591 MHz were similar, but no changes were detected for 2.450 MHz, from which the authors suggested that the effect is frequency-dependent.

The experiments were performed on anesthetized rats, with consequent lowering of brain temperatures. Whether similar results would occur in the absence of anesthesia has not been determined. Although some points in those studies are open to question, the positive findings are worthy of further investigation. Albert et al. (1981) noted that prenatal development of the mammalian brain depends on migration of nerve cells, and that a readily identifiable cell class in the cerebellum called Purkinje cells arises during the second half of gestation. They reported lower mean counts of Purkinje cells in 40-day-old rat pups exposed to RFR for 5 days in the womb to 2.45-GHz RFR at 10 mW/cm^2 than for sham-exposed pups, results are open to question because of the large variations in SAR (0.8 to 6 W/kg, 2 W/kg estimated mean) due to movement of the dams during exposure. Merritt and Frazer (1975) obtained negative findings in mean brain concentrations of various neurotransmitters and their metabolites assayed in mice exposed to predominantly electric or magnetic fields at 19 MHz. However, the authors noted that at 19 MHz, a mouse absorbs very little energy from either field.

Lai et al. (1988) investigated the effects of exposure to 2.45-GHz CW and pulsed RFR at whole-body SARs of about 0.6 W/kg on choline uptake in various regions of the rat brain, using circularly polarized RFR in individual cylindrical waveguides and plane-polarized RFR in a miniature anechoic exposure chamber. The spatial mean SAR in the rat head was about 0.77 W/kg in the waveguides (with the rat facing the source), and 0.56 W/kg in the anechoic chamber. Both positive and negative findings were obtained. The authors suggested that the differences in results for CW and pulsed RFR in certain brain regions could be ascribed to the RFR auditory effect. They did not suggest specific mechanisms for results in other regions where CW and pulsed RFR had similar effects.

In summary, histochemical changes in the CNS were seen at relatively low SARs by Sanders and coworkers (1985) and Lai et al. (1988), but their significance with regard to possible human health hazards is not clear and needs further investigation. Taken collectively with other experimental results in which the effects observed were ascribed to local increases in brain temperature, it seems unlikely that exposure to RFR levels that do not increase local brain temperatures would cause deleterious histopathologic or histochemical effects in the human central nervous system.

4.5.4 EEG- and Evoked-Response Changes

Investigations have been carried out to ascertain the effects of RFR on the electroencephalogram (EEG) and on the evoked responses (EVs) by visual or auditory stimuli. Basic problems with most of the early studies, such as those of Baranski and Edelwejn (1968, 1975) and Takashima et al. (1979), were the use of metallic electrodes to record EEGs or EVs during exposure to RFR; their presence probably caused enhancement of the incident fields at the electrode sites, and artifactual data in the recordings due to pickup of the fields by the leads from the electrodes to the instrumentation. The findings of early studies can be discounted because of such use. On the other hand, EEG measurements taken after completion of RF exposure may be less definitive because of interpretation problems stemming from the time consumed in attaching the electrodes and the variability in their placement. Moreover, any transient effects that may occur during RF exposure would disappear when exposure ceases. The latter points are applicable to the negative findings of Kaplan et al. (1982) on monkey EEGs.

In several studies, such as those by Tyazhelov et al. (1977) and Chou and Guy (1979a), effort was made to minimize artifact occurrence by design of electrodes and leads from materials having high resistivities comparable to those for tissue. When such electrodes were implanted before exposure and were present during exposure, no significant differences in EEGs or evoked responses between control and RFR-exposed animals were obtained, as exemplified in a study by Chou et al. (1982). Thus, there is little or no evidence that the EEG or evoked responses of people will be affected by RFR at levels below current exposure guidelines.

4.5.5 Calcium Efflux

Increased release of calcium from brain tissue of newly hatched chickens was observed when the tissue was exposed to 147-MHz or 450-MHz RFR at levels in the range 1-2 mW/cm^2 only if the RFR was amplitude-modulated at certain relatively low frequencies. The authors reported that, under specified exposure conditions, more calcium ions were exchanged between the exposed brain hemisphere and the fluid bathing it in comparison with the unexposed hemisphere (the "calcium-efflux effect"). The maximum effect was seen for modulation at 16 Hz and no effect was seen for unmodulated (CW) 147-MHz or 450-MHz RFR. Bawin et al. (1975) described the basic experimental protocol for chick brain studies. Subsequently, others conducted studies with chick brain (Blackman et al., 1979, 1985), rat brain (Shelton and Merritt, 1981; Merritt et al., 1982), cat brain (Adey et al., 1982), subcellular fragments isolated from synapse regions of nerve cells (synaptosomes) (Lin-Liu et al., 1982), frog heart (Schwartz et al., 1990), toad heart (Wood et al., 1992), atrial strips of frog heart (Schwartz and Mealing, 1993), and cultured cells (Dutta et al., 1984, 1989, 1992, 1994). These various studies were conducted under a number of experimental conditions and produced a variety of results which permit the overall conclusion that, when observable, the calcium efflux phenomenon is relatively small in magnitude and has proven difficult to replicate. The results from the Bawin and Blackman groups led to their conclusion that calcium efflux effects occurred only within narrow ranges of modulation frequency ("frequency windows") and power density ("power windows"). Blackman conducted an extensive series of calcium efflux studies using low frequency magnetic fields without any RFR. The low frequency studies showed a harmonic series of frequency windows and multiple power windows and indicated that there was an interaction between the applied magnetic fields and static magnetic fields such as the earth's magnetic field. The validity of the calcium efflux effect has remained in contention. Some observers hold that small, variable effects such as those reported in the various calcium efflux experiments are not truly effects of exposure to RFR but instead are over-interpretations of noisy data or perhaps the result of procedural errors.

The calcium efflux effect is relatively important for discussions of RFR biological effects because, if valid, it is an effect which does not involve temperature elevation; that is, it is a non-thermal effect. Calcium ions are required for several cell functions and also serve as one of the principal agents for regulation of cell biochemical activity. For this reason, an effect on calcium metabolism might be an indication of interference in cell functions. However, there has so far not been a demonstration of any physiological change nor any harmful effect of RF exposure caused by altered cellular calcium. There is no experimental evidence that the effect, if it does exist, is harmful to humans or intact animals.

To better appreciate the quality of the data and reasons for controversy, specific findings from some of the literature on calcium efflux and RF exposure are reported in the following paragraphs.

Bawin et al. (1975) also compared the effects of RF exposure on calcium efflux with those from chick brains that were intentionally poisoned with sodium cyanide which stops metabolic activity, including

membrane transport processes. The results showed that for 16-Hz modulation, although the mean effluxes from the normal and poisoned brains were both significantly higher than their respective controls, the differences between poisoned and normal brains for each exposure condition were not statistically significant. The absence of any differences between normal and poisoned brains appeared to indicate that no calcium had moved across cell membranes, i.e., that the calcium efflux effect did not involve transmembrane calcium transport.

Bawin and Adey (1976) performed experiments to ascertain whether the previously observed calcium efflux from amplitude-modulated 147-MHz fields was due to the carrier frequency or to the amplitude modulation itself. They exposed chick-brain preparations to sinusoidal fields at frequencies of 1, 6, 16, or 32 Hz [in the extremely low frequency (ELF) and sub-ELF ranges] instead of to 147-MHz fields amplitude-modulated at those frequencies. The exposures were for 20 minutes at peak electric fields of 5, 10, 56, and 100 V/m. The results indicated that the effect with ELF and sub-ELF fields was opposite to that with amplitude-modulated 147-MHz fields: decreases rather than increases of calcium efflux were obtained, with maximum effect with 6 and 16 Hz at 10 V/m. These data also indicated the existence of a field-amplitude "window": calcium-efflux decreased with exposure to 6 and 16 Hz field by a statistically significant amount at 10 and 56 V/m, but not at lower and higher levels (5 or 100 V/m).

Other research, such as the study in chick brain with amplitude modulated 147 MHz fields by Albert et al., 1987, and the studies with pulse-modulated RFR and rat brains by Shelton and Merritt (1981) and Merritt et al. (1982) reported no effects on calcium. Several of the recent studies that reported positive findings suggest that magnetic fields, primarily at power line frequencies (as well as the earth's direct current [dc] field), contribute to the effect.

Adey et al. (1982) presented the results of a study of $^{45}Ca^{++}$ efflux from the cortex of the paralyzed but awake cat. No experimental data obtained for the RFR-exposed and sham-exposed animals were given (other than mention of their use in a curve-fitting method) nor were they directly compared for statistically significant differences. The absence of such data imply that the differences in calcium efflux between the RFR-exposed and sham-exposed cats were in the mean-squared deviations from the ideal efflux curve and the cyclic variations about the idealized linear fits to the data, but not between the values themselves at corresponding times, nor their means over time.

Blackman et al. (1982), noting that their calcium-efflux changes (increases) in chick brains were opposite in sign to those found by Bawin and coworkers (reductions) at frequencies in the same range, hypothesized that magnetic fields, primarily those at powerline frequencies (as well as the earth's DC field), contributed significantly to the effect. However, the experimental findings of Blackman et al. (1985b) with magnetic fields were obscure.

Blackman et al. (1991) subsequently reported that calcium efflux can be reduced, enhanced, or nullified by appropriately varying the temperature of the chick-brain samples before and during exposure. The authors hypothesized the existence of a temperature-window effect (one that occurs only within a narrow temperature range). However, it is noteworthy that little if any efforts were taken in previous investigations by Blackman and coworkers to measure or control sample temperatures although environmental temperatures were controled. An alternative, opposing hypothesis is that altered calcium efflux is a temperature effect not related to RF exposure.

In view of the apparently contradictory findings about the RFR-induced calcium-efflux effect, the controversy over its existence is likely to be unresolved in the foreseeable future. However, if the effect is indeed proved to exist, there is no experimental evidence that any health effects are associated with the calcium efflux effect.

4.5.6 Cellular Effects of Modulated RFR

The possibility of modulation-dependent effects has been studied in a variety of test systems. These studies are controversial because many of the results lead to a conclusion that biological effects can occur athermally and particularly as a result of amplitude modulation at low frequency. Although the Sutro Tower DTV transmissions would not incorporate this mode of modulation, this topic is worthy of discussion as an illumination of current RFR research.

Byus et al. (1984) found reduced enzyme activity in lymphocytes exposed to a 450 MHz RFR modulated at 16, 40 and 60 Hz, but not at other modulation frequencies.

A body of research with modulated RF fields concerns the growth-regulating enzyme ODC (ornithine decarboxylase). Byus et al. (1988) examined three cell lines (liver tumor, ovary cells and skin cancer cells) for effects of modulated RFR on ODC. For 16-Hz modulation, ODC activity was increased in all three cell lines. ODC activity in liver cells was increased by modulation at 10, 16 and 20 Hz, but not at 5, 60 and 100 Hz, demonstrating a frequency "window" centered near 16 Hz. Byus et al. (1988) investigated whether the ODC effect was related to enhanced DNA synthesis, but found no effects of 450 MHz fields (1mW/cm^2, amplitude modulated) on DNA synthesis in hepatoma cells. Cain et al. (1997) studied the effects of RFR modulated by the pulsed, digital signal used for a type of cellular telephone communications known as TDMA. Results reported in abstract form indicated no effects in one cell line (the C6 rat brain cancer line), but a 50% reduction in ODC activity for a mouse fibroblast cell line only when exposed to the higher level of exposure and only at 3 and 4 hours after stimulation of ODC activity, but not at 2 hours. However, this "higher" level of exposure, 8.40 mW/cm^2,was identified with a low (nonthermal) SAR of 7.8 μW/g. Exposures at 0.78 μW/g had no effect. It can be noted that the reported transient reduction in ODC activity would suggest an inhibition of growth and is contrary to most reported effects of RFR on ODC.

Litovitz et al. (1993) used a mouse fibroblast cell line in experiments which confirmed the occurrence of enhanced ODC activity, but in this case using 915 MHz RFR and modulation frequencies near 60 Hz. The most important outcome of the Litovitz (1993) experiments was the observation that an effect on ODC activity required that the modulation frequency be undisturbed for a minimum of about five to ten seconds. There was, for example, no effect on ODC activity when the frequency was shifted from 55 to 65 Hz more rapidly than once every five seconds. This result showed that the characteristic time for the interaction of the modulated field with the biological system was several seconds, much longer than the time needed for most biochemical activities. Later studies on the same cell line from the Litovitz laboratory showed that the most favorable condition for an increase in ODC activity was an 8 hour exposure to a 60-Hz amplitude modulated RFR, with a weaker effect of 50 Hz square-wave modulation (i.e., 50-Hz pulses), and no effects for amplitude modulation by speech, 60-Hz FM modulation, nor for CW signals (Penafiel et al., 1997). The experiments which made comparisons of various types of modulation were conducted with 835 MHz RFR and for exposures that lasted from 2 to 24 hours and the positive findings occurred with a relatively high degree of statistical reliability, for example, $P<0.01$ in many cases.

Overall, data from two laboratories are in general agreement that ODC activity levels are transiently affected by low-frequency modulated RFR in the absence of any noticeable heating. There is no evidence to suggest that the changes in ODC activity are linked to harmful effects on cells or potentially harmful effects in animals or human beings.

Other research also may be evidence for modulation-dependent effects. Czerska et al. (1992) observed that pulsed RFR enhanced a naturally-occurring cell transformation in white blood cells to greater extent than CW fields. An electrophysiological study of heart cells showed that pulse-modulated RFR had effects which differed from the effects of CW RFR (Seaman and DeHaan, 1993). Dutta et al. (1994) reported modulation-dependent effects on a cell enzyme and Samosy et al. (1991) reported that pulsed fields caused effects on cell surface charge and cell shape which were different from effects produced by CW fields of similar strength. Low frequency modulation was reportedly required for an effect of nonthermal microwaves on the chemical binding ability of two brain neurotransmitters according to a recent research in the Russian scientific literature (Iurinskaia et al., 1996). Not all studies have found modulation-dependent effects. For example, Pakhomov et al. (1995) found no differences in effects on heart cell electrical activity for a variety of pulse modulated fields.

4.5.7 Behavioral, Neurochemical and Neuropharmacologic Effects of Pulsed and CW Microwaves on Rats

Lai and colleagues studied rat brain responses to pulsed and CW RFR in experiments on rat behavior (typically, success in learning a maze) and rat brain neurochemistry (for review Lai, 1992). The overall outcome of research conducted over a period of over 10 years is that both pulsed (2 usec, 500 pps) and CW microwaves (2450 MHz), at levels generally accepted to be nonthermal (1 mW/cm2, SAR = 0.6 W/kg), affect specific learning behaviors and neurotransmitter activity. Effects were found for the neurotransmitter acetylcholine in certain brain regions but not others. The distinction between the effects of CW and pulsed RFR depended on which brain regions were examined. Neurochemistry research indicated that effects on particular brain opioids caused effects on those nerve cells which respond to the transmitter substance acetylcholine. Because of similar findings in studies of stress in rats, Lai and his coauthors suggested that the first event to influence the nervous system was a stress-like response similar to the responses caused by crowding and loud noise. Among the findings from this series of experiments was the report by Lai et al. (1984) that pulsed microwave exposures at a level causing a low level of whole-body heating (SAR = 0.6 W/kg) appeared to affect CNS control of body temperature in rats given pentobarbital, a drug that inhibits normal thermoregulatory response. Another finding was that MW-exposed rats had body temperatures higher than those of sham-exposed rats, but because body temperature changes depended on whether the head or rump was more strongly irradiated, Lai et al. (1984) concluded there were both thermal and central nervous system effects. A later study by Lai et al. (1989) demonstrated three effects on the central nervous system of rats exposed to pulsed microwaves: 1) biochemical changes in choline uptake and its blockade by naltrexone (a blocker of central nervous system acetylcholine responses); 2) neurophysiological changes (changed numbers of muscarinic receptors); and 3) impaired rat behavior (learning on the radial arm maze). The tests were done at one SAR level (SAR = 0.6 W/kg, 2450 MHz) for various exposure durations and repetitions. Drugs which block central nervous system acetylcholine receptors also blocked the effect of pulsed microwaves on acetylcholine receptors (Lai et al., 1991). Lai et al. (1994) provided more evidence for a microwave effect on acetylcholine receptors because the inhibition in maze performance caused by microwaves could be reversed by a drug (physostigmine) which activates acetylcholine receptors and thereby counteracts the microwave inhibition of responses to acetylcholine. RFR exposures in this study were to a pulsed microwave signal (0.6 W/kg) for 45 min prior to performance on the maze. Although subtle thermal effects cannot be ruled out in all aspects of this research, the overall results indicate that pulsed and CW RFR affect rat behavior and neurochemistry in the absence of biologically important changes in temperature. There is no evidence that the effects observed in

these laboratory studies are pathological. It should be noted that the experiments done by Lai et al. have not been replicated by other researchers.

4.6 IMMUNOLOGY AND HEMATOLOGY: *IN VITRO* STUDIES

Many reports indicate that RFR has specific effects on the immune systems of mammals. Most reported effects were detected after exposure at power densities of about 10 mW/cm^2 and higher; a few effects have been found from exposure to levels as low as about 0.5 mW/cm^2. In most of the studies, the mechanisms for the effects were not investigated, and many of the results were not consistent with one another.

Early studies were done to determine whether RF exposure can transform cultures of lymphocytes (one type of leukocyte or white blood cell) into lymphoblasts (cells in active mitosis or division). In such studies, samples of lymphocytes taken from the body were cultured, exposed to RFR (or exposed and then cultured), and examined for possible effects induced by the RFR. Usually, such cells were cultured with and without a mitogen, a chemical agent that can stimulate transformation of cells into lymphoblasts. In later studies, more subtle effects on various types of leukocytes were sought, as were possible effects of RF exposure on red blood cells (erythrocytes).

4.6.1 Leukocyte Studies

Much early work investigating possible effects of RFR on cultures of white blood cells exposed *in vitro* suffered from inadequate control of cell temperature during exposure. In later studies in which effective temperature control was exercised, no significant differences were found in various endpoints between RFR-exposed cultures and control cultures that were held at the same temperature. In studies where the culture temperature was elevated by RFR, the effects observed were clearly of thermal origin.

Among the endpoints were the percentages of cell viability; lymphoblast transformation; synthesis of DNA, RNA, and total protein. Also assayed were production of alpha-interferon induced in cells infected with influenza virus, and mitogen-induced production of gamma-interferon.

Smialowicz (1976) exposed suspensions of mouse-spleen cells to 2.45-GHz RFR at 10 mW/cm^2 (SAR about 19 W/kg) for 1, 2, or 4 hours. Other suspensions held at 37°C (without RF exposure) for the same durations served as controls. Following treatment, the specimens were cultured with and without each of four different mitogens. No significant differences were seen between RFR-exposed and control specimens treated for corresponding durations. The percentage viability of the specimens, measured right after each treatment, also showed no significant differences between exposed and control cultures.

Hamrick and Fox (1977) exposed cultures of rat lymphocytes with and without a mitogen to 2.45-GHz RFR at 5, 10, or 20 mW/cm^2 (0.7, 1.4, or 2.8 W/kg) for 4, 24, or 44 hours, and assayed them for lymphoblast transformation by the uptake of tritium-labeled thymidine in the cells. The differences in thymidine uptake between the mitogen-stimulated cultures and non-stimulated cultures for each RFR level and duration were large as would be expected, but the differences between the RFR- and control cultures were nonsignificant.

Roberts et al. (1983) exposed human mononuclear leukocyte cultures to 2.45-GHz RFR for 2 hours at 4 W/kg, with no attempt to remove heating due to the RFR. Other cultures were sham exposed, and untreated cultures served as controls. Mean viability of all three groups increased and decreased with time,

but the differences among the groups were not significant. Similar results were obtained at 0.5 W/kg and at intermediate SARs. There were also no significant differences among the groups in DNA, RNA, and total protein synthesis, or in the production of alpha-interferon induced by infecting gell cultures with influenza virus or in mitogen-induced production of gamma-interferon. Roberts et al. (1987) obtained similar negative findings with influenza-infected human mononuclear leukocyte cultures exposed to pulsed or CW 2.45-GHz RFR at 4 W/kg.

Lyle et al. (1983) investigated whether 450-MHz RFR at 1.5 mW/cm^2 (SAR not indicated) would affect the toxic activity of a specific type of murine T-lymphocytes (effector cells) against a specific kind of lymphoma cells (target cells). The authors prepared radioactively tagged mixtures of the two cell types and performed 4-hour gamma-counting assays while the mixtures were being exposed to the RFR. The results indicated that the toxic activity of the effector cells was reduced by 17-24%. However, similar percentages (15-25%) were observed in 4-hour assays done in the absence of the RFR if the effector cells had been exposed to the RFR for 4 hours before mixing them with the target cells. This result led the authors to surmise that the changes in cytotoxicity observed in the previous experiments were due to actions of the RFR on the effector cells. After exposing effector cells to the RFR for 4 hours, the authors also did the 4-hour assays of mixtures (in the absence of RFR) at 1, 4, 9, and 12.5 hours. They observed 20%, 13%, 12%, and no inhibition, indicating diminution of the effect of the RFR on the effector cells with the time interval after exposure.

Sultan et al. (1983a) investigated the effects of combinations of RFR with hyperthermia on the antigen-antibody activity of B-lymphocytes. Mouse B-lymphocytes were exposed to 2.45-GHz RFR at levels in the range 5-100 mW/cm^2 (2.25-45 W/kg) at temperatures within the range 37°C-42.5°C. Antigen-antibody activity was seen in more than 90% of cells exposed to RFR and heat-treated at 37°C, but in less than 60% of cells heat-treated at 41°C, and in less than 5% of those heat-treated at 42.5°C. The authors concluded that the mechanisms of such inhibition activity are thermal in origin, with no apparent effects of 2.45-GHz RFR for exposed and control samples held at the same temperature. Similar results were obtained by Sultan et al. (1983b) with 147-MHz amplitude-modulated RFR in the range 0.1-48 mW/cm^2 (0.004-2.0 W/kg).

In summary, much of the early work seeking possible effects of exposing suspensions of various classes of leukocytes to RFR suffered from the lack of adequate control of cell temperature during exposure. In subsequent studies in which effective control over suspension temperature during RF exposure was exercised, the differences in results between exposed and control cultures treated for the same durations and held at the same temperature were not significant. In studies where elevation of culture temperature by RFR or conventional means did affect leukocytes adversely, the effects were clearly of thermal origin.

4.6.2 Erythrocyte Studies

Sought in various studies of RFR interactions with samples of red blood cells (RBCs) taken from animals or humans were hemolysis (loss of hemoglobin) and alterations in cell membrane function, particularly effects on movement of sodium ions (Na^+) and potassium ions (K^+) across the cell membrane.

Peterson et al. (1979) heated suspensions of rabbit RBCs by conventional means or with 2.45-GHz RFR at 10-140 mW/cm^2 (46-644 W/kg) while continuously monitoring the suspension temperatures, and assayed the suspensions for loss of hemoglobin (Hb) and K^+ after either treatment. For RBCs warmed to only 3.7°C above room temperature with 10 mW/cm^2 (46 W/kg) or by conventional heating for 45 minutes, no

additional Hb or K^+ was released. By contrast, when RBCs were rapidly warmed from room temperature to 37°C by either technique, the heated erythrocytes lost significantly more of both Hb and K^+ than RBCs held at room temperature. The other results showed that both Hb and K^+ were lost in equal amounts in all experiments in which RFR-heated and conventionally heated RBCs were warmed at the same rate to the same final temperature, indicating that the effect was thermal.

The authors noted that their results were in agreement with those of Liu et al. (1979), who reported no significant differences in loss of Hb or K^+ from rabbit RBCs heated to 37°C by 2.45-GHz, 3-GHz, or 3.95-GHz RFR or by conventional techniques, and with similar results of Hamrick and Zinkl (1975) with 2.45-GHz, 3 GHz, and conventional heating. However, they indicated that their findings differed from those of Baranski et al. (1971, 1974) and Ismailov (1971), who reported increased hemolysis and efflux of K^+ from rabbit RBCs exposed to 1-GHz or 3-GHz RFR at power densities as low as 1 mW/cm^2.

Peterson et al. (1979) also exposed samples of human RBCs to 37°C with 2.45-GHz RFR at 90 mW/cm^2 (412 W/kg) for 8 minutes and maintaining them there for 37 minutes by exposure at 30 mW/cm^2 (137 W/kg). Unlike the results for the rabbit RBCs, no significant differences among the groups were seen in either hemolysis or K^+ release. Absence of those effects in human RBCs may indicate that RFR-induced changes in rabbit blood may not be reflected in similar effects with human blood.

Brown and Marshall (1986) sought nonthermal effects of RFR on growth and differentiation of the murine erythroleukemic (MEL) cell line. Noting that MEL cells form Hb and exhibit other forms of erythroid differentiation in response to the inducer hexamethylene bisacetamide (HMBA), they exposed HMBA-cultured MEL cells for 48 hours to 1.18-GHz RFR at an SAR of 18.5 W/kg, 36.3 W/kg, or 69.2 W/kg. During exposure, the incubation temperature was held at 37.4°C. Control cultures were held at the same temperature in a water bath. No significant differences were found among the cultures exposed at each RFR level and their corresponding control cultures in rate of cell growth, cell differentiation, or the amounts of hemoglobin produced. Also, the mean values for each endpoint did not vary significantly with RFR level.

Subtle effects on the membranes of RBCs were sought by Olcerst et al. (1980), Allis and Sinha (1981, 1982), Shnyrov et al. (1984), Liburdy and Penn (1984), Liburdy and Vanek (1985), and Kim et al. (1985), who used various treatments and detection methods. In most such studies, RFR effects at the temperatures where phase transitions in the membranes are believed to occur were investigated, with mixed results. (Phase transitions are changes of state involving energy input or output that does not increase or decrease temperature, such as at the freezing and boiling points of water.) However, any relationship between such effects and possible health consequences has not been established.

In summary, in early studies of RF exposure of erythrocytes, hemolysis and potassium-ion (K^+) efflux were reported for rabbit erythrocytes exposed at average power densities as low as 1 mW/cm^2. However, in later investigations, Hb and K^+ losses from rabbit erythrocytes resulting from heating with RFR to 37°C did not differ significantly from the losses obtained with conventional heating. The threshold SAR for effect was found to exceed 46 W/kg.

4.7 IMMUNOLOGY AND HEMATOLOGY: *IN VIVO* STUDIES

Studies of immunological effects of RFR in live animals can be divided into those in which changes in specific immunological parameters were sought--the subject of this section--and those in which effects of

RFR on the health of the subjects and their resistance to disease were examined, discussed in the next section.

4.7.1 Effects of Exposures on Immunological Parameters

Studies seeking changes in immunological parameters from exposing animals to RFR yielded mixed results. Some researchers, such as Huang et al. (1977), Huang and Mold (1980), Wiktor-Jedrzejczak et al. (1977), and Sulek et al. (1980) found that RF exposure of mammals increased the proliferation of leukocytes (white blood cells), or subclasses of leukocytes (such as T-lymphocytes or B-lymphocytes), or production of antibodies (relative to controls) in response to antigens injected into the animals, but with few exceptions the measured or estimated SARs were well in excess of 1 W/kg. However, other researchers, such as Lin et al. (1979), Smialowicz et al. (1981, 1982a, 1982b), Ortner et al. (1981), and Wong et al. (1985) obtained negative results. In most such studies, cells cultured without or with mitogens (chemical agents that stimulate mitosis) were assayed. Smialowicz et al. (1983) found that exposure of mice to 2.45-GHz RFR at 30 mW/cm^2 (21 W/kg) transiently decreased the activity of natural killer (NK) cells in the spleen against mouse-lymphoma cells, but the effect was not seen at 15 mW/cm^2 or 5 mW/cm^2 (10.5 W/kg or 3.5 W/kg). Collectively, the positive findings were probably thermally induced, rather than due to any nonthermal interactions of RFR.

4.7.2 Effects of Chronic Exposure on Health, Longevity, and Resistance to Disease

More directly relevant to possible RFR effects on the human immune system would be studies in which animals are chronically exposed to RFR (preferably over virtually their entire lifetimes) to determine whether such exposure adversely affects their health, longevity, and resistance to natural disease or experimental challenge with various microorganisms or toxins therefrom. Some studies indicated that animals exposed to RFR for relatively short periods can withstand bacterial infection better than sham-exposed animals. However, few studies involving chronic RF exposure have been carried out or repeated by other laboratories.

The Prausnitz and Susskind (1962) study (discussed in Section 4.3.3), in which 200 mice were exposed for 4.5 minutes a day, 5 days a week, for 59 weeks to 9.3-GHz pulsed RFR at roughly 45 W/kg appeared to show that such exposure was beneficial, but probably because the resulting 3.3°C daily rise in body temperature was protective against the presence of pneumonia in the mouse colony. Liddle et al. (1987) found that the survival rate of mice injected with *Staphylococcus* and then exposed to 2.45-GHz RFR at 10 mW/cm^2 (6.8 W/kg) for 5 days (4 hours a day) in ambient temperatures in the range 19-40°C was higher than in sham-exposed mice, supporting the beneficial effects of RFR found by Prausnitz and Susskind (1962). Above 31°C, the survival rates of the RFR-exposed mice dropped sharply, to 0% at 37°C, and similar results were seen for the sham-exposed mice above 34°C (no survivors at 40°C), both findings ascribed to hyperthermia.

Probably the most comprehensive chronic RF exposure investigation to date was a University of Washington study (also discussed in Section 4.3.3), in which 100 rats each were concurrently sham-exposed and exposed to 2.45-GHz RFR at 0.4 to 0.15 mW/cm^2 over the lifetimes of the exposed animals (except those withdrawn for interim tests and those that expired before completion of the exposure regimen). Immunologic tests of 10 each RFR-exposed and sham-exposed rats withdrawn after 13 months showed counts of splenic T-lymphocytes and B-lymphocytes that were significantly higher for the RFR than the sham group, an effect ascribed to stimulation of the lymphoid system by the RFR. However, this

effect was absent in similar tests on treatment completion; the absence was ascribed to immunosenescence. Rat longevity was not affected by the RFR at corresponding times during the exposure regimen.

Toler et al. (1988) concurrently exposed 100 rats each to 435-MHz pulsed RFR for about 22 hours a day, 7 days a week, for 6 months at 1 mW/cm^2 average power density (time-averaged SAR about 0.3 W/kg). Blood samples drawn at various times were assayed for the stress hormones ACTH, corticosterone, prolactin in the plasma; for plasma catecholamines; and for hematologic parameters, including hematocrit and various blood cell counts. Heart rates and arterial blood pressure were also monitored. The results indicated no significant RFR-induced differences between groups in any of those endpoints.

In comparison with the above negative findings with nonthermal exposures and beneficial effects of hyperthermic exposures, Liddle et al. (1994) found a significant reduction of about 20% in lifetime among mice exposed to 2.45 GHz CW RF at 10 mW/cm^2 (SAR = 6.8 W/kg, a mildly hyperthermic dose rate), but a slightly longer lifespan for exposure to 3 mW/cm^2 (SAR = 2.0 W/kg), although the lifespan enhancement was not at a statistically signficant level. In the work by Liddle et al. (1994) groups of 25 female mice were exposed from age 35 days to the end of life for 1 hour per day, 5 days per week. Mean lifespan was reduced from 706 days to 572 days for the 10 mW/cm^2 group and increased to 738 days for the 3 mW/cm^2 group. The causes of death were not determined so it is not possible to determine if any particular diseases occurred differentially between exposed and control animals or if the differences in lifespan reflected a difference in aging of the animal as a whole. This study had two interesting outcomes: mildly hyperthermic whole body exposures might adversely affect lifespan and an exposure just one-third as great showed a trend toward enhanced lifespan. If accurate, these findings imply complex effects on the organism in the absence of severe stress or significant changes in body temperature. The observation of slightly increased lifespan in mice exposed at 3 mW/cm^2 is noteworthy when considering two other studies (Chou et al., 1992; Spaulding et al., 1971) where small, statistically nonsignificant increases in lifespan also were observed among the exposed rats and mice. But, note for comparison the study by Szudzinski et al. (1982) (section 4.5) which showed reductions in the lifespan of mice treated with a chemical which causes skin cancer in conjunction with RFR at a frequency and specific absorption rates similar to those used by Liddle et al. (1994). In view of recent attention to the possibility of damage to DNA by RFR (Lai and Singh, 1996; 1996) it is possible to speculate that lifespan effects might occur during nonthermal exposures as a result of subtle DNA damage such as that associated with neurodegenerative diseases.

The contrasts among the various lifespan studies discussed above involve not only different exposure intensities, but pulsed versus CW waveforms, frequency, and different animal species. These differences prevent drawing a general conclusion about effects on lifespan, but there appears to be a tendency towards enhanced lifespan for mildly thermal exposures but reduced lifespan when RF exposure is combined with chemical carcinogens. There is little or no evidence for effects on lifespan or immune function at exposure levels below the protective exposure guidelines. These effects are relatively slight and it is not known if there are any such effects in humans exposed to still lower levels of RFR.

4.8 PHYSIOLOGY AND BIOCHEMISTRY

The literature on physiological and biochemical effects associated with exposure to RFR is extensive. Many of the reported effects were associated with other events (e.g., changes in hormonal levels or stress adaptation), some are questionable for various reasons, and the medical significance of others is unclear.

4.8.1 Metabolism and Thermoregulation

Several studies were done in which effects of RFR were sought on the metabolic and thermoregulatory systems of primates. Bollinger (1971) exposed rhesus monkeys to 10.5-MHz or 19.3-MHz RFR (in the Sutro Tower Digital TV transmitters frequency band), each monkey at successively higher levels up to 600 mW/cm^2 (estimated SARs about 0.2 and 0.6 W/kg), or to 26.6-MHz RFR at up to 300 mW/cm^2 (about 0.6 W/kg). No thermal stress, heart-rate increases, or other influences on electrical events of the heart cycle due to the RFR were observed. Also, no significant differences were found between exposed and control monkeys in hematologic and blood-chemistry analyses done before and after exposure. Differences in the mean counts of monocytes and eosinophils were seen, but were ascribed to conditions unrelated to the RFR. Examinations for gross pathology and histopathology indicated no RFR-induced abnormalities.

Frazer et al. (1976) measured the skin and rectal temperatures of rhesus monkeys during exposure to 26-MHz RFR at 500, 750, or 1000 mW/cm^2 and found that the monkeys were in thermal equilibrium even at 1000 mW/cm^2. Similar findings with 20-MHz RFR at 1270 mW/cm^2 were obtained by Krupp (1977). Krupp (1978) also conducted a follow-up study on 18 of the previously exposed rhesus monkeys and found no RFR-related variations from normal values of hematologic and biochemical blood indices or in the physical conditions of the monkeys.

Ho and Edwards (1979) used oxygen-consumption rate as an indicator of stress in mice exposed to 2.45-GHz RFR in a waveguide system that permitted continuous monitoring of whole-body SAR during exposure. Exposures were for 30 minutes, during which oxygen-consumption rates and SARs were determined at 5-minute intervals. The results showed decreased mean SARs from 56 to 39 W/kg during exposure. Clearly the mice sought to diminish their thermal burdens by altering their body configurations during exposure so as to minimize their RFR-absorption rates; their rates of oxygen consumption also decreased, and returned to normal after exposure cessation.

Stern et al. (1979) trained fur-clipped rats in a cold chamber to press a lever that turned on an infrared lamp. During exposure to 2.45-GHz RFR at increasing levels in the range 5-20 mW/cm^2 (1-4 W/kg), the rats progressively decreased the rate at which they turned on the lamp.

Adair and Adams (1980), in one of several studies of voluntary and physiological thermoregulation, trained squirrel monkeys to regulate the ambient temperature (T_a) in an exposure chamber by adjusting the flow of air at various temperatures into the chamber, and monitored their skin and rectal temperatures. During exposure to 2.45-GHz RFR at 7 mW/cm^2 (1.1 W/kg) and higher, the monkeys altered the air flow to reduce the T_a, and their skin and rectal temperatures remained stable, even at 22 mW/cm^2 (3.3 W/kg). Bruce-Wolfe and Adair (1985) also showed that squirrel monkeys were able to vary the RFR level as a source of thermalizing energy and to select air streams at 10°C and 50°C to regulate the T_a, and thereby maintain normal rectal temperature.

Adair et al. (1985) exposed trained squirrel monkeys for 15 weeks, 40 hours a week, at 1 mW/cm^2 or 5 mW/cm^2 (0.16 or 0.8 W/kg) and environmental temperatures of 25°C, 30°C, or 35°C. At 1 mW/cm^2 and 25°C or 30°C, the monkeys made no change in preferred T_a. However, at 1 mW/cm^2 and 35°C or at 5 mW/cm^2 and all three environmental temperatures, the monkeys selected cooler environments (T_a s 1°C to 3°C lower). Before, during, and after treatment, physiological changes were assessed periodically. Foot sweating was higher at 35°C, but was not enhanced by exposure to the RFR at either level. Colonic temperature was not affected, but skin temperature varied in an unpredictable manner. Some decreases in

body mass were seen at 5 mW/cm^2. No significant RFR-induced changes in blood indices or in other physiological characteristics were found.

Lotz (1985) exposed rhesus monkeys to 225-MHz RFR (near their whole-body resonance) at levels in the range 1.2-15.0 mW/cm^2 (0.8-10.2 W/kg) and to 1.29-GHz RFR (well above resonance) at 20, 28, and 38 mW/cm^2 (2.9, 4.0, and 5.4 W/kg). The criterion for RFR tolerance was defined as a rectal temperature not exceeding 41.5°C. The average rectal-temperature increases with 225-MHz RFR at 2.5 and 5.0 mW/cm^2 (1.7 and 3.4 W/kg) were 0.4°C and 1.7°C, but the monkeys could not tolerate exposure for more than 90 minutes to 7.5 mW/cm^2 (5.1 W/kg) or higher. No changes were observed in circulating cortisol levels for exposures at 5 mW/cm^2 or less. The mean rectal-temperature rises with 1.29-GHz RFR at 20, 28, and 38 mW/cm^2 (2.9, 4.0, and 5.4 W/kg) were 0.4, 0.7, and 1.3°C. These results confirmed that RF exposure near resonance is most effective for producing hyperthermia.

In summary, it is evident that heating is the basis for the various effects of RFR on the autonomic thermoregulatory systems of mammals and on their behavioral thermoregulatory responses to RFR. Especially noteworthy are the results for primates because of their far greater physiological similarities to humans than the other animals studied.

4.8.2 Endocrinology

Exposure of mammals to RFR has yielded rather inconsistent effects on the endocrine system. In general, effects are apparently related to either the heat load associated with RFR or the stress induced by RFR, and possibly to other circumstances.

Lotz and Michaelson (1978) first "gentled" rats before RF exposure by weighing and handling each rat at least 4 times a week, and equilibrating it by taking its colonic temperature and putting it into an exposure cage for 3-5 hours for several days. Three-hour tests of the effects of such equilibration showed a rapid rise of colonic temperature and the corticosterone (CS) level in the blood to a plateau during the first 30 minutes, followed by a return to baseline values by the end of the 180 minutes, thus demonstrating the need for such equilibration prior to exposure. The authors then exposed unanesthetized gentled rats to 2.45-GHz RFR for 30, 60, or 120 minutes at levels up to 60 mW/cm^2 (9.6 W/kg), and measured their colonic temperatures and CS levels after exposure. Thirty minutes after exposure at 13 mW/cm^2 (2.1 W/kg), the mean colonic temperature showed a small but significant rise, and at the higher RFR levels, it increased in rough proportion to the level. The mean CS level rose slightly for up to 120 minutes after exposure at 13 mW/cm^2, up to 60 minutes at 20 mW/cm^2, and 30 minutes at 30 mW/cm^2. All other rises in CS level were significant and highly correlated with the colonic temperature rises. The estimated thresholds for adrenal-axis stimulation were 30-50 mW/cm^2 (4.8-8.0 W/kg) for 60-minute exposures and 15-20 (2.4-3.2 W/kg) mW/cm^2 for 120-minute exposures. The latter range is somewhat less than half the resting metabolic rate of the rat.

Cairnie et al. (1980) showed that exposure of unanesthetized mice for 16 hours to 2.45-GHz RFR at 50 mW/cm^2 (60 W/kg) produced significantly higher rectal temperatures, but the testis temperatures of the exposed mice did not differ significantly from those for the sham-exposed mice, indicating that the thermoregulatory system of the testes was able to compensate for the increased thermal burden from RFR at close to lethal levels. The authors also exposed conscious mice for various durations to 2.45-GHz RFR at levels in the range 21-37 mW/cm^2, after which they examined testicular cells for damage and counted total and abnormal sperm. The corresponding ranges of whole-body and testicular SARs were 25.3-44.5

W/kg and 8.4-14.8 W/kg, respectively. There were no significant differences between RFR-exposed and sham-exposed mice in mean percentages of damaged testicular cells, sperm counts, or percentages of abnormal sperm.

Lotz and Podgorski (1982) implanted a catheter in the jugular veins of rhesus monkeys for monitoring levels of cortisol, thyroxine (T_4), and growth hormone (GH). They collected blood samples from each monkey hourly for 24 hours and measured colonic temperature with an indwelling probe. At the same clock time during the 24-hour period, each monkey was exposed for 8 hours to 1.29-GHz RFR at 20, 28, or 38 mW/cm^2 (2.1, 3.0, or 4.1 W/kg). The authors noted that the resting metabolic rate (RMR) of a rhesus monkey is 2.4 W/kg.

For sessions at 20 mW/cm^2 and 28 mW/cm^2, the mean plasma-cortisol levels did not differ significantly from those for sham exposure, but they rose significantly above control level during sessions at 38 mW/cm^2. The levels then diminished to control values, indicating that the effect was transient. The authors suggested that a threshold existed between 28 and 38 mW/cm^2 (3.0 and 4.1 W/kg), that the rises were associated with rectal-temperature elevations of about 1.7°C, and that the results support the hypothesis that adrenocortical effects of RFR are thermally induced. For all RFR levels, no significant differences in mean GH or T_4 levels were seen at corresponding times during RFR and sham sessions.

Lebovitz and Johnson (1983) exposed unanesthetized male rats for 9 days (6 hours a day) to 1.3-GHz RFR at a whole-body SAR of 6.3 W/kg, which produced a mean core-temperature rise of 1.5°C. Following treatment completion, groups of rats were weighed and euthanized at intervals corresponding to 0.5, 1, 2, and 4 cycles of spermatogenesis. Spermatids resistant to homogenization were counted in the right testis, and daily sperm production was calculated. The left testis was processed for examination by light microscopy. There was no significant difference in the weight of seminal vesicles between RFR-exposed and sham-exposed rats, indicating that the RF exposure was not deleterious to testosterone production, a finding supported by histological evaluations by light microscopy. Lebovitz and Johnson (1987) also investigated the same endpoints in unrestrained rats exposed once for 8 hours to 1.3-GHz CW RFR at 9 W/kg, selected to yield a core-temperature rise of 4.5°C, said to be lethal for chronic exposure. In addition, they assayed trunk blood for follicle-stimulating hormone and leutinizing hormone. No significant differences were found in the endpoints, except for a decline in epididymal sperm count 26 days (2 cycles of spermatogenesis) after the RF exposure. However, they remarked that, in view of the negative results in the many other endpoints examined, that single positive result is highly questionable. They also indicated that a differential sensitivity of germ cells at this stage of maturation had been reported for conventional heating of the testes.

Lu et al. (1985), noting inconsistencies in the findings of various investigators, described their studies with male Long-Evans rats from two different suppliers. Among their findings was that exposure of rats from both suppliers to 2.45-GHz RFR for 2 hours at 25, 30, or 40 mW/cm^2 yielded higher levels of the hormone T_4 in blood serum than for sham exposure, but were not significantly affected at 20 mW/cm^2 or lower. However, because they had found that the normal T_4 concentrations for the rats were higher than for the rats from one supplier, those and other results were reevaluated by separately comparing T_4 levels for the RFR-exposed rats from each supplier with sham-exposed rats from that supplier. When that comparison was made, they found no significant RFR-induced changes in T_4 concentration. They remarked: "From the viewpoint of environmental health, changes in serum thyroxine cannot be used as an indicator of a past history of microwave exposure due to its limited magnitude of response and its sensitivity to extraneous factors."

Accinni et al. (1988) investigated possible effects of RF exposure on the rabbit kidney *in vivo*. They exposed the renal areas of white rabbits to 27.12-MHz RFR with a standard diathermy apparatus. The electric and magnetic field intensities, measured with portable E-field and H-field probes within the empty exposure cage, were 800-1100 V/m and 1.5-3 A/m. The exposures were done on 5 consecutive days a week for 3 weeks. Rectal temperatures increased during exposure to final temperatures that never exceeded 41°C. On treatment end, the kidneys were removed from 7 RFR-exposed and 2 sham-exposed rabbits to search for early changes. The kidneys of the remaining rabbits were excised 5 months after treatment to search for any long-term effects.

In the kidneys removed at treatment end, mild to moderate changes in the juxtamedullary zone were observed by light and electron microscopy. Those changes were characterized by small areas of tubular degeneration and atrophy, and by focal and segmental glomerular degeneration and sclerosis. By electron microscopy, various lesions and early regenerative features were detected in the tubules. No tubular lesions were found in the kidneys of the rabbits examined 5 months later, a finding ascribed by the authors to the active regenerative processes detected in the earlier phase. The authors remarked that the effects of RFR heating in their study, which yielded temperatures that never exceeded 41°C, were not likely to have had a determinant role in causing the observed renal lesions.

Most of the studies of possible effects of RFR on endocrine systems were conducted on rodents. Studies that reported positive findings also yielded indications that the effects were largely due to increases in the thermal burdens of the animals. In many studies, observed alterations in endocrine function may have been significantly influenced by stresses in the animals. For this reason, the results of studies that reduced stress by acclimating animals to handling and the experimental situation are notable.

Although some effects of RF exposure on the endocrine system appear to be straightforward and predictable from physiological considerations, other, more subtle effects may be worthy of additional study (e.g., those related to the interactions among the pituitary, adrenal, thyroid, and hypothalamus glands, and/or their secretions). Part of the problem in interpreting such results appears to be related to the uncertainties about stress mechanisms and various accommodations to such mechanisms. Animals placed in novel situations are much more prone to exhibit stress responses than those adapted to experimental situations.

In summary, because the effects of RFR on the endocrine systems of animals are largely ascribable to increased thermal burdens, to stresses engendered by the experimental situation, or to both, there is no clear evidence that such effects would occur in humans exposed to RFR at levels which do not produce significant increases in body temperature.

4.8.3 Cardiovascular Effects

Few investigations have been carried out on possible effects of RFR on the human heart. However, various studies have been performed on hearts (or parts thereof) excised from animals, and others have been conducted on the hearts of live animals.

4.8.3.1 *In Vitro* Studies

Frey and Seifert (1968) exposed excised beating frog hearts to 10-μs pulses of 1.425-GHz RFR at 60 mW/cm^2 peak. Because the RFR pulses were triggered at the peak of the P wave of the electrocardiogram

(EKG), and at 100 and 200 milliseconds (ms) after the peak, the time-averaged power density was very small. The results for the zero and 100-ms delays were inconclusive, but a significant increase in heart rate (tachycardia) was seen for the 200-ms delay. The tachycardia was a possible artifact of exposure from metal electrodes. Clapman and Cain (1975) and Liu et al. (1976) endeavored to reproduce the results above, but could not do so. Moreover, Liu et al. (1976) opened the thorax of frogs and exposed the heart of each frog *in situ* to 100-μs pulses of 1.42-GHz or 10-GHz RFR. Again, negative results were obtained.

Lords et al. (1973) exposed turtle hearts submerged in Ringer's solution to 960-MHz CW RFR, usually for 30 minutes, in a capacitor system at applied powers in the range 0-500 mW. Bradycardia was seen from about 50 to 200 mW, and tachycardia at higher powers. The authors estimated that about 3.3% of the power was absorbed by the heart and that the temperature increase in the heart at 100 mW was about 0.2°C. They also found that conventionally heating the Ringer's solution (without RFR) produced tachycardia, so they hypothesized that the bradycardia seen in the lower power range resulted from RFR-induced neurotransmitter release by remnants of the sympathetic and parasympathetic nervous systems in the heart preparation. Tinney et al. (1976) confirmed the hypothesis of Lords et al. (1973). They showed that the RF exposure did not produce bradycardia if propranolol hydrochloride (which blocks the sympathetic nervous system) or atropine (which blocks the parasympathetic nervous system) is added to Ringer's solution. Reed et al. (1977) also observed bradycardia in isolated rat hearts exposed to the same frequency for 10 minutes at SARs in the range 1.5-2.5 W/kg, but not if they used the same blocking agents.

Galvin et al. (1981b) isolated cardiac muscle cells from the quail heart and exposed them in suspension for 90 minutes to 2.45-GHz CW RFR within a special water-filled-waveguide system at 37°C. The exposures were at mean SARs of 1, 10, 50, or 100 W/kg. After exposure, suspension samples were examined for the integrity of the cardiac cells, using the trypan-blue exclusion test. (An intact cell will exclude this vital stain.) The remainder of each suspension was centrifuged, the fluids were assayed for the release of enzymes creatine phosphokinase (CPK) and lactic acid dehydrogenase (LDH), and the residual pellets were resuspended. Pellets from some experiments were processed for examination by electron microscopy.

Trypan-blue exclusion was not affected by exposure at 1 W/kg, but the suspensions exposed at 10, 50, and 100 W/kg exhibited successively larger increases in percentages of cells permeable to the stain relative to their respective control suspensions. CPK release was not affected at any SAR. However, LDH release increased monotonically with SAR, but the increases relative to controls were nonsignificant except at 100 W/kg. By electron microscopy, the ultrastructure of heart cells exposed at 1, 10, and 50 W/kg, as well as control cells, appeared normal. However, cells exposed at 100 W/kg showed increased cytoplasmic vacuolization and chromatin clumping, but the intercellular junctions remained intact. Evidently the positive results of this study were thermally induced.

Galvin et al. (1982a) also isolated atria of spontaneously beating rat hearts, suspended the atria in tubes continuously perfused with solution, and exposed the specimens individually for 30 minutes to 2.45-GHz CW RFR at 2 or 10 W/kg in the water-filled waveguide system at 37°C or 22°C. Contractile force and beat rate were recorded periodically before, during, and after exposure. At 37°C and 10 W/kg, the beat rate of each specimen expressed as a percentage of its own rate at the start of exposure was not significantly different from 100%. At 22°C also, there were no significant differences in average beat rates between corresponding control atria. Average contractile force at 37°C was 640 mg for control atria and those exposed at 2 and 10 W/kg. At 22°C, it was 1,200 mg. These findings for isolated heart atria support the conclusion of Galvin and McRee (1981a), discussed below, that exposure of intact animals to CW RFR at the stated RFR levels has no influence on the myocardium or its neural components.

Yee et al., 1984 demonstrated that faster decreases in beat rate of RFR-exposed isolated frog hearts than those in the absence of RFR occurred only in hearts whose beat rates were recorded with a glass electrode filled with a potassium-chloride solution or with a metal-wire electrode; such effects were absent with an ultrasound probe, a tension transducer, or a glass electrode filled with physiologic (Ringer's) solution.

In summary, the authors of an early study reported the induction of tachycardia in isolated beating frog hearts by RFR pulses in synchrony with the EKG, but other researchers could not confirm this finding in either isolated hearts or in live animals. In some studies, RFR was reported to produce tachycardia (heart-rate increase) in excised frog or turtle hearts, whereas bradycardia (heart-rate decrease) was reported in others. Such positive findings were shown to be suspect, because of the use of attached or indwelling electrodes that probably introduced artifacts. Various kinds of electrodes were investigated, and special types were developed that were not perturbed by RFR or did not perturb the local RF fields. Studies in which such nonperturbing electrodes were used showed that heart rates were altered only at RFR levels that produced rises in temperature or otherwise added thermal burdens to the animal.

In other studies, effects of RF exposure were sought on the release of the enzymes creatine phosphokinase (CPK) and lactic acid dehydrogenase (LDH), and on the integrity of the cells from cardiac muscles isolated from the quail heart, using the trypan blue exclusion test. Stain exclusion was not affected by exposure at 1 W/kg, but muscles exposed at 10, 50, and 100 W/kg showed successively larger increases in percentages of cells permeable to the stain. The release of CPK was not affected at any SAR. However, the release of LDH increased with SAR, but the increases were not significant relative to controls except at 100 W/kg. In yet another study, RF exposure had no effect on beat rates or contractile forces of heart atria isolated from rats. Thus, little or no reliable experimental evidence exists that excised hearts or tissues therefrom are affected by exposure to RFR other than by thermal effects.

4.8.3.2 *In Vivo* Studies

Presman and Levitina (1963a, 1963b) were two early studies in which live rabbits were exposed for 20 minutes to 2.4-GHz CW RFR at 7-12 mW/cm^2 or to 3-GHz pulsed RFR at 3-5 mW/cm^2 average power density, 4.3-7.1 W/cm^2 pulse power density. During each exposure and for 10 minutes before and afterward, the EKGs of the rabbits were recorded with plate electrodes.

Neither tachycardia nor bradycardia was observed during exposure of the entire dorsal surface, but tachycardia was seen during the first 5 minutes after exposure, followed by bradycardia. However, dorsal exposure of the head only or of the back only produced significant tachycardia during exposure, with head exposure yielding the greater effect. Bradycardia was also seen during exposure in three ventral aspects. No original data were presented, only relative differences of means. The presence of metal electrodes during exposure undoubtedly introduced artifacts of sufficient size to render the results meaningless.

Kaplan et al. (1971) and Birenbaum et al. (1975) tried to replicate the studies of Presman and Levitina (1962a) but were unable to do so. Kaplan et al. (1971) found no significant difference between the heart rates of rabbits during or after exposure and the rates during no exposure. Birenbaum et al. (1975) found that changes in heart rate and respiration rate were the same for RFR and infrared radiation (IR), but subcutaneous temperature increased more rapidly and rose to higher values for the IR exposures.

Phillips et al. (1975) exposed rats to 2.45-GHz RFR for 30 minutes at 0, 4.5, 6.5, or 11.5 W/kg. Nonsignificant bradycardia was seen at 4.5 W/kg; mild but significant bradycardia developed within 20

minutes at 6.5 W/kg, followed by recovery in 2 hours; pronounced bradycardia occurred abruptly at 11.1 W/kg, after which the heart rates rose to values well above those of controls and persisted at the higher levels to the end of the test period. Incomplete heart block was evident, with recovery within 60 minutes after exposure end.

Chou et al. (1980) exposed rabbits dorsally or ventrally 20 minutes per day for 10 days to CW or pulsed 2.45-GHz RFR at 5 mW/cm^2 (average). Highest SARs for dorsal exposure were 0.86 W/kg in the brain and 0.09 W/kg in the heart; for ventral exposure, they were 0.24 and 0.30 W/kg. The rabbits were also exposed dorsally to RFR pulses synchronized to heart rate with a delay time of 0, 0.1, or 0.2 seconds relative to the R wave of the EKG (measured with carbon-loaded Teflon electrodes). No significant differences were seen between heart rates during the periods of exposure and nonexposure. Rabbits were also exposed to CW RFR at 80 mW/cm^2.

Galvin and McRee (1981) surgically produced myocardial ischemia (MI) [inadequate blood supply to the heart] in cats and sham exposed or exposed the hearts to 2.45-GHz CW RFR at 30 W/kg with an applicator. Cats without MI were similarly treated. Before and during the exposure of each cat, mean arterial blood pressure, cardiac output, heart rate, and EKG were measured, and blood samples were assayed for concentration of plasma protein and CPK activity. After exposure, the hearts were excised and assayed for creatine phosphokinase (CPK) activity. The results for both the MI and non-MI cats indicated no significant differences in mean arterial blood pressure, cardiac output, or heart rate between RFR-exposed and sham-exposed groups, and no synergy of ischemia and RF exposure. The excised hearts of the RFR and sham groups showed no significant differences in plasma or tissue CPK activity. Thus, localized exposure of either the undamaged or ischemic heart to the RFR *in vivo* had no effect on the myocardium or its neural components--results at variance with those for excised hearts exposed to RFR.

Galvin and McRee (1986) cannulated a femoral artery in anesthetized rats to permit continuous recording of mean arterial blood pressure and removal of blood samples. After the rats recovered, the mean arterial blood pressure and various cardiovascular, biochemical, and hematologic indices of each rat were recorded during exposure to 2.45-GHz CW RFR. No significant differences were found between RFR-exposed and sham-exposed rats in initial heart rates or in any of the blood parameters assayed, but during the first hour of exposure, the mean heart rate of the RFR group decreased about 10%, a significant drop, and remained there (with smaller variations) during the rest of the period. The authors surmised that the bradycardia was due to reduction of metabolic rate to compensate for the heat from the RFR.

In summary, various studies in which cardiovascular effects were sought from exposure of animals to RFR *in vivo* yielded negative findings except at RFR levels that clearly were hyperthermal, as evidenced by much increased respiratory rates and other manifestations of heat stress. Also, in a study of cats surgically given myocardial ischemia (MI), no significant differences were seen for both MI and non-MI cats between RFR-exposed and sham-exposed groups in mean arterial blood pressure, cardiac output, or heart rate. Also, no synergy of ischemia and RF exposure was found, an indication that humans with heart problems are most unlikely to be affected by exposure to RFR at or below the ANSI/IEEE (1992) or FCC (1996) maximum allowable levels.

4.9 RFR EFFECTS ON NATURALISTIC BEHAVIOR, REFLEX ACTIVITY, LEARNING, AND PERFORMANCE OF TRAINED TASKS

Numerous studies have been conducted on possible effects of RF exposure on various kinds of animal behavior. Representative papers on this topic have been selected for discussion in this section. Possible interactive effects of exposure to RFR and drugs on behavior and physiologic responses of animals are discussed in Section 4.10.

4.9.1 Rodents

Justesen and King (1970) trained food-deprived rats to lick a specially designed nozzle a prescribed number of times (usually 40, termed an FR-40 schedule) to receive a drop of dextrose-water solution. The rats were then exposed to 2.45-GHz RFR at temperatures ranging from 22°C to 26°C within a modified commercial microwave oven. Each session consisted of alternating 5-minute intervals of RFR and no RFR at equivalent power densities up to 15 mW/cm^2 (mean SAR about 45 W/kg) for 60 minutes total. The FR schedules were made more complex by the use of a 525-Hz tone as alternating reinforcement and extinction components presented at random intervals during a session. At 4.6 W/kg, the mean number of reinforcement responses decreased to about a third of the number in the absence of RFR, decreases primarily ascribed to cessation of responding, especially near session end, probably associated with warming of the rats. At 0.8 or 1.6 W/kg and with more complex FR-20 schedules, none of the rats responded much above baseline levels. Rectal temperatures taken after exposure at 4.25 W/kg showed rises of more than 2°C. Histological examination of the RFR-exposed rats and other untreated rats showed no significant differences indicative of adverse effects of the RFR

Hunt et al. (1975) sham-exposed and exposed rats to 2.45-GHz RFR for 30 minutes at 6.3 W/kg within a modified microwave oven, after which their exploratory movements were recorded. Both groups showed decreasing activity. The RFR group's activity diminished faster than for the sham group, but the activities of both groups became comparable toward session end. Rats were also trained to swim a 6-meter channel forth and back repeatedly during a 24-hour period, and each rat was scored for its median swim speed for each successive block of 20 traverses after exposure for 30 minutes at 6.3 or 11 W/kg. Right after exposure at 6.3 W/kg, the mean speed of the RFR group was comparable to that of the sham group for the first 200 traverses, it then decreased for the next 100 traverses, but then became comparable to the sham group again. The colonic temperatures of the 11-W/kg group immediately after exposure indicated severe hyperthermia (41°C or higher) despite partial relief by water immersion, and its performance after exposure was clearly impaired by the hyperthermia. However, testing after a 1-day delay showed recovery.

The performance of the 6.3-W/kg group was similar to that of the sham group for about 200 traverses, but was below mean control speed for about the next 100 traverses, after which both groups performed comparably once more. Colonic temperatures measured immediately after treatment showed that those exposed at 11 W/kg were rendered severely hyperthermic (41°C or higher), with partial relief by water immersion for those tested right after exposure. At 11 W/kg, the performance of the group tested right after exposure was clearly impaired by the hyperthermia, despite the partial relief from water immersion. However, when the 11-W/kg group was tested 1 day later it showed recovery from the hyperthermia and yielded results similar to those of the 6.3-W/kg group.

The authors also trained water-deprived rats to press a lever in a complex task to obtain small quantities of saccharin-flavored water, after which groups were exposed for 30 minutes at 0, 6.5, or 11 W/kg on

successive days and tested immediately each day for 30 minutes. The results were given in terms of the percentages of errors of omission or commission versus elapsed time at 5, 15, and 25 minutes of testing. Following sham exposures, the mean errors of omission were 10-15%; after 6.5 W/kg, they were 36% at 5 minutes of testing, but dropped to the sham-exposure range at 15 and 25 minutes. After 11 W/kg, however, the percentages were all much higher than sham-exposure values. No significant differences were found in mean errors of commission among the treatments.

Monahan and Ho (1976) demonstrated that mice tend to lean against the waveguide wall instead of stay in the middle of the waveguide. Thus with this behavior they minimize their absorption of 2.45-GHz CW RFR in a waveguide held at 24°C because the electric field diminishes to zero at the wall. The absorption decreases were determined from the decreases of SAR with time as calculated from measurements of forward and reflected power in the waveguide.

Lin et al. (1977), Schrot et. al. (1980), Gage and Guyer (1982), and Lebovitz (1981, 1983) studied the effects of RFR on rats trained to perform various behavioral tasks. The results of Lin et al. (1977) with food-deprived rats exposed to 918-MHz RFR at 10, 20, or 40 mW/cm^2 (2.1, 4.2, or 8.4 W/kg) showed significant changes in performance rate only for 8.4 W/kg, at which those rats exhibited heat stress, including panting, fatigue, and foaming of the mouth. Schrot et. al. (1980) found that rats trained in a more complex task involving responses to auditory stimuli and sequences of lever presses responded with higher error-responding rates, lower sequence-completion rates, and changes in the normal acquisition pattern when exposed to pulsed 2.8-GHz RFR at an average power density of 10 mW/cm^2 (1.7 W/kg) than at 5 mW/cm^2 (0.85 W/kg); below 5 mW/cm^2, the rats but were basically unaffected. Gage and Guyer (1982) trained rats to do a complex task to receive food pellets, after which they exposed the rats to 2.45-GHz RFR for 15.5 hours at 8 or 14 mW/cm^2 (1.6 or 2.8 W/kg) at ambient temperature at 22°C, 26°C, or 30°C without access to food or water. At treatment end, the rats were given water for 10 minutes and then tested. At each temperature, their response rates decreased with increasing RFR level.

Lebovitz (1981) concurrently sham-exposed and exposed groups of food-deprived rats in individual waveguides to 1.3-GHz pulsed RFR. Each waveguide contained a bar and means for illuminating the bar as a visual cue and for delivering a food pellet. Prior to exposure, food-deprived rats were trained to press their bars in a complex schedule of illumination to receive food pellets.

One group each was exposed at 1.5, 3.6, or 6.7 W/kg. The response rates at various intervals of the group exposed at 1.5 W/kg for 8 weeks were stable and not significantly different from those of its sham group at corresponding intervals. This was also true for the group exposed for 9 weeks at 3.6 W/kg, also showed no significant differences in SD response rates. The group exposed for 6 weeks of at 6.7 W/kg also showed no significant differences in overall response rates, but its response rates at specific intervals were significantly lower than for its sham group, differences ascribed to fewer in bar pressings near the end of those behavioral sessions. The author noted that 6.7 W/kg is the approximate resting metabolic rate for a 240-g rat, so exposure at that level doubled its heat dissipation requirements. Thus, he concluded that thermal factors were probably involved in those differences

Lebovitz (1983) obtained similar findings with 1.3-GHz CW RFR at 5.9 W/kg and 6.7 W/kg pulsed RFR. (The small SAR difference was necessitated by equipment limitations.) The author noted the thermal basis for the behavioral changes and a threshold of about 3.5 W/kg irrespective of whether the RFR was CW or pulsed.

D'Andrea et al. (1986a) exposed one group of rats to 2.45-GHz CW RFR at 0.5 mW/cm^2 (0.14 W/kg) for 7 hours a day on 90 consecutive days, totaling 630 hours. Another group was sham exposed. No significant differences were found in daily measures of body masses or intake of food and water. Monthly tests of each rat for its threshold reactivity to electric footshocks of varied intensity within a gridded-floor chamber showed no significant differences from those for sham-exposed rats. After the 90 days of treatment, half the rats in each group were assessed for open-field behavior, shuttlebox performance, and lever pressing for food pellets on an interresponse time schedule. Major changes were seen in daily trials of the open-field tests, but no significant differences were ascribable to RF exposure.

Shuttlebox performance was tested using a tone and a white light as a compound conditional stimulus (CS), and electric shock as an unconditional stimulus (UCS). Two days after shuttlebox testing, the rats were deprived of food and were trained to press a lever twice within a progressively tighter specific time interval between the presses to obtain a food pellet. The shuttlebox responses were highly variable, but overall, the differences were not statistically significant. The results of this study and of two similar studies in the same laboratory (D'Andrea et al., 1986b; DeWitt et al., 1987) were not fully consistent but showed little if any statistically significant differences between RFR-exposed and sham-exposed rats. However, the authors suggested that the threshold for behavioral responses to 2.45-GHz RFR in rats may be in the range 0.5-2.5 mW/cm^2 (0.14-0.70 W/kg).

Mitchell et al. (1988) exposed rats to 2.45-GHz CW RFR at 10 mW/cm^2 (2.7 W/kg) for 7 hours in individual plastic cages that permitted free movement. Right after treatment, each rat was tested for vertical and horizontal spontaneous locomotor activity, acoustic startle response, and retention of a shock-motivated passive avoidance task. Lower activity was seen in the RFR-exposed rats than the sham-exposed rats, especially during the second half of test sessions. The mean of startle responses of the RFR-exposed rats was significantly lower than for the sham-exposed rats. Immediately after the startle-response test, each rat was placed in the lighted smaller part of a gated, two-chamber shuttle box, the larger part of which was dark and equipped to deliver an electric shock. If the rat moved into the larger chamber within 2 minutes, it was given a 1-second shock; if it stayed in the smaller chamber for more than 2 minutes, it was removed and not tested further. A week later, retention of the shock experience was tested in the box. The differences in avoidance activity between the RFR and sham groups were not significant.

Akyel et al. (1991) trained rats on three behavioral schedules to press a lever for food pellets, after which each rat was exposed once a week for 10 minutes to pulses of 1.25-GHz RFR at 1-MW peak forward power. The average forward power was held constant, during any session, at 4, 12, 36, or 108 W, obtained by changing the pulse repetition frequency. Each rat was given all four RFR levels in a weekly quasi-random order. The corresponding whole-body doses or specific absorptions (SAs) and whole-body SARs respectively ranged from 0.5 to 14.0 kJ/kg and 0.84 to 23.0 W/kg. Testing of each rat was begun in less than 80 seconds after exposure end. At the three lower RFR levels, no significant differences in any of the behavior schedules were seen. At the highest level (14.0 kJ/kg, 23.0 W/kg), however, the rats trained on two of the schedules failed to reach baseline performance and those on the third schedule displayed variable effects. Those exposures caused an average rise of 2.5°C in colonic temperature. The authors concluded that those behavioral changes were thermally induced.

Walters et al. (1995) exposed rats to ultrawideband (UWB) RFR pulses of duration 7-8 nanoseconds at very high peak powers at a repetition rate of 60 pps for 2 minutes, and measured their rectal temperatures just before and after RFR- and sham exposure. The bandwidth was about 0.25-2.50 GHz and the peak electric field strength was 250 kV/m at 0.25 GHz, (far-field equivalent power density 1.7 GW/cm^2). One

behavioral test was swimming performance to exhaustion, given the rats one day before treatment and again three hours after treatment. In the test, each rat was required to swim in cylindrical container at a water depth of 45 cm until the time in seconds when the rat was unable to break the water surface for 10 seconds. For the test, a lead weight was affixed to the tail of each rat corresponding to 3% of its body weight.

The rats were also given a functional observational battery (FOB) of tests consisting of 29 physiological and behavioral measures that yield continuous, rank order, count, descriptive, and quantal data. Blood was collected at 2 and 48 hours after treatment and assayed for four serum enzymes shown to be sensitive to narrow-band microwave-induced stress. Brain slices from RFR-exposed and sham-exposed rats were stained and examined by light microscopy for expression of c-fos protein (the product of the c-fos proto-oncogene). A group of rats injected with hypertonic saline solution served as positive controls.

No significant differences were evident between RFR-exposed and sham-exposed rats in the swimming tests or in any of the other tests. The authors remarked that c-fos protein expression would be expected to be the most sensitive test for RFR effect, because it is induced in response to many stimuli including light, heat stress, electrical stimulation of the brain, and cerebral ischemia. Negative results were also obtained in rhesus monkeys by Sherry et al. (1995) with the same RFR source (next section).

In summary, many of the studies on avoidance behavior indicate that RFR could be a noxious or unpleasant stimulus. There is much evidence, however, that changes in such behavioral patterns induced by RFR are responses by the thermoregulatory systems of the animals, either to minimize absorption of heat in normal or warm ambient environments (including high levels of humidity) or to obtain warmth in relatively cold environments. Behavioral effects involving pulsed microwaves can be due to the auditory response of the animal. The results of studies on disruption of performance or learned behavior by RFR were variable. However, most of the findings showed that the behavioral changes were ascribable to the added thermal burden imposed by the RFR.

4.9.2 Nonhuman Primates

Galloway (1975) trained rhesus monkeys in a discriminative behavioral task to press one or more of three levers on panels when the panels were selectively lit, to receive a food pellet. After training, the head of each was exposed to 2.45-GHz RFR at estimated mean head SARs of 7, 13, 20, 27, and 33 W/kg. The RFR was administered for 2 minutes just before each behavioral session but terminated earlier if the monkey began to convulse. In addition, monkeys were exposed at 13 W/kg for 5 daily 1-hour schedules of 2 minutes on and 1 minute off, totaling 40 minutes of exposure per day. Convulsions occurred for all exposures at 33 W/kg, and often at 20 W/kg. No effects on discriminative behavior were evident at the lower RFR levels which is a relatively large change in effects.

In a repeated-acquisition test, each monkey was required to press the correct lever for each of four illumination stimuli in the proper sequence, which was changed each day. In sessions just before RF exposure, a slight learning trend (diminishing error rate) was seen, but the changes were too small to ascribe significance thereto. This was also true for the results at all RFR levels except 33 W/kg, for which the error rate at session start was highest. Thus, except possibly for the latter result, the RFR had no effect on this behavioral paradigm.

Cunitz et al. (1975) trained a 3-kg and a 5-kg rhesus monkey on a complex serial reaction program, after which the head of each monkey was inserted through a hole in the bottom of a 383-MHz resonant cavity, with the monkey facing a diamond array consisting of the ends of four light pipes mounted through the cavity's side wall. To light any pipe, the monkey was required to move a lever to the left, right, up, or down to correspond with the position of that pipe end in the diamond. Each monkey was exposed to 383-MHz RFR at successively higher input powers up to 15 W (at estimated head SARs up to 33 W/kg and 20 W/kg respectively for the two monkeys). The larger monkey was also exposed at 17.5 W (23 W/kg). The lowest SARs for diminished performance by the two monkeys were about the same: 22 and 23 W/kg.

Scholl and Allen (1979) trained rhesus monkeys in a visual-tracking task that required each monkey to move a lever to hold a continuously moving spot within a prescribed clear area on the screen of a display monitor. The spot was moved electronically in a specific pattern, and lever responses generated continuous difference signals (errors). The central 15% of the screen was clear and comprised the on-target area. The monkey received a 0.1-second electric shock for each 1 second accumulated outside the clear area.

After training, the monkeys were exposed to horizontally polarized, 1.2-GHz CW RFR at 10 mW/cm^2 and 20 mW/cm^2 (measured at the center of the head in the absence of the monkey) for 2 hours a day at 2-day intervals until each was exposed for 120 minutes at each level. This polarization and frequency were selected to provide half-wave resonant absorption in the monkey head. The corresponding head SARs were 0.8 and 1.6 W/kg. Each daily session comprised 40 work trials of 1.5 minutes each, alternating with similar rest periods. The endpoint scored was the adjusted root mean square (ARMS) of the tracking error for each trial, expressed as a percentage of the total target area. Of 720 data points collected during a total of 36 hours of RF exposure, only 4 points were outside the 95% confidence limits, fewer than expected by chance. Clearly, the performances of the monkeys were not diminished by the exposure. Whether the apparent performance improvement observed was RFR-related could not be ascertained.

De Lorge (1976) trained 3 rhesus monkeys to perform a task in which each was required to press a lever in front of its right arm, thereby producing either a low-frequency tone to signal that no food pellet is available, or a higher-frequency tone for which the monkey had to press a lever in front of its left arm to receive a pellet. During 1-hour sessions, pellets were made available at variable intervals (VIs) around 30 seconds (VI-30-s schedule). During the 2-hour training sessions, pellets were made available on a VI-60-s schedule. After training, the monkeys were exposed frontally to 2.45-GHz RFR at levels in the range 4-72 mW/cm^2 measured at head height. The estimated head SARs were 0.4-7.2 W/kg. Sessions of VI-30-s behavior were conducted on each monkey lasting 1 hour, during 30 minutes of which it was being exposed at a head SAR of 0.4 or 1.6 W/kg. At either RFR level, the performances of the monkeys were not affected by the RFR, which led to the use of the VI-60-s schedule during 2-hour test sessions. In the 2-hour sessions, the monkeys were exposed for 1 hour at levels in the range 1.6-7.2 W/kg. One of them was also exposed at 1.6 W/kg during the entire 2-hour sessions. No significant departures from control rates were exhibited by 2 monkeys at 6.2 W/kg, and at 5.2 W/kg for the third monkey. At 6.2 W/kg, the performance of that monkey was about 80% of its mean control performance.

De Lorge (1979) also trained four squirrel monkeys in 1-hour sessions to press either the right or the left lever on top of a chair to obtain a food pellet. Initially, each successive lever press resulted in the alternate activation of a red light and a blue light in front of the monkey. When consistent performance was achieved, the contingencies were changed so that press of the left lever was rewarded only when the blue light was on, with right-lever presses continuing to alternate the red and blue lights. Each monkey was then exposed from above to 2.45-GHz RFR at levels in the range 10-75 mW/cm^2. SARs were estimated to have

been 0.5 to 3.75 W/kg. In 41 daily sessions, exposures were done during the middle 30 minutes of 1-hour test sessions, with the other two 15-minute periods used to obtain baseline data. In the next 53 sessions, the session duration was 2 hours, and the RF exposures were during the middle 1 hour. Neither the 30-minute nor the 60-minute exposure regimens caused any obvious permanent physical changes in any of the monkeys.

Among the various performance measures, only the rate of right-lever responses showed an RFR-induced change. This measure showed a slight trend toward lower rates with increasing RFR level, to a minimum of about 90% of the mean control value at 60 mW/cm^2 (3.0 W/kg), and a slightly higher value (92%) at 70 mW/cm^2 (3.5 W/kg). The behavioral effects of 1-hour exposures were similar to those of 30-minute exposures but more pronounced. No consistent behavioral changes occurred below 50 mW/cm^2 (2.5 W/kg); above that level, the effects increased with RFR level. The author concluded that the behavioral changes seen in the squirrel monkeys were temporary and clearly related to hyperthermia. Consistent changes were seen when rises in rectal temperature exceeded 1°C, which corresponded to a threshold between 40 and 50 mW/cm^2 (2.0-2.5 W/kg).

De Lorge (1984) similarly trained food-deprived rhesus monkeys to do a task in which each monkey was to press a lever in front of its right hand (an observing response), which produced a low tone to signal that no food pellet will be delivered, or a high tone to signal availability of a pellet. No tones were presented without a lever press, and right-lever presses during the presence of either tone did nothing. After stable performance was attained, each monkey was frontally exposed during 1-hour sessions to vertically polarized 225-MHz CW RFR (near whole-body resonance), or to pulsed RFR at 1.3 GHz or 5.8 GHz (both above whole-body resonance). The SAR ranges were 2.0-4.4 W/kg for 225 MHz, 2.6-12.4 W/kg for 1.3-GHz, and 0.34-4.7 W/kg at 5.8 GHz. Reductions in observing-response rates occurred at levels of 1.3-GHz RFR above a threshold of 6.5 W/kg.

For example, one monkey exhibited decreases in observing-response rates during exposure to 1.3-GHz RFR at 50 mW/cm^2 (6.5 W/kg) and higher, and the rate reduction became larger toward the latter part of each session as the RFR level was raised. Also, the observing-response patterns became increasingly erratic during sessions--an effect most pronounced at 225 MHz (near resonance), at which they paused for as much as 15 minutes and often stopped responding at all for the last half of a session at 10 mW/cm^2 (4 W/kg).

As a more definitive index of behavioral change than the individual cumulative records, the author plotted the mean and standard error (SE) of the ratio of the observing-responses during each RFR session at each frequency to the values during the preceding sham session versus the power density. The results for each frequency yielded a threshold power density for significant behavioral alterations that rose with frequency: 7.5 mW/cm^2 at 225 MHz, 63 mW/cm^2 at 1.3 GHz, and 140 mW/cm^2 at 5.8 GHz. However, the corresponding threshold whole-body SARs varied up and down with frequency: respectively 3 W/kg, 8.2 W/kg, and 4.3 W/kg, presumably because of the frequency-dependent differences in penetration depth and absorption properties.

The detection-response rates on the food lever were not consistently affected by RF exposure at any frequency. No effect was observed for 225 MHz or 5.8 GHz; for 1.3 GHz, a decreased detection-response rate was observed occasionally only at 83 mW/cm^2 or higher. However, plots of the mean ratio of detection-response latencies during RF exposure to those during sham exposure versus power density showed values slightly but significantly higher than 1 at all three frequencies and at most power densities.

At each frequency, the mean ratio changed both upward and downward with power density, but with an overall downward trend.

Postreinforcement pause (a pause after a reinforced detection-response) was also affected. The mean ratio for exposure to 225-MHz RFR to that for sham exposure was 1.0 in the range 5-7.5 mW/cm^2, but rose significantly to 1.5 at 10 mW/cm^2. With 1.3 GHz, the changes were both upward and downward, but nonsignificant up to 63 mW/cm^2, at which the mean ratio was 1.3. Above 63 mW/cm^2, it decreased to 1.1 at 93 mW/cm^2; but the latter ratio was still significantly larger than 1.0. The only significant change for 5.8 GHz was at 150 mW/cm^2, to 1.06, a smaller increase than for the other frequencies.

The mean colonic temperature at the start of the 1-hour sessions rose an average of 0.15°C during the sham-exposure sessions. For 225 MHz, the rises were linear with RFR level, from 0.8°C at 5 mW/cm^2 to 2.1°C at 10 mW/cm^2. For 1.3 GHz, they were smaller, and for 5.8 GHz, they were even more gradual.

The author's estimates of the absolute thresholds for the disruption of observing-response rates for each frequency were: 8.1 mW/cm^2 for 225 MHz, 57 mW/cm^2 for 1.3 GHz, 67 mW/cm^2 for 2.45 GHz (from de Lorge, 1976), and 140 mW/cm^2 for 5.8 GHz--values that increased with RFR frequency. However, the corresponding SARs, 3.2 W/kg, 7.4 W/kg, 6.7 W/kg, and 4.3 W/kg, varied both upward and downward with frequency, perhaps reflecting penetration-depth differences and absorption properties again.

D'Andrea et al. (1989) trained food-deprived rhesus monkeys to operate three levers (left, right, center) in various sequences to obtain food pellets. The sessions were 60 minutes long. The task during each session comprised three successive 10-minute schedules of lever presses, followed by repetition of the same three schedules.

In the first 10-minute schedule, the monkey was required to withhold responding for 8 seconds after the start of a tone, and then to respond only within the next 4 seconds; the correct response during those 4 seconds was 2 presses of the left lever within 2 seconds of each other. The authors called this an interresponse-time (IRT) schedule. The second 10-minute period was devoted to a time-discrimination (TD) schedule, in which a press of the center lever in the presence of blue light randomly yielded white light of short or long duration. At the end of either duration, the white light was replaced with red and green light. When the red and green light were present, the monkey, to obtain a pellet, had to press the right lever if the preceding white light was of short duration, or the left lever if the preceding white light was of long duration. During the third 10-minute period, a fixed-interval (FI) schedule was used in which the monkey was presented with a continuous tone of higher frequency, and its first press of the right lever after 55 seconds yielded a pellet.

During the 60-minute sessions, each monkey was sham exposed or exposed from above to pulses of 1.3-GHz RFR at a pulse power density of 131.8 W/cm^2. The peak SAR was 15.0 W/kg in the head and 8.3 W/kg in the whole body. The pulse repetition rates ranged from 2 pps to 32 pps, with corresponding average power densities of 0.92 mW/cm^2 to 14.80 mW/cm^2. No significant differences were seen between sham exposures and RF exposures in any of the behavioral responses.

Sherry et al. (1995), in a study similar to Walters et al. (1995) with rats, used the Primate Equilibrium Platform (PEP) to investigate whether exposure to the RFR from an ultrawideband (UWB) transmitter would alter the behavior of rhesus monkeys. The PEP provides a continuous compensatory task: The animal is seated in a restraint chair that rotates on the pitch axis about the animal's center of gravity.

Random perturbations in pitch are generated by a computer at unpredictable intervals. Large variations in platform position occur with no monkey present, with a standard deviation of 12-15°. While seated in the restraint chair, the monkey is required to manipulate a joystick control to compensate for the variations in pitch. Whenever the platform position deviates by more than 15° from the horizontal, the monkey is given a mild electric shock to the tail. A well-trained monkey can reduce the variation to 2-4° and receive less than 1 shock per hour.

Six unanesthetized adult male rhesus monkeys were trained to perform the PEP task. Although there were characteristic baseline differences among them, the level achieved by each was extremely stable. For this study, the monkeys were tested for 1 hour per test at least four months before exposure to RFR and at least twice weekly during the experiments. The RF exposures were done within an anechoic chamber, with each monkey in a restraining chair facing the boresight of the transmitter antenna. The RFR consisted of 2-5 nanosecond pulses at a repetition rate of 60 pps administered for 2 minutes (7200 pulses total). The bandwidth of each pulse was 100 MHz to 1.5 GHz, with the highest power in the band 250-500 MHz and a peak E-field strength of 250 kV/m. Each monkey was exposed twice, with 6 days between exposures, and was tested for 1 hour after each exposure and at 24-hour intervals for the next five days.

At the maximum field strength available and the highest repetition rate, no core-temperature increase was detected, so no SAR data could be collected. However, theoretical calculations using the pulse characteristics of the RFR yielded a whole-body SAR of 0.5 W/kg. The only significant effects were for individual differences among the subjects and changes of each during the 12 five-minute intervals of each hour of test, with no effects due to the RF exposures per se. The authors remarked that the changes with time during the 12 five-minute intervals were due to a tendency for performance to decline during the course of test sessions, independent of exposure.

The authors also indicated that time constraints in the use of the equipment did not permit sham exposures to control for the passage of time. Last, they mentioned the concerns about human exposure to ultrashort-risetime RFR raised by Albanese et al. (1994) after completion of this study, and the response to Albanese et al. (1994) by Merritt et al. (1995). Merritt et al. (1995) indicated that the experimental results of this study and the one by Walters et al. (1995) with rats (see Section 4.9.1) do not support any special human-safety concerns about such RFR.

In summary, RF exposure can alter the behavior of non-human primates, but primarily at levels high enough to produce behavioral thermoregulatory responses (voluntary or non-voluntary), but there is little or no experimental evidence of adverse effects at thermogenic levels that are within the compensatory capabilities of the thermoregulatory systems of the animals. It is worthy of emphasis that the behavioral findings of the primate studies are more relevant than those with the other animal species with regard to possible effects of RFR on human behavior, because the tasks the primates had to learn were far more complex, and because their physiologies and intelligences are much closer to those of humans. It is also noteworthy that reasonably accurate thresholds for RFR-induced behavioral changes were determined for each primate species, and that those thresholds served as the primary basis for the ANSI/IEEE (1992) human-exposure guidelines.

4.10 RFR AND DRUGS

Thomas et al. (1979) trained food-deprived rats to press a bar for a food pellet. After stable baseline patterns were achieved, an effect-versus-dose function for the psychoactive drug chlordiazepoxide