about 1 - 2 feet) if the base station is placed on an office desk. See figure 2. The following power density levels were obtained :

Table 1: 2.4 GHz cordless phone base station power density levels in the same room

	US GIGASET (2.4 GHz)*	**GERMANY GIGASET (DECT)****
Distance	DFHSS (Digital Frequency Hopping Spread Spectrum) digital pulsed 100 Hz frequency range 2450 MHz	DECT (Digital Enhanced Cordless Telecommunication) digital pulsed 100 Hz frequency range 1880 MHz
30 centimeter - 12.5“	673,000 µW/m^2	405,000 µW/m^2
50 centimeter – 19.8”	280,000 µW/m^2	146,000 µW/m^2
1 meter - 39.4”	72,000 µW/m^2	36,000 µW/m^2
2 meter - 78.8“	23,000 µW/m^2	9,100 µW/m^2

*this study, **OEKO-TEST 1996

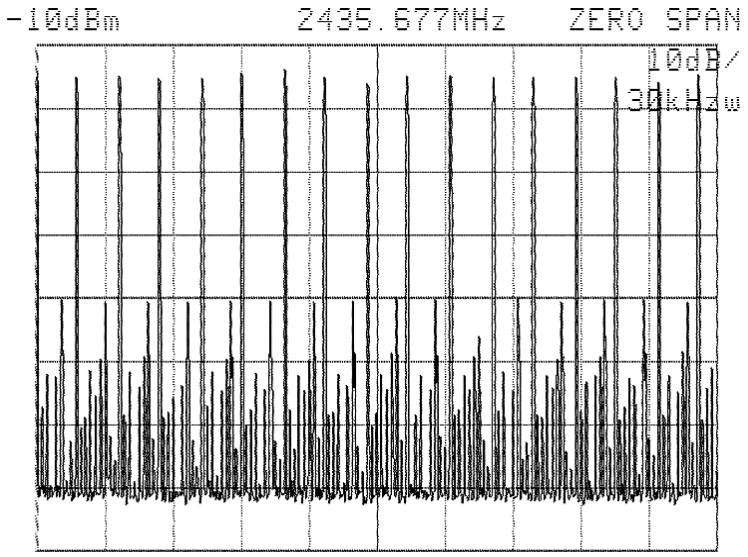
The results of the US GIGASET showed similar power densities when compared with the power densities reported for the GIGASET sold in Germany with DECT technology. Physical barriers such as e.g. wood framed walls, cabinets, closets have only a limited shielding effect inside a building. To evaluate a real life radiation exposure, the base station was placed on a desk in a bedroom (home office) and the actual power densities were measured in the different rooms. In this experimental test set, the real life effect of such a cordless phone installed in an average home and its associated radiation exposures were evaluated. The following values were obtained during our test set. The measurements showed a significant exposure for the occupants (see also table 2 and floor plan in appendix, figure 4)

Table 2: 2.4 GHz cordless phone base station power density levels in the house

Room	**Power Density (maximum pulse peak value)**
Office with phone	33,800 µW/m^2
Master bedroom	13,500 µW/m^2
Bedroom 2	5,400 µW/m^2
Bedroom 3	680 µW/m^2
Living room	140 µW/m^2
Family room	50 µW/m^2
Outside	9 µW/m^2

Besides the permanent emission from such a base station, the pulsed nature of the signal was analyzed and is displayed in figure 3. The spectrogram shows the periodic pulsed signal. The dynamic range of the power density covers the full range scale from minimum (pause) to maximum (pulse) and is sending out pulses every 10 milliseconds (ms) or 100 Hz (Hertz).

Figure 3: Pulsed signal of 2.4 GHz DFHSS technology – ZERO SPAN (GIGASET cordless phone base station)



Summary

The levels encountered are considerably high for an indoor source, which emits permanently. The radiation peak values in the same room are higher than those encountered in proximity to cellular base stations located at pole mount or roof top positions. Even in the master bedroom and in the second bedroom, the power density levels were in the range of or above the 95. percentile radiation level just recently obtained from a study of cellular phone tower measurements in residential areas (HAUMANN 2002). For comparison, thermal (guidelines), other non-thermal (recommendations), and cellular tower exposure reference values are listed in the table 3 below.

Table 3: Comparison of Standard Threshold Values and Recommendations

Comparison of Standard Threshold Values and Recommendations (electromagnetic fields, non ionizing radiation)	**Total Power Density**
Standards, > 2,000 MHz (e.g.)	
FCC/ANSI - USA	10,000,000 µW/m^2
Germany, England, Finland and Japan	10,000,000 µW/m^2
Belgium	1,200,000 µW/m^2
Switzerland and Italy	90,000 µW/m^2
Recommendations / References (e.g.)	
Ecolog Study, Germany (ECOLOG 2000)	10,000 µW/m^2
Cellular tower radiation - high exposure level, 95. percentile (HAUMANN 2002)	6,300 µW/m^2
Salzburg, Austria (RESOLUTION 2000)	1,000 µW/m^2
EU Parliament (STOA 2001)	100 µW/m^2
Cellular tower radiation – background level, 20. percentile (HAUMANN 2002)	15 µW/m^2
Low exposure, Oeko-Test (OEKO TEST 2001)	10 µW/m^2
Nighttime exposure, Baubiology Standard (SBM 2000)	0.1 µW/m^2
Natural cosmic microwave radiation (MAES 2000)	< 0.000001 µW/m^2

Many European researcher, physicians, environmental professionals and toxicologist signed a resolution requesting the immediate stop of the DECT technology. This petition was delivered to the Germany Environmental Minister Mr. Jürgen Trittin in October of 1999 (RESOLUTION 1999). The Germany magazine "Oeko-Test" (equivalent to the US magazine Consumer Test) had 16 DECT cordless phones tested, published the measurement results, and rated all phones as not recommendable due to the constant emission of high levels of pulsed radiation (OEKO-TEST 1999).

Conclusions
As long as the only base for official standards on high frequency radiation are thermal effects and heating of the body tissue (**FCC, ICNIRP, ANSI, IEEE, NCRP**) there is no need for the industry to invest into saver products. More and more scientists state that the view of energy absorption only is insufficient to describe the possible effects on human health. Potential biological effects need to be considered due to

1.) Non-thermal or low intensity levels of HF radiation,
2.) Chronic versus acute exposure and,
3.) Pulsed HF radiation, which is reported to be more bioactive than constant wave HF radiation.

The human body reacts much more complex than acknowledged in the thermal model and is very sensitive to extreme periodic stimuli. The biological system takes the "energy" as well as the "information" which is brought e.g. by the continuous pulsed modulation pattern.

Much experimental evidence of non-thermal influences of microwave radiation on living systems has been published in the scientific literature during the last 30 years – relating both to *in vitro* and *in vivo* studies – and were reviewed just recently by the STOA commission of the European Parliament (STOA 2001). From the use of microwave wireless technologies e.g. the following non-thermal biological effects have been reported:

- Changes in the electrical activity in the human brain,
- Increase in DNA single and double strand breaks from HF exposure to 2.45 GHz,
- Increased lymphoma rates (2 fold) in transgenic mice exposed twice a day exposed to 30 minutes of cell phone (GSM) signals over 18 month,
- Increased permeability of the blood-brain barrier in rats,
- Observation of an increase in resting blood pressure during exposure,
- Increased permeability of the erythrocyte membrane,
- Effects on brain electrochemistry (calcium efflux),
- Increase of chromosome aberrations and micronuclei in human blood lymphocytes,
- Synergistic effects with cancer promoting and certain psychoactive drugs,
- Depression of chicken immune systems,
- Increase in chicken embryo mortality,
- Effects on brain dopamine/opiate electrochemistry,
- Increases in *DNA* single and double strand breaks in rat brain,
- Stressful effects in healthy and tumor bearing mice,
- Neurogenetic effects and micronuclei formation in peritoneal macrophage.

In this review study, a threshold of 1000 $\mu W/m^2$ was evaluated for non-thermal biological effects. For locations with any long-term exposure, a further safety factor of 10 was recommended for pulsed cellular phone radiation sources as cellular phone base stations. In this case, the power densities should not exceed 100 $\mu W/m^2$.

The constant High-Tec HF radiation brought into the US homes and offices by 2.4 GHz digital technology cordless phones is definitely a big step in the wrong direction in terms of environmental health protection and radiation exposure prevention. This reveals a complete misunderstanding of progress for our new millennium.

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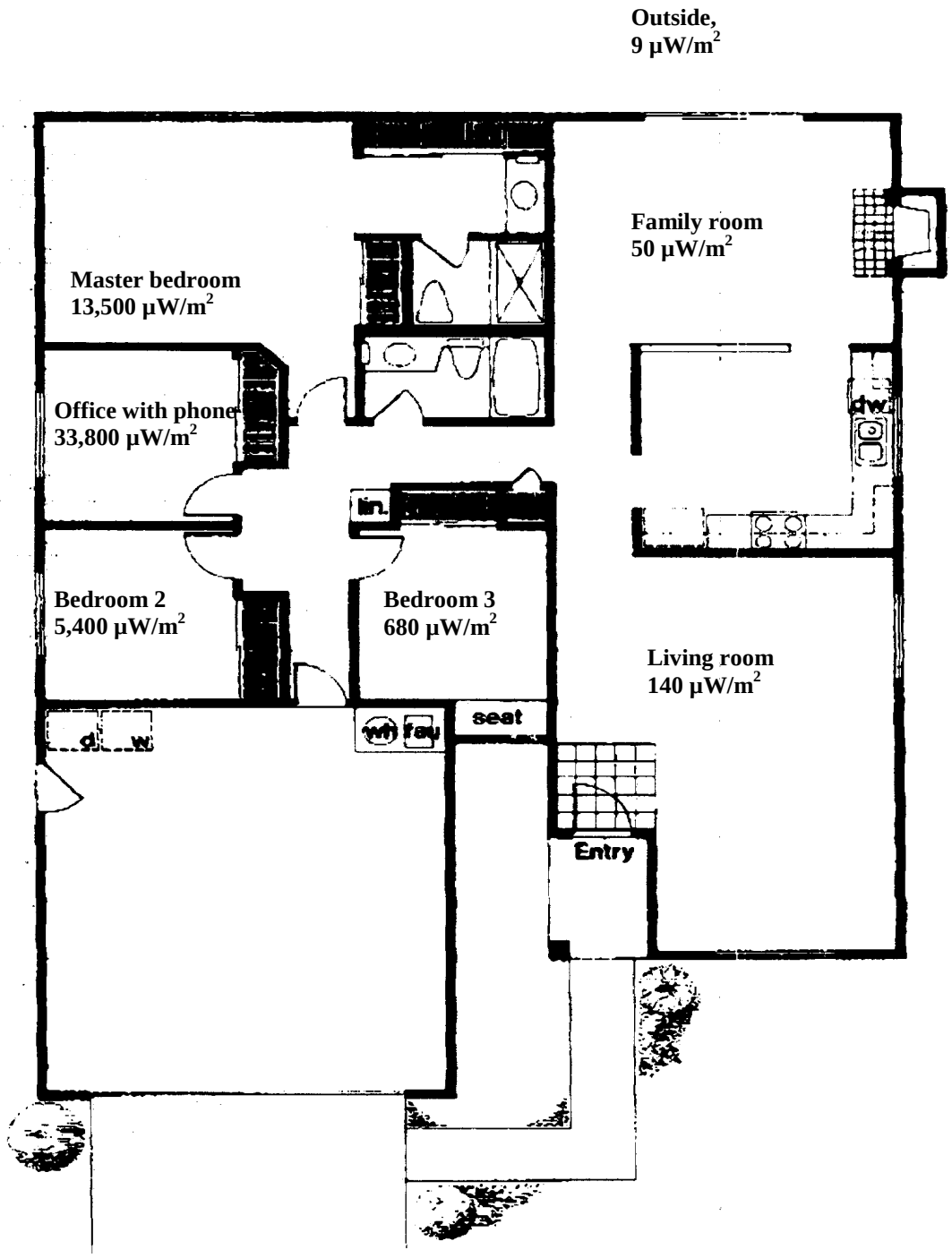
Appendix

Figure 4: Floor plan with exposure data



RADIATION RESEARCH **147**, 631–640 (1997)
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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: $\varepsilon_\tau' = 34.3$, $\varepsilon_\tau'' = 21.3$ (from ref. *19*).
Fat at 900 MHz and 37°C: $\varepsilon_\tau' = 9.94$,
$\varepsilon_\tau'' = 3.46$ (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.

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

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.

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			Renal disease[a]		Other disease[b]	Undiagnosable[c]
		Lymphoblastic	Nonlymphoblastic	Total	Alone	Total		
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.

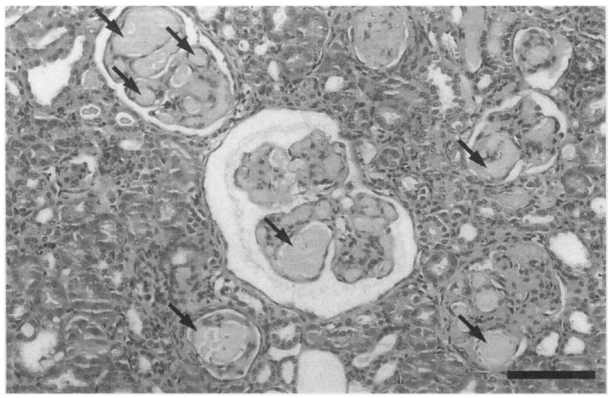

FIG. 1. Histological appearance of the distinctive renal disease in Eμ-*Pim1* mice. The photomicrograph of a hematoxylin and eosin-stained section of a kidney from such a mouse killed when terminally ill shows dilated glomerular capillaries (some examples marked with arrows) filled with amorphous eosinophilic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

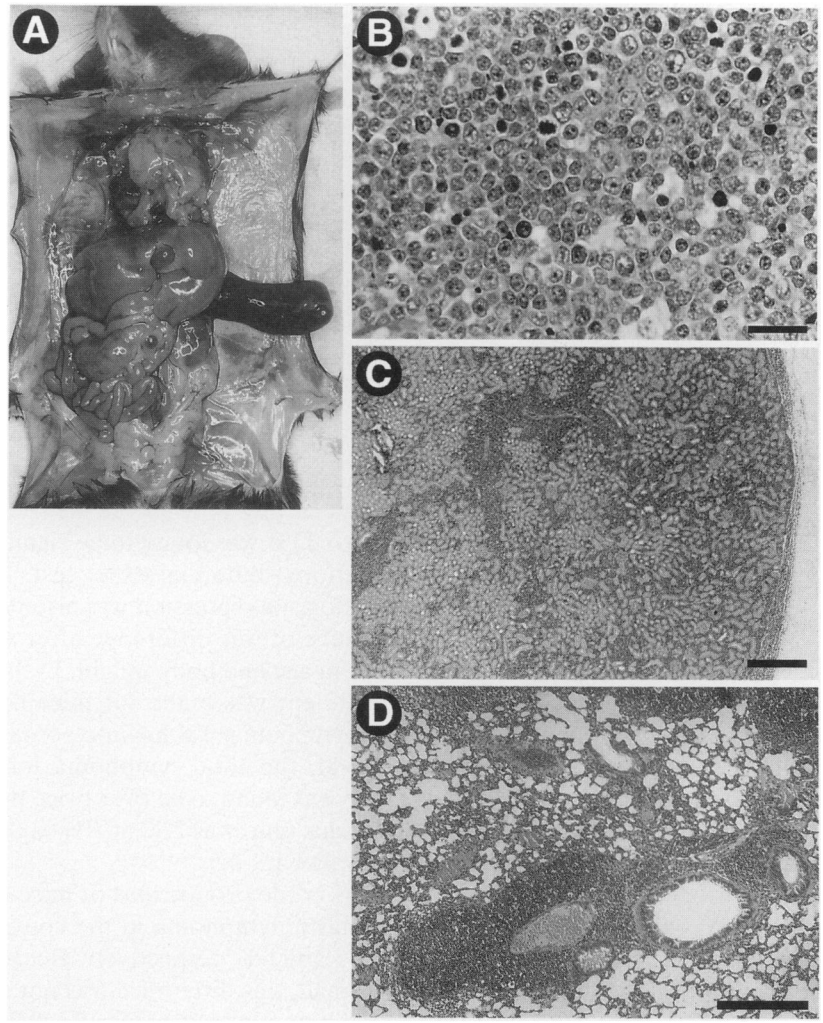

FIG. 2. A typical case of advanced lymphoblastic lymphoma of thymic origin in an Eμ-*Pim1* mouse. The panels show (A) a postmortem dissection exposing greatly enlarged thymus, spleen and lymph nodes, and hematoxylin and eosin-stained sections revealing (B) a mass of lymphoblasts, with frequent mitotic 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-

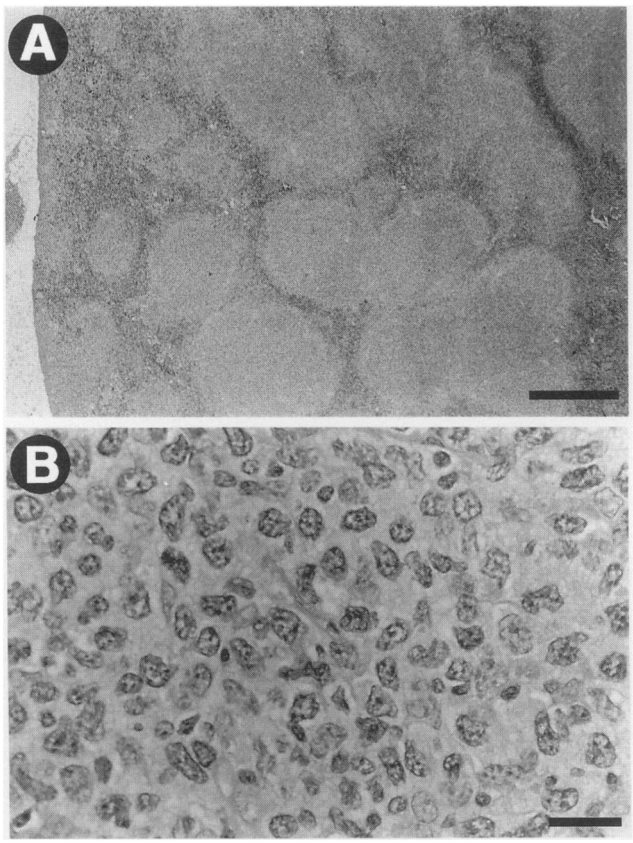

FIG. 3. A representative case of follicular lymphoma in an Eμ-*Pim1* mouse. Photomicrographs of hematoxylin and eosin-stained tissue sections show (A) a low-power view of the spleen in which numerous large tumor nodules have replaced the normal small islands of white pulp (scale bar, 500 μm), and (B)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

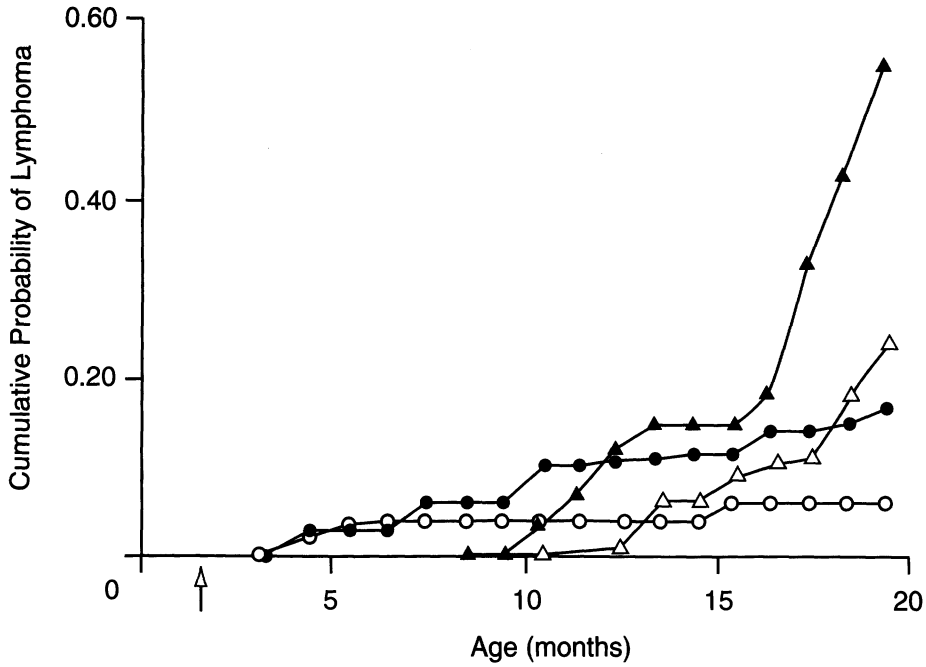

FIG. 4. Cumulative probability of development of lymphoma with age in Eμ-*Pim1* mice. (●, ○) Lymphoblastic lymphoma and (▲, △) non-lymphoblastic lymphoma in RF-field-exposed (●, ▲) and sham-exposed (○, △) animals. The cumulative probability values were calculated by adjusting the crude incidence of lymphoma 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.

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Long-Term, Low-Level Microwave Irradiation of Rats

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Our goal was to investigate effects of long-term exposure to pulsed microwave radiation. The major emphasis was to expose a large sample of experimental animals throughout their lifetimes and to monitor them for effects on general health and longevity.

An exposure facility was developed that enabled 200 rats to be maintained under specific-pathogen-free (SPF) conditions while housed individually in circularly-polarized waveguides. The exposure facility consisted of two rooms, each containing 50 active waveguides and 50 waveguides for sham (control) exposures. The experimental rats were exposed to 2,450-MHz pulsed microwaves at 800 pps with a 10-μs pulse width. The pulsed microwaves were square-wave modulated at 8-Hz. Whole body calorimetry, thermographic analysis, and power-meter analysis indicated that microwaves delivered at 0.144 W to each exposure waveguide resulted in an average specific absorption rate (SAR) that ranged from 0.4 W/kg for a 200-g rat to 0.15 W/kg for an 800-g rat.

Two hundred male, Sprague-Dawley rats were assigned in equal numbers to radiation-exposure and sham-exposure conditions. Exposure began at 8 weeks of age and continued daily, 21.5 h/day, for 25 months. Animals were bled at regular intervals and blood samples were analyzed for serum chemistries, hematological values, protein electrophoretic patterns, thyroxine, and plasma corticosterone levels. In addition to daily measures of body mass, food and water consumption by all animals, O_2 consumption and CO_2 production were periodically measured in a sub-sample (N=18) of each group. Activity was assessed in an open-field apparatus at regular intervals throughout the study. After 13 months, 10 rats from each group were euthanized to test for immunological competence and to permit whole-body analysis, as well as gross and histopathological examinations. At the end of 25 months, the survivors (11 sham-exposed and 12 radiation-exposed rats) were euthanized for similar analyses. The other 157 animals were examined histopathologically when they died spontaneously or were terminated *in extremis*.

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Statistical analyses by parametric and non-parametric tests of 155 parameters were negative overall for effects on general health, longevity, cause of death, or lesions associated with aging and benign neoplasia. Positive findings of effects on corticosterone level and immune system at 13 months exposure were not confirmed in a follow-up study of 20 exposed and 20 control rats. Differences in O_2 consumption and CO_2 production were found in young rats. A statistically significant increase of primary malignancies in exposed rats vs. incidence in controls is a provocative finding, but the biological significance of this effect in the absence of truncated longevity is conjectural. The positive findings need independent experimental evaluation. Overall, the results indicate that there were no definitive biological effects in rats chronically exposed to RF radiation at 2,450 MHz.

Key words: SAR, longevity, health, tumor incidence

INTRODUCTION

Advances in dosimetry, and a better understanding of energy absorption by biological tissues, have eliminated many concerns regarding effects of radio-frequency electromagnetic radiation [Tyler, 1975; Elder and Cahill, 1984; NCRP, 1986; Polk and Postow, 1986; Lin, 1989; Gandhi, 1990]. Despite this lessening of concern for low-level, acute exposures, lack of data on long-term, low-level radiation has fueled public and scientific concerns. In this context, officials of the United States Air Force sought to support research in this area to provide data for use in the development of environmental impact studies for present and planned Air Force systems.

The goal of the project was to investigate effects on health of long-term exposure to low-level, pulsed microwave radiation. The approach was to expose a large population of experimental animals to microwave radiation throughout most of their lifetimes and to monitor them for effects on general health and longevity.

Although the initial impetus for the study was the question of environmental impact of the Air Force PAVE PAWS system, early on it was decided not to study a replica of the PAVE PAWS emissions, but to create a generalized level of radiation that would provide whole-body exposure based on the maximum of permissible absorption [ANSI C95.1-1982, 1983; IEEE C95.1–1991,1992] at the resonant frequency in human beings (0.4 W/kg), as scaled to the proportions of the experimental animal of choice.

Following a period of pilot studies and training of technicians, exposures to microwaves commenced on September 1, 1980, and concluded September 27, 1982. The 100 experimental and 100 sham-exposed animals underwent the longest near-continuous exposure ever completed. The findings were reported in a series of 9 Air Force technical reports, which are available through the National Technical Information Service (Springfield, Virginia). Interested readers should refer to the technical reports for details [Guy et al., 1983a, b, 1985; Chou et al, 1983; Johnson et al., 1983, 1984; Kunz et al., 1983, 1984, 1985].

METHODOLOGY

Experimental Design

Exposure criteria. Much of the past work on chronic exposure of large numbers of text animals has been based on anechoic chambers, metal capacitor plates, or

resonant cavities. With these methods, the energy coupled to each animal is a function of the group size, group orientation, and the orientation of each animal within the group, as well as of the presence and location of water and food dispensers. Because estimates of energy absorption are uncertain, quantitative extrapolation of biological results from laboratory animals to human beings is virtually impossible. In addition, the cost in time and resources of even simple experiments involving chronic exposures of animal populations in large anechoic chambers is prohibitive.

For this study, we chose a system of cylindrical, wire-mesh waveguides to expose a large number of animals to a common source while independently maintaining relatively constant and quantifiable coupling of electromagnetic energy to each animal regardless of position, posture, or movement [Guy and Chou, 1977; Guy et al., 1979]. The system, which consists of a number of independent waveguides, allows individual animals to be continuously exposed under normal laboratory conditions while living unrestrained and with continuous access to food and water.

A frequency of 2450 MHz was selected so each rat would have approximately the same size-to-wavelength ratio as a human being exposed at 450 MHz, the frequency near which PAVE PAWS operates. The initial consideration was to produce the same average SAR in test animals as predicted for man exposed to a 1-mW/cm^2, 450-MHz RF field. To simulate radar exposure, pulse modulation was used (10 μs pulse, 800 pps). In addition to the pulse modulation, we decided to square-wave modulate the microwaves. The inclusion of square-wave modulation was prompted by the evidence of altered movement of Ca^{++} ions in chicken and cat brains exposed to ELF-modulated RF fields [Adey, 1981]. Because the demonstrated effects are most pronounced when the modulation frequencies correspond to the dominant EEG frequency, we selected a modulation frequency of 8 Hz because it is at the peak of the rat's hippocampal theta rhythm [Coenen, 1975].

Rationale of biological assessment. Not only were reported biological effects from low-level microwaves selected as end points (e.g., alterations of hematopoietic, immunologic, and specific blood chemistry indices), but assays for effects on general health, metabolism, and life span were also included (references listed in later sections). In addition, end points were considered that could be assessed without seriously compromising the health of the animal, the value of concurrent measurements, or the power of the statistical evaluations on the chosen end points. Only male rats were used to minimize statistical variation, i.e., to avoid the hormonal variations characteristic of female rats. Use of female rats would have required a substantial increase in the number of animals. A total of 155 parameters was studied. The end points selected are shown in Table 1. Due to space limitations, details of rationale and methods of biological assessments cannot be provided here. The original NTIS reports should be consulted.

Statistical considerations. For any failure-time end point, such as time to death, time to cancer diagnosis, or time to some specified change in animal mass or blood chemistry, an initial sample size of 100 in each group was calculated to be sufficient for detection, at the .05 significance level, of a 50% increase (or 33% reduction) in instantaneous failure rate with a probability (power) of 90%. For any normally distributed end point (including transformations on failure-time variables), a sample size of 100 per group permits the detection, at the .05 level of significance, of a difference between groups of 40% of one standard deviation, with a power of 90%. Adjustment for a differential effect due to the altered experimental procedure for

TABLE 1. Endpoints Selected to Study the Effects of Long-Term, Low-Level RF Exposure on Rats

Category	Parameters	No.
Behavior	Open field behavior (activity, quadrant change, urination, defecation)	4
Corticosterone	Serum corticosterone	1
Immunology	Mitogen stimulation (PHA,LPS,ConA,PWM,PPD), B-cell, T-cell, %CRPC, total CRPC, plaque	10
Hematology	WBC, RBC, HCT, Hgb, MCV, MCH, MCHC, neutrophils, lymphocytes, eosinophil, monocytes	11
Blood chemistry	Glucose, BUN, creatinine, Na, K, Cl, CO_2, uric acid, total bilirubin, direct bilirubin, Ca, phosphorus, alkaline phosphatase, LDH, SGOT, SGPT, cholesterol, triglycerides, total protein, albumin, globulin	21
Protein electrophoresis	Albumin fraction, alpha-1 and 2 fractions, beta fraction, gamma fraction	4
Thyroxine	Thyroxine	1
Urinalysis	Urine observation	1
Metabolism	Body mass, food consumption, water consumption, O_2 consumption, CO_2 production, respiratory quotient, metabolism quotient	7
Total body analysis	Body mass, moisture, protein nitrogen, crude fat, nonprotein nitrogen, total ash, mineral contents (aluminum, antimony, arsenic, barium, beryllium, bismuth, boron, cadmium, calcium, chromium, cobalt, copper, iron, lead, magnesium, manganese, molybdenum, nickel, phosphate, potassium, selenium, silver, sodium, strontium, tin, titanium, vanadium), fatty acids (palmitic, palmitoleic, stearic, oleic, linoleic, linolenic)	39
Organ mass	Heart, brain, liver, kidneys, testicles, adrenals	9
Histopathology	All tissues and organs	46
Longevity	Survival days	1
Total		155

the subset of 36 rats subjected to metabolic rate measurements had very little effect on the power calculations made, nor did adjustment for an interim euthanasia of 20 animals.

Differences between the two groups on single measurements were assessed by Student's *t* tests, in some cases after transformation to improve the normality of the data. Reported *P* values must be considered in the light of the multiple end points analyzed. Logical groupings of variables were compared across groups of the multivariate Hotelling's T^2 statistic.

Differences in tumor prevalence or incidence were assessed with time-adjusted analyses. The occurrence of neoplastic and non-neoplastic lesions was recorded along with the age of the animal and whether the animal had died spontaneously or was euthanatized. Survival curves of the exposed and sham-exposed animals were estimated by product-limit estimates [Kaplan and Meier, 1958] and compared by the log-rank statistic [Mantel, 1966]. The histopathological data were grouped with respect to age, at 6-month intervals, and the data were divided into neoplastic and non-neoplastic diagnoses. The incidence of neoplastic or non-neoplastic lesions was given as the proportion of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals examined pathologically (denominator). For tissues that required gross observation for detection of lesions (i.e., skin or subcutaneous tumors), for lesions that appeared at several sites (i.e., multiple

lymphomas), or for tissues that were examined histologically only when lesions were detected grossly, the denominator consisted of the number of animals necropsied in that experimental group.

The analysis of the lesions involved a 4-way table with factors of age at death, treatment condition, mode of death (terminated or spontaneous), and organ. The tables were then collapsed with respect to individual organs. From these tables, the Mantel-Haenszel estimate of the odds ratio was computed, and the chi-square statistic was used to test whether the odds ratio was significantly different from unity [Mantel and Haenszel, 1959]. This statistic reflects the difference in prevalence of lesions, over time, between the exposed and sham-exposed animals, and is appropriate if the lesions are "incidental" (do not affect the animal's survival). The time to a malignant lesion was also analyzed with survival-analysis techniques, as would be appropriate if lesions were fatal. If an animal had malignant lesions, its time-to-tumor was taken as its survival time. If there were no malignant lesions present, the time-to-tumor was considered censored (i.e., the time to appearance of a tumor is assumed to be longer than the time to death). The log-rank statistic was used to compare the times to tumor of the exposed animals with those of the sham-exposed animals [McKnight and Crowley, 1984].

Final protocol. During the first year of the study, the rats were bled from the orbital artery every 6 weeks, with the first bleeding during the 7th week of exposure. In addition to the hematological and serum-chemistry evaluation of blood collected during the first bleeding, corticosterone levels were determined in all samples having adequate amounts of serum. In subsequent bleedings, corticosterone and thyroxine levels were determined only quarterly, whereas the hematology and serum chemistry were evaluated for each sample (every 6 weeks). This frequency of bleeding was considered sufficient to detect the onset of most degenerative or disease states that would occur during the lives of the individual rats without unduly stressing the animals. Every 3 months a urinalysis was done on all rats, the first during the fourth week of exposure. This frequency of biochemical evaluations increased the opportunity to detect subclinical abnormalities and to follow their pathophysiological course. Open-field assessment was conducted every 6 weeks.

During the second year of the study, the frequency of bleeding was reduced to 12-week intervals, and the corticosterone analysis was eliminated except just prior to euthanasia of remaining animals at the end of the 2 years; urinalysis was done every 2 weeks, and open-field analysis was conducted quarterly.

Facilities

Animal facility. To maintain the colony of rats used in this study in the healthiest possible state, free of chronic disease and other problems common to rats, two specific-pathogen-free (SPF) rooms in the Division of Animal Medicine were acquired (Fig. 1). Access to the clean hall is via a shower room, through which all personnel must pass to shower and don autoclaved garments. A walk-in autoclave connected the cage-washing facility with the clean hallway so that, once washed, all materials entering the clean hall must have passed through autoclaving before being returned to the animal rooms. All soiled cages and waste collectors left the clean rooms via the dirty hallway and were then taken to the cage-washing facility.

Each alcove housed 20 waveguides mounted on four horizontal shelves, five waveguides per shelf. The exposure and sham-exposure waveguides were randomly

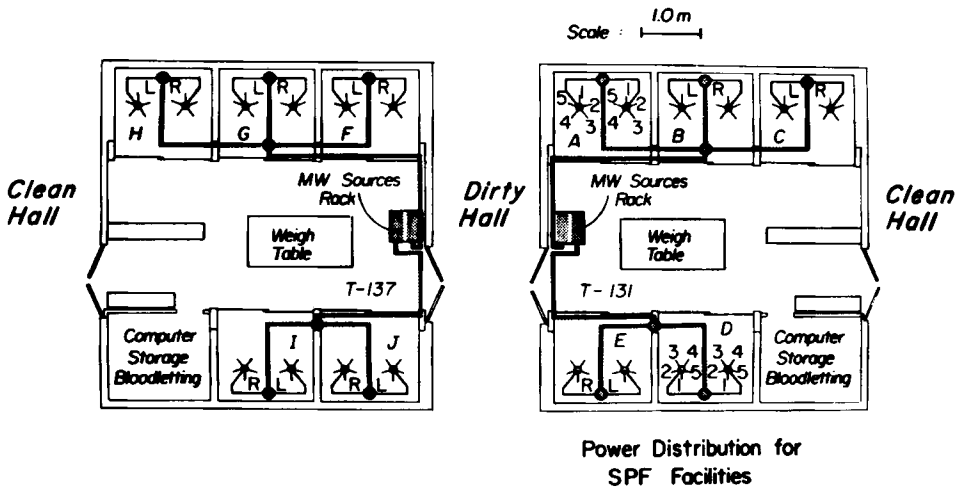

Fig 1. Top view of exposure rooms (T-131 and T-137) with alcove designations and associated exposure-cell identification system.

arranged, except that only sham-exposure waveguides could be in the center position because of a sliding-glass-door operation. One of the alcoves in each room was equipped as a metabolism alcove, in which O_2 consumption and CO_2 production were measured. The sixth alcove in each room was partitioned off as a procedures area that was used for bleeding and as housing for the main data-collection computer and miscellaneous supplies.

In the SPF rooms the airflow rate was programmed for 22 exchanges each hour, to maintain positive-pressure flow. Over the course of the project, ambient temperatures were balanced between the workspaces and alcoves to maintain a fairly constant 21±1°C environment in the facility. Humidity was in the range of 30–70%. Sound-pressure measurements indicated an average level in the central workspace of approximately 60 dBA (relative to 20 $\mu N/cm^2$) and alcove levels that were 6 to 10 dBA lower, depending on position within the alcove. Light-intensity measurements during the light cycle (0700–1900) indicated a 13-lux average workspace level and 6-lux average alcove level.

Microwave exposure system. As shown in Figure 2, when an animal housed in a plastic cage was exposed in the circular waveguide to microwaves fed into the terminal (P_{IN}), some energy (P_A) was absorbed by the animal, some (P_W) was absorbed by the walls of the chamber, some was reflected in the form of both right-hand (P_{RR}) and left-hand (P_{RL}) circularly polarized waves that couple back to the probes on the feed section of the waveguide, and some (P_{TA} and P_{TB}) were absorbed at the termination terminals. The reflected component P_{RR} was measured as CP_{RR} at the reflecting arm of the bidirectional coupler, which was placed between the source and the input probe (C is the coupling coefficient of the bidirectional coupler). The reflected component P_{RL} was measured directly at the other terminal of the transmitting transducer. The power level of the incident energy launching the right-hand circularly polarized waves was measured (as CP_{IN}) at the incident-wave terminal of the coupler. The power level of energy transmitted beyond the animal was measured at the terminals (P_{TA} and P_{TB}) of the termination transducer. The sum

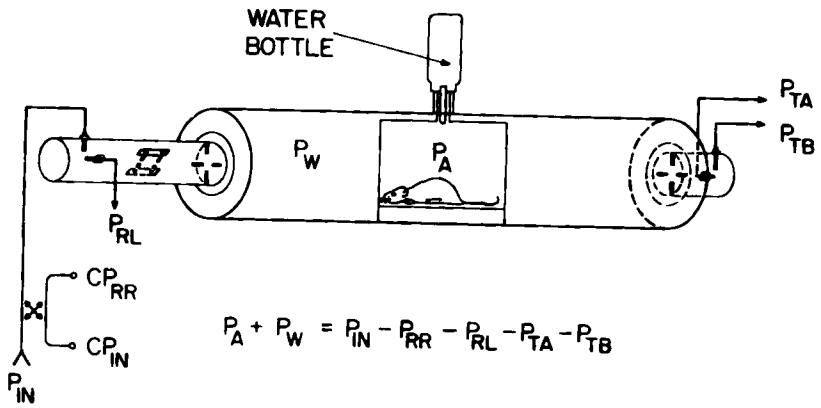

Fig. 2. The exposure chamber was a circularly polarized waveguide operating at 2,450 MHz. A rat, housed inside a plastic cage, was exposed in the 20.3-cm diameter wire-mesh tube. A circular polarizer, at the left end, converted the linearly-polarized TE_{11} mode to the circularly-polarized TE_{11} mode. Tuning stubs inside the polarizer matched the impedance of the propagating modes. Transmitted microwaves were terminated in the transducer at the right side of the tube.

of power levels of energy absorbed by the animal and the chamber walls can be obtained from the equation in Figure 2.

The water bottle in each waveguide was electrically decoupled from the animal by two concentric 1/4-wavelength choke sections so that the tip of the water nozzle had an extremely high impedance, virtually preventing conduction currents between it and any contacting object. The theory of the waveguide operation has been described elsewhere [Guy et al., 1979].

Microwave generation and distribution. Each exposure room was equipped with two 2,450-MHz pulsed microwave sources (Epsco, model PG5KB, Trenton, NJ), each source capable of providing an average output power of 20 W and a peak power of 5 kW. These generators were controlled by a microprocessor to deliver repetitive pulse trains as shown in Figure 3.

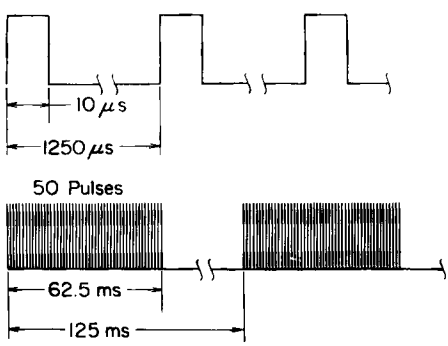

Fig. 3. Modulation characteristics of the microwave pulses: 8 groups per second, 50 10-μs-wide pulses per group, with a repetition rate of 800 pps. The period was 125 ms; with pulse onsets separated by 1.25-ms intervals. This is the equivalent of an 800-pps source square-wave modulated at 8 Hz.

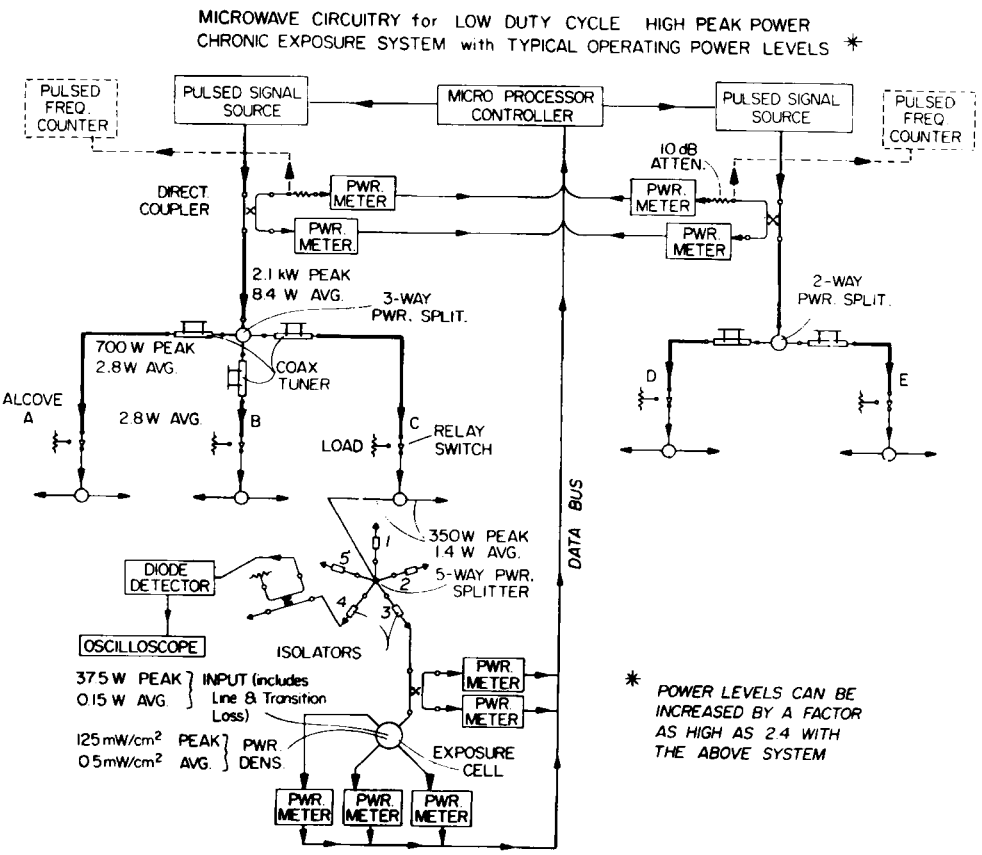

Fig. 4. Schematic of the microwave-distribution system in room T-131. The microwave energy was divided by means of low-loss coaxial cable via a 3-way splitter. Then the microwaves were fed through a single-pole double-throw (SPDT) coaxial relay to a 2-way splitter. Microwaves from each arm of the 2-way splitter were fed to a 5-way splitter; thus, the power-level of microwave radiation was again equally divided and transmitted through isolators to the two groups of five active exposure waveguides in each alcove. The distribution system of the second generator in each room was similar except that the microwaves were initially split in two ways to energize two alcoves. Power levels of forward and reflected energy at each generator output terminal was measured and recorded through a directional coupler and digital power meters interfaced with a microprocessor.

The microwaves from one generator in each equipment rack were transmitted to three alcoves (Fig. 4). The power levels of input, reflected, and transmitted energy associated with one exposure waveguide per room were monitored to obtain a recording of the average absorption loss of the waveguide-rat assembly; the average SAR could be calculated from the known waveguide loss and the mass of the rat. Each room contained a total of nine power meters, two each for the incident and reflected energy at each generator and five for the incident, reflected, and transmitted energy at the multiple terminals of the respective waveguides. The average SAR in the experimental animal was determined from the power meters. Throughout the chronic study, the monitoring system was connected each day to a different exposure waveguide so that every waveguide was monitored 1 day every 50

days over the course of the experiment. There was insignificant down time due to microwave power failure. Spare generators were available for this rare occurrence.

Dosimetry

Dosimetry studies conducted in preparation for this experiment were directed toward determining the power level for each waveguide that would best simulate with rats the exposure of man to an RF field. To determine the conditions necessary for simulating such exposure, the relation between the input power and the average and distributed SAR in the body of an exposed rat living in the exposure waveguide had to be quantified.

A microprocessor-controlled, twin-well calorimetry system was developed to measure the average SAR in rat carcasses. The average SARs for live exposed rats over the first year of exposure are shown in Figure 5. The results show that the SARs calculated from the data on the live animals are very close to but slightly less than the values calculated from measurements on rat carcasses. The results of this study have been published by Chou et al. [1984]

To best simulate the exposure of human beings, from child to adult, to radiation at the maximal levels allowed by ANSI C95.1-1982 [1982], the input power level for each alcove cluster was set so that the average input power was 0.144 W, which resulted in an initial average SAR of 0.4 W/kg in young rats of 200-g body mass.

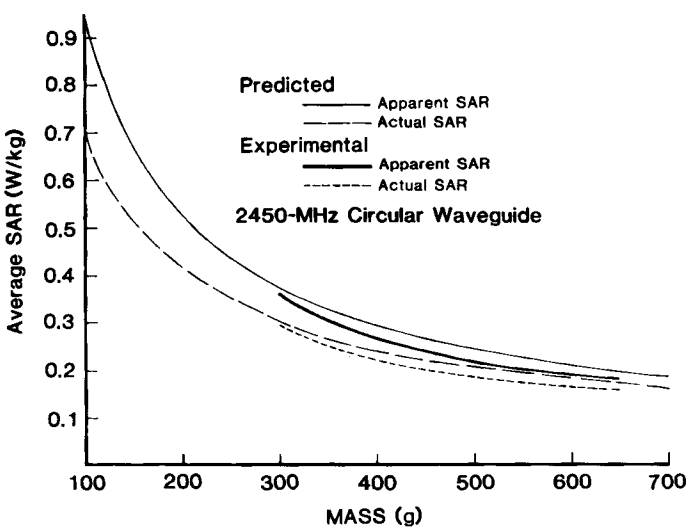

Fig. 5. Average SAR values measured for rat carcasses (Predicted) compared with average SAR values measured for free-roaming exposed rats (Experimental); SARs were averaged over weekly periods during first-year chronic exposure (input power 0.144 W to the waveguide). The predicted actual SAR was measured calorimetrically on rat carcasses of various body masses; bodies were exposed in five orientations in the waveguide: center, far corner, side, transverse, and diagonal positions. Predicted apparent SAR was measured by power meters on rat carcasses. Experimental apparent SAR was measured by power meters in live rats. Assuming that a live rat would spend equal time in each of the five orientations, the experimental actual SAR data were calculated from the apparent SAR and correction factors. The correction factors were the ratios between actual SAR and apparent SAR measured on rat carcasses.

Experimental Animals and Exposure Regimen

Two hundred male, Cesarean-derived, barrier-reared, Sprague-Dawley rats were obtained at 3 weeks of age from Camm Laboratory (Wayne, New Jersey): the rats were randomly assigned to exposure and control groups. Exposure began at 8 weeks of age, 21.5 h/day, 7 days a week, for 25 months. Maintenance procedures were done between 8 A.M. and 12 A.M. to minimize circadian-rhythm effects. The two and one-half hours off-time was used for cage cleaning, measurements of body mass, food and water consumption, blood letting, and other biological procedures.

Biological Assessment

Behavior testing. Behavior is a valuable end point for assessing neurological effects of exposure to microwaves [Lovely et al., 1977; cf. Shandala et al., 1979]. Constraints of both design and logistics, however, made selection of appropriate tests for this project a difficult task. Tests should not jeopardize the health of the animals or the reliability of data obtained from other measures. A test protocol must not entail differential treatment of an animal based on its performance (e.g., shock intensity or reward magnitude) and thereby produce secondary effects as artifacts that must be distinguished from any primary (microwave) effect. In addition, all testing must be performed within the SPF environment and in such a manner so as not to interfere with the normal daily maintenance procedures or exposure protocols.

The risk of physical harm to the animals eliminated many standard behavioral tests, so we chose a simple behavioral test based on quantification of a naturally-occurring behavior. Open-field or exploratory behavior has long been used as a sensitive endpoint in pharmacology and teratology, and it is accepted as a measure of general arousal or anxiety [Walsh and Cummins, 1976]. In addition, East European researchers have used the open-field test extensively in biological studies of microwaves [Shandala et al., 1979].

The open-field test is not the most impressive of the behavioral tests considered; however it is simple in nature and does not rely on elaborate or time-consuming training procedures or shock-motivated performance, and it can be routinely administered by laboratory personnel under the rigid SPF protocol.

An open-field apparatus with infrared-light-emitting sensors was used. This apparatus provided a readout of both motion activity and the coordinates in the field. The latter information was used to indicate an animal's field position in one of the possible quadrants. In addition, at the end of each test session the apparatus was inspected for urination and defecation.

Evaluation of the immune system. Alterations in the immune system due to microwave exposure have been reported and disputed in the literature [cf., e.g., Mayers and Habeshaw, 1973; Czerski et al., 1974; Huang et al., 1977; and Wiktor-Jedrzejczak et al., 1977]. The conflicting results justified an assay of immunocompetence in this study. The immune-system evaluation consisted of several basic tests that were designed to detect immunological effects that might result from exposure to RF fields:

a. Blood lymphocyte evaluation of the numbers of B- and T-cell, antigen-positive lymphocytes, and complement-receptor-bearing lymphocytes.

b. Spleen lymphocyte evaluation for response to the following mitogens: phytohemagglutinin (PHA), concanavalin A (ConA), pokeweed mitogen (PWM), lipopolysaccharide (LPS), and purified protein derivative of tuberculin (PPD).

c. Direct plaque-forming cell assay (with spleen cells) and serum-antibody titration of exposed rats immunized with the T-dependent antigen sheep red-blood cells (SRBC).

The following immunological tests were performed at the 13-month interim euthanasia of 10 animals from each treatment group, and after 25 months of exposure with the final euthanasia of 10 animals from each group; response of splenic lymphocytes to various mitogens, plaque-forming ability, complement-receptor formation, and enumeration of B- and T-cells.

Blood sampling for corticosterone and health profile. Pituitary-adrenal axis activity as indexed by plasma corticosterone levels has long been interpreted as an indicator of general arousal, i.e., alerting borne of anxiety, fear, or stress. If long-term exposure to pulsed RF fields disrupts normal physiological functions or is psychologically disturbing to the animal, an increased basal level of corticosterone can be expected [Lotz and Michaelson, 1978]. The endocrine system can provide evidence of summation of multiple, otherwise subthreshold, effects. Individual corticosterone data are of value for correlation with results from individual animals or subpopulations that might exhibit abnormal indices of blood chemistry or a high incidence of tumors, and also as a measure of a possible nonspecific microwave effect.

The research protocol required the rapid collection of blood from all test animals in a 2-h period per day over 4 days for each blood sampling. The collection procedure was designed to be as rapid and atraumatic as possible. To prevent artifactual elevation of corticosterone, blood samples for serum corticosterone were drawn within 2 min after a rat was removed from its cage [Zimmermann and Crutchlow, 1967; Davidson et al., 1968]. The animals were rapidly anesthetized by a mixture of halothane, nitrous oxide, and oxygen; blood samples were drawn by the relatively atraumatic retro-orbital technique. Alternate eyes were sampled for blood in successive samplings so as to minimize ocular damage. A single blood sample, 1.8 to 2.0 ml, was taken at each session for all determinations.

Metabolism. An important consideration in performing the long-term exposure of rats is that the nominal 0.4-W/kg average SAR, initially is about 5% of the average metabolic rate of an active, young 200-g rat and about 10% of its resting rate. This SAR may be as high as 15% of the average metabolic rate of a lethargic, old, 600-g rat and 25% of its resting rate. The decision was, therefore, made to use a constant power density, which resulted in a declining SAR as the animals matured.

Exposure to microwave radiation for long periods could have different consequences for longevity, either life-shortening or life-lengthening, depending on the energy-budgeting option [Sacher and Duffy, 1978]. Therefore, given the importance of the metabolic versus extrinsic-budget question, the protocol provided the following animal measurements:

a. Daily-lifetime body mass measures, i.e., growth.
b. Daily-lifetime food and water consumption.
c. 24-h cycles of oxygen consumption and carbon dioxide production, measured at regular intervals throughout the life span.
d. Periodic assessment of thyroxine level.
e. Periodic assessment of urine production.
f. Total-body analysis at spontaneous death or termination.

Despite the importance of direct metabolic measurements through respiratory gas-exchange analysis, two factors precluded their application to all 200 animals:

(1) physical as well as financial constraints made it impossible to instrument all 200 waveguides, and (2) rotating all animals through a few instrumented waveguides would have an associated animal-transfer-management risk and a subsequent loss of data. In addition, were such a mass rotation attempted, the need to allow each animal a minimum of 2 days in the instrumented waveguide to adapt to the new environment would have led to a rotation schedule allowing data to be obtained, at most, twice a year from an animal, which would have been too infrequent. Therefore, we selected a subset of the exposed and control samples for rotation through waveguides adapted for the measurement of oxygen consumption and carbon dioxide production. This procedure did not result in loss of overall statistical power, and it produced more frequent measures on the specific animals involved. Given the modular arrangement of the rooms, 36 animals (18 exposed and 18 sham-exposed) were measured for respiratory gas exchange.

Histopathology. As part of a general health screen at time of animal procurement, 10 rats, 21 days old, received gross and histopathological examination. After 13 months, 10 exposed and 10 sham-exposed rats were randomly-selected and euthanatized for examination; at 25 months, the surviving 12 exposed rats and 11 sham-exposed rats were euthanatized and examined. The other 157 animals were examined when they died spontaneously or were terminated *in extremis* during the study.

A pathologist (L.L.K.), without knowing the identity of the rats, provided evaluative data to the technical personnel of the Bioelectromagnetics Research Laboratory, who were responsible for computer entry and quality control. Statisticians then evaluated the data, and the final results were reviewed by the pathologist for appropriate interpretative comments.

The occurrence of neoplastic and non-neoplastic lesions was recorded along with the age and the cause of death of each animal, whether the animal was euthanatized or had died spontaneously. The data on pathology were collected to permit comparison of survival curves of exposed and sham-exposed animals, age-associated lesions, and incidence of tumor metastases, as well as the number of lesions per rat.

RESULTS

Behavioral Evaluations

Figure 6 shows data from the 14 sessions of open-field assessment; except for the first test session, 2 years of exposure to the low-level, pulsed-microwave radiation did not lead to significant behavior alterations as measured by activity, defecation, or urination. During the first test session, the general activity level of the exposed animals was significantly lower ($t = -2.24$, $P = .026$, $df = 195$), by approximately 9%, than that of the sham-exposed animals. The open-field activity pattern during the course of this study resembles that normally observed as a function of age and experience, and it apparently was not affected by a lifetime of exposure to the low-level pulsed microwaves (Hotelling's T^2 statistic $F = 8.73$, $P = .40$, $df = 8,168$).

Plasma Corticosterone

Analysis of the data obtained during the five sampling periods (Fig. 7) indicates that serum corticosterone levels were not dramatically altered in either the

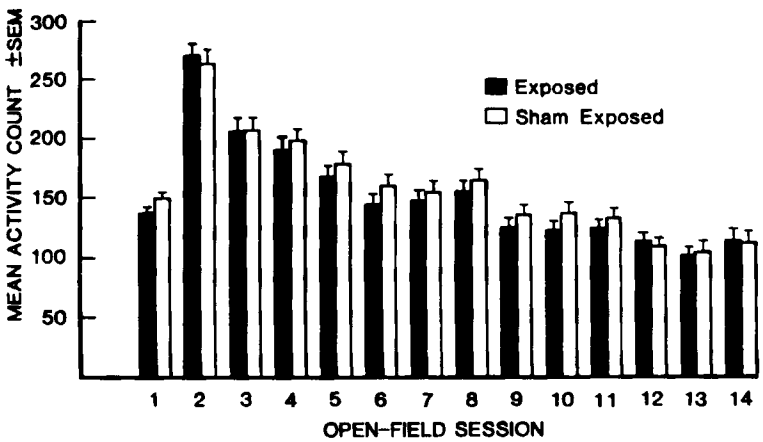

Fig. 6. Comparison by treatment group of mean levels of activity throughout the 14 open-field assessment sessions.

exposed or sham-exposed rats. The multivariate statistical analyses of the data ($\underline{F}$ = 1.38, $\underline{P}$ = .24, df = 5,133) indicate that no overall effects of microwave radiation were measurable by levels of serum corticosterone.

When the serum corticosterone values of exposed and sham-exposed animals were compared for each session, a *t* test indicated that exposed animals had relatively elevated serum corticosterone levels at the time of the first sampling session ($\underline{t}$ = 2.06, $\underline{P}$ = .04, df = 154), and that sham-exposed animals had elevated levels at the time of the third session ($\underline{t}$ = -2.25, $\underline{P}$ = .026, df = 161). Exposed and sham-exposed animals had comparable levels of corticosterone on all other regular sampling sessions.

The finding of elevated corticosterone was tested in a follow-up study [Chou et al., 1986]. Two groups of 20 animals each were exposed for 6 and 12 months, respectively, under the same exposure parameters as in the original study. An equal

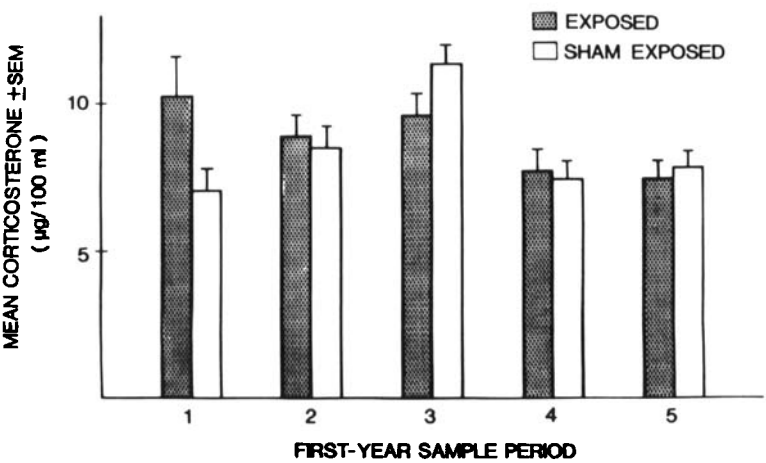

Fig. 7. Comparison of mean corticosterone levels from five quarterly determinations during the first year of the project.

number of sham-exposed rats served as controls. Corticosterone measured at 6 weeks, 6 months and 12 months did not show any statistically significant differences ($P > .05$) between 20 control and 20 exposed rats.

Immunological Competence

When compared with sham-exposed rats after 13 months of exposure (Fig. 8), exposed animals had a significant increase in both splenic B-cells ($t = 3.76$, $P = .002$, df = 16) and T-cells ($t = 3.48$, $P = .003$, df = 16). This apparent general stimulation of the lymphoid system in exposed animals was not detected in the animals after 25 months of exposure: Comparison of exposed and sham-exposed rats at euthanasia of survivors did not reveal any significant differences in the percentage or total numbers of B and T cells per spleen.

No significant differences were seen between exposed and sham-exposed rats in the percentage of complement-receptor-positive cells in the spleen at either the interim or final euthanasia. These findings indicate no difference between the treatment groups for lymphocyte maturation.

The plaque assay performed on exposed animals immunized with SRBC in the 13-month exposure rats exhibited a slight but statistically insignificant increase in plaques per spleen relative to the sham-exposed. This difference reversed after 25 months when exposed animals showed a slightly lower and statistically insignificant number of plaques per spleen. This assay indicated no statistically significant alteration of the reticuloendothelial system, which first processes antibodies in the presence of T-cells, because the SRBC antigen is T-cell dependent.

The mitogen-stimulation studies following 13 months of exposure revealed significant differences between groups in their responses to various B- and T-cell specific mitogens. The radiated animals had a nonsignificant increase in response to PHA but a significant increase in response to LPS (mean of 6.06 vs. 3.67, $t = 2.35$, $P = .032$) and PWM (mean of 6.41 vs. 4.61, $t = 2.43$, $P = .027$). As compared with sham-exposed animals, exposed animals also had a significantly increased response to ConA (mean of 17.0 vs. 10.7, $t = 2.65$, $P = .018$) and a decreased response to PPD (mean of 2.74 vs. 6.98, $t = -2.65$, $P = .018$). These results indicate a selective effect of exposure on the lymphoreticular system's response to mitoge-

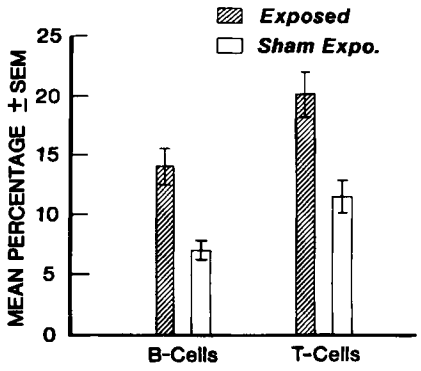

Fig. 8. Mean percentages of B-cells and T-cells within culture population of splenic lymphocytes for exposed and sham-exposed groups.

nic stimulation. Mitogen-response data were not available from the 25-month exposure studies because the lymphocyte cultures failed to grow.

In a follow-up study [Chou et al., 1986], no significant differences between 20 exposed and 20 sham-exposed rats were observed in the proliferation of thymocytes to ConA, PHA, and PWM after 6- and 12-months of RF exposure. The same lack of differences was found for splenocytes stimulated by LPS, PHA, PPD, ConA, and PWM. Flow cytometry revealed no group alterations in the number and frequency of B- and T-cells. However, after 12 months of exposure, a reduction in cell surface expression of Thy 1.1 (T-cell related) surface antigen, and a reduction in the mean cell-surface density of s-Ig (B-cell related) on small lymphocytes in spleen were observed. The stimulatory effect observed in the original study was not confirmed.

General Health Profile

In an attempt to detect and document any effects on the general health of the exposed animals, the following biochemical and hematological parameters were monitored: serum chemistry components, hematological constituents, protein electrophoretic patterns and fractions, and thyroxine levels. Multivariate analyses with Hotelling's T^2 statistic on a truncated data set (outliers removed) indicated no overall

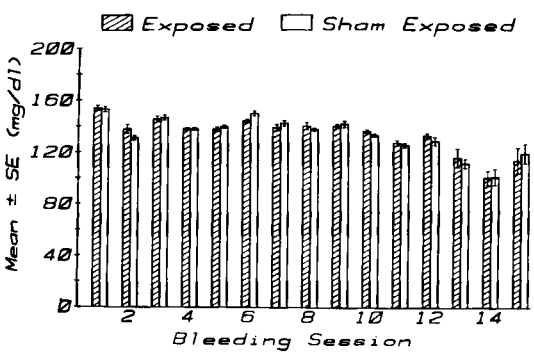

Fig. 9. Comparison of serum glucose for exposed and sham-exposed animals for 15 sampling sessions.

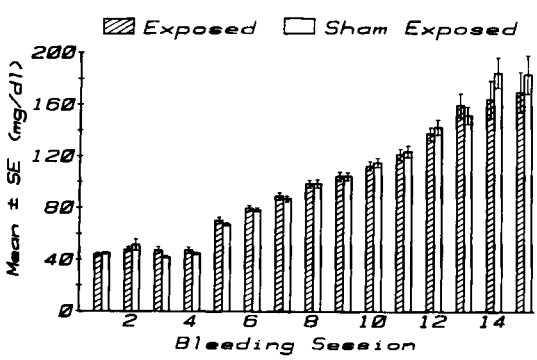

Fig. 10. Comparison of serum cholesterol levels of exposed and sham-exposed animals from 15 sampling sessions.

differences among all parameters between exposed and sham-exposed samples. Figures 9 and 10 present two representative examples of glucose and cholesterol levels from 36 sets of data. Individual *t* tests of all parameters across all 15 sampling sessions indicated a significant reduction in the absolute eosinophil counts of exposed rats during session 2, and marginally significant reductions in absolute neutrophil count during sessions 2 and 3. None of the other comparisons was significant. Therefore, these findings indicate that after the 25-month exposure no consistent effects were produced in bone-marrow erythropoietic cells or in the juxtaglomerular apparatus of the kidney and its production of erythropoietins.

Twenty-one serum chemical constituents were measured in serum samples collected during all 15 sampling sessions. The serum-chemistry tests were sensitive enough to detect population changes due to aging. Statistical analysis of the data by Student's *t* tests did not indicate any differences between exposed and sham-exposed animals.

Electrophoresis of the serum proteins revealed no significant changes in the electrophoretic patterns and absolute protein fractions between the population groups. Both groups showed a gradual decrease in the albumin/globulin ratio with increasing age, and the overall level of globulin fractions observed in these barrier-sustained animals was lower than that reported in conventional-colony animals. The microwave exposure had no apparent effect on the functioning of various organ systems that contributed to serum-protein concentrations.

Thyroxine levels did not differ significantly between exposed and sham-exposed animals (Fig. 11). Thus, exposure had no effect on the hypothalamic-pituitary-thyroid feedback mechanism. The absolute level of serum thyroxine developed to a maximum in young animals and decreased gradually as they aged. The correlation of this age-related decrease in thyroxine levels with increasing cholesterol (Fig. 10) and triglyceride levels in both test and sham groups shows it to be a reliable indicator of metabolic activity in the rat.

The major conclusion that can be reached from the evaluations of hematology, serum chemistry, protein electrophoretic patterns and fractions, and thyroxine levels is that any significant variations of the parameters observed during the lifetime of the exposed animals were to be expected as a function of aging.

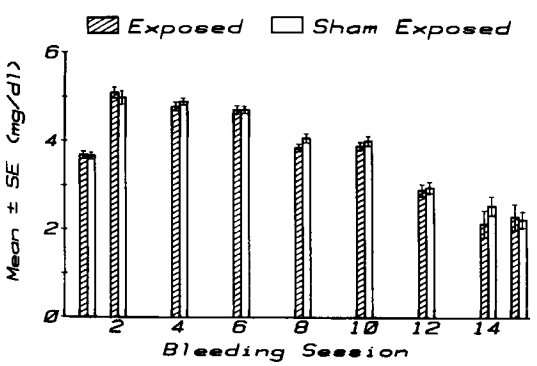

Fig. 11. Comparison of thyroxine data for exposed and sham-exposed animals for blood sampling sessions for which analysis was made.

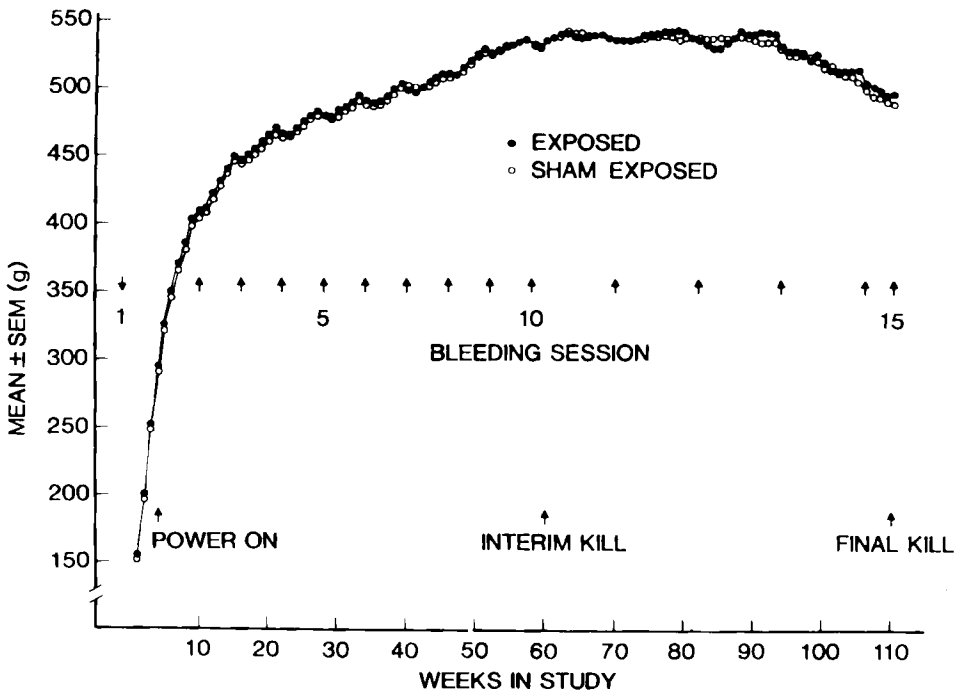

Fig. 12. Mean weekly body mass throughout 25-month study. Arrows indicate periodic bleeding sessions as well as other significant events during the course of the study.

Metabolism

Body mass and consumption of food and water. Growth curves for microwave-exposed and sham-exposed animals throughout this study (Fig. 12) are in general agreement with those reported for the Sprague-Dawley rat [Berg, 1960; Masoro, 1980]. The asymptotic body mass was somewhat lower than expected, possibly because of a periodic "stunting" effect coincident with the start of the regular blood-sampling sessions.

The average daily food intake of approximately 25 to 26 g is higher than that usually reported for the rat [Brobeck, 1948; Hamilton, 1967; Jakubczak, 1976] and indicated by the feed manufacturer (12 to 15 g/day). These food-intake norms, however, are for animals housed in a standard animal facility maintained at a higher ambient temperature (25 °C). The amount of food eaten by the animals in our facility, which was maintained at 21 ± 1 °C, is in agreement with that reported for animals housed at lower ambient temperatures [Brobeck, 1948; Hamilton, 1967; Jakubczak, 1976] and in other studies in our laboratory that had used the waveguide apparatus [Lovely et al., 1977]. Throughout the 25 months, no overall differences were observed between treatment groups in either food or water consumption.

The similarity in overall patterns of growth, food and water consumption, and body-mass loss and recovery in exposed and sham-exposed samples indicates that no effects of microwave irradiation were apparent in these measures of long-term energy balance.

Total body analysis. With one exception, the combined analyses of organ mass, general carcass composition, fatty-acid profile, and mineral content provided no evidence that metabolic processes were adversely affected in the animals exposed for 13 or 25 months to microwave radiation. A highly significant elevation of adrenal mass was indicated by the 75% increase observed for exposed rats as compared with sham-exposed animals. However, when the animals with benign tumors in the adrenal gland were separated from those without tumors, the difference became insignificant. For animals with tumors, the adrenal mass was significantly higher in the exposed group than in the sham group. This analysis indicated that the increase in adrenal mass was related to the tumors and was independent of the metabolic processes in the rats. The mean adrenal mass in exposed animals without tumors was slightly larger, but statistically insignificant, as compared with that of the sham-exposed rats. This increase in mass was attributed to one animal with a hyperplastic adrenal cortex, which was secondary to a pituitary tumor.

O_2 consumption and CO_2 production. Differences between exposed and sham-exposed rats occurred in O_2 consumption and CO_2 production in younger rats (body mass 300–400 g) but not in the more mature animals (17–24 months old, body mass 550–600 g). The average hourly O_2 consumption for the young rats during the nocturnal period (1900–0600 lights off) was significantly different between the treatment conditions (Hotelling T^2 statistic, $F = 2.29$, $P = 0.025$, df = 11,44). Although individual t tests of hourly CO_2 productions of the young animals did not show consistent significant difference between treatment groups, the Hotelling T^2 statistic was significant during the diurnal (1300–1900 lights on) period ($F = 2.73$, $P = .023$, df = 6,49) and even more significant during the night time hours ($F = 2.91$, $P = .006$, df = 11,44). The effects observed in the young animals were less pronounced during the second round (36 days later) of measurements. On an hour-to-hour basis, the mature animals' metabolic measures appeared less variable than those of the young. The young animals demonstrated more marked responses to the lights-off condition and generally higher levels on each measure during the night time hours, i.e., the active portion of the rats' circadian cycle. This apparent synchronization of metabolic activity with the light-dark cycle has been noted by others investigating the variation of activity, food and water consumption, and energy balance patterns as a function of photoperiod [Zucker, 1971; Besch and Woods, 1977].

Gross Pathological and Histopathological Evaluation

Longevity. Product-limit estimates and log-rank statistics were used to estimate and compare survival curves of exposed and sham-exposed animals (Fig. 13). Evaluation of the curves revealed that the median survival time was 688 days for exposed animals and 663 days for the sham-exposed. Despite subtle differences in the survival curves in the early and late stages of the study, statistical analysis indicated no significant differences during any phase of the life span of the animals. Statistical evaluation indicated no association between a specific cause of death and treatment condition; however, for cause of death due to urinary tract blockage (9 in exposed group and 19 in sham group), there is some indication that survival times were longer in the exposed animals.

Histopathology. Parasitic, bacterial, mycoplasmal, and viral agents were monitored during the 25-month period. A low-level (15%) infestation of the colony with pinworms, *Syphacia muris*, occurred but no histological lesions were attributed to these nematodes. The microflora of the animals was altered over the course of the experiment by the sporadic occurrence of *Proteus* sp. (*mirabilis, vulgaris*, and

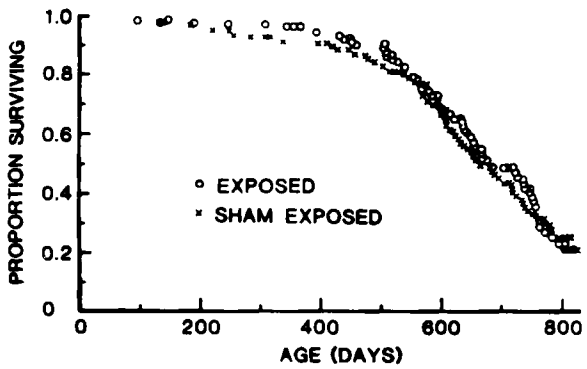

Fig. 13. Survival data for microwave-exposed and sham-exposed animals throughout the 25-month study.

vulgaris), *Staphylococcus epidermidis*, *Neisseria* sp., *Escherichia coli*, and *Klebsiella* sp. These intestinal flora became opportunistic organisms in the few cases of preputial adenitis and wound infections that occurred. *Mycoplasma* sp. was not isolated, either by culture or serology, and serological monitoring failed to reveal any significant elevations in titers of any of the common rodent viruses. There were no underlying diseases that complicated or produced erroneous results in the gross or histopathological evaluations of the experimental animals.

The histopathology data were grouped with respect to the animal's age, at 6-month intervals, and the data were divided into neoplastic and non-neoplastic diagnoses. The documentation of morphological lesions showed 2,184 pathological changes in the 200 animals examined. The non-neoplastic lesions comprised 1,992 of the observed changes, with 217 unique combinations of organs and lesions. The neoplastic lesions accounted for 192 of the observations, with 83 unique combinations of organs and types of neoplasms.

Chronic glomerulonephropathy was the most frequent cause of death and one of the most consistently encountered non-neoplastic lesions. Statistical analysis (Mantel-Haenszel estimate and chi-square statistics) indicated that glomerulonephropathy was less frequently observed in the exposed than in the sham-exposed animals ($P = .04$, $df = 1$). Analysis of the other non-neoplastic lesions did not indicate that the specific lesions were more likely in either treatment condition. To detect a progressive development of the chronic glomerulonephropathy, the severity of the lesions was also evaluated. This analysis revealed no significant differences between the treatment condition and the severity of non-neoplastic lesions.

The neoplastic lesions were identified as benign or malignant, with the malignant lesions classified as primary or metastatic. A summary of these combinations is presented in Table 2, which indicates the total number of primary and metastatic malignancies and benign lesions observed in both exposed and sham-exposed animals. The incidence of neoplastic lesions corresponds with that normally reported for the Sprague-Dawley rats: Only two tumors were present in rats younger than 12 months, and tumor incidence rapidly increased after 18 months of age [MacKenzie and Garner, 1973; Altman et al., 1985]. The endocrine system had the highest incidence of neoplasia in the aging rats, as is expected in this animal. The incidence of benign pheochromocytoma of the adrenal medulla was much higher in the exposed group than in the controls (7 out of 100 vs. 1 out of 100). However, Fisher's exact test did not show a statistically significant effect ($P = .065$).

TABLE 2. Neoplastic Lesions Per Organ System

Organ	Lesions	Exposed			Sham-exposed		
		B	P	M	B	P	M
Adrenal	Adenoma	0	0	0	1	0	0
	Carcinoma	0	0	0	0	1	0
	Cortical adenoma	10	0	0	10	0	0
	Cortical carcinoma	0	3	0	0	0	0
	Myelomonocytic leukemia	0	0	0	0	0	1
	Malignant lymphoma	0	0	1	0	0	0
	Pheochromocytoma	7	0	0	1	0	0
Blood vessel	Hemangiosarcoma	0	1	0	0	0	0
Bone marrow	Leukemia	0	0	0	0	0	1
	Myelomonocytic leukemia	0	0	1	0	0	1
	Malignant lymphoma	0	1	0	0	0	0
Brain	Myelomonocytic leukemia	0	0	0	0	0	1
	Malignant lymphoma	0	0	2	0	0	0
Cervical	Myelomonocytic leukemia	0	0	0	0	0	1
Lymph node	Lymphocytic lymphoma	0	0	0	0	1	0
	Malignant lymphoma	0	0	0	0	0	1
Colon	Malignant lymphoma	0	0	1	0	0	0
Duodenum	Myelomonocytic leukemia	0	0	1	0	0	0
	Malignant lymphoma	0	0	1	0	0	0
	Squamous cell carcinoma	0	0	1	0	0	0
Edipidymis	Squamous cell carcinoma	0	0	1	0	0	0
Eye	Leukemia	0	0	0	0	0	1
Heart	Myelomonocytic leukemia	0	0	1	0	0	1
	Malignant lymphoma	0	0	1	0	0	0
	Neurinoma	1	0	0	2	0	0
Kidney	Leukemia	0	0	0	0	0	1
	Myelomonocytic leukemia	0	0	1	0	0	1
	Malignant lymphoma	0	0	1	0	0	0
	Nephroblastoma	1	0	0	1	0	0
Liver	Adenoma	2	0	0	0	0	0
	Carcinoma	0	0	0	0	1	0
	Hepatocellular adenoma	1	0	0	0	0	0
	Leukemia	0	0	0	0	0	1
	Myelomonocytic leukemia	0	0	2	0	0	1
	Malignant lymphoma	0	0	1	0	0	1
	Squamous cell carcinoma	0	0	1	0	0	0
Lung	Leukemia	0	0	0	0	0	1
	Myelomonocytic leukemia	0	0	1	0	0	0
	Malignant lymphoma	0	0	1	0	0	0
Lymph node	Myelomonocytic leukemia	0	1	2	0	1	0
	Malignant lymphoma	0	0	1	0	0	0
	Transitional cell carcinoma	0	0	1	0	0	0
Mesentery	Transitional cell carcinoma	0	0	1	0	0	0
Nasal cavity	Leukemia	0	0	0	0	0	1
Pancreas	Adenoma	0	0	0	1	0	0
	Islet-cell adenoma	1	0	0	1	0	0

Continued

TABLE 2. Continued.

Organ	Lesions	Exposed			Sham-exposed		
		B	P	M	B	P	M
Pancreas	Squamous cell carcinoma	0	0	1	0	0	0
Parathyroid	Malignant lymphoma	0	0	1	0	0	0
Parotid SG	Myelomonocytic leukemia	0	0	1	0	0	0
Peritoneum	Liposarcoma	0	1	0	0	0	0
Pituitary	Adenoma	17	0	0	21	0	0
	Carcinoma	0	2	0	0	0	0
Preputial gland	Malignant lymphoma	0	0	1	0	0	0
Skeletal muscle	Myelomonocytic leukemia	0	0	1	0	0	0
Skin	Auditory sebaceous sq carcinoma	0	1	0	0	0	0
	Basal cell carcinoma	0	1	0	0	0	0
	Basal cell tumor	1	0	0	0	0	0
	Keratoacanthoma	1	0	0	1	0	0
	Malignant lymphoma	0	0	1	0	0	0
	Pilomatricoma	1	0	0	0	0	0
	Sebaceous adenoma	2	0	0	0	0	0
Spleen	Myelomonocytic leukemia	0	0	1	0	0	1
	Malignant lymphoma	0	0	1	0	0	0
Stomach	Malignant lymphoma	0	0	1	0	0	0
	Squamous cell carcinoma	0	1	0	0	0	0
	Squamous cell papilloma	3	0	0	4	0	0
SubQ tissue	Fibroma	1	0	0	0	0	0
	Fibrosarcoma	0	1	0	0	0	0
	Lipoma	1	0	0	0	0	0
	Neurinoma	0	0	0	1	0	0
Testes	Benign interstistial cell tumor	1	0	0	0	0	0
	Squamous cell carcinoma	0	0	1	0	0	0
Thymus	Myelomonocytic leukemia	0	1	0	0	0	0
	Lymphocytic lymphoma	0	1	0	0	0	0
	Malignant lymphoma	0	0	0	0	1	0
Thyroid	Adenoma C-cell	10	0	0	9	0	0
	Carcinoma C-cell	0	2	0	0	0	0
	Leukemia	0	0	0	0	0	1
	Malignant lymphoma	0	0	1	0	0	0
Ureter	Malignant lymphoma	0	0	1	0	0	0
Urin/bladder	Transitional cell carcinoma	0	1	0	0	0	0
	Transitional cell papilloma	1	0	0	0	0	0
Zymbal's gland	Leukemia	0	0	0	0	0	1
Total		62	18	36	53	5	18

This table lists neoplastic lesions found per organ system. These lesions may be benign (B), a primary malignancy (P), or a metastatic malignancy (M) arising from a primary malignancy in another organ system (i.e., a malignant neoplasm may occur as a metastatic malignancy in many organs of a single animal, but as a primary malignancy in only one organ system of an animal).

The low incidence of neoplasia with no significant increase in any specific organ or tissue required the data to be collapsed and evaluated with respect to occurrence per se of neoplasms, with no attention given to the site or organ of occurrence. For benign lesions, as shown in Table 3, the Mantel-Haenszel (M-H) estimate of the odds ratio was 1.04. The chi-square statistic, which tests whether the relative risk is 1, was .001 ($P = .97$, df = 1); therefore, we found no evidence that either group had an excess of benign lesions. For total neoplastic incidence including benign and malignant lesions, statistical evaluation revealed no significant difference between the exposed and sham-exposed groups ($\chi^2 = 0.32$, $P > .05$).

A similar set of tables was prepared for primary malignant neoplastic lesions and is presented in Table 4. When all age categories for the primary malignant lesions were considered, the M-H estimate of the odds ratio was 4.27 and the chi-square statistic was 7.66 ($P = .006$, df = 1). With the first three age categories combined and the analysis repeated, the M-H statistic was 4.38 and the chi-square statistic was 7.9 ($P = .005$, df = 1). When the first four age categories were collapsed (leaving two categories: 1–24 and 25–30 mo), the M-H statistic was 4.47 and the chi-square was 6.97 ($P = .008$, df = 1). When age at death was ignored completely, the M-H estimate of the relative risk was 4.46 and the chi-square was 8.00 ($P = .005$, df = 1). It is interesting that the estimate of the odds ratio and the chi-square statistic are both insensitive to the way the data were grouped with respect to age at death.

A survival-type analysis also was done with time of death as a surrogate for time to tumor development if a primary malignant lesion were present. If no primary malignant lesions were found, time to tumor was considered censored at the time to death. From that analysis, the log-rank statistic is 7.63 with a P value of .006. This analysis indicates that the primary tumors occurred earlier in exposed rats than in sham-exposed animals.

DISCUSSION

We investigated the effects on health of long-term exposure to low-level, pulsed, microwave radiation. Among the 155 parameters studied, most of them showed no

TABLE 3. Incidence of Benign Neoplasms at Death

Age	Benign neoplasms	No. of animals	
		Exposed	Sham
Age considered (mo)			
1–6	Yes	0	0
	No	3	3
7–12	Yes	0	3
	No	5	5
13–18	Yes	1	5
	No	24	18
19–24	Yes	16	11
	No	19	24
25–30	Yes	22	19
	No	10	12
Age not considered			
	Yes	39	38
	No	61	62

TABLE 4. Incidence of Primary Malignant Lesions at Death

Age	Primary malignant lesions	No. of animals	
		Exposed	Sham
Age considered (mo)			
1–6	Yes	0	0
	No	3	3
7–12	Yes	0	0
	No	5	8
13–18	Yes	2	2
	No	23	21
19–24	Yes	9	1
	No	26	34
25–30	Yes	7	2
	No	25	29
Age not considered			
	Yes	18	5
	No	82	95

significant differences associated with exposure during the 25-month period. However, a few parameters showed positive effects. There was a statistically significant increase in the mean of the serum corticosterone level in exposed rats at the time of the first blood sampling, and there was a significantly lower level at the third session of measurement as compared with sham-exposed animals. The other significant effects involved the immune response of the rats at 13 months of exposure and the O_2/CO_2 metabolism in young rats.

The early finding of elevated corticosterone levels was not found in the later sessions of the 25-months study. The failure to repeat may be due to maturational differences, to the decreasing SAR as animals grew, or to a combination of the two. The lack of a significant difference in the total number of B and T cells in the terminal-euthanasia animals of the original study may also be the result of aging, the onset of immunosenescence, or the declining SARs. The role of a decreased SAR in animals across time should be considered if a similar study is conducted. One could avoid this problem by increasing the power level to keep the SAR constant.

A follow-up study was conducted to confirm both corticosterone and immune system effects [Chou et al., 1986]. Neither effect was confirmed in two groups of 20 animals each exposed for 6 and 12 months, respectively, under the same exposure condition as the original study. An equal number of sham-exposed animals served as controls. The sample size of 20 animals per group was chosen to have good statistical power (80%) to detect the same magnitude of differences observed in the original study. The failure to confirm indicates that the original findings are not robust.

The lack of discernible differences in O_2 consumption and CO_2 production in the mature animals at this level of microwave exposure is in agreement with the results of Phillips et al. [1975]. They exposed male adult rats to various intensities of 2,450-MHz microwaves. Animals receiving 27 cal/min (~ 2W) showed no difference from controls. The microwave exposure in our study resulted in an energy deposition of 1.5–2.0 cal/min (144 mW) throughout the lifetime of the animal,

well below levels employed by Phillips et al. Under the ambient environmental conditions of temperature, humidity, and airflow, the rate of energy deposition used in our study was not sufficient to produce robust changes in the metabolism of the mature rat exposed to microwave irradiation. Changes in the O_2 consumption and CO_2 production were observed in young, exposed animals—and these changes were more pronounced during the first round of the measurements—are consistent with the fact that the rate of energy absorption in our waveguide apparatus decreases with increasing body mass. Due to the fast growth rate of the rats (Fig. 12), the animals were subjected to higher SARs only during the first month.

The incidence of benign pheochromocytome of the adrenal medulla was higher but not statistically significantly so in the exposed group. However, we note that the incidence of this tumor in the exposed group does not exceed the incidence of tumors reported in the literature for this strain of rat housed under specific pathogen-free conditions [Anver et al., 1982]. Strict comparisons of these data with those from other laboratories cannot be made, however, because the animals were not subjected to parallel conditions. A reference control—large numbers of untreated rats except for observation of longevity and post-mortem analysis—would be desirable in future studies.

The finding of a near fourfold increase of primary malignancies in the exposed animals is provocative. These data cannot be considered as an artifact because different statistical analyses led to similar results. Although the overall difference in numbers of primary malignancies is statistically significant, the biological significance of this difference is open to question. First, detection of this difference required the collapsing of sparse data without regard for the specific type of malignancy or tissue of origin. Also, when the incidence of the specific primary malignancies in exposed animals was compared with specific tumor incidence reported in the literature, the exposed animals had an incidence similar to that of untreated control rats of the same strain maintained under similar SPF conditions. It is important to note that no single type of primary malignancy was enhanced in the exposed animals. From the standpoint of carcinogenesis and under the assumption that the initiation process is similar for both benign and malignant tumors, benign neoplasms have considerable significance. That treatment groups showed no difference in incidence of benign tumors is an important element in defining the promotion and induction potential of microwave radiation for carcinogenesis.

Morphologically, carcinogenesis proceeds through transitory or progressive states of growth, including hyperplasia and/or dysplasia, benign neoplasia, and finally overt malignant neoplasia. This morphological continuum, which often, but not always occurs, is the basis for grading systems and staging systems in common usage in medical pathology. Although the exact cause of cancer remains illusive, there is considerable morphological and biochemical evidence that neoplasms in humans and animals progress through a series of stages and ultimately become completely autonomous, invade surrounding tissue, and metastasize widely. Although there are readily recognizable histopathologic differences between the cancer cell and the normal cell, the biochemical differences, especially relating to the molecular biology of DNA and RNA synthesis, protein and polypeptide synthesis, enzyme activity, and membrane receptions to ultrastructural and cellular components is far from being completely understood [Busch, 1974, 1979].

The incidence of benign pheochromocytomas of the adrenal medulla was higher in the exposed group than in the controls; however, no other single type of tumor was significantly increased by the treatment, even though the primary malignancies of all types is significantly elevated in the exposed group. In considering this issue, one perspective to keep in mind is that, with the induction of cancer by a carcinogen, tissue-specific effects are usually induced, so that an agent is not usually considered carcinogenic unless it induces a significant response in any one tissue. The U.S. Environmental Protection Agency Guidelines for carcinogenicity risk assessment states, "A statistically significant excess of tumors of all types in the aggregate, in the absence of a statistically significant increase of any individual tumor type, should be regarded as minimal evidence of carcinogenic action unless there are persuasive reasons to the contrary" [U.S. EPA, 1986].

The combining of malignant tumors from all sites for statistical comparison of incidence in the exposed and control groups is questionable as to its biological relevance. A major factor that one must consider is the different response found in this study from what is expected in a chemical carcinogenesis study. There was no discernible induction of benign tumors in the organs that were apparently developing malignant neoplasms. Considering that the majority of the 155 parameters evaluated showed no differences, and especially that longevity was not affected, the biological significance of the increased primary malignancies is unknown. Chance variations may be the reason for difference in numbers of malignancies [Ward, 1983].

Scientists of the Georgia Institute of Technology have performed a complementary study, also supported by the Air Force; 200 rats were exposed to 435-MHz fields in circular, parallel-plate waveguides, 22 hr/day for 6 months. No significant differences in blood-borne end points were found [Toler et al., 1988]. To explore the possibility of RF-induced tumor initiation or promotion, the Georgia Tech group exposed a large population (200 exposed and 200 sham-exposed) of mammary-tumor-prone mice to 435 MHz fields for 21 months. This study was specifically designed to examine the effects of low-level, pulsed RF fields on cell growth and differentiation, unlike our project which was designed to study effects on general health and longevity. Their experiment is completed and the data are being analyzed. It will be interesting to compare their results with ours.

CONCLUSIONS

Microwave exposure of 100 male rats (and 100 sham-exposed controls) at SARs of 0.4 to 0.2 W/kg (pulsed, 2,450-MHz circularly-polarized microwaves at 21.5 h/day, for 25 months) showed no biologically significant effects on general health, serum chemistry, hematological profiles, longevity, cause of death, and lesions associated with aging and benign neoplasia. Statistically significant effects were found in corticosterone levels and immunological parameters at 13 months exposure, but these findings were not confirmed in a follow-up study. O_2 consumption and CO_2 production were lower in exposed young rats. These effects were not observed in mature rats. The findings of an excess of primary malignancies in exposed animals is provocative. However, when this single finding is considered in light of other parameters, it is conjectural whether the statistical difference reflects a true biological influence. The overall results indicate that there are no defintive, biologi-

cally significant effects on rats chronically exposed to this form of microwave irradiation. Positive findings need further independent experimental evaluation.

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Counter-motions

Counter-motions in accordance with § 126 of the German Stock Corporation Act (Aktiengesetz – AktG) submitted to the extraordinary shareholders' meeting of Deutsche Telekom AG to be held in Hanover, Germany, on November 19, 2009.

The calling of the Corporation's extraordinary shareholders' meeting was announced in the electronic Federal Gazette on October 5, 2009. Together with this announcement, the management's motion for resolution concerning the only item on the agenda was published. To the extent that any related counter-motions within the meaning of Section 126 AktG submitted have to be disclosed, we list them below, stating the name of the respective shareholder and any reasons given.

If you wish to authorize the proxies appointed by the Corporation to act as your proxy, please note the following: You can also use the voting instructions form issued or the Internet dialog to issue instructions to the Corporation's proxies in connection with the counter-motions given below.

You can endorse counter-motions that are exclusively aimed at rejecting the motion of the management by instructing to vote "no" to the management's motion for resolution in the respective agenda items. Counter-motions that do not just reject the motion of the management altogether, but which are aimed at bringing about amended resolutions, are identified below using letters. To issue instructions to the Corporation's proxies for the event that the counter-motions identified using letters are put forward for approval at the shareholders' meeting, please also indicate your vote by checking the box / clicking on the check box next to the letter of the counter-motion on your voting instructions form / in the Internet dialog.

If a counter-motion which you wish to vote on is identified differently in the list below, please enter this manually on the voting instructions form in one of the four fields provided specifically for this purpose and check the relevant box to indicate your vote. The voting instruction options in the Internet dialog will be automatically amended accordingly.

If you are using your voting instructions form to issue instructions to a bank or shareholders' association (or a person or association that has a status equal to banks pursuant to § 135 or pursuant to § 135 AktG in conjunction with § 125 (5) AktG, each in conjunction with § 20 Introductory Act of the Stock Corporation Act (EGAktG) as amended by the Act Implementing the Shareholder Rights Directive (ARUG)) and wish to have your voting rights also exercised on any of the counter-motions identified with letters (or identified differently), please verify beforehand not only whether and on what conditions the proxy is prepared to represent your voting right, but also, if relevant, to what extent the proxy is also prepared to represent your voting right also in connection with the counter-motions concerned.

If you have any questions regarding the shareholders' meeting, please do not hesitate to contact the dedicated hotline on +49 (0) 228 181-78895 Monday through Friday (except on public holidays) from 8.00 a.m. to 6.00 p.m.

Last update: November 06, 2009

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SOCIAL BOOKMARKS

SOCIAL BOOKMARKS

The Shareholder Bernd Reichel, Hanover, has submitted the following counter-motion on item 1 on the agenda:
"[…] Therefore, I reject this proposal and recommend other shareholders to vote against it and suggest that the Supervisory Board, Board of Management, employees, works council members and Verdi get down to work and start thinking about how to retain more customers. […]"

The Shareholder Karlheinz Kensch, Aalen has submitted the following counter-motion on item 1 on the agenda:

"[…] as a shareholder of the company and with reference to §§ 125 and 126 of the German Stock Corporation Act (AktG), I will propose the following counter-motion at Deutsche Telekom AG's extraordinary shareholders' meeting on November 19, and will call upon the shareholders in attendance to support my motion.

The following is being presented for resolution: The Spin-off and Take-over Agreement concluded on September 3, 2009 with T-Mobile Deutschland GmbH with its registered offices in Bonn. The planned/concluded agreement contains under item 3.1 the provision that "any and all tangible and intangible fixed assets, shown both as assets and liabilities, including contractual relations and other legal relations and legal positions of any kind, receivables and liabilities, uncertain liabilities, potential liabilities and future and conditional receivables and liabilities for which the legal basis has already been created, irrespective of whether these must or may be recorded in the balance sheet or have actually been recorded in the balance sheet" shall be transferred unless expressly exempted from the transfer. Item 5 of the agreement then specifies that Deutsche Telekom shall receive a new share in T Mobile Deutschland GmbH of EUR 980,000,000.

Reasoning:
Grounds include the health risks to humans and animals, as well as damage to vegetation as a result of the HF transmission technologies used, such as microwave radio, mobile communications, DECT, WLAN, etc.
Although cell phone manufacturers recommend that their devices are not used in buildings or at locations with "poor" reception, such safety information and warnings found in the cell phone descriptions are not heeded when it comes to aggressive sales – on the contrary; the focus now with "home zone" agreements is to sell customers these dubious communications technologies, planned and developed for "outdoor use," as a replacement for their existing fixed-network lines.

Although DETAG is aware of the patents (Siemens DECT DE 103 45 529 B3 2005.04.14 and Swisscom WLAN WO 2004/075583 A1) and the explanations they include of the health risks of these technologies, which resulted in the granting of "new, reduced-radiation" technologies, DETAG and T Mobile continue to sell phones featuring the "old" technology without mentioning the "reduced radiation" of devices manufactured under the patents.
Some of these health risks and damaging effects have been known to DETAG for decades:
Ecolog Institut for T-Mobile 2000: http://www.ecolog-institut.de/fileadmin/user_upload/Publikationen/MOBILFUNK_2000_Mobil_incl_E.pdf
Warnings from the Federal Office for Radiation Protection: http://www.bfs.de/de/elektro/hff
Warnings from Dr. Volkrot: http://www.diewellenbrecher.de/pdf/volkrodtrichtfunk.pdf and http://www.diewellenbrecher.de/pdf/waldsterben1987.pdf
Bundesrat notification (document 478/09) on implementing the EU guideline
A6-0089-2009 https://www.umwelt-online.de/PDFBR/2009/0478_2D09.pdf

The following links contain further information:
Ärzte und Mobilfunk ("Doctors and mobile communications"): http://www.aerzte-und-mobilfunk.net/

Kompetenzinitiative (“Competence Initiative“): http://www.kompetenzinitiative.de/
Diagnose Funk ("Mobile diagnoses"): http://www.diagnose-funk.ch/
Microwave-related illnesses: http://www.diewellenbrecher.de/
Austrian insurance: http://www.diagnose-funk.org/assets/2009-7-21_df_bp_auva-report.pdf
Real estate value loss: http://www.diagnose-funk.ch/recht/wertverluste/index.html

It is not possible to insure against the risk of damage as, since 2002, insurers refuse to conclude insurance against the risk of claims for compensation resulting from these technologies, on the grounds that such risk cannot be calculated (they also make reference to asbestos and creosote). Internationally, multiple lawsuits have been launched relating to health problems. Several countries, including ones that share a border with Germany, have already specified lower threshold values. Legal rulings in many countries (Germany too) have resulted in the prevention or dismantling of some transmitters, including transmitters belonging to DETAG.

This potential for claims for damages in the future reveals a massive deficiency in the agreement, as it contains no provisions relating to this matter. In particular, the agreement contains no risk provisions for future claims for damages, no regulation on indemnity from liability vis-à-vis end customers, and no indemnity from liability vis-à-vis landlords who provide buildings or parts of buildings for antenna sites or who rent property to Deutsche Telekom or T Mobile.

These risks are uncertain and unacceptable, and are being completely ignored. Furthermore, a review must be carried out to determine whether it is even permissible to transfer the existing, functioning, wired nationwide network coverage infrastructure – which is embedded in legislation and originates from the days of Deutsche Bundespost (state-owned and funded at least partially by tax money) – in its entirety to T Mobile, which is designed purely for maximizing profit. There are concerns that the wired infrastructure will neither be maintained in future on cost grounds nor be switched over to fiber optic cable but, instead, that it will be replaced by further mobile solutions that are damaging to health (see above). In the event of damage being sought, the lack of risk provisions and indemnity from liability at T Mobile would result in insolvency, and the victims will not receive any compensation. The destroyed wired infrastructure will then have to be rebuilt out of the general public's pocket.

Therefore, I reject this proposal and recommend other shareholders to vote against it and suggest that the Supervisory Board, Board of Management, and employees get down to work and make the relevant modifications to the agreement (risk provisions for T Mobile or its indemnity from liability).[…]"

The Shareholder Franz Simon Haider, Ratingen, has submitted the following counter-motion on item 1 on the agenda:

" […] as a shareholder of the company and with reference to §§ 125 and 126 of the German Stock Corporation Act (AktG), I will propose the following counter-motion at Deutsche Telekom AG’s extraordinary shareholders’ meeting on November 19, and will call upon the shareholders in attendance to support my motion.

Reasoning:

1. The motion to merge the two business units, T-Home and T-Mobile Deutschland, is far too complicated and is not suitable for forward-looking, positive business development. I therefore call upon the Board of Management and the Supervisory Board to convene a new extraordinary shareholders' meeting based on a significantly simpler motion.

2. According to 1.6 of the existing motion for the shareholders' meeting, the redefined T-Home business area as set out in the Spin-off and Take-over Agreement does not include the Products and Innovation unit according to the organizational structure in place as of July 1.

Under a responsible business strategy for T-Home, responsibility for Products and Innovation as an integral part of an organization should fall completely within in the T-Home organizational structure.

3. I have analyzed the development of Deutsche Telekom's business over several years with the conclusion that the Group's problems have always arisen from an overly complication organization, and that the company is not able to gain the commitment of its employees or convince them regarding a unified direction or strategy, or to leverage their full potential – see e mail concept, see problems in the United States, in the UK and in Poland. This means that Deutsche Telekom's problems do not lie in its organization but, instead, in the motivation of employees, above all in the Product Development department and the Innovation department.[…]"

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