STUDIES OF GENOTOXICITY AND APOPTOSIS USING HUMAN LYMPHOCYTES OR MURINE NEUROBLASTOMA CELLS EXPOSED *IN VITRO* TO RADIOFREQUENCY FIELDS CHARACTERISTIC OF MOBILE PHONES.

A thesis submitted for the degree of Doctor of Philosophy

by

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Abstract

The aim of the study was to investigate whether non-thermal levels of radiofrequency (RF) fields, characteristic of some mobile phones, might be directly genotoxic when applied *in vitro* to unstimulated G_0 or stimulated human lymphocytes. Also, the study aimed to investigate the possibility that RF fields might act epipigenetically when combined with x-rays, by modifying their effect when applied *in vitro* to G_0 lymphocytes. In addition, the possibility of RF fields inducing apoptosis in murine neuroblastoma (N2a) cells was also examined.

G_0 lymphocytes from 4 donors were exposed for a total of 24 h to a continuous or an intermittent RF signal. The signals were 935 MHz GSM (Global System for Mobile Communication) Basic, 1800 MHz GSM Basic, 935 MHz continuous wave (CW) carrier frequency, and 935 MHz GSM Talk. Stimulated lymphocytes were exposed for a total of 48 h to intermittent 1800 MHz RF signals that were GSM Basic or the carrier frequency only. The RF fields used for the 24 h exposure of N2a cells were all at 935 MHz and consisted of GSM Basic, GSM Talk and a CW signal. The chosen Specific energy Absorption values of the signals were either 1 or 2 W/kg. These values are near the upper limit of actual energy absorbed in localised tissue by a person from some mobile phones. The field was applied to G_0 human lymphocytes either alone or combined with an exposure to 1 Gy x-rays given immediately before or after the RF field. A dose of 4 Gy x-rays was used as a positive control for apoptosis induction in N2a cells and in the study with stimulated lymphocytes no x-rays were used.

The lymphocytes were assayed by several standard methods to demonstrate genotoxicity. Unstable chromosome aberrations (stimulated lymphocytes and those exposed in G_0), sister chromatid exchanges (SCE) and cytokinesis blocked micronuclei (MN) (lymphocytes exposed in G_0). In addition the SCE and MN assays allowed nuclear division indices (NDI) to be calculated as NDI defines the cell cycle progression of lymphocytes after PHA stimulation and how this might be affected by RF exposure. N2a cells were assessed by fluorescence microscopy for levels of apoptosis at a number of time points post RF field or x-ray exposure, between 0 and 48 h. Three independent assays that detect different stages of the apoptotic pathway were used, the Annexin V binding, caspase activation and *in situ* end labelling.

By comparison with appropriate sham exposed samples no effect of RF fields alone could be found in G_0 or PHA stimulated lymphocytes exposed *in vitro*. Also, RF fields did not modify any measured effects of x-rays either given before or after RF exposure. No statistically significant difference in apoptosis levels were observed between RF exposed and sham exposed N2a cells in either a proliferating or differentiated state for any assay at any time point post exposure.

These data suggest that RF exposures characteristic of GSM mobile phones are not directly genotoxic nor do they influence the genotoxicity of the well established clastogenic agent, x-radiation. Also, that apoptosis levels in proliferating and differentiated murine neuroblastoma cells are not significantly affected by RF field exposure. Within the experimental design used in these investigations, in all instances, no effect from the RF signal was observed.

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List of Abbreviations

A

AIF	apoptosis inducing factor
AM	amplitude modulation
ANOVA	analysis of variance

C

^{o}C	degrees Celsius
CA	chromosome assay
CDMA	Code Division Multiple Access
CHO	Chinese hamster ovary
CO_2	carbon dioxide
CTF	comet tail factor
Cu	copper
CW	continuous wave

D

DAMPS	Digital Advanced Mobile Phone System
DAPI	4', 6-diamidino-2-phenylindole
DCS	Digital Cellular System
DF	degrees of freedom
DMEM	Dulbecco's Modified Eagles Medium
DNA	deoxyribonucleic acid
drib	2-deoxy-D-ribose
DSB	double strand break
DTX	discontinuous transmission
dUTP	deoxyuridine triphosphates

E

ELF	extremely low frequency
EMF	electromagnetic fields

F

FBS	foetal bovine serum
FDMA	Frequency Division Multiple Access
FITC	fluorescein isothiocyanate
FM	frequency modulated
FMCW	frequency modulated continuous wave
FPG	fluorescence plus Giemsa

G

GHz	gigahertz
GMSK	Gaussian minimum shift keying
GSM	Global system for Mobile Communications
Gy	Gray

H

h	hour(s)
HPA	Health Protection Agency
HSP	heat shock protein
HVL	half value layer
Hz	Hertz

I

IAEA	International Atomic Energy Agency
ICNIRP	International Commission on Non-Ionising Radiation Protection
IEGMP	Independent Expert Group on Mobile Phones
iDEN	integrated Digital Enhanced Network
IT'IS	Information Technologies in Society Foundation

J

J/m2	Joules per metre squared

K

kg	kilogram
kHz	kilohertz

M

M	molar
MEM	Minimum Essential Medium
MHz	megahertz
min	minute
mL	millilitre
mm	millimetre
mM	millimolar
MMC	mitomycin C
MN	micronucleus assay
MTLs	mean tail lengths
MX	3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone

N

N2a	murine neuroblastoma Neuro2a cells
NDI	Nuclear Division Index
NRPB	National Radiological Protection Board

P

PARP	poly ADP ribose polymerase
PBS	phosphate buffered saline
PHA	phytohaemagglutinin
PI	propridium iodide
PS	phosphatidylserine

PW	pulsed wave

R

RF	radiofrequency
RPM	radical pair mechanism
RSC	The Royal Society of Canada
RTL	radial transmission lines

S

SAR	specific energy absorption rate
SCE	sister chromatid exchange
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SD	standard deviation
SE	standard error
SSB	single strand break
SSI	Swedish Radiation Safety Authority
sXc	system for the exposure of cells

T

TDMA	Time Division Multiple Access
TdT	deoxynucleotidyl transferase
TEM cell	transverse electro-magnetic cell
TUNEL	terminal deoxynucleotidytransferase dUTP nick end labelling
TV	television

U

UMTS	Universal Mobile Telecommunications System
UV	ultra violet
UVC	ultra violet C
µg	microgram

V

VHF	very high frequency

W

W	Watts
W-CDMA	Wideband Code Division Multiple Access
WHO	World Health Organisation

Y

YO-PRO®-1	Quinolinium, 4-0(((3-methyl-2(3H)-benzoxazolylidene)methyl)-1-03-(trimethylammonio)propyl)-, diiodide

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Authors Declaration

I declare this thesis entitled ‘Studies of genotoxicity and apoptosis using human lymphocytes and murine neuroblastoma cells exposed *in vitro* to radiofrequency fields characteristic of mobile phones’ submitted to Brunel University for the degree of Doctor of Philosophy has not previously formed the basis for any award at this University or any other similar institution. All the work described in this thesis is my own, with the exception of the method for calculating errors on the Nuclear Division Index devised by Dr. R Haylock and Dr. E Ainsbury, (Health Protection Agency). Where the data came from a joint research project this is indicated in the text, but my results alone are summarised. All the assistance I have received has been clearly acknowledged and all the sources of information used have been specifically referenced.

Some of the work presented in this thesis has been published in peer reviewed journals:

Stronati, L., Testa, A. Moquet, J., Edwards, A., Cordelli, E., Villani, P., Marino, C., Fresegna, A.M., Appolloni, M. and Lloyd, D. (2006) 935 MHz cellular phone radiation. An *in vitro* study of genotoxicity in human lymphocytes. *International Journal of Radiation Biol*ogy. 82(5), 339-346.

Moquet, J., Ainsbury, E., Bouffler, S. and Lloyd, D. (2008) Exposure to low level GSM 935 MHz radiofrequency fields does not induce apoptosis in proliferating or differentiated murine neuroblastoma cells. *Radiation Protection Dosimetry*. 131(3):287-296.

1. Literature Review

1.1 Introduction

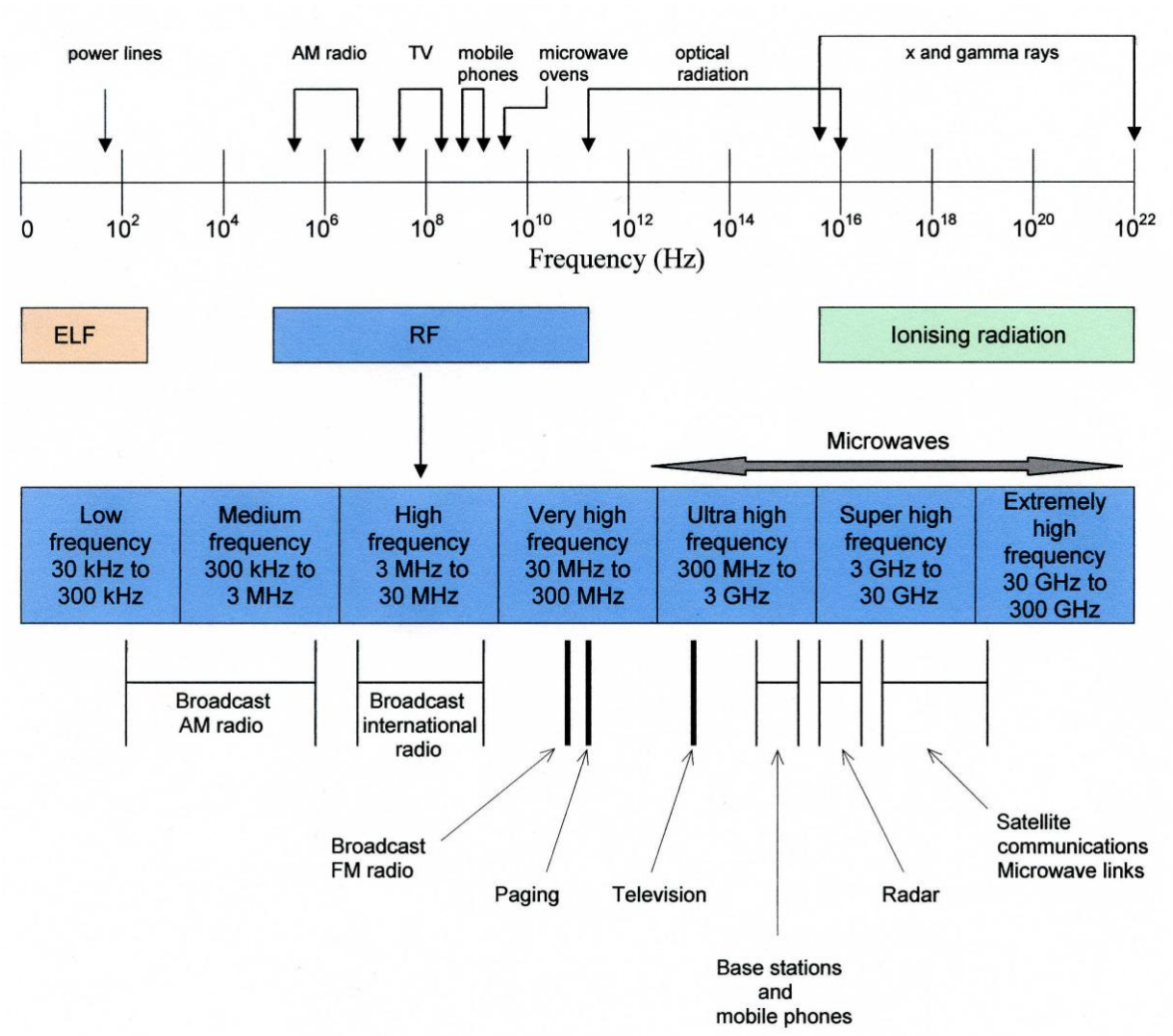

Figure 1.1. The electromagnetic spectrum with the radiofrequency section enlarged.

The electromagnetic spectrum is shown in Figure 1.1. It extends from extremely low frequency fields (ELF) to ionising gamma radiation. X and gamma rays have frequencies of > $3x10^{15}$ Hertz (Hz), and when they pass through matter the energies of their photons are high enough to cause ionisation by liberating atomic electrons from molecules leaving them positively charged; hence they are known as ionising radiation. The energy deposited in cells by this type of radiation, is sufficient to break chemical bonds and damage DNA. The health

effects that can be caused by exposure to ionising radiation are well understood. At the other end of the spectrum ELF fields have a frequency of > 0 to 300 Hz. ELF fields are produced in the generation and distribution of electricity. The only established mechanism for ELF fields interacting with biological material is through the induction of electric currents in the body, although there are a number of other proposed mechanisms. Radical pair production is considered a plausible possibility (Timmel and Henbest 2004). A radical pair is produced by the transfer of an electron from a donor to an acceptor molecule upon excitation e.g. by the absorption of light or ionising radiation. The transfer between a pair of molecules results in each having an unpaired electron, thus making them highly reactive. After the creation of a radical pair, magnetic fields can alter the dynamics of the transitions between the spin states of their unpaired electrons and influence chemical reactions. There is some evidence that this occurs in biological systems particularly in the magnetic sense of some animals (Ritz et al. 2004). It has been suggested that the radical repair mechanism (RPM) is located in the eye and is mediated by blue-light photoreceptors known as cryptochromes (Gegear et al. 2008). Cryptochromes have been discovered in the human eye (Thompson et al. 2003), but is not known to what extent the RPM occurs. However, health concerns persist and much of the research on ELF fields has paralleled that on the health effects of RF fields.

RF fields have a frequency of about $3x10^4$ to $3x10^{11}$ Hz and Figure 1.1 shows that within this range there are many applications generating RF fields, including mobile phones which transmit and receive at frequencies of about 900, 1800 and 2100 MHz. Mobile telecommunication is often considered a recent phenomenon, but as early as the 1920s the United States police pioneered the use of VHF (very high frequency) radios in cars and in the late 1940s the first vehicle-based mobile radio-telephone systems became available. By the early 1980s the first analogue mobile phone, the so called first generation (1G), systems began operation and in 1985 the first mobile phone call was made in the UK. Six years later the first digital call was made, using a second generation (2G) phone and a GSM (global system for mobile communication) network. In 2001 a third generation (3G) network for multimedia phones that can accommodate Web-based applications was launched in Japan.

It is GSM technology which supports most of the world's mobile phone networks and by 2008, three billion ($3x10^9$) people were using the GSM network, this came just 4 years after the first billionth user (GSM Association 2008) and illustrates just how rapidly mobile phone use has become a common aspect of everyday life. The RF wave used by a mobile phone is known as the carrier wave, which by itself carries no information. To make it useful for communication, information has to be imposed upon the carrier wave by a process known as modulation. The modulation takes the continuous carrier wave and alters it at a rate that is slower than its original frequency either by pulsing (digital modulation), varying the amplitude (amplitude modulation) or varying its phase (phase modulation). The RF signal can then be allocated to many different users by a number of different strategies, the most common being GSM, FDMA (frequency division multiple access), TDMA (time division multiple access) and CDMA (code division multiple access).

At present, safety guidelines for RF exposure are based upon well characterised heating effects on biological tissue, summarised by UNEP/WHO/IRPA 1993, Nageswari 2003 and more recently by the International Commission on Non-Ionising Radiation Protection (ICNIRP) 2009. ICNIRP has recommended guidelines for limiting exposure of the general public to RF fields (ICNIRP 1998). These limits are defined in terms of a measure of 'absorbed dose' for RF fields, which is the Specific energy Absorption Rate (SAR). SAR is measured by the power absorbed (in Watts) per kilogram of tissue. The average whole body limit is an SAR of 0.08 W/kg and the localised SAR limit for the head and trunk is 2 W/kg; mobile phones are designed to operate within these limits. The SAR values are averaged over any 6 minute period and the localised SAR is averaged over any 10 grams of continuous tissue. These limits are based on a 'worst case' estimate of the SAR received during a call because it assumes the mobile phone is transmitting at a maximum power of 2 W, which is only ever reached at long distances from the base station. Mobile phones do not operate at a fixed power output during a call and depending on the quality of the radio link to the base station the power output can drop to 2 mW; a factor of 1000 lower than the maximum. A maximum temperature rise in the head while using a mobile phone, at the 2 W/kg maximum, has been calculated to be about 0.1°C (IEGMP 2000).

In contrast, base stations with a single transmitter radiate power of about 10 W. Figure 1.2 illustrates how the RF beam spreads out from the antenna.

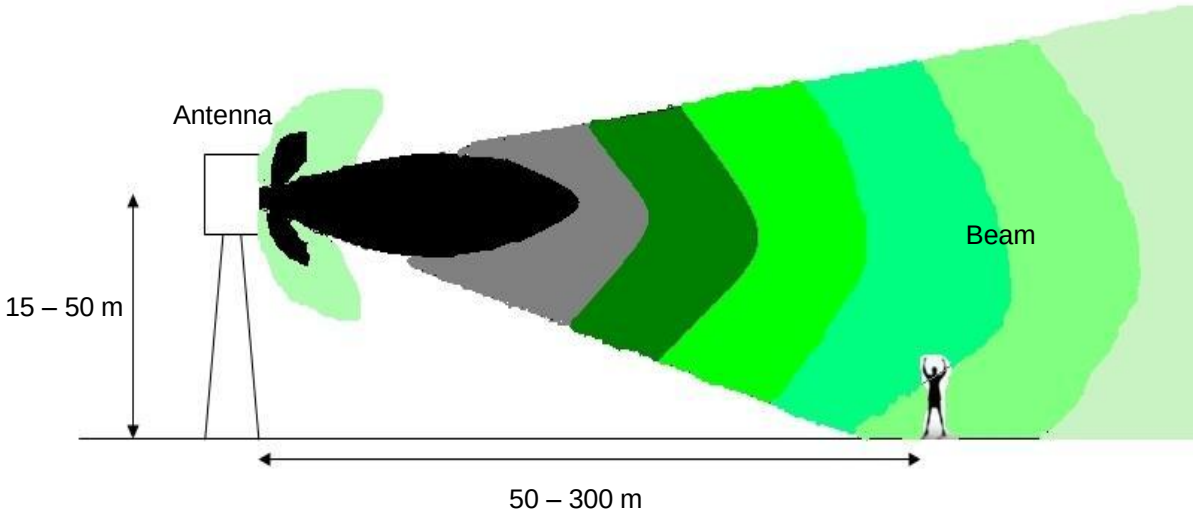

Figure 1.2. Simplified diagram of the RF beam from a base station antenna, showing how it spreads out and does not reach the ground until the distance from the tower is at least 50 m. The signal strength reduces with distance from the antenna – indicated by lighter shading, although in reality the beam is not so well defined as shown here, but consists of a series of lobes.

Provided the antenna is elevated at least 15 m, a person standing at ground level less than 50 m from the base station will not be within the RF field. As the RF beam does not reach ground level for at least 50 m, the RF fields from the base station antennae themselves are more evenly spread over the whole body for a person standing as shown in Figure 1.2, but are 50 to 100 times smaller than from a 2.2 cm mobile phone antenna held close to the head. Heating effects are consequently about 5000 times smaller (IEGMP 2000). The ICNIRP limits are designed to avoid identified health hazards such as excessive heating and ICNIRP (ICNIRP 2004) considered the data on postulated non-thermal effects, such as cancer induction or DNA damage, insufficient to give a quantitative risk assessment.

For several years the potential adverse health effects of exposure to RF fields have been considered by national and international bodies such as the World Health Organisation (UNEP/WHO/IRPA 1993), the Royal Society of Canada (RSC.EPR99-1 1993), the Independent Expert Group on Mobile Phones

(IEGMP 2000), the National Radiological Protection Board (NRPB 2004 a and b), the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR 2007) and the Swedish Radiation Safety Authority (SSI 2003 and 2007). Apart from well characterised heating effects no realistic and agreed biophysical mechanism for the interaction of RF fields with biological systems has been established (Foster 2000), but the potential effects of low level exposures remain an area of controversy and uncertainty, constantly fuelled by the media and public apprehension. The evidence considered by, for example IEGMP (IEGMP 2000) and NRPB (NRPB 2004 a and b) led to the conclusions, that there are no clear health risks associated with mobile phone use *per se*; except the risk of accidents when driving whilst using a mobile phone. However a somewhat precautionary approach was recommended and the committees stated that there remains a need for further biological research to provide a more robust evidence base. Health concerns have been raised by some epidemiological evidence, which suggests that long-term (>10 y), low-level RF exposure from regular usage of mobile phones involving fields not thought to cause a significant temperature rise in biological tissue, may be associated with cancer and in particular, brain tumours (Hardell et al. 2006). However, due to the long-term nature of epidemiological studies no definitive evidence to support or refute an association between cancer and RF exposure is yet available. It is known that many carcinogens cause damage to DNA (Loeb and Harris 2008) therefore one approach is to study the possible genotoxicity of RF fields using standard *in vitro* assays for chromosomal breakage. Apoptosis is also known to play a role in carcinogenesis (Zörnig 2001) and specifically brain carcinogenesis (Wechsler-Reya 2001). These ideas form the rationale for the work described in this thesis.

RF fields are described as non-ionising because they have insufficient energy to dislodge electrons from atoms. To effect a genotoxic change the electromagnetic wave must deposit enough energy to alter the DNA either directly or indirectly. Sagripanti and Swicord (1986) suggested DNA in solution could absorb RF energy directly, but the positive data they observed were most likely the result of free radicals released by the copper electrodes immersed in the solution of DNA. An hypothesis for indirect damage to DNA by an RF field could be that reactive species may be generated elsewhere in the cell as a

result of a biochemical reaction initiated by RF exposure. The main well established and uncontentious mechanism by which RF fields affect biological systems is by heating. Acute exposures to RF fields above the recommended exposure limits that cause a rise in body temperature of >1°C, e.g. overexposure of persons working close to radio or radar antennas that are in operation, have been shown to cause thermal injury to skin such as erythema. In addition, symptoms such as fatigue, vertigo, headaches and eye irritation have also been reported (NRPB 2003). Cellular responses to hyperthermia (temperature >39°C) include the induction of apoptosis, DNA damage in cycling cells and sensitisation to chemical mutagens or ionising radiation (Roti-Roti 2008). Asanami and Shimono 1997 observed heating alone to be genotoxic when mice were exposed to 40°C for 1 to 2 h. In bone marrow sampled 24 h post exposure the micronucleus level in polychromatic erythrocytes was significantly above that found in control animals. Jorritsma and Konings 1984 have shown that exposure of Ehrlich ascites and HeLa S_3 to temperatures of 43°C to 45°C for 1 to 5 h caused DNA strand breaks to increase. Heating was also shown to enhance the action of a known genotoxic agent; x-rays. Cells held at 45°C for 15 to 60 mins and then irradiated with 6 Gy x-rays showed significantly more strand breaks than cells exposed to x-rays alone. In addition, the genotoxic action of mitomycin C (MMC) was shown to be enhanced in mice held at 37°C for 4 h, giving final body temperatures of 40°C to 43°C and measured by micronucleus levels in polychromatic erythrocytes (Asanami and Shimono 1999). Sakaguchi et al. 1995 observed an increase in the level of apoptosis, which persisted for 12 to 48 h post exposure, in cells (spleen, lymph nodes and thymus) taken from rats treated with whole body hyperthermia of 41.5°C for 2 h compared to control animals. A significantly increased level of apoptotic cells have also been observed in HL-60 cells following heating at 45°C for 20 to 45 mins (Lim et al. 2006).

Apart from heating, there are no plausible or fully accepted modes for mechanisms whereby RF fields can induce genetic damage or moderate apoptosis. Nevertheless effects studies have been undertaken and *in vivo* and *in vitro* studies of genotoxicity and apoptosis make an important contribution to evaluating the potential risk of RF fields from mobile phones. A range of cell types from prokaryotes and eukaryotes have been studied including plant as

well as animal cells. Whole animals or cells have been exposed to a range of RF frequencies, some not in the mobile phone range, but still providing useful information on possible genotoxicity. A number of different mobile phone technologies have been used, with and without modulation and continuous or intermittent signals. For the purpose of this review, investigations using mammalian cells that look for the induction of DNA strand breaks, chromosomal aberrations, micronuclei or sister chromatid exchanges have been considered together with *in vitro* studies to assess apoptosis. Heating features as the one certain factor that causes positive effects and thermal models predict a maximum temperature rise in the head of about 0.1°C as a result of the energy absorbed when using a mobile phone (IEGMP 2000). Therefore, an acceptable level of heating in any study would need to be close to 37.0°C with, ideally, a temperature rise not greater than 0.1°C. However frequently in the studies reviewed in this chapter the specification of heating has been criticised for being inadequately engineered or described. Thus, positive effects simply due to thermal processes cannot be ruled out.

1.2 Studies investigating the induction of single or double strand breaks using the comet assay

The comet assay can be used with any cell or tissue type, providing isolated single cells can be produced, to examine DNA damage and, by timed repeated assays, its repair. Cells are embedded in an agarose gel on a microscope slide and a lysis step removes non-DNA material such as proteins. The DNA is unwound and exposed to an electric field under neutral or alkaline conditions. DNA fragments stream out towards the positive electrode, producing a characteristic ‘comet’ shape, the amount of migration is proportional to the number of strand breaks. Results from computerised image analysis can be expressed as tail-to-head ratio, percentage of DNA in the tail or most commonly the tail moment (tail length x tail intensity) and the normalised comet moment (Σ[(amount of DNA at distance X) x (distance X)] / total DNA). Visual scoring is more subjective and the endpoints include tail length or the assignment of cells into 5 groups according to the amount of DNA judged to be in the tail. Alkali

lysis is used to assess single strand breaks (SSBs) and neutral lysis to assess double strand breaks (DSBs), however this distinction may not be so clear cut (Östling and Johanson 1984). SSBs and DSBs are regarded as the primary DNA lesions produced by ionising radiation, and possibly electromagnetic fields (EMF), and they repair on timescales of a few minutes and a few hours respectively.

The first publication that claimed SSBs could be induced by RF field exposure of whole animals was from Lai and Singh in 1995 and again in 1996 and 1997, when they reported more studies that also extended to increases in DSBs after RF exposure. In these studies rats were exposed to RF fields of 2450 MHz in circular waveguides for 2 h (hours), either to a continuous wave (CW) or a pulsed wave (PW) signal, with an average SAR of 0.6 and 1.2 W/kg and a brain SAR of 0.5 - 2.0 W/kg. Lai and Singh's studies indicated a significant increase in DNA strand breaks, as measured visually by comet tail length, in brain cells immediately, (CW only), and 4 h after exposure. In rats treated with free radical scavengers (melatonin or N-tert-butyl-a-phenylnitrone), before and after RF exposure, this effect was not seen.

The authors postulated that DNA strand breaks after RF exposure could be due to a direct effect of the RF field on DNA molecules and/or on the DNA repair mechanism (1996) and that free radicals may play a role (1997). However, these results are difficult to reconcile with the current knowledge of DNA damage induced by physical and chemical agents, as Williams (1996) commented. Malyapa et al. (1997a) also noted that Lai and Singh suggested that the DNA damage they found may be due to an indirect effect, where time is needed for the damage to be expressed. Malyapa et al. (1997a) agree with Williams (1996) that this does not conform to the conventional concept of DNA damage and its repair from other types of radiation, where DNA damage is highest shortly after exposure and the effect of repair is to reduce the amount of damage seen with time, not to increase it. Also, one would expect unrepaired / misrepaired damage resulting from an impairment of the DNA-repair mechanism to be expressed as an increase in chromosomal aberrations and micronuclei. The majority of studies using these endpoints after RF exposure

have not reported any increase (these studies will be discussed in subsequent paragraphs).

Malyapa et al. (1998) carried out a study to investigate Lai and Singh's 1995 claim, that low intensity exposure to 2450 MHz fields causes DNA SSBs. They exposed rats to a 2450 MHz CW signal for 2 h, at an SAR of 1.2 W/kg. No overall differences were found in the number of SSBs, measured by image analysis of comet length and the normalised comet moment, in cells from the exposed or sham exposed animals at 0 and 4 h post exposure. Rats that had been asphyxiated by carbon dioxide (CO_2) showed more intrinsic DNA damage and variation between experiments and the authors believe that the damage was due to delay between asphyxiation and removal of the brain and the variability in the time taken in the CO_2 chamber. The authors concluded that their study does not confirm the findings of Lai and Singh (1995) and the effects found in their own study were not due to RF fields. It is important to note that in Lai and Singh's papers the time between the animal being sacrificed and the removal of the brain is omitted. In addition, measuring comet length and normalised comet moment by computerised image analysis, Lagroye et al. (2004) have found no difference in SSB levels in brains taken from rats 4 h after exposure to a 2450 MHz PW signal for 2 h when compared to sham exposed control rats.

The alkaline comet assay has also been used to examine SSBs in the DNA of cells exposed *in vitro* to RF fields. Different cell types (rodent and human), different frequencies and modulations, SARs and exposure times have all been used. Malyapa et al. (1997a) carried out an *in vitro* study to determine if a 2450 MHz CW signal causes SSBs, as measured by image analysis of comet length and normalised comet moment, in cultured mammalian cells. They exposed exponentially growing human glioblastoma cells U87MG and mouse C3H 10T½ cells to RF fields in radial transmission lines (RTLs), for 2, 4 and 24 h. The RTLs gave a reasonably uniform RF exposure. SARs were calculated as 0.7 and 1.9 W/kg and the temperature was maintained in the RTLs at 37.0 ± 0.3°C. Sham exposures were also carried out. No significant differences were found between the test group and the controls assayed immediately for all exposures and after 4 h for the 2 h exposure. The authors concluded that 2450 MHz RF

fields do not cause DNA damage in cultured mammalian cells under the experimental conditions used.

In another study Malyapa et al. (1997b) exposed mouse C3H 10T½ cells and human glioblastoma cells U87MG to an 835.62 MHz FMCW (frequency modulated continuous wave), and an 847.74 MHz CDMA signal. Exposures were for various periods up to 24 h, with an SAR of 0.6 W/kg. The temperature was monitored continuously and maintained at 37.0 ± 0.3°C. Positive controls were irradiated with gamma radiation and the alkaline comet assay was used to measure DNA strand breaks, as in Malyapa et al. (1997a). No significant differences between cells exposed to FMCW or CDMA signals compared to sham exposed controls were found and the authors concluded that mobile phone frequencies do not appear to damage DNA. Also, using mouse C3H 10T½ fibroblasts Li et al. (2001) reported on exposures to 835.6 and 847.7 MHz FDMA signals at SARs of 3.2 and 5.1 W/kg for 2, 4 and 24 h. During the exposure the temperature was maintained at 37.0 ± 0.3°C. The alkaline comet assay was used to detect DNA strand breaks, using image analysis to measure comet moment and length, immediately after exposure, as well as 4 h after the 2 h exposure. No excess of DNA SSBs was detected. In addition, using a 2.1425 GHz signal Sakuma et al. (2006) exposed human glioblastoma cells (A172) continuously for 2 or 24 h. Exposures were to the unmodulated CW signal at an SAR of 80 mW/kg or to a W-CDMA (wideband code division multiple access) modulated signal with an SAR of 80, 250 or 800 mW/kg. The alkaline comet assay was used to detect DNA strand breaks by measuring the tail length, moment and percentage of DNA in the tail by computerised image analysis software. Again no effect of the RF field was observed. Exposure of human IMR-90 fibroblasts to the CW and W-CDMA signal delivering an SAR of 80 mW/kg for 2 or 24 h gave the same result.

The work of Phillips et al. (1998) used the alkaline comet assay, in a way similar to Malyapa et al. (1997b), to look for primary DNA damage in Molt-4 lymphoblastoid cells exposed to a 813.56 MHz iDEN (integrated digital enhanced network) signal and a 836.55 MHz TDMA signal. At low SARs (2.4 or 2.6 μW/g) a significant decrease in DNA migration through the gel was seen for both types of signal compared to sham exposed cells. At high SARs (24 or 26

μW/g) cells exposed to the iDEN signal showed a statistically significant increase in DNA migration, but the cells exposed to the TDMA signal showed a significant decrease in DNA migration compared to sham exposed cells. The authors suggested the decrease in migration was a 'protective' effect produced by the TDMA signal modulation. Unfortunately the data of Phillips et al. (1998) does not indicate a consistent pattern of DNA damage and these contradictory results may be due to experimental inconsistencies. Hook et al. (2004) have also used Molt-4 lymphoblostoid cells to study the possible genotoxic effects of RF fields. Exponentially growing cells were exposed for 24 h to four different signals. A 847.74 MHz CDMA (SAR 3.2 W/kg), a 835.62 MHz FDMA (SAR 3.2 W/kg), a 813.56 MHz iDEN (SAR 2.4 or 24 mW/kg) and a 836.55 MHz TDMA (SAR 2.6 or 26 mW/kg) signal. The temperature was maintained at 37.0 ± 0.3°C. Unlike the work of Phillips et al. (1998) no significant differences in SSBs, measured by image analysis of comet length and normalised comet moment, were observed in exposed, sham exposed and control cells.

Vershaeve et al. (1994) reported that after exposing human blood lymphocytes *in vitro*, 5 cm from a base station antenna to a 954 MHz GSM signal for 1 to 2 h, cells from 30 out of 32 donors showed increased strand breakage assayed by visual scoring of comet length. The cells were exposed in a cooled box to maintain the temperature at 17°C. The sham exposed cells were placed in a metal can, but seemingly not mu-metal, to shield them from the EMF while also inside the cooled box. Unfortunately, there are uncertainties about the precise dosimetry and the possibility of thermal effects that make the results questionable. Maes et al. (1997), from the same laboratory, using whole blood samples exposed to a 935.2 MHz GSM signal for 2 h and an SAR of 0.3 to 0.4 W/kg did not detect SSBs by the alkaline comet assay measured by visually scoring tail length, tail moment and tail DNA content. Unfortunately the temperature of the blood was not reported.

Vijayalaxmi et al. (2000) also used human blood samples to compare several comet assay protocols to detect SSBs by measuring tail length, tail moment and tail intensity by computerised image analysis. Samples were exposed to a 2450 MHz PW signal for 2 h. The calculated average SAR was 2.135 W/kg, but SARs ranged from 8.175×10^{-5} to 5.015 W/kg within the flask. The temperature of the

medium was reported as 36.9 ± 0.3°C. Comet assays were performed immediately and 4 h post exposure. No evidence for an increase in SSBs after RF exposure was detected. Although Vijayalaxmi et al. (2000) used the same comet assay procedures as Lai and Singh (1995, 1996, and 1997) the cell types were different, whereas Malyapa et al. (1998) used the same cell type as Lai and Singh but a slightly different comet assay protocol. It is unclear whether the differences in the comet assay procedure and/or different cell types are responsible for the different results.

In a series of experiments McNamee et al. (2002a, b and 2003) used human blood lymphocytes to investigate the possible induction of SSBs immediately after RF field exposure. All the studies used visual scoring of comets to determine the percentage of DNA in the tail, comet length and tail moment. In the first study (2002a) a 1900 MHz CW signal was used; in the second (2000b) a 1900 MHz PW signal was used and in the third (2003) both types of signal were used. For all three studies the SARs were 0.1, 0.26, 0.92, 2.4 and 10 W/kg. The reported temperature in the first two studies was 37.0 ± 0.5°C and the exposure time was 2 h. For the third study the reported temperature was 37.0 ± 1.0°C. The authors reported no increase of SSBs compared to sham exposed controls in all cases.

Zeni et al. (2005) have also evaluated the possible *in vitro* genotoxic effects of RF field exposure using human lymphocytes. With a 900 MHz GSM GMSK (Gaussian minimum shift keying) or a TDMA modulated signal with SARs of 0.3 and 1 W/kg unstimulated whole blood cultures were exposed for 2 h. The temperature was maintained at 37.0 ± 0.1°C. Immediately after exposure the induction of DNA damage was assessed using the alkaline comet assay. No significant difference in the number of SSBs, measured by image analysis of the percentage of DNA in the comet tail and the normalised comet moment, was observed compared to sham exposed controls for any exposure. This finding was supported by similar results for chromosome aberrations and SCE assays performed at the same time.

A study by Diem et al. (2005) produced some intriguing results using an 1800 MHz signal. Human fibroblasts and rat granulosa cells (GFSH-R17) were

assessed immediately by the alkaline and neutral comet assays after the following exposure regimes: 1800 MHz CW signal, either continuous or intermittent (5 min on/10 min off), delivering an SAR of 2 W/kg, 1800 MHz GSM Basic intermittent signal (SAR 2 W/kg) and 1800 MHz GSM Talk continuous signal (SAR 1.2 W/kg). The duration of the exposures was 4, 16 or 24 h in total. During that time the cells were maintained at a temperature of 37°C, which rose by no more than 0.06°C. In this study the comets were visually classified into five groups, depending on the amount of DNA in the tail. Then, using an uncommon method for analysing the comet data, the five groups were modified by transformation factors to derive a single comet tail factor (CTF). This does not appear to be the same as the more usual markers for DNA strand breaks. The two cell types showed a significant increase in CTF compared to the sham exposed controls after 16 and 24 h exposures; although there was no significant difference between the two time points. Exposures that had been intermittent or GSM Talk produced a higher level of damage than the CW signal. The authors concluded the major part of the observed induction of SSBs and DSBs was the result of intermittent exposure to RF fields. It is unlikely that the results are due to thermal effects, because the exposure system used gives good exposure and environmental control and if there were 'hotspots' in the cell layer this would have been most evident using the continuous exposure regimes. No mechanism has been proposed to explain why an intermittent exposure is apparently more genotoxic than one that is continuous. Moreover, it is not known why the duration of the exposure beyond 16 h, in this case, did not increase further the damage observed. The authors suggest the effect of RF fields may be cell type dependent and varies with duration of the on/off cycles.

This work on intermittent exposures has attracted considerable attention particularly as it was reproduced several times by the same group over several years and with other end points (discussed later), in addition to comets. Thus, Ivancsits et al. (2003) suggested that exposure to intermittent extremely low frequency electromagnetic fields (ELF-EMF), causes DNA damage. Ivancsits et al. (2005) also suggested human fibroblasts, but not lymphocytes, show a response to ELF-EMF and this could be why many studies that have used lymphocytes have shown no effect of RF fields (e.g. Tice et al. 2002 and McNamee 2002a/b and 2003). However, independent studies to replicate and

extend the work of Diem et al. (2005) and Ivancsits et al. (2003) found no effect of RF-EMF (Speit et al. 2007) or ELF-EMF (Scarfi et al. 2005). Vijayalaxmi et al. (2006) have commented on the work of Diem et al. (2005) and Ivancsits et al. (2005) expressing concern about possible mis-interpretation of the data. Their concern is related to the use of arbitrary transformation factors to derive a CTF. Vijayalaxmi et al. suggest confounding factors, such as changes in the cell cycle and/or apoptosis as well as DNA damage between the sham and exposed cells, can change the tail factor value and result in mis-interpretation of data. Also, as human lymphocytes in G_0 are non-dividing this may account for their lack of response. Further doubt has been cast on the findings of Diem, Ivancsits and colleagues following allegations of misconduct and the withdrawal of a recent publication. This will be more fully discussed later in this thesis (section 6.1.1).

1.2.1 Conclusion

Most of the studies in which an effect of RF fields has been reported have produced results that are inconsistent (Lai and Singh 1995, 1996 and 1997; Phillips et al. 1998), or the possibility of thermal effects cannot be ruled out (Verschaeve et al. 1994). The study by Diem et al. 2005, reported consistent results using a well characterised exposure system. However, independent researchers have been unable to reproduce the findings (Speit et al. 2007). A number of publications have reported no effect of RF fields. In some the frequencies used were not in the mobile phone range (Malyapa et al. 1997a and 1998; Vijayalaxmi et al. 2000), or the temperature was not reported (Maes et al. 1997). The studies by Malyapa et al. 1997b and Sakuma et al. 2006 found no effect of mobile phone frequency RF fields in human glioblastoma cells after exposures of 2 to 24 h. This was confirmed using a second cell type, but not by any other assay. In experiments by Zeni et al. 2005 and McNamee et al. 2002a, b and 2003 human lymphocytes were exposed for 2 h to 900 and 1900 MHz RF fields respectively. Good exposure systems were used and a range of SARs were studied. No effect of RF fields was reported and this was confirmed by other assays performed at the same time. Table 1.1 summarises the relative strengths of all the studies reviewed in Section 1.2.

Table 1.1. Table summarising the relative strengths of the studies reviewed in Section 1.2.

☼ = poor; ☼☼ = adequate; ☼☼☼ = good

? = insufficient information to form a judgement

A positive response reported: Y = yes, N = no

Study	Positive response	Statistical strength	Sufficient controls	Thermal control	Relevant frequency	Relevant SAR
Diem et al. 2005	Y	☼☼	☼☼☼	☼☼☼	☼☼☼	☼☼☼
Hook et al. 2004	N	☼☼	☼☼	☼☼	☼☼☼	☼☼☼
Lagroye et al. 2004	N	☼☼	☼☼	☼☼	☼	☼☼☼
Lai and Singh 1995	Y	☼☼	☼☼	?	☼	☼☼☼
Lai and Singh 1996	Y	☼☼	☼☼	?	☼	☼☼☼
Lai and Singh 1997	Y	☼☼	☼☼	?	☼	☼☼☼
Li et al. 2001	N	☼☼	☼☼	☼☼	☼☼☼	☼
McNamee et al. 2002a	N	☼☼☼	☼☼☼	☼☼	☼☼☼	☼☼☼
McNamee et al. 2002b	N	☼☼☼	☼☼☼	☼☼	☼☼☼	☼☼☼
McNamee et al. 2003	N	☼☼☼	☼☼☼	☼☼	☼☼☼	☼☼☼
Maes et al. 1997	N	☼☼☼	☼☼	?	☼☼☼	☼☼☼
Malyapa et al 1997a	N	☼☼	☼☼☼	☼☼	☼	☼☼☼
Malyapa et al 1997b	N	☼☼	☼☼☼	☼☼	☼☼	☼☼☼
Malyapa et al 1998	N	☼☼	☼☼	☼☼	☼	☼☼☼
Phillips et al. 1998	Y	☼☼	☼☼	?	☼☼☼	☼☼☼
Sakuma et al. 2006	N	☼☼	☼☼	☼☼	☼☼☼	☼☼☼
Speit et al. 2007	N	☼☼☼	☼☼☼	☼☼☼	☼☼☼	☼☼☼
Vershaeve et al. 1994	Y	☼	☼☼	☼	☼☼	?
Vijayalaxmi et al. 2000	N	☼☼	☼☼☼	☼☼	☼	☼☼☼
Zeni et al. 2005	N	☼☼☼	☼☼☼	☼☼☼	☼☼☼	☼☼☼

1.3 Studies investigating the induction of chromosome aberrations

Since the 1920s it has been known that ionising radiation can damage chromosomes (Müller 1927) and produce visible chromosome alterations. Several theories have been proposed to account for this and these include the Breakage and Reunion theory (Sax 1941), the Exchange Theory (Revel 1955) and the Molecular Theory (Chadwick and Leenhouts 1978). The idea that DSBs are the precursor lesions for chromosome aberrations is supported by indirect evidence (Natarajan and Zwanenburg 1982, Bryant 1984, Ager et al. 1991) and alterations such as the dicentric and acentric fragments are primarily the product of mis-repair and unrepaired DSBs (Darroudi et al. 1989). The frequency per cell of chromosome aberrations produced by ionising radiation is dose dependent.

A few researchers have looked for chromosomal aberrations produced *in vivo* in individuals occupationally exposed to RF fields. Garaj-Vrhovac et al. (1990a) found an increase in chromatid breaks, chromosome breaks, dicentrics and acentric fragments in such individuals, whereas similar studies by Garson et al. (1991) and Maes et al. (1995) reported no increase above control levels. However, all these studies have been criticised on the grounds of insufficiently precise dosimetry, their small sample sizes and lack of accounting for different life-style factors. Another more recent *in vivo* study by Maes et al. (2006) found no RF effect as measured by chromosome aberrations in 49 individuals occupationally exposed to RF fields when compared to 25 unrelated non-exposed control persons. This was also so for the SCE and alkaline comet assays carried out on the 25 control subjects and 16 of the occupationally exposed persons.

Studies using *in vitro* irradiation of cultured rodent cells or human lymphocytes are far more numerous. Chen et al. (1974) exposed Chinese hamster ovary (CHO) cells and human amnion cells to a 2450 MHz signal, at various intensities, for between 4 and 20 min (minutes). Chromosomal aberrations were found to be induced in both cell types, but the levels were higher in the CHO

cells. Control samples exposed to thermal heating did not show an enhanced level of chromosome aberrations and the authors concluded a non-thermal effect of RF exposure had been observed. The lack of technical detail in the report, however, makes the interpretation of the data difficult. In contrast Alam et al. (1978) investigated the effects of 2450 MHz RF fields on CHO cells exposed for 30 min at a temperature of 29°C. They showed no increase in chromosome aberrations, but cells exposed at elevated temperatures of 49°C showed significant increases in chromatid aberrations and other cellular damage, such as nuclear vacuoles and de-condensed chromosomes. The authors believed this to be due to a field-induced hyperthermic effect and thus not a genotoxic effect.

Yao (1982) exposed rat kangaroo RH5 and RH16 cells to a 2450 MHz CW signal to assess growth rates and chromosome aberrations after RF field exposure. A converted 2450 MHz microwave oven was used as an environmentally controlled waveguide. The temperature of the medium was measured with, as the author describes it, an ordinary thermometer and this was used to calculate the temperature of the exposed cells. A calculated SAR of 15.2 ± 1.82 W/g is reported. The cells, grown continuously in the RF field for over 20 or more passages, showed reduced growth rates and an increase in chromosome aberrations. When the cells were taken out of the RF field these effects were reversed. Given the description of the apparatus and the dosimetry information it is difficult to determine the precise nature of the RF exposure, which casts doubt on the experimental results.

Lloyd et al. (1984) examined human blood lymphocytes that had been exposed to 2450 MHz fields at temperatures below 36°C, with SARs of 104 and 193 W/kg. They looked for unstable chromosome and chromatid aberrations, but found that chromosome damage in exposed cells did not exceed that of controls. As an extension to the 1984 study, Lloyd et al. (1986a) exposed human blood to SARs of 0, 4, 40, 100 and 200 W/kg using a 2450 MHz RF field for 20 min; the same blood donor was used as in the 1984 study. Blood temperature rose during the exposure from 37°C to 40°C. No extra chromosomal damage was found in exposed cells compared to controls.

In a series of papers Garaj-Vrhovac et al. (1990b, 1991 and 1992) have reported an increase in chromatid and chromosome aberrations in V79 Chinese hamster cells (1990, 1991) and human lymphocytes (1992) that was independent of the RF exposure time using a 7700 MHz CW signal. The authors suggest their results confirm this type of RF field induces damage in the structure of DNA and as the exposure system was maintained at 22°C this was not a thermal effect. However, an increase in temperature cannot be ruled out because a surface probe was used to measure the temperature and exactly how the measurements were made is not clear. The studies also fail to mention the SAR and exactly how the cells were held during the exposure.

Maes et al. (1993) exposed human blood lymphocytes to a 2450 MHz RF signal for 30 and 120 min at 36.1°C. The temperature was monitored by a thermistor, which had been put in the blood sample, giving feedback to a micro computer. A marked increase in chromosome aberrations as well as micronuclei was reported, but these increases could be due to secondary experimental factors. The presence of a metal thermistor in the sample could have caused localised heating of cells by acting as an antenna during the RF exposure. The SAR calculation was conducted in a separate experiment not involving RF fields, whereby an electric resistor was moved through the medium. As this was not done during the RF exposure the assumed uniform SAR distribution may be incorrect.

Subsequently, Maes et al. (1995) exposed human lymphocytes to a 954 MHz GSM signal from an antenna 5 cm away with an SAR of 1.5 W/kg, while the temperature was maintained at 17.0 ± 1.0°C. Control samples were kept inside a metal can, but not mu-metal, to shield them from the RF field. The data indicated that chromosomal aberrations were higher in the RF exposed cells and in cells kept in the metal can, than other control cells which had been kept in another room. The authors suggested the cells in the metal can had been insufficiently shielded from the electromagnetic waves, so the results were directly due to the magnetic component of the field or a secondary effect caused by the metal can, which behaved as an antenna. As the cytogenetic effect was seen in cells exposed 5 cm from the RF field antenna the authors concluded there was no evidence of a serious health hazard to the general

public living tens of meters away from 954 MHz RF-emitting pylons. The study, however, claimed a slight *in vitro* effect and indirect mechanisms for the action of RF fields with biological samples were suggested. Shortcomings in the instrumentation and the method of temperature measurement could mean that thermal effects cannot be ruled out.

Further investigations by Maes et al. (1997, 2000 and 2001) were carried out using human blood lymphocytes exposed to RF fields. In the first study (1997) a 935.2 MHz GSM CDMA signal was used at an SAR of between 0.3 and 0.4 W/kg, with an exposure time of 2 h. The temperature during the exposure was not stated. In the second study (2000) a 455.7 MHz signal was used, although the type was not given, with an SAR of 6.5 W/kg at a temperature of 17.0 ± 1.0°C and an exposure time of 2 h. The third study (2001) used a 900 MHz GSM CW signal at an SAR of 0.4 to 10 W/kg for an exposure time of 2 h. Again the temperature during the exposure was not given. In contrast with earlier work in the same laboratory all three studies showed no evidence for the induction of chromosome aberrations in human lymphocytes.

More studies examining the possible induction of chromosome aberrations after RF exposure have been carried out by Vijayalaxmi et al. (1997b, 2001a and b). In the first study (1997b) blood was exposed to a 2450 MHz CW signal with a mean SAR of 12.5 ± 0.1 W/kg. The exposure to RF was intermittent, with a sequence of 30 min RF on and 30 min RF off for a total of 90 min. During the exposure the temperature of the sample did not rise above 39°C. The second study (2001a) exposed blood diluted in culture medium to an 835.62 MHz FDMA signal with a mean SAR of 4.4 or 5.0 W/kg for 24 h. In the third study (2001b) diluted blood was exposed to an 847.74 MHz CDMA signal with an SAR of 4.9 or 5.5 W/kg for 24 h. The reported temperature was 37.0 ± 0.3°C. In all three studies no evidence for the induction of chromosome aberrations by RF fields was observed.

1.3.1 Conclusion

All the *in vivo* studies of occupationally exposed individuals are unreliable due to imprecise dosimetry and poor statistical strength (Garaj-Vrhovac et al. 1990a; Garson et al. 1991; Maes et al. 1995 and 2006). The majority of *in vitro* cellular studies conducted before the year 2000 investigated the effect of RF fields at a frequency of 2450 MHz and greater, which are not in the mobile phone range (Chen 1974; Alam et al. 1978; Yao 1982; Lloyd et al. 1984 and 1986a; Garaj-Vrhovac et al. 1990b, 1991 and 1992; Maes et al. 1993; Vijayalaxmi et al. 1997b). In other studies RF fields in the range 455 MHz to 954 MHz have been used (Maes et al. 1995, 1997, 2000 and 2001; Vijayalaxmi et al. 2001a and b). Maes et al. 1995 found a 954 MHz RF field induced chromosome aberrations in human lymphocytes, but this was not repeated in their later studies with similar frequencies. In addition all of the studies by Maes et al. can be criticised for either a lack of information about the sample temperature or poor thermal control. Vijayalaxmi et al. (2001a and b) found no effect of 835 and 847 MHz RF fields, however too few cells were scored for chromosomal aberrations to provide reliable statistics. Table 1.2 summarises the relative strengths of all the studies reviewed in Section 1.3.

Table 1.2. Table summarising the relative strengths of the studies reviewed in Section 1.3.

☼ = poor; ☼☼ = adequate; ☼☼☼ = good

? = insufficient information to form a judgement

A positive response reported: Y = yes, N = no

Study	Positive response	Statistical strength	Sufficient controls	Thermal control	Relevant frequency	Relevant SAR
Alam et al. 1978	N	☼	☼☼	?	☼	?
Chen et al. 1974	Y	☼	☼	?	☼	?
Garaj-Vrhovac et al. 1990a	Y	☼	☼	?	☼	?
Garaj-Vrhovac et al. 1990b	Y	☼☼	☼☼	?	☼	?
Garaj-Vrhovac et al. 1991	Y	☼	☼	?	☼	?
Garaj-Vrhovac et al. 1992	Y	☼	☼	?	☼	?
Garson et al. 1991	N	☼	☼	?	☼	?
Lloyd et al. 1984	N	☼☼	☼☼	☼☼	☼	☼
Lloyd et al. 1986	N	☼☼	☼☼	☼☼	☼	☼
Maes et al. 1993	Y	☼	☼☼	☼	☼	?
Maes et al. 1995	Y	☼☼	☼☼	☼	☼☼☼	☼☼☼
Maes et al. 1997	N	☼	☼☼	?	☼☼☼	☼☼☼
Maes et al. 2000	N	☼☼	☼☼	☼	☼☼	☼
Maes et al. 2001	N	☼☼	☼☼	?	☼☼☼	☼☼☼
Maes et al. 2006	N	☼	☼	?	☼☼	?
Vijayalaxmi et al. 1997b	N	☼	☼☼☼	☼	☼	☼
Vijayalaxmi et al. 2001a	N	☼	☼☼☼	☼☼	☼☼☼	☼☼
Vijayalaxmi et al. 2001b	N	☼	☼☼☼	☼☼	☼☼☼	☼☼
Yao 1982	Y	☼	☼	?	☼	☼

1.4 Studies investigating the induction of micronuclei

The micronucleus assay is often used as a surrogate for chromosome aberration induction because it requires a less skilled examiner at the microscope than aberration analysis and data acquisition is more rapid and thus less costly. Micronuclei are formed from acentric chromosomes, chromatid fragments or whole chromosomes that are not included in the daughter nuclei at cell division. These fragments or whole chromosomes form a separate object that stains chromatin positive, the micronucleus, encapsulated in its own nuclear membrane within the cytoplasm of the cell. Ionising radiation is known to induce micronuclei, the levels of which are dose dependent. With cells such as lymphocytes the micronucleus assay is fairly robust in that the inclusion of cytochalasin B in the culture medium and confining scoring to binucleated cells ensures that cell cycle control is maintained. Hence the micronucleus yield is not diluted by cells that have failed to divide or have passed beyond their second metaphase (Fenech 2000).

The micronucleus assay has been used to look for a genotoxic effect in humans exposed occupationally to RF fields. Garaj-Vrhoac et al. (1990a) found not only chromosomal aberrations to be increased in workers, but also micronuclei. This is supported by the study of Fucic et al. (1992) who reported an increase in micronuclei levels in the lymphocytes of exposed workers. However, the studies can be criticised on the grounds of insufficient dosimetry, small sample size and failure to account for potentially confounding life-style factors. A recent study by Yadav et al. (2008) found an increased frequency of micronuclei in exfoliated buccal epithelial cells in 85 persons exposed *in vivo* to RF fields compared to age and sex matched control subjects. Within the exposed group a positive correlation was observed between the frequency of micronuclei and the duration of the exposure up to 4 years, although a slight decrease was seen for subjects whose exposure was greater than 4 years. Although the work of Yadav et al. (2008) can be criticised for having a relatively small number of subjects it does lend support to a recent large epidemiological case-control study by Sadezki et al. (2008), involving 460 cases and 1266 controls, which found an association between mobile phone use and parotid gland tumours.

Unfortunately, this study relied on subjects' recall of mobile phone use, so an inaccurate estimation of use may have confounded the finding.

Research involving the short and long term exposure of normal and cancer prone animals, either to the whole or part of the body, have tried to overcome the shortcomings of the human studies. Vijayalaxmi et al. (1997a) used tumour-prone C3H/HeJ mice to investigate the induction of micronuclei in the peripheral blood and bone marrow of these animals by an RF field, because if animals are predisposed to develop tumours they may also be sensitive to RF exposure. The mice were exposed to a 2450 MHz CW signal at an SAR of 1 W/kg for 1.5 years, 20 h per day, 7 days per week. In the paper the authors stated that there was no increase in the levels of micronuclei in the two cell types when compared to controls. However, a correction was published by Vijayalaxmi et al. (1998), which reported a tiny but significant increase of 0.05% in micronucleus levels, when a comparison was made with sham exposed mice and historical controls. The authors stated it would be premature to conclude that RF fields acted as a weak mutagen especially as there was no significant increase in the number of tumours seen in the RF exposed mice (Frei et al. 1998b).

Two additional studies using normal rats have been reported by Vijayalaxmi et al. (2001c and 2003). In the first (2001c), male Sprague-Dawley rats were exposed continuously for 1 day to a 2450 MHz CW signal at a whole body average SAR of 12 W/kg. No increases in micronuclei were seen in the peripheral blood or the bone marrow cells. In the second study (2003) Fisher rats received a chronic exposure to the head, which also included an exposure *in utero* from day 13 of gestation until weaning. The exposure was to a 1600 MHz communication signal, used in Iridium satellite phones, for 2 h per day, 5 days a week for 2 years. The SARs used were 0.16 and 1.6 W/kg. Again, no increase in micronuclei was observed in the bone marrow cells of exposed animals when compared to sham exposed and cage-control animals.

Trosic et al. (2002) have also investigated the induction of micronuclei in the peripheral blood cells of normal rats exposed to RF fields. In this study Wistar rats were exposed to a 2450 MHz CW signal at 1 or 2 W/kg; rats were exposed 2 h per day for 2, 8, 15 and 30 days. Only in the rats exposed for 2 h a day for 8

days was a significant increase in the mean number of micronuclei observed, although the range spanned that found in the control animals. However, the mean micronucleus levels returned to normal after 15 and 30 days of RF exposure. Trosic et al. (2002), also, observed an increase in the number of immature erythrocytes in the peripheral blood at the start of the experiment. The authors have suggested that the rat spleen eliminates micronucleated cells from the peripheral blood and an adaptive or recovery mechanism operated to explain their results. In contrast, Juutilainen et al. (2007) found no increase in micronucleus frequencies after long term RF field exposure. In this study, female CBA/S mice were exposed for 1.5 h per day, 5 days per week, for a total of 78 weeks to a 902.5 MHz CW signal giving an SAR of 1.5 W/kg or a pulsed signal with an SAR of 0.35 W/kg. For the first 3 weeks only, the animals were given a total of 4 Gy x-rays, (1.33 Gy per week), with the exception of the cage-controls. A second study group, which comprised female transgenic mice and non transgenic litter mates, was exposed to a GSM or DAMPS (digital advanced mobile phone system) signal, both delivering an SAR of 0.5 W/kg for a total of 52 weeks (1.5 h per day, 5 days per week). In addition, three times a week the mice were exposed to an ultraviolet (UV) radiation dose (240 J/m^2, minimum erythema dose), excluding the cage-control animals. For all mouse strains and exposure conditions no significant effects of RF fields was found on the frequency of micronuclei in polychromatic erythrocytes or in normochromatic erythrocytes. The authors suggest that as UV radiation is absorbed in the skin the blood forming organs would not be directly affected so this study reflects any effect of the RF field alone, whereas the study using x-rays would also shown any enhancement of genomic instability by RF fields. The study as a whole did not demonstrate any acute or persistent effects on micronuclei from RF fields, also no persistent effect of x-rays and sham RF exposure was observed.

An extensive study by Görlitz et al. (2005) has looked for the induction of micronuclei in four cell types from B6C3F-1 mice after RF field exposure. Mice were exposed (whole body) for 2 h per day for 1 or 6 weeks to a 900 MHz GSM or 1800 DCS (digital cellular system) signal that were modulated using GSM Basic or DTX (discontinuous transmission). The average whole body SARs for the 1 week exposures were 3.7, 11.0 and 33.2 W/kg and for the 6 week

exposures they were 2.8, 8.3 and 24.9 W/kg. These exposure levels were sufficiently low to ensure that there would be no thermal effects. Bone marrow cells (1 week study), erythrocytes (6 week study), keratinocytes and spleen lymphocytes (both studies) were assayed for micronuclei. None of the RF field exposures induced micronuclei in any cell type when compared to sham treated controls. This study does not support the adaptive/recovery mechanism suggested by Trosic et al. (2002).

Just as for chromosome aberrations, many more studies investigating the induction of micronuclei have used *in vitro* exposure to cells, either cultured rodent cells or human blood lymphocytes. Garaj-Vrhovac et al. (1991) reported an RF field time dependent increase in the number of V79 Chinese hamster cells with one, two, three and four micronuclei, in addition to the increases in chromosome aberrations discussed earlier. Bisht et al. (2002) used C3H 10T½ mouse fibroblasts exposed *in vitro* to either an 835.62 MHz FDMA of an 847.62 MHz CDMA signal. The SARs for the FDMA signal were 3.2 and 5.1 W/kg and for the CDMA signal, 3.2 and 4.8 W/kg. In all the experiments the exposure times were 3, 8, 16 and 24 h and the temperature was maintained at 37.0 ± 1.0°C. The authors reported no induction of micronuclei by the different exposure regimes.

Zotti-Martelli et al. (2000) used signals of 2450 and 7700 MHz, the latter being much higher than those used for mobile phone communication, to examine the incidence of micronuclei in human lymphocytes exposed *in vitro*. Blood samples were exposed for 15, 30 and 60 min, but the SAR was not stated. Significant increases in the levels of micronuclei, when compared to controls, were observed. As an example, the longest exposure time of 60 mins to a 2450 MHz signal at the highest power used, gave a frequency of 11.5 ± 0.7 micronuclei per 1000 cells compared to 2.5 ± 0.7 in the control culture. By comparison, a net increase of 9 micronuclei per 1000 cells would be expected in irradiated lymphocytes from an x-ray dose of ~75 mGy (Prosser et al. 1988). Zotti-Martelli et al. concluded that microwaves at both frequencies were able to cause cytogenetic damage at high power density and long exposure time; which supported the work of Garaj-Vrhovac et al. (1992). Also, the authors claimed the observed increases in micronuclei were not due to hyperthermia, but the

temperature of the blood samples was not measured at the time of the RF exposure. Instead the temperature variation was measured in water samples under the same experimental exposure conditions. However, the conductivity of water differs significantly from whole blood, so that more energy from the RF field could have been absorbed by the blood. Therefore the blood may have been at a higher temperature than the water and thus the increase in micronuclei could be related to heating. More recently Zotti-Martelli et al. (2005) have reported another study using human lymphocytes exposed *in vitro* to RF fields. An 1800 MHz CW signal was used to expose whole blood for 60, 120 and 180 min. The temperature of the samples was reported as 21 to 22°C, but the SAR was not given. A low but significant increase in the frequency of micronuclei that was dependent on exposure time was reported. However, the temperature was again measured in water samples so, as before, the increase in micronuclei could have been related to heating.

Tice et al. (2002) published in detail the findings of a study first reported by Hook et al. (1999a and b) at the 21st Annual Meeting of the Bioelectromagnetics Society on the incidence of SSBs and micronuclei after the exposure of human lymphocytes to RF fields. Lymphocytes were exposed *in vitro* to 837 MHz analogue, CDMA and TDMA signals, as well as a 1909.8 MHz GSM personal communications systems signal. The SARs for the 837 MHz signals were 1, 2.5, 5 and 10 W/kg, while for the 1909.8 MHz signal the SARs were 1.6, 2.9, 5 and 10 W/kg. In all the experiments the temperature was 37.0 ± 0.1°C and the duration of the exposure was either 3 or 24 h. Exposures of 3 h duration, at all the SARs and signal types tested, did not induce micronuclei when the samples were compared to unexposed controls. However, when the exposure was for 24 h at 10 W/kg a significant increase in micronuclei was observed for all signal types. At 5 W/kg a significant increase only occurred for the analogue and TDMA signals; below 5 W/kg no increases in micronucleus levels were observed. The authors conceded that although the measured temperature never rose above 37.5°C higher localised ‘hot-spot’ temperatures could have been produced. This could explain the increase in micronuclei observed at high SARs and the longer exposure time. Concurrent experiments looking for SSBs showed no evidence of an RF field effect. Other studies, where the temperature was properly maintained during the RF exposure, found no increase in the

levels of micronuclei and SSBs following *in vitro* exposure of human lymphocytes (McNamee et al. 2002a and b, 2003) nor micronuclei and chromosome aberrations (Vijayalaxmi et al. 2001a and b).

In vitro experiments using human lymphocytes have been reported by d'Ambrosio et al. (2002), who also investigated the possible effects of the phase modulation of the RF signal on micronucleus levels. Lymphocyte cultures were set up and exposed for 15 min to either a 1748 MHz GMSK CW or PW signal, the culturing process was then continued for 72 h. A maximum SAR of about 5 W/kg and a maximum temperature of 35.7 ± 0.1°C was reported by the authors. When compared to controls the micronucleus level was unaffected if the exposure was to the CW signal, but a significant increase was observed in cells exposed to the PW signal. The authors have suggested that human lymphocytes show a different response, which depends on the phase modulation of the RF field. This work tends to support the findings of Lai and Singh (1995). However, there are technical issues that call the results into question. In the paper it is not stated how the culture flasks were supported or how their positions were reproduced in the waveguide, which can alter the SAR between experiments. Also, the SAR value quoted in the paper was an average for the flask, plus its support, plus the cells and the medium, the volume of which varied slightly within each flask. This leads to the possibility of heterogeneity of SAR within the culture flask, so the SAR at the precise location of the cells may have been higher than 5 W/kg. A second method to calculate SAR relied on measurements at nine points within the flask that differed in some cases by more than 50%, which suggests there could have been thermal 'hot spots' within the culture flask. In addition to these technical issues there were no replicate exposures for an individual and no repeated experiment with any donor. No sham exposures were performed and the exposed and non-exposed cells were handled differently.

Zeni et al. (2003) have reported *in vitro* experiments using stimulated and unstimulated human lymphocyte cultures during the RF field exposure employing the following regimes: Stimulated lymphocytes were exposed to a 900 MHz CW or GSM modulated intermittent signal (6 min RF on, 3 h RF off for 14 cycles) with an estimated SAR of 1.6 W/kg. Alternatively, unstimulated

lymphocytes were exposed to a 900 MHz GSM intermittent signal (6 min RF on, 3 h RF off for 8 cycles) with an estimated SAR of 1.6 W/kg. In addition, stimulated lymphocytes were exposed to a 900 MHz GSM continuous signal for 1 h per day for 3 days with an estimated SAR of 0.2 W/kg. The temperature rose from 36.6°C when the signal was off to a maximum of 37.1°C when the signal was on. Assays for micronuclei showed no significant difference for any exposure regime when compared to unexposed controls. A later comprehensive study by Zeni et al. (2008) used lymphocytes from 6 donors to investigate genotoxic effects of intermittent 1950 MHz UMTS (Universal Mobile Telecommunication System) RF fields with an SAR of 2.2 W/kg on unstimulated G_0 and phytohaemagglutinin (PHA) stimulated lymphocytes at different stages of the cell cycle. The exposure of cells at different stages of the cell cycle was achieved by placing them in the RF field at various times before and after stimulation by PHA. Hence, a 24 h RF exposure of G_0 lymphocytes, followed by a further 44 h after PHA stimulation, exposed cells in G_0, G_1, S and G_2 stages of the cell cycle. Alternatively, a 44 h exposure following PHA stimulation, exposed cells in G_1, S and G_2. The intermittent exposure switched between 6 min RF on, 2 h RF off for total times of 24 to 68 h. The incidence of micronuclei was not significantly increased in PHA stimulated lymphocytes exposed in different stages of the cell cycle compared to sham exposed control cultures. Also, the RF exposure of unstimulated G_0 lymphocytes caused no significant change in micronucleus frequency or DNA strand breaks. Overall, no effect of an intermittent RF field was indicated by this study.

There are other studies using human lymphocytes, such as those by McNamee et al. (2002b and 2003), who used a PW signal and found no effect using the micronucleus assay and the comet assay to detect SSBs. Also, Vijayalaxmi et al. (1997b) using a CW intermittent signal found no effect using the micronucleus and chromosome aberration assays. Vijayalaxmi (2006b) exposed G_0 lymphocytes, from several donors, for 2 h to a 2450 MHz and an 8.2 GHz PW RF signal delivering an SAR of 2.13 and 20.71 W/kg respectively. No significant differences were seen in micronucleus levels compared to sham exposed control cells. Also, to look for any differences in response of PHA stimulated lymphocytes, cell cultures were exposed for 2 h to the 8.2 GHz signal 24 h after the culture was initiated. After a total culture time of 72 h

analysis of micronucleus levels showed no significant difference from sham exposed cultures. The results were also confirmed using the chromosome aberration assay. Scarfi et al. (2006) published an inter-laboratory study that exposed human G_0 lymphocytes, from 10 donors, for 24 h to a 900 MHz GSM modulated signal at SARs of 0, 1, 5 and 10 W/kg, under controlled temperature conditions. The two research groups involved in the study confirmed each others findings, that there was no evidence of a genotoxic effect of RF fields using the micronucleus assay.

1.4.1 Conclusion

The *in vivo* human studies, (Garaj-Vrhovac et al. 1990a; Fucic et al. 1992; Yadev et al. 2008), are not statistically robust and the RF dosimetry was inadequate. Other *in vivo* studies have examined the effect of RF exposure on animals such as rats and mice. However some studies investigated RF fields not in the mobile phone range (Vijayalaxmi et al. 1997a, 2001c; Trosic et al. 2002), or the measurement of temperature was not described (Vijayalaxmi et al. 2003). Two large studies by Görlitz et al. 2005 and Juutilainen et al. 2007 investigated the effects on mice exposed to 900 / 1800 MHz and 902.5 MHz RF fields respectively. In both studies no effect of the RF field on micronucleus levels was detected. Several *in vitro* studies have also investigated RF fields outside the mobile phone range (Garaj-Vrhovac et al. 1991; Zotti-Martelli et al. 2000; Vijayalaxmi 2006b). In three studies investigating human lymphocytes exposed to 837 - 1800 MHz RF fields an increase in micronucleus levels was observed (d'Ambrosio et al. 2002; Tice at al. 2002; Zotti-Martelli et al. 2005). However, poor temperature control was a feature of all three studies. Publications that reported no effect of RF fields (835 – 1950 MHz) on micronucleus levels in human lymphocytes include Vijayalaxmi et al. 2001a and b; McNamee et al. 2002a, b and 2003; Zeni et al. 2003 and 2008; Scarfi et al. 2006. Good exposure systems were used and the temperature was well controlled. No effect of RF fields was confirmed by other assays performed at the same time (McNamee et al. 2002a, b and 2003; Zeni et al. 2008) or by two laboratories (Scarfi et al. 2006). Table 1.3 summarises the relative strengths of all the studies reviewed in Section 1.4.

Table 1.3. Table summarising the relative strengths of the studies reviewed in Section 1.4.

☼ = poor; ☼☼ = adequate; ☼☼☼ = good

? = insufficient information to form a judgement

A positive response reported: Y = yes, N = no

Study	Positive response	Statistical strength	Sufficient controls	Thermal control	Relevant frequency	Relevant SAR
Bisht et al. 2002	N	☼☼	☼☼	☼☼	☼☼☼	☼☼
d'Amdrosio et al. 2002	Y	☼☼	☼	☼	☼☼☼	☼
Fucic et al. 1992	Y	☼	☼	?	☼	?
Garaj-Vrhovac et al. 1990a	Y	☼	☼	?	☼	?
Garaj-Vrhovac et al. 1991	Y	☼	☼	?	☼	?
Görlitz et al. 2005	N	☼☼	☼☼	☼☼☼	☼☼☼	☼☼
Juutilainen et al. 2007	N	☼☼	☼☼	☼☼	☼☼☼	☼☼☼
McNamee et al. 2002a	N	☼☼☼	☼☼☼	☼☼	☼☼☼	☼☼☼
McNamee et al. 2002b	N	☼☼☼	☼☼☼	☼☼	☼☼☼	☼☼☼
McNamee et al. 2003	N	☼☼☼	☼☼☼	☼☼	☼☼☼	☼☼☼
Scarfi et al. 2006	N	☼☼☼	☼☼☼	☼☼☼	☼☼☼	☼☼
Trosic et al. 2002	Y	☼☼☼	☼☼	?	☼	☼☼☼
Tice et al. 2002	Y	☼☼	☼☼☼	☼	☼☼☼	☼☼☼
Vijayalaxmi et al. 1997a	N	☼☼☼	☼☼☼	☼☼	☼	☼☼☼
Vijayalaxmi et al. 1997b	N	☼	☼☼☼	☼	☼	☼
Vijayalaxmi et al. 2001a	N	☼☼	☼☼☼	☼☼	☼☼☼	☼☼
Vijayalaxmi et al. 2001b	N	☼☼	☼☼☼	☼☼	☼☼☼	☼☼
Vijayalaxmi et al. 2001c	N	☼☼	☼☼☼	?	☼	☼
Vijayalaxmi et al. 2003	N	☼☼☼	☼☼☼	?	☼☼☼	☼☼☼
Vijayalaxmi 2006b	N	☼☼	☼☼☼	☼☼	☼☼☼	☼
Yadav et al. 2008	Y	☼	☼	?	☼	?
Zeni et al. 2003	N	☼☼☼	☼☼	☼☼☼	☼☼☼	☼☼☼
Zeni et al. 2008	N	☼☼☼	☼☼☼	☼☼☼	☼☼☼	☼☼
Zotti-Martelli et al. 2000	Y	☼	☼	☼	☼	?
Zotti-Martelli et al. 2005	Y	☼☼	☼	☼	☼☼☼	?

1.5 Studies investigating the induction of sister chromatid exchanges (SCE)

The SCE assay detects reciprocal exchanges of DNA between homologous sites on two sister chromatids of replicating chromosomes. It is a sensitive indicator for the genotoxic action of some agents, particularly many mutagenic chemicals. However, not all agents that are known to be genotoxic induce SCEs; e.g. ionising radiation and bleomycin are poor inducers of SCEs in lymphocytes exposed in the G_0 stage of their cell cycle.

McRee and MacNichols (1981), using 10 week old CD-1 mice exposed to a 2450 MHz signal 8 h per day for 28 days with an average SAR of 21 W/kg, compared the SCE in bone marrow cells of the exposed, sham exposed and cage-control mice. No significant difference in the numbers of SCEs was detected.

More studies have used *in vitro* exposure of human lymphocytes to assess the possible induction of SCEs by RF fields. Lloyd et al. (1984 and 1986a) reported no trend in the SCE levels seen in human lymphocytes exposed to a 2450 MHz signal at different SARs, which concurred with their results discussed earlier for chromosome aberrations. Maes et al. (1993) not only reported on chromosome aberrations and micronuclei induction, but also SCE levels after RF exposure of G_o lymphocytes in whole blood. Although the authors reported increased levels of chromosome aberrations and micronuclei the SCE levels were unaffected. Khalil et al. (1993) claimed to have found a weak increase in the levels of SCEs and chromosome aberrations in human lymphocytes exposed *in vitro* to RF fields, but also suggest that this may be a temperature effect. Antonopoulos et al. (1997) exposed cultured human lymphocytes for 56 h and then measured the level of SCEs in the exposed and control cultures. Three types of signal were used: firstly, a 380 MHz PW trans-European trunked radio signal with an SAR of 0.08 W/kg; secondly, a 900 MHz PW digital communication system signal with an SAR of 0.208 W/kg; and thirdly an 1800 MHz PW GSM signal with an SAR of 1.7 W/kg. The temperature was reported to be 37.0 ± 0.1°C. No differences in the frequencies of SCEs between the exposed and control

cultures were observed. Incidentally, there were also no differences in cell cycle progression between exposed and control cultures measured at several time points between 48 and 68 h.

1.5.1 Conclusion

The majority of studies have investigated RF field effects at frequencies not in the mobile phone range (Khalil et al. 1993; Lloyd et al. 1984 and 1986a; McRee and MacNichols 1981; Maes et al. 1993). The one study that produced a positive response (Khalil et al. 1993) is unreliable because of poor temperature control and low statistical strength. In the study by Antonopoulos et al. 1997, the effect of RF fields with frequencies of 900 and 1800 MHz were investigated. Cultured human lymphocytes showed no difference in SCE levels between the sham and exposed samples. Table 1.4 summarises the relative strengths of all the studies reviewed in Section 1.5.

Table 1.4. Table summarising the relative strengths of the studies reviewed in Section 1.5.
☼ = poor; ☼☼ = adequate; ☼☼☼ = good
? = insufficient information to form a judgement
A positive response reported: Y = yes, N = no

Study	Positive response	Statistical strength	Sufficient controls	Thermal control	Relevant frequency	Relevant SAR
Antonopoulos et al. 1997	N	☼☼	☼☼	☼☼	☼☼☼	☼☼☼
Khalil et al. 1993	Y	☼	☼	☼	☼	?
Lloyd et al. 1984	N	☼☼	☼☼	☼☼	☼	☼
Lloyd et al. 1986a	N	☼☼	☼☼	☼☼	☼	☼
McRee and MacNichols 1981	N	☼	☼☼☼	☼☼	☼	☼
Maes et al. 1993	N	☼	☼☼	☼	☼	?

1.6 Studies investigating RF field exposure in combination with another well established physical or chemical mutagen

Many more studies investigating SCE induction, and indeed other end points too, have involved RF exposures in combination with other well known genotoxic agents. While RF fields may not be directly genotoxic they may be operating indirectly in a more subtle dose modifying manner. One plausible possibility has been raised that RF fields may potentiate the effect of other environmental mutagens, perhaps by compromising the fidelity of repair of initial DNA damage. An enhanced tendency to mis- or non-repair will be expressed as a greater frequency of visible chromosomal alterations or strand breaks in the various experimental assays described earlier.

Underlying mechanisms by which a RF field could operate in this manner are far from clear and essentially unexplored. One might postulate that RF, possibly via the radical pair process altering the dynamics of electron spin states, could lead to a point mutation, a single base change, in the DNA molecule. This might then be expressed as an altered protein. If this were to occur in one or more essential enzymes in the DNA repair process, or compromise the ability of necessary molecules to cross the nuclear membrane, the resultant effect might be sub-optimal repair of DNA lesions produced by exposure to a chemical mutagen or ionising radiation. Alternatively rather than involving DNA mutations, RF fields may cause changes in protein conformation. A protein can adopt different conformations each with a different energy. Protein confirmations of similar energy could interact to form coupled states. If these coupled states were separated by energies corresponding to frequencies of about a gigahertz it may be possible that RF fields, in the mobile phone range, could interact with these protein molecules. If this interaction caused changes in protein folding this could then give rise to biological effects. There is some experimental evidence to support this idea from three research groups, Bohr and Bohr 2000, Laurence et al. 2000 and Astumian 2003.

Ciaravino et al. (1987) investigated the induction of SCEs in CHO cells. A 2450 MHz PW signal was used at an SAR of 33.8 W/kg for 2 h. During the exposure

the temperature increased from 37°C to 40°C. The cells were exposed to RF fields with or without mitomycin C (MMC) being present in the culture at a concentration of $1x10^{-8}$ M. Appropriate sham exposed controls and temperature controls were also included. After exposure the cells were washed to remove the MMC. No significant difference in the SCE levels were observed between the simultaneous exposure to RF fields and treatment with MMC compared to the chemical alone. Ciaravino et al. (1991) repeated their earlier study with MMC using the same 2450 MHz PW signal at an SAR of 33.8 W/kg, with a temperature rise of 37.0 to 39.7°C. The findings matched their earlier study. Included in this second study was another genotoxic chemical, Adriamycin, an antibiotic that intercalates in DNA, which has a different mode of action to MMC, a DNA cross-linker. Two different concentrations of Adriamycin were used, $7.75x10^{-7}$ M and $1x10^{-6}$ M, during the RF field exposure. Again no significant difference between the RF / chemical induction of SCEs and the temperature control / chemical induction of SCEs was observed.

Another series of studies using CHO cells was reported by Kerbacher et al. (1990). The CHO cells were exposed to a 2450 MHz PW signal at an SAR of 33.8 W/kg. During the 2 h exposure the temperature rose from 37 to 40°C. The CHO cells were simultaneously treated with either Adriamycin or MMC. No difference in the number of chromosome aberrations was found between samples treated with MMC alone or in combination with RF fields. In tests using different concentrations of Adriamycin in combination with RF exposure no difference was observed relative to the chemically treated and non-RF exposed controls in most samples. In one sample, however, a small but statistically significant increase was observed, but this was also seen in a waterbath heated control suggesting the result could be attributable to an increase in temperature rather than being directly related to the RF field exposure.

A series of four studies by Maes et al. (1996, 1997, 2000 and 2001) investigated the interaction of RF fields of different frequencies and modulations with MMC. In all the studies the authors used human lymphocytes and did not expose the cells simultaneously to the RF signal and MMC, but used a sequential exposure of RF followed by the chemical. In the first study (1996), human blood was exposed for 2 h, 5 cm from a base station antenna emitting a

954 MHz GSM signal. The calculated SAR was 1.5 W/kg and a temperature of 17.0 ± 1.0°C was reported. Immediately following the RF exposure cells were cultured either with or without MMC (0.05 µg/mL or 0.1 µg/mL). The authors did not observe a direct effect of the RF fields on cell proliferation nor on SCE frequency. They did report however, those cells that were exposed to the RF field and subsequently to MMC showed a significantly higher frequency of SCEs compared to chemical treatment alone; indicating a synergistic action of RF fields and MMC. As the cells were exposed to a base station antenna the measurements of the SAR were difficult and the authors acknowledge that the electric and magnetic fields were not stable. Therefore thermal effects cannot be ruled out.

In the second study (Maes et al. 1997) blood was exposed to a 935.2 MHz GSM CDMA signal at an SAR of between 0.3 and 0.4 W/kg; the temperature was not given. Again no effect of the RF fields alone was seen, but a weak synergistic effect between the RF field and MMC was observed. This was lower than in the previous study and only significant in 2 of the 4 donors. The authors suggested the difference between the two studies may be the result of the difference in power and mode of exposure. However, in the second study the control level of SCEs was low compared to the control level normally found in that laboratory (Maes 1993 and 1996).

Maes et al. (2000), in their third study, placed human blood samples 5 cm away from the antenna of a car phone in a cooled box, with a temperature of 17.0 ± 1.0°C, for 2 h. The RF signal was 455.7 MHz and the calculated SAR was approximately 6.5 W/kg. After the RF exposure cells were either irradiated to a range of x-ray doses (0.05, 0.1, 0.2, 0.3 and 1 Gy) at room temperature before initiating the culture or cells were cultured with / without MMC (0.5 or 0.1 µg/mL). Again there was no effect of the RF fields alone and the combination of RF and x-rays did not show a synergistic effect as tested by chromosome aberration analysis. When RF exposure was followed by MMC treatment some differences in the levels of SCEs were seen compared to control samples, but the results were not consistent. This study used exposure conditions that were not well characterised and the approximation of the SAR does not preclude the presence of a thermal effect at the cellular level.

In the fourth study (Maes et al. 2001) blood lymphocytes were exposed for 2 h in a transverse electro-magnetic (TEM) cell. A 900 MHz GSM signal was used in different modes: continuous, 'pseudo-random' (simulating conversation) or 'dummy bursts' (simulating standby mode). SARs of 0.4, 2, 3.5, 5.5 and 10 W/kg were used to test the effect of RF fields using chromosome aberration analysis. To study the interaction of RF fields and a physical mutagen a 2 h RF exposure was used, with SARs of 2 or 3.5 W/kg, followed by a 1 Gy x-ray exposure. Alternatively, to study the co-genotoxicity of RF fields and a chemical mutagen cells were cultured in the presence of MMC (0.1 µg/mL) following the RF exposure. The chromosome aberration and the SCE analyses showed no evidence for any of the signals having an effect when compared to appropriate controls. The authors did find fluctuations in the response and speculated that perhaps electromagnetic fields had a subtle biological effect. However, different donors were used for the different exposure regimes and the fluctuations seen may, in part, be due to inter-individual variation.

Although the results of the four studies by Maes et al. (1996, 1997, 2000 and 2001) sequentially progressed from suggesting a synergistic effect of RF fields to no effect, a more recent study by Baohong et al. (2005) produced some intriguing data, using isolated human lymphocytes, to investigate possible dose modifying effects of RF fields with four different chemicals. In this study an 1800 MHz GSM signal was used at an SAR of 3 W/kg while the temperature was maintained at 37.00 ± 0.08°C in all cases. The chemicals used were MMC (a DNA cross-linker), bleomycin (a radiomimetic agent), methyl methanesulfonate (MMS, an alkylating agent) and 4-nitroquinoline-1-oxide (4NQO, a UV mimetic agent). Each chemical was used at four increasing concentrations that did not induce cell death. The RF exposure took place either before, during or after exposure to the chemical. The cells were exposed to the chemical mutagen for 3 h and to an RF signal for 2 hours. The combined RF field and chemical exposure was for 3 h with the RF on for 2 h, although it is not clear from the paper which of the 3h was to the chemical only. Detection of SSBs was performed using the alkaline comet assay by computer aided measurement of tail length and moment, either immediately after exposure or 21 h later. RF field exposure alone, RF with bleomycin or methyl methanesulfonate in any combination did not induce SSBs when compared to sham exposed controls.

However, RF fields with MMC or 4-nitroquinoline-1-oxide in any combination produced an enhanced amount of DNA damage compared to sham exposed controls that was significantly different at 0 and 21 h. However, surprisingly there appeared to be no significant increase in DNA damage with an increasing concentration of chemical.

The authors suggest that the results may be related to the different modes of action of the four chemical mutagens and to different DNA repair pathways. No mechanism is proposed to explain why there is no significant dose response effect with the chemicals or why DNA damage seems to increase at 21 compared to 0 h post exposure. Also, no explanation is proposed as to why the amount by which RF fields enhances the DNA damage caused by chemicals is similar irrespective of the exposure conditions. The equipment used in this study provides good exposure and environmental control therefore it seems unlikely the observations can be the result of increased temperature. Although the study is supported by earlier work in the same laboratory using a 2450 MHz signal (Zhang et al. 2002 and 2003) in the recent study only one donor was used. No independent validation of this work has yet been carried out.

A second study by Baohong et al. (2007) investigated the potential combined effects of UVC (ultraviolet C) and an 1800 MHz RF field delivering an SAR of 3 W/kg on human lymphocytes from three donors. Four experimental groups consisted of a control group, a RF exposure only group, a UVC exposure only group and a UVC plus RF field group. Several doses of UVC were used ranging from 0.25 to 2.0 J/m^2 as well as 0, 1.5 and 4 h RF exposures; the temperature was maintained at 37.00 ± 0.08°C. The alkaline comet assay determined tail length to use as an index of DNA damage. Mean tail lengths (MTLs) showed no significant difference between the control groups and the RF only groups. As expected the 1.5 h post exposure to UVC only produced MTLs significantly greater than the appropriate control group, but not at 0 or 4 h post exposure. In the UVC plus RF field group, a 1.5 h exposure to microwaves produced a lower number of MTLs compared to the UVC only group, at all doses. MTLs were significantly lower at 0.75, 1.0 and 1.5 J/m^2. After a 4 h RF exposure the MTLs were higher than the corresponding UVC only values and this was significant at 0.25, 0.5 and 1.0 J/m^2. The authors suggest that the RF exposure may inhibit the incision or ligation step in nucleotide excision repair. However, given that

not all the doses of UVC combined with RF exposure produced a significant effect the authors concede that more studies are need to further investigate the hypothesis.

Recently Manti et al. (2008) exposed human G_0 lymphocytes to a 1.95 GHz UMTS signal with SARs of 0.5 and 2 W/kg, for 24 h after cells had been given a 4 Gy dose of x-rays. In this study four donors were used and all types of chromosomal aberrations involving chromosomes 1 and 2 were scored using bi-coloured fluorescence *in situ* hybridisation. Although there were significant differences in the fraction of aberrant cells between donors, no significant variation due to RF exposure was found. ANOVA (analysis of variance) testing revealed a significant increase of 0.11 exchanges per cell at 2 W/kg when compared to the 0 W/kg control. However, of the three donors for whom data were available at 0.5 W/kg, two had a lower exchange frequency compared to the 0 W/kg sample; for the other donor the frequencies were similar. At all SARs there was significant variation in the exchange frequencies between the four donors.

Another recent study, by Zhijian et al. (2009), used human G_0 leukocytes exposed to RF fields in combination with x-rays. In this study leukocytes from 4 donors were exposed to an 1800 GSM signal, delivering an SAR of 2 W/kg. The total exposure time was for 24 h, but the RF field was switched on and off intermittently (5 min RF on, 10 min RF off). The sXc exposure system was maintained at 37.0 ± 0.1°C and the temperature difference between the RF and sham exposed samples did not exceed 0.1°C. Following the RF field exposure, the samples were given doses of x-rays of 0, 0.25, 0.5, 1.0 and 2.0 Gy. DNA damage was assessed using the alkaline comet assay from 0 to 240 min post exposure. Computer image analysis was used to determine the percentage of DNA in the tail of 200 comets per donor per data point. In all 4 donors, no significant difference was observed between RF alone and sham exposed samples. As expected a dose effect relationship was seen for the different x-ray doses. However, there was no significant differences in the x-ray induced DNA damage between RF exposed samples and the appropriate sham RF control. The authors concluded that an intermittent RF exposure had no direct effect on DNA damage or a synergistic effect when combined with x-rays.

Few studies have looked at co-genotoxicity of RF field exposure *in vivo*. The study by Maes et al. (2006) described in Section 2.3 took blood from RF exposed and unexposed subjects and included MMC at 0.1 µg/mL in the SCE cultures (total exposure time 72 h) and 25 µg/mL for the comet assay (1 h exposure time to whole blood). No evidence of a RF field mediated change in sensitivity to MMC was detected by the comet or the SCE assays. A study by Verschaeve et al. (2006) used female rats to investigate *in vivo* co-genotoxicity of RF fields and the chemical mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). The rats were exposed to a 900 MHz mobile phone signal, amplitude modulated at 217 Hz, for 2 h per day, 5 days per week for 2 years; the average whole body SAR was 0.3 or 0.9 W/kg. MX was added to drinking water throughout the experiment at a concentration of 19 µg/mL. Blood was taken at 3, 6 and 24 months to assess the number of micronuclei in erythrocytes and DNA damage was determined using the alkaline comet assay. Again no evidence of co-genotoxicity was found.

1.6.1 Conclusion

In the earlier studies RF frequencies of greater than 2100 MHz were used (Ciaravino et al. 1987 and 1991; Kerbacher et al. 1990). Also in a number of investigations the temperature was poorly controlled or the information was not provided in the publication (Ciaravino et al. 1987 and 1991; Kerbacher et al. 1990; Maes et al. 1996, 1997, 2000, 2001 and 2006). More recent studies (Baohong et al. 2005 and 2007; Manti et al. 2008; Zhijian et al. 2009) have investigated the effect of RF fields in combination with a mutagen on human lymphocytes using exposure systems with good thermal control. In general most studies have combined RF with one other agent. Baohong et al. has merit in having found a positive response with 1800 MHz RF fields in combination with UVC (2007), MMC and 4-nitroquinoline-1-oxide (2005) by measuring DNA strand breaks. In both studies the results were not confirmed by another assay for genotoxicity. Manti et al. 2008 reported a small, but significant increase in chromosomal aberrations after lymphocytes were exposed to 4 Gy x-rays and then 1950 MHz RF fields. However, Zhijian et al. 2009 found no effect on the levels of DNA strand breaks induced by 2 Gy x-rays in cells previously exposed

to 1800 MHz RF fields. Table 1.5 summarises the relative strengths of all the studies reviewed in Section 1.6.

Table 1.5. Table summarising the relative strengths of the studies reviewed in Section 1.6.
☼ = poor; ☼☼ = adequate; ☼☼☼ = good
? = insufficient information to form a judgement
Positive response reported: Y = yes, N = no

Study	Positive response	Statistical strength	Sufficient controls	Thermal control	Relevant frequency	Relevant SAR
Baohong et al. 2005	Y	☼☼	☼☼	☼☼☼	☼☼☼	☼☼
Baohong et al. 2007	Y	☼☼	☼☼☼	☼☼☼	☼☼☼	☼☼
Ciaravino et al. 1987	N	☼☼	☼☼	☼	☼	☼
Ciaravino et al. 1991	N	☼☼	☼☼	☼	☼	☼
Kerbacher et al. 1990	N	☼☼	☼☼	☼	☼	☼
Maes et al. 1996	Y	☼☼	☼☼	☼	☼☼☼	☼☼☼
Maes et al. 1997	Y	☼☼	☼☼	?	☼☼☼	☼☼☼
Maes et al. 2000	N	☼☼	☼☼	☼	☼☼	☼
Maes et al. 2001	N	☼☼	☼☼	?	☼☼☼	☼☼☼
Maes et al. 2006	N	☼☼	☼☼	?	☼☼	?
Manti et al. 2008	Y	☼☼☼	☼☼☼	☼☼☼	☼☼☼	☼☼☼
Verschaeve et al. 2006	N	☼☼	☼☼	☼☼	☼☼☼	☼☼☼
Zhijian et al. 2009	N	☼☼	☼☼	☼☼	☼☼☼	☼☼☼

1.7 Studies investigating *in vitro* RF field exposure and levels of apoptosis

Apoptosis, or programmed cell death is a normal component of a number of biological processes including normal cell turnover, development and functioning of the immune system and embryonic development. However, the abnormal regulation of apoptosis has been implicated in diseases such as cancer, Parkinson's and Alzheimer's. Normal levels of apoptosis may be

distorted by being either reduced or enhanced. Apoptosis plays an important role in protecting an organism against cancer by removing cells with damaged DNA or impaired cell cycle regulation. Reduction of the normal apoptotic process could facilitate the proliferation of abnormal cells. It has been shown that some tumour cells are able to block apoptosis by a number of molecular mechanisms, such as the over expression of anti-apoptotic proteins or the down-regulation of pro-apoptotic proteins. Alternatively, changes to cell signalling pathways can also perturb the normal regulation of apoptosis (Elmore 2007). In contrast, excessive apoptosis, as a response to damaging stimuli, can cause tissue damage and if enough cells die this can result in temporary or permanent dysfunction of the affected organ (Linnik et al. 1995; Leist and Nicotera 1998). Therefore, cellular studies of apoptosis may provide an understanding of how RF fields could induce cancer or tissue damage.

A number of stimuli cause cells to enter apoptosis, which follows a sequence of controlled steps (Elmore 2007). An early event in the apoptotic pathway is the translocation of phosphatidylserine (PS) from the inner to the outer face of the plasma membrane, while DNA fragmentation is a typical late-stage event and a central feature of the process is a cascade of proteolytic enzymes called caspases. Assays to detect these distinct events in the apoptotic pathway have been used to study the possible induction of apoptosis by RF fields in a number of different cell types. Three of the most commonly used apoptosis assays include the Annexin V binding assay, which detects cells with exposed PS; the caspase activation assay, which can detect specific or all caspase enzymes; and the *in situ* end labelling assay, in particular TUNEL (terminal deoxynucleotidytransferase dUTP nick end labelling), which detects DNA fragmentation. The activation of caspase enzymes leads to the cleavage of specific proteins such as poly ADP ribose polymerase (PARP) that can also be used to assess apoptosis. Apoptotic cells are permeable to the fluorescent nuclear dye YO-PRO®-1 iodide and uptake measurements can be used to assess apoptosis, while DAPI (4’,6-diamidino-2-phenylindole) staining is used to look at characteristic morphological changes.

Belyaev et al. (2005) exposed lymphocytes from healthy donors and persons who claimed to be hypersensitive to electromagnetic fields to a 915 MHz RF

signal with an SAR of 37 mW/kg for 2 h. At 24 and 48 h post exposure apoptosis was assessed by morphological cellular changes and DNA fragmentation. No significant differences in apoptosis levels were detected by comparison with unexposed controls. An extensive study by Capri et al. (2004a) also used lymphocytes from young and old donors and exposed them to several 1800 MHz GSM modulated signals; GSM Basic, Talk and DTX. All the exposures were to an intermittent signal, 10 min RF on and 20 min RF off, for a total of 44 h with an SAR of 2 W/kg (Basic and Talk) and 1.4 W/kg (DTX). The lymphocytes were also exposed to RF fields with or without 2-deoxy-D-ribose (dRib) treatment. Immediately after RF exposure spontaneous and dRib induced apoptosis was detected using the Annexin V binding assay. The authors found in all cases that the susceptibility of the cells to apoptosis was unaffected by exposure to RF fields. These data supported those obtained in a study by Lagroye et al. (2002), where human lymphocytes were exposed with and without dRib to very similar RF fields and SARs, although in this work the signal was continuous rather than intermittent. Also, in a second study by Capri et al. (2004b), in which human lymphocytes were exposed to a 900 MHz GSM or CW signal (SAR 70 to 76 mW/kg) for 1 h per day for 3 days with or without dRib treatment, apoptosis levels assayed by Annexin V binding were again found to be unaffected by RF fields.

Recently Palumbo et al. (2008) have also investigated caspase-3 activation following RF field exposure. A modulated 900 MHz GSM signal with an average SAR of 1.35 W/kg was used in the study and the temperature was maintained at 36.7 ± 0.3°C. Exponentially growing Jurkat T cells and both quiescent and proliferating human lymphocytes, from 4 donors, were exposed to the RF field for 1 h. Caspase-3 activity was measured 0, 3 and 6 h post exposure in five replicate experiments with Jurkat T cells, while lymphocytes, in both states, were assessed after 6 h. A small but statistically significant increase in caspase-3 activity was observed 6 h post exposure in the Jurkat T cells and proliferating lymphocytes when compared to sham exposed controls. The G_0 lymphocytes showed no significant difference in caspase-3 activity. As caspase-3 is thought to be involved in processes other than apoptosis, such as differentiation and cell proliferation, Palumbo et al. (2008) went on to investigate Annexin V binding with Jurkat T cells and proliferating lymphocytes, as well as PARP cleavage in

the Jurkat cells. Using the same RF field exposure the proliferating lymphocytes were assessed 6 h and the Jurkat cells at 6 and 24 h post exposure for Annexin V binding. Following three repeat experiments no significant difference in the percentage of Annexin V positive cells was observed compared to sham exposed controls. Also, no evidence of increased PARP cleavage following the RF field was detected. Although this study supports the observation that RF fields do not induce apoptosis, the authors are unsure of the biological significance of an apparent increase in caspase-3 activity induced by an RF field. They suggest further studies are needed to investigate caspase-3 activity in other experimental systems and to understand if the observed increase is possibly an 'abortive apoptotic response or a proteolytic mechanism to modulate specific signalling pathways.'

Hook et al. (2004) using human Molt-4 cells showed that the frequency of apoptotic cells, measured by Annexin V binding, was not affected by RF field exposure. Four types of RF signals were used with different frequencies and modulation forms. A 847.74 MHz CDMA signal (SAR 3.2 W/kg), a 835.62 MHz FDMA signal (SAR 3.2 W/kg), a 813.56 MHz iDEN signal (SAR 2.4 or 24 mW/kg) and a 836.55 MHz TDMA signal (SAR 2.6 or 26 mW/kg. Cells were exposed to the CDMA and FDMA signals for 2 h and to the TDMA and iDEN signals for 2, 3 and 21 h. Immediately after the RF exposure the cells were assayed for apoptosis and the cells exposed to the iDEN and TDMA signals were also assayed at 4 h post exposure. In parallel with the apoptosis studies cells were assessed for DNA strand breaks using the alkaline comet assay already discussed in Section 1.2. The RF fields of all four signal types did not induce alterations in the level of DNA damage or induce apoptosis. Two further studies have also used the Annexin V binding assay immediately after RF field exposure. Lantow et al. (2006) exposed human Mono Mac 6 cells to an 1800 MHz GSM DTX signal at 2 W/kg for 12 h and Lagroye et al. (2002) exposed human U937 lymphoma cells to a 900 MHz GSM signal at an SAR of 0.7 W/kg for 48 h. Again, in both studies, no significant induction of apoptosis was detected.

Using murine L929 fibroblasts that were stimulated to divide by adding fresh growth medium or stressed by adding medium without serum, Höytö et al.

(2008) have studied caspase-3 activation after a 1 h RF exposure. Two RF fields were used, an 872 MHz pulse modulated and a CW signal, both delivering an SAR of 5 W/kg. The waveguide exposure system gave a uniform SAR and the temperature was controlled at 37.0 ± 0.3°C. Three independent replicate experiments were performed and as expected the stressed, serum deprived, cultures produced a greater increase in caspase-3 activity compared to the stimulated cells. However, no significant differences in caspase-3 activity was observed between cells exposed to a RF field and the appropriate sham exposed control culture; although both stimulated and stressed cells showed a lower, but not significant, level of caspase-3 activation after the CW exposure compared to the pulse modulated signal.

In contrast to these negative results other researchers using different cell types and exposure conditions have reported RF-induced apoptosis. Caraglia et al. (2005) exposed human oropharyngeal epidermal KB cells to a 1.95 GHz RF signal, at an SAR of 3.3 mW/kg for 1, 2 and 3 h. By using the Annexin V binding assay immediately after exposure a time dependent increase in the level of apoptosis was found; with 20% of cells apoptotic from a 1 h exposure rising to 45% from a 3 h exposure. In addition a time dependent decrease in the heat shock protein HSP90 was also observed. Taken together, the authors considered the increase in apoptosis rate was attributable to the inactivation of the Ras $\longrightarrow$ Erk survival signalling with enhanced deregulation of Ras and Raf1. However, Lee et al. (2006) found no change in HSP90 protein expression, as well as HSP70 and HSP27, in Jurkat T cells or rat primary astrocytes following a 1 h exposure to a 1763 MHz RF field at SARs of 2 and 20 W/kg 6, 12 or 24 h post exposure. Previously Marinelli et al. (2004) had exposed human T-lymphoblastoid leukaemia cells CCRF-CEM to a 900 MHz CW signal for 2 h at an SAR of 3.5 mW/kg and shown an increase in the percentage of cells undergoing apoptosis. Here the increase was thought to be the result of early activation of the p53 dependent, as well as the p53 independent, apoptotic pathways. However, at longer exposure times (24 and 48 h) the effect decreased, although the differences between the exposed and unexposed cells were still statistically significant.

Most of the published studies have considered effects on haemopoietic cells and other non brain derived cell. Given the concern that mobile phone use may be associated with brain effects, neural cells have also been used to assess apoptosis after RF field exposure. Several recent studies that have used neuroblastoma cells have failed to demonstrate apoptosis after exposure to RF fields. Gurisik et al. (2006) exposed the human neuroblastoma cell line SK-N-SH for 2 h to a 900 MHz signal at an SAR of 0.2 W/kg. Apoptosis was assessed 24 h post exposure by using flow cytometry to measure the uptake of the nuclear dye YO-PRO®-1 iodide by apoptotic cells. No significant differences between the sham and RF exposed cells were observed. Merola et al. (2006) exposed the human neuroblastoma cell line Lan-5 to a 900 MHz GSM signal in a wire-patch cell, at an SAR of 1 W/kg. Following a 24 or 48 h exposure to the RF signal, again, no significant differences in apoptosis between the exposed and sham exposed cells were found using the caspase activation assay. This was confirmed by the PARP test following RF exposure times of 48 and 72 h. Joubert et al. (2006) also exposed another human neuroblastoma cell line, SH-SY5Y, for 24 h to a 900 MHz GSM and a CW signal, in a wire-patch cell, with SARs of 0.25 and 2 W/kg respectively. Three methods were used to detect apoptosis, DAPI staining, caspase-3 activity and TUNEL at 0 and 24 h post exposure. No significant differences in the levels of apoptosis were observed between exposed and control cells with any signal, time point or assay used.

Although only a few studies have used neuroblastoma cell lines to examine RF induced apoptosis several more have used other brain-derived cell lines. Most have found that RF fields failed to induce apoptosis. Firstly, Hirose et al. (2006) used proliferating human glioblastoma A172 cells, as well as human fibroblasts (IMR-90), to assess apoptosis levels using the Annexin V binding assay immediately after exposure to 2.1425 GHz W-CDMA or CW signals associated with mobile phone base stations. No significant difference in the percentage of apoptotic cells in the A172 cells after a 24 or 48 h exposure to the W-CDMA signal, using SARs of 80, 250 and 800 mW/kg, were observed; nor when the exposure was a CW signal delivering an SAR of 80 mW/kg. Concordant results were also found using the IMR-90 fibroblasts. Moreover no significant difference was observed in the expression levels of p53 or in the gene expression of subsequent downstream targets of p53 signalling. Therefore, the authors

concluded that low level RF fields up to SARs of 800 mW/kg did not induce p53 dependent apoptosis.

In two recent studies, Joubert et al. (2007 and 2008), exposed primary rat cortical neurones to RF fields for 24 h and used several methods to assess apoptosis at 0, and 24 h post exposure. Joubert et al. (2007) used a GSM 900 MHz signal at an SAR of 0.25 W/kg and assessed apoptosis using the three assays in their previous neuroblastoma study (Joubert et al. 2006), namely, DAPI staining, caspase-3 activity and TUNEL. They were unable to demonstrate any difference in apoptosis between the exposed and unexposed cells. This was in agreement with Lagroye et al. (2002) who found that apoptosis levels measured at 4, 8 and 24 h post exposure, by the Annexin V binding assay, were not increased in primary rat astrocytes after a 1 h exposure to a 900 MHz GSM field with an SAR of 2 W/kg. However, in the second study, Joubert et al. (2008), using a 900 MHz CW signal with an SAR of 2 W/kg, did report a significant difference in the apoptosis frequency between field exposed and the sham exposed cells assayed by DAPI staining and TUNEL. During the RF exposures a temperature rise of 2°C was noted and therefore control experiments with neurons exposed to 37 and 39°C were also performed. As the apoptosis rate in the RF exposed cells was also significantly different from these controls Joubert et al. (2008) concluded they may have seen an effect of RF fields. A further two assays for apoptosis: caspase-3 activation and apoptosis inducing factor (AIF) labelling, were also carried out on exposed, sham exposed and control cells. Although no increase in the caspase-3 activity was found, cells with AIF positive labelling were increased in the RF exposed neurons from between 3 to 7 fold compared to the sham exposed and control cells. However, the authors concede that localised thermal effects of the RF field could not be ruled out.

The apoptotic process is controlled by a highly complex cascade of molecular events and some of the studies discussed above have also investigated RF effects on the expression of genes involved in apoptosis and cell survival signalling (Hirose et al. 2006, Marinelli et al. 2004, Caraglia et al. 2005). Other studies have also investigated RF effects on gene expression. Ivaschuk et al. (1997) using differentiated PC12 rat pheochromocytoma cells of neural origin

found no alteration in the expression of c-fos after a 20 min 836 MHz RF exposure at three power densities (0.09, 0.9 and 9.0 mW/cm^2). A transient decrease in c-jun expression was seen at a power density of 0.9 mW/cm^2 after a 40 min exposure, but an intermittent exposure of 20 min RF field on and 20 min off, (with a maximum total time of 100 min) did not alter the expression of c-fos or c-jun compared to sham controls. Buttiglione et al. (2007) exposed human neuroblastoma cells to a 900 MHz GSM modulated RF field delivering an SAR of 1 W/kg between 5 min to 24 h. Erg-1 transcription was found to be significantly above control levels for exposures of 5, 15 and 30 min. In addition, a significant number of apoptotic cells were observed after a 24 h exposure, as measured by flow cytometry as well as a decrease in the apoptosis inhibitor Bcl-2, but not Bax, which is an effector of apoptosis. Several other studies (Lee et al. 2005, Pacini et al. 2002, Zhao et al. 2007) have also reported an effect of RF fields on the up-regulation of genes associated with apoptosis. However, in the study by Lee et al. (2005) an average SAR of 10 W/kg was used, which may have resulted in localised temperature hot-spots, whereas the dosimetry and exposure conditions in the studies by Pacini et al. (2002) and Zhao et al. (2007), which consisted of a mobile phone being placed near the cells, were not well controlled.

Using a well defined exposure system, Czyz et al. (2004) and Nikolova et al. (2005), have reported a series of experiments in which embryonic stem (ES) cells were exposed to 1.71 GHz GSM modulated RF fields. In the first study (Czyz et al. 2004) found low and transient increases in c-jun, c-myc and p21 following exposure to a GSM 217 Hz modulated signal in p53 deficient cells, but not in the wild type, while exposure to a GSM Talk signal produced no significant response in either cell type. The second study (Nikolova et al. 2005) used neural progenitor cells differentiated from the wild type ES cells exposed to a GSM 217 Hz modulated intermittent signal (5 min on, 30 min off for a total of 6 or 48 h). Again small transient changes in some apoptosis and cell cycle related genes were observed after RF exposure, as well as DSBs, but not in apoptotic cell frequencies, cell proliferation or chromosome aberrations. Two recent studies by Huang et al. (2008a and b) have also investigated levels of gene expression and DNA strand breaks following RF exposure in Jurkat T cells (2008a) and immortalised mouse auditory hair cells (2008b). In both studies

cells were exposed to a 1763 MHz CDMA RF signal delivering 10 W/kg (Jurkat T cells) or 20 W/kg (auditory hair cells). The exposure time for the auditory hair cells was 24 and 48h and 24 h only for the Jurkat T cells. In both studies no significant change was observed in the level of SSBs, as measured by comet tail moment and tail length, in the RF exposed cells compared to sham exposed samples. Analysis of full genome microarrays for human or mouse, as appropriate, revealed some changes in the levels of gene expression compared to the sham exposed controls, however, in both studies this represented less than 0.1% of the total genes examined and the changes in gene expression were not consistent with a functional category such as genes involved in apoptosis. These studies would tend to suggest that any RF induced low level transient changes in gene expression are not detected by physical alterations such as DNA damage or changes in the numbers of cells undergoing apoptosis.

1.7.1 Conclusion

In many studies reviewed in section 1.7, RF fields relevant to mobile phones have been used and delivered by exposure systems giving good temperature control. However exceptions to this are Lee et al. 2005; Pacini et al. 2002; Zhao et al. 2007; Joubert et al. 2006 and 2008. Criticisms for lack of statistical strength, use of a high SAR and/or insufficient controls can be levied against a number of studies that reported both no or a positive response (Belyaev et al. 2005; Buttiglione et al. 2007; Caraglia et al. 2005; Gurisik et al. 2006; Huang et al. 2008b; Ivaschuk et al. 1997; Marinelli et al. 2004; Pacini et al. 2002; Zhao et al. 2007). The majority of the more robust studies have found no effect of RF fields with the exception of Czyz et al. 2004 and Palumbo et al. 2008. Czyz et al. 2004 reported that a 1.71 GHz GSM RF field produced low transient increases in some of the genes associated with apoptosis. Palumbo et al. 2008 investigated the effect of a 900 MHz GSM signal on levels of apoptosis in Jurkat T cells. The caspase 3 assay produced a positive response, but this was not confirmed by two further assays for apoptosis. Table 1.6 summarises the relative strengths of all the studies reviewed in Section 1.7.

Table 1.6. Table summarising the relative strengths of the studies reviewed in Section 1.7.

☼ = poor; ☼☼ = adequate; ☼☼☼ = good

? = insufficient information to form a judgement

A positive response reported: Y = yes, N = no

Study	Positive response	Statistical strength	Sufficient controls	Thermal control	Relevant frequency	Relevant SAR
Belyaev et al. 2005	N	☼	☼	☼☼☼	☼☼☼	☼☼☼
Buttiglione et al. 2007	Y	☼☼	☼	☼☼	☼☼☼	☼☼☼
Capri et al. 2004a	N	☼☼	☼☼	☼☼☼	☼☼☼	☼☼☼
Capri et al. 2004b	N	☼☼	☼☼	☼☼☼	☼☼☼	☼☼☼
Caraglia et al. 2005	Y	☼	☼	☼☼	☼☼☼	☼☼☼
Czyz et al. 2004	Y	☼☼	☼☼	☼☼☼	☼☼☼	☼☼☼
Gurisik et al. 2006	N	☼☼	☼	☼☼	☼☼☼	☼☼☼
Hirose et al. 2006	N	☼☼	☼☼	☼☼	☼☼☼	☼☼☼
Hook et al. 2004	N	☼☼	☼☼	☼☼	☼☼☼	☼☼☼
Höytö et al. 2008	N	☼☼	☼☼	☼☼	☼☼☼	☼
Huang et al. 2008a	N	☼☼	☼☼	☼☼	☼☼☼	☼☼
Huang et al. 2008b	N	☼☼	☼☼	☼☼	☼☼☼	☼
Ivaschuk et al. 1997	Y	☼☼	☼	☼☼	☼☼☼	?
Joubert et al. 2006	N	☼☼	☼☼	☼	☼☼☼	☼☼☼
Joubert et al. 2007	N	☼☼	☼☼	☼☼	☼☼☼	☼☼☼
Joubert et al. 2008	Y	☼☼	☼☼	☼	☼☼☼	☼☼☼
Lagroye et al. 2002	N	☼☼☼	☼☼	☼☼☼	☼☼☼	☼☼☼
Lantow et al. 2006	N	☼☼	☼☼	☼☼☼	☼☼☼	☼☼☼
Lee et al. 2005	Y	☼☼	☼☼	☼☼	☼	☼
Marinelli et al. 2004	Y	☼	☼	☼☼	☼☼☼	☼☼☼
Merola et al. 2006	N	☼☼	☼☼	☼☼	☼☼☼	☼☼☼
Nikolova et al. 2005	N	☼☼	☼☼	☼☼☼	☼☼☼	☼☼☼
Pacini et al. 2002	Y	☼	☼	☼	☼☼☼	☼
Palumbo et al. 2008	Y	☼☼	☼☼	☼☼	☼☼☼	☼☼☼
Zhao et al. 2007	Y	☼☼	☼☼	☼	☼☼☼	?

1.8 Summary

A large number of genotoxicity as well as apoptosis studies, most of which have been *in vitro* rather than *in vivo*, have used various RF fields related to mobile phones with many different SARs and exposure conditions. The quality of the dosimetry described in recent papers tends to be more reliable than in the earlier studies, where heating may have been a confounding factor. Some studies have produced positive results, although these are in the minority. A meta-analysis of data from 63 publications by Vijayalaxmi and Prihoda (2008) concluded that for some exposure conditions a statistically significant increase in DNA damage occurred for some end-points, but there was also evidence for publication bias. The literature is full of failure to reproduce positive results elsewhere and sometimes in the same laboratory.

As the previous 36 pages have demonstrated the literature is extensive, varied and at times contradictory. In an attempt to pull the essential factors together, the literature review has been summarised in the following tables. These are based on a number of different factors including: the exposure system and conditions, the cell type and endpoints used, together with the consistency or transience of the effect and number of donors or experiments. Studies investigating the genotoxicity or co-genotoxicity of RF fields are summarised in Table 1.7 and Table 1.8 respectively. Table 1.9 summarises studies investigating apoptosis and RF fields.

Table 1.7. Summary table of studies investigating genotoxicity of RF fields against a number of different factors. Numbers in italics refer to the studies listed in references

Factor	Studies showing a positive effect	Studies showing no effect
Frequency	In the majority of studies, prior to the year 2000, a frequency of 2450 MHz or higher was used [20, 41, 42, 43, 77, 78, 79, 99, 165, 170, 197]. These are above the frequencies used for mobile phone communication. Frequencies in the mobile phone range include: 1909 [163], 1800 [28,206, 207], 1748 [25], 954 [100,168], 836 [130] and 813 [130] MHz. However, only one or two studies have been carried out at each of these frequencies.	Studies prior to 2000 used frequencies of 2450 [2, 76, 90, 91, 98, 106, 108,171, 181] or 835/847 [107] or 900/935 [3, 102] MHz. Post 2000 the frequencies used include: 1900/1950 [96, 97, 98, 200], 1800 [46, 153], 1600 [185], 900/935 [74, 104, 147, 198, 199], 835/847 [12, 53, 86, 107, 183] MHz. Several studies have been carried out for a given frequency.
Signal	In most studies a CW signal has been used [42, 43, 72, 77, 78, 165, 170, 206, 207]. While in others it was a PW signal [77, 78, 79, 99]. Fewer studies have used a modulated signal such as GSM [28, 100, 163, 168], iDEN [130], TDMA [163], CDMA [163], GMSK [25]. Mainly only 1 or 2 signal types used.	In some studies a CW [12, 70, 90, 96, 98, 106, 107, 108, 144, 153, 171, 184, 172] or PW [3, 70, 76, 97, 97, 182, 188] signal has been used and in others a modulated signal. The types of modulation include: GSM [3, 70, 102, 104, 147, 153, 202], iDEN [53], TDMA [53, 199], CDMA [12, 53, 183], GMSK [199], FDMA [53, 86, 107, 182], DSC [46] and UTMS [200]. Mainly only 1 or 2 signal types used.
SAR	Many early studies (before 2000) lack dosimetry information. In studies that state the SAR, the value is usually 2 W/kg and below [28, 77, 78, 79, 100, 130, 163, 165, 170]. A few report SARs of 5 and 10 W/kg [25, 99,163].	Some early studies (before 2000) lack dosimetry information. In most studies SARs of 5 W/kg or below were used [3, 12, 46, 53, 70, 76, 86, 96, 97, 98, 102, 104, 106, 107, 108, 144, 147, 153, 181, 182, 183, 184, 188, 198, 199, 200]. Few studies report using SARs of 10 W/kg and greater [46, 90, 91, 96, 98, 104, 147, 171, 184, 188].
Exposure system	A range of exposure systems have been used. They include a converted microwave oven [197], a base-station antenna [100, 165], anechoic chambers [206], TEM cells [130, 163] and waveguide systems [20, 25, 28, 41, 42, 43, 77, 78, 79, 99, 170].	A range of exposure systems have been used. They include a base-station antenna [100], anechoic chambers [144, 147, 171, 181], TEM cells [53, 102, 105, 198, 200], radial transmission lines [12, 86, 106, 107, 182, 183, 185] and waveguide systems [70, 96, 90, 91, 96, 97, 98, 108, 153, 184, 188, 199].
Exposure time	The majority of studies that exposed cells *in vitro* have used times ranging from less than 15 min to 2 h [20, 25, 28, 41, 42, 43, 99, 100, 130, 165, 168, 206,207]. Few have used longer exposure times of up to 24 h [28, 130,163]. *In vivo* exposure of animals have taken place over 2 h [77, 78, 79], or number of days up to a period of 1.5 y [165, 170].	In most studies an *in vitro* exposure times of 2 or 24 h [12, 76, 86, 96, 97, 98, 102, 103, 104, 106, 107, 108, 144,147, 153, 171, 181, 182, 183, 184, 199, 200] have been used, although some exposures have been for 68 h [3, 198, 200]. *In vivo* exposures of animals have taken place over a number of days up to a period of 1.5 y [46, 70, 98, 185].
Continuous or intermittent	The overwhelming majority have used a continuous exposure regime [20, 25, 28, 41, 42, 43, 77, 78, 79, 99, 100, 130, 163, 168, 197, 206, 207], although *in vivo* animal exposures have been intermittent (e.g. 2 h per day) [165, 170]. Only one study exposed cells *in vitro* to an intermittent signal [28].	The vast majority of *in vitro* cellular studies used a continuous exposure regime [3, 12, 76, 86, 90, 91, 96, 97, 98, 102, 103, 104, 106, 107, 108, 144, 147, 181, 182, 183, 184, 188, 198, 199, 200]. Only a few have used an intermittent exposure [153, 171]. *In vivo* animal exposures have been intermittent (e.g. 2 h per day) [46, 70, 185].

Table 1.7 continued.

Factor	Studies showing a positive effect	Studies showing no effect
Temperature control	Most studies before the year 2000 either do not mention temperature or the method of control was poor [20, 41, 42, 43, 72, 77, 78, 79, 99, 100, 130, 168, 170, 197]. Post 2000, all the studies have attempted to control the temperature. However, for most studies the presence of hot-spots in the samples remains a possibility [25, 163, 165, 206, 207].	In the studies before the year 2000 the temperature was not always mentioned [2, 98, 102, 108, 171]. Post 2000 the temperature has been controlled in the majority of studies [3, 12, 86, 96, 97, 98, 103, 106, 107, 147, 153, 181, 182, 183, 184, 188, 198, 199, 200].
Sham exposure	In most studies sham exposures were used as controls [25, 28, 41, 42, 43, 77, 78, 79, 99, 100, 130, 163, 170, 206, 207].	The majority of studies used sham exposures as controls [3, 12, 46, 53, 70, 76, 86, 90, 91, 96, 97, 98, 102, 103, 104, 106, 107, 108, 144, 153, 171, 181,182, 183, 184, 185, 188, 198, 199, 200].
Cell type / whole animal	*In vitro* studies have mainly used human lymphocytes (mainly G_0) [25, 43, 72, 99, 100, 163, 168, 206, 207], but also lymphoblastoid cells [130] and fibroblasts [28]. Animal cell types include Chinese hamster [20, 41, 42], rat granulosa [28] and rat kangaroo cells [197]. *In vivo* studies have used humans (lymphocytes) occupationally exposed to RF [39, 40], and rats (brain [77, 78, 79] and peripheral blood [165]) and mice (peripheral blood [165, 170] and bone marrow [170]).	*In vitro* studies have mainly used human lymphocytes (mainly G_0) [3, 90, 91, 96, 97, 98, 102, 103, 104, 147, 171, 181,182, 183, 198, 199, 200], but also fibroblasts [144, 153], lymphoblastoid [53] and glioblastoma [106, 107, 144] cells. Animal cell types include Chinese hamster [2, 153], and mouse 10T½ fibroblasts [12, 86, 106, 107]. *In vivo* studies have used humans (lymphocytes) occupationally exposed to RF [44, 100], rats (brain [76, 108], peripheral blood [184] and bone marrow [184, 185])) and mice (bone marrow [46, 98], spleen [46], erythrocytes [46, 70] and keratinocytes [46]).
End point	DNA strand breakage (0 to 21 h post exposure) [28, 77, 78, 79, 130, 168], chromosome aberrations [20, 40, 41, 42, 43, 72, 99, 100, 197] and micronucleus assays [25, 39, 40, 42, 43, 99, 163, 165, 170, 196, 206, 207] are the most common. In most studies only one endpoint was used.	DNA strand breakage (0 to 4 h post exposure) [53, 76, 86, 96, 97, 98, 102, 106, 107, 108, 144, 153, 181, 199, 200], chromosome aberrations [2, 44, 90, 91, 100, 102, 103, 104, 171, 182, 183, 188, 199], SCE [2, 3, 90, 98, 102, 199] and micronucleus assays [12, 46, 70, 97, 98, 144, 147, 153, 171, 184, 185, 188, 198, 199, 200]. In some studies only one endpoint was used, while in other several were used.
Consistency	In some studies a consistent pattern of increased damage was not seen or the increases were small [130, 170]. Also, in some studies, where more than one endpoint was assessed, not all showed a positive effect [99, 163].	The studies consistently showed no effect of RF fields [2, 3, 12, 44, 46, 53, 70, 76, 86, 90, 91, 96, 97, 98, 100, 102, 103, 104, 106, 107, 108, 144, 147, 153, 171, 181, 182, 183, 184, 185, 186, 188, 198, 199, 200].
Study size	Studies with human lymphocytes mainly used blood from 2 donors [99, 206, 207], (range 1 to 9) [25, 72, 100, 163]. Generally 3 replicate experiments [41, 42, 43], (range 1 to 8) [20, 28, 130] were performed using cultured cells. *In vivo* animal studies used 8 to 9 rats [77, 78, 79, 165] or 62 mice [170] per experimental group. The number occupationally exposed people studied ranged from 10 to 85 per group [39, 40, 170, 196].	Studies with human lymphocytes mainly used blood from 4 donors [102, 103, 104, 182, 183, 198], (range 1 to 15) [3, 90, 91, 96, 97, 98, 102, 147, 171, 181, 199, 200]. Generally 3 replicate experiments were performed using cultured cells [12, 86, 106, 107, 144, 153]. *In vivo* animal studies used 4 to 130 rats [76, 103, 184, 185] or 20 mice [46, 70] per experimental group. The number occupationally exposed people studied ranged from 6 to 40 per group [44, 100].

Table 1.8. Summary table of studies investigating the co-genotoxicity of RF fields against a number of different factors. Numbers in italics refer to the studies listed in references

Factor	Studies showing a positive effect	Studies showing no effect
Frequency	Frequencies include: 2450 [71], 1950 [110], 1800 [9, 10], 954 [101], 935 [102] and 455 [103] MHz.	Frequencies include: 2450 [21, 22], 1800 [204] and 900 [104, 169] MHz.
Signal	In the majority of studies a GSM [9, 10, 101], CDMA [102] or UMTS [110] signal was used	CW [104] and PW [21, 22] were used as well as GSM [169, 204] signals.
SAR	The SARs used were mainly at or below 3 W/kg [9, 10, 101, 102, 110], although SARs of 6.5 [103] and 33.8 [71] were also used.	The SARs used were mainly at or below 3 W/kg [104, 169, 204], although SARs of 5.5 [104], 10 [104] and 33.8 [21, 22] W/kg were used.
Exposure time	Most exposure were for 2 h [9, 71, 101, 102, 103], but 1.5 [10] and 24 [110] h exposures occurred.	Exposures were for 2 [21, 22, 104, 105] or 24 h [204], except the animal exposures that took place over a 2 y period [169].
Continuous or intermittent	Continuous exposures [9, 10, 71, 101, 102, 103, 110].	Continuous exposure [21, 22, 104, 105]. Intermittent exposure of cells (5 min RF on, 10 min RF off, for 24 h) [204] and the whole animal exposure, (2 h per day) [169].
Temperature control	Prior to 2000 the temperature of the samples is not stated or the temperature control was not good [71, 101, 102]. Post 2000 the temperature was controlled [9, 10, 103, 110].	In all the *in vitro* studies that occurred before 2001 the temperature control is not stated or not very good [21, 22, 104, 105]. Post 2001 the temperature control is good [204].
Sham exposure	RF exposed samples were compared with sham exposed samples [9, 10, 71, 101, 102, 103, 110].	RF exposed samples were compared with sham exposed samples [21, 22, 104, 105, 169, 204].
Co exposure	Mainly MMC [9, 71, 101, 101, 103], but also Adriamycin [71], MMS [9], 4NQO [9], bleomycin [9], UVC [10] or x-rays [110].	Mainly MMC [21, 22, 104, 105], but also MX [169], Adriamycin [22] or x-rays [104, 204].
Cell type / whole animal	In the majority of studies G_0 human lymphocytes were used [9, 10, 101, 102, 103, 110]. Chinese hamster cells were also used [71].	The *in vitro* studies used G_0 human lymphocytes [104, 105, 204] and Chinese hamster cells [21, 22]. The *in vivo* study used rat peripheral blood [169].
End point	Mainly DNA strand breaks [9, 10, 103] and SCE [101, 102, 103]; some studies used the CA assay [71, 110]. In some studies more than one end point was used.	In most studies the end point was SCEs [21, 22, 104, 105]. Other end points included chromosome aberrations [104], micronuclei [169] and DNA strand breaks [169, 204]. In some studies more than one end point was used.
Consistency	Within most studies the results were not always consistent, (e.g. only one sample or one SAR gave positive) [6, 7, 65, 94, 101].	The studies consistently showed no effect of RF fields [21, 22, 104, 105, 169, 204].
Study size	Studies with human lymphocytes mainly used blood from 4 donors [102, 103, 110], (range 1 to 8) [9, 10, 101]. 2 to 7 replicate experiments were performed using cultured cells [71].	Studies with human lymphocytes used blood from 2 to 8 donors [104, 105, 204]. 4 replicate experiments were performed using cultured cells [21, 22].

Table 1.9. Summary table of studies investigating apoptosis and RF fields against a number of different factors. Numbers in italics refer to the studies listed in references

Factor	Studies showing a positive effect	Studies showing no effect
Frequency	In the majority of studies 900 MHz was used [15, 69, 111, 128], but also a 1950 MHz signal [18].	The most commonly used frequencies were 900 [17, 48, 67, 68, 75, 115] and 1800 [16, 75, 80] MHz, but others include: 2142 [50], 1710 [125], 915 [11], 872 [55], 847 [53] and 835 [55] MHz.
Signal	CW [69, 111] or GSM [15, 128] signals were used.	In the majority of studies GSM signals [11, 16, 17, 48, 50, 55, 67, 68, 75, 80, 115, 125] were used, but also CDMA [53] and WCDMA [50].
SAR	SARs were at or below 3.5 W/kg [15, 18, 69, 111, 128].	All SARs were at or below 2W/kg [11, 16, 17, 48, 50, 55, 67, 68, 75, 80, 115, 125], except for one study (5 W/kg) [53].
Exposure time	Mainly 3 h or below [15, 18, 69, 111, 128], but also 24 [15, 69] and 48 h [69].	Some 2 h or less [11, 48, 55, 75] and some 24 [50, 67, 68 115] or 48 h [50, 75, 115, 125]. Exposure times of 6 h [125], 12 h [80], 44 h [16] and 3 days [17] have also been used.
Continuous / intermittent	Continuous exposures [15, 18, 69, 111, 128].	Continuous [11, 16, 48, 50, 53, 55, 67, 68, 75, 80, 115] and intermittent [17, 125] exposures.
Temperature control	In most studies the temperature was controlled [15, 18, 111, 128].	The temperature was controlled [11, 16, 17, 48, 50, 53, 55, 67, 68, 75, 80, 115, 125].
Sham exposure	RF exposed samples were compared with sham exposed samples [15, 18, 69, 111, 128].	RF exposed samples were compared with sham exposed samples [11, 16, 17, 48, 50, 53, 55, 67, 68, 75, 80, 115, 125].
Cell type	Human: G_0 and stimulated lymphocytes [128], Jurkat T cells [128], neuroblastoma [15], T-lymphoblastoid leukaemia [111] and oropharyngeal epidermal KB cells [18]. Rat: primary cortical neurones [69].	Human: G_0 lymphocytes [11, 16, 17, 75], fibroblasts [50], Molt 4 [53], Mono Mac 6 [80], glioblastoma [50] and neuroblastoma [48, 67, 115] cells. Rat: primary cortical neurons [68] and astrocytes [75]. Mouse: fibroblasts [55] and embryonic stem cells [125].
End point / time post exposure	DAPI staining [69], Annexin V [18], caspase-3 [69, 128], TUNEL [69] and FACS analysis [15, 111]. Usually only one assay was used in a study. Most assays were performed immediately after exposure, although a few were also carried out at 3 [128], 6 [128] and 24 h [15, 69] post exposure.	The most commonly used assays were Annexin V [16, 17, 50, 53, 75, 80], caspase-3 [55, 67, 68, 115], and TUNEL [67, 68]. Others included: DAPI staining [67, 68], FACS analysis [125], PARP [67] and YO-PRO-1 [48]. In some studies only 1 assay was used. In others 2 or more assays were used. The assays were performed immediately or 24 h after exposure. In a few studies times of at 4 [53], 8 [75] or 48 [11] h were also used.
Consistent / transient	Positive results were either very small or transient or inconsistent [15, 18, 69, 111, 128].	The studies consistently showed no effect of RF fields [11, 16, 17, 48, 50, 53, 55, 67, 68, 75, 80, 115, 125].
Study size	1 to 5 replicate experiments [15, 18, 69, 111, 128] or 4 blood donors [128] were used.	In most studies results from 3 replicate experiments were used [50, 55, 67, 80, 115], (range 3 to 6) [48, 68] and 7 [11], 18 [16, 75] or 31 [17] blood donors.

As indicated in the summary tables, in many of the studies the RF signals and frequencies have not been related to those most commonly used in current mobile phone technology, namely GSM signals at 935 or 1800 MHz. In addition, the tables show a number of different exposure systems have been used and that temperature was not always well controlled. However, in most studies a sham exposure has been used as a control and the SAR has been at or below the maximum SAR received by a person when making a mobile phone call. Therefore the first aim of this study was to use a well characterised exposure system that had good temperature control and was able to deliver several different GSM signals, at relevant frequencies and SARs. Most studies showing no or a positive effect of RF have employed short continuous exposure times of 2 h or less, therefore the aim of the present study was to use a longer exposure time of 24 h, and was either continuous or intermittent. Table 1.8 summarises studies that have investigated whether RF fields related to mobile phone communication can influence the genotoxic effects of another mutagen (usually the chemical MMC) *in vitro*. Most of the recent studies have suggested a positive result, although this tended to be either inconsistent between samples or a transient effect. Therefore, the aim of the present study was to investigate the co-genotoxicity of RF fields and x-rays.

Tables 1.7 and 1.8 show that human lymphocytes have commonly been used to investigate the genotoxicity of RF fields and have shown both no effect and positive results. These summary tables also show that generally only one or two assays have been used to assess genotoxicity. Therefore, the aim of the present study was to use the human lymphocyte as the cell of choice from 4 donors and to assess genotoxicity using three standard cytogenetic tests. The chromosome aberration (CA), the micronucleus (MN) and the sister chromatid exchange (SCE) assays. The MN and SCE techniques also provided data that could be used to measure the speed of *in vitro* cellular proliferation. In addition, PHA stimulated lymphocytes were also exposed for 48 hours to RF fields to investigate any genotoxic effects on cycling cells. Table 1.9 shows that a number of cell types have been used to investigate the effect of RF fields on apoptosis. However, apoptosis has usually been assessed using one or two tests, within a study, at one or two time points post exposure. Therefore, the aim of the present study was to investigate the potential of RF fields to cause

cellular injury by the induction of apoptosis in murine N2a neuroblastoma, a cell type relevant for brain effects, in both proliferating and differentiated states. The N2a cells were assessed for apoptosis induction following a time course of 0 to 48 hours post exposure. Three independent assays for apoptosis were used the Annexin V assay, caspase activation and in situ end labelling as each detect a different stage of the apoptotic pathway.

In the following section (2.2) a detailed description of the GSM signals used in the present experiments is given. It should be noted that in many of the previous studies reviewed here in Section 1 there was insufficient information provided to devise similar data on signal structure. Moreover many of the reviewed studies did not use GSM.

2. Materials and Methods

2.1 Exposure system

Two RF exposure systems were used delivering RF fields of 935 and 1800 MHz respectively. Much of the work described in this thesis was done at 935 MHz, because the 1800 MHz system was only available, on loan, for a limited time. The systems, sXc (system for the exposure of cells), were developed and extensively tested by the Information Technologies in Society Foundation (IT'IS) Zurich, Switzerland. A full account of the apparatus is described by Schuderer et al. (2004 a and b). Both the sXc900 and the sXc1800 exposure set ups, shown in Figures 2.1 and 2.2 respectively, are based on two identical rectangular waveguide cavities, which resonate at 935 and 1800 MHz respectively.

Figure 2.1. The sXc900 exposure system showing the waveguides inside the incubator and the controlling computer.

Figure 2.2. The sXc1800 exposure system showing the waveguides inside the incubator.

When experiments were being carried out one cavity provides the RF exposure and the other acts as a sham control. The waveguide cavities prevent extraneous fields and also any 'cross fire' from the active cavity to the sham. The 'leakage' from the active waveguide to the sham cavity is below -30 decibels. Therefore, the field in the sham cavity will be more than 1000 times smaller than the field in the active waveguide. To guarantee both the sham and RF exposed samples were subject to the same environmental conditions the waveguides were housed inside a 37.0 ± 0.1°C tissue culture incubator with a humidified atmosphere of 5.0 ± 0.2% CO_2 in air.

A holder and distance spacer was specially designed by IT'IS to allow Petri dishes to be accurately positioned inside each waveguide chamber; Figure 2.3 shows the sXc900 dish holder and Figure 2.4 the holder for the sXc1800 equipment. Four 35 mm Petri dishes could be placed in the sXc1800 waveguides whereas the sXc900 waveguides, having a larger cross-section, allowed for eight dishes to be exposed simultaneously.

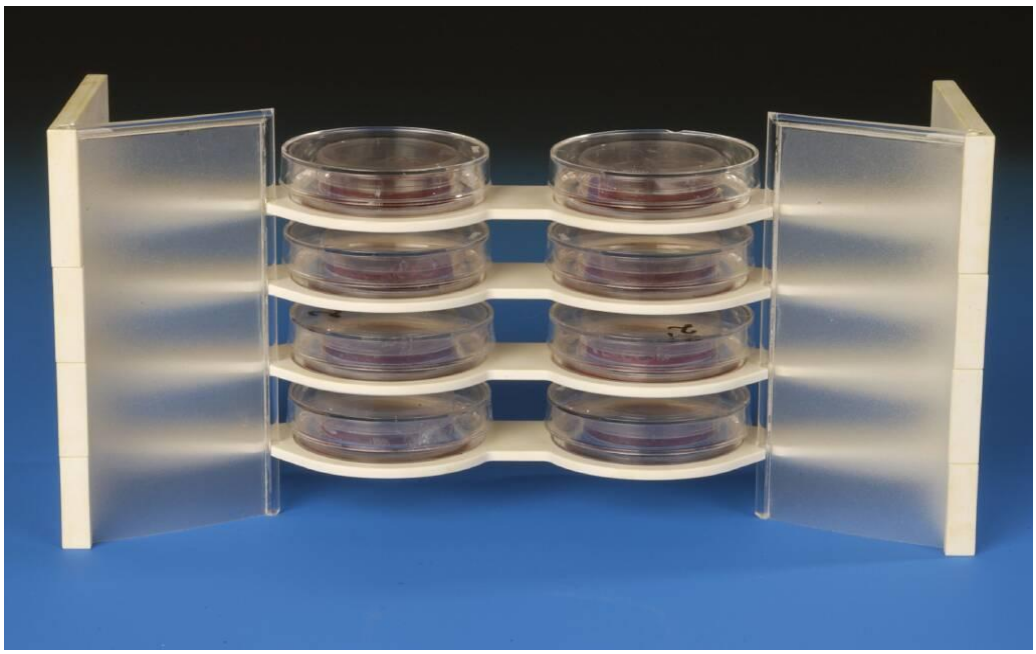

Figure 2.3. Dish holder for the sXc900 system. Blood in 35 mm Petri dishes placed inside 60 mm dishes filled with distilled water.

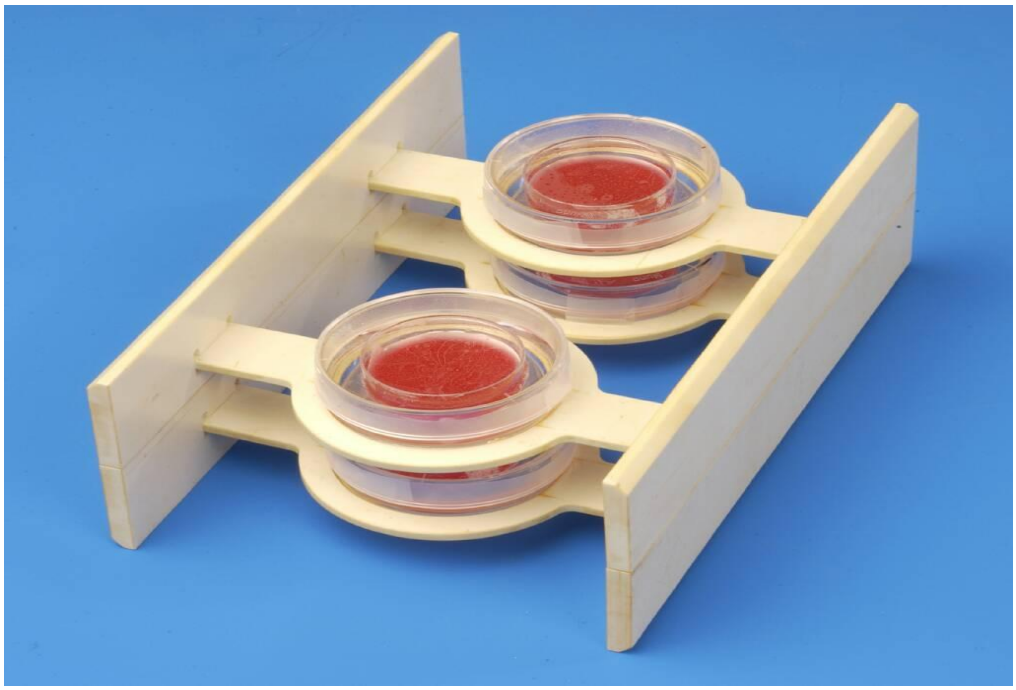

Figure 2.4. Dish holder for the sXc1800 system. Blood in 35 mm Petri dishes placed inside 60 mm dishes filled with distilled water.

The exposure of blood lymphocytes in suspension occurred in the E-field (electric field) maximum. To increase the uniformity of the SAR, 3.1 mL of whole blood was placed in 35 mm Petri dishes, which in turn were placed in the centre of a 60 mm diameter Petri dish and the space between the two dishes filled with 4.9 mL of distilled water. Exposure of monolayer neuroblastoma cell cultures occurred in the H-field (magnetic field) maximum in 35 mm Petri dishes containing 3.1 mL of medium. The optimum volumes and geometry providing homogeneous field strengths in the samples were designed and tested by the IT'IS engineers. For cells in suspension the non-uniformity of the SAR distribution was less than 40% and 54% in the sXc1800 and 900 systems respectively, providing the cells were not in the meniscus area. In tests, which allowed whole blood to settle for 1 h in the 35 mm Petri dishes, total white cell counts detected no cells in the meniscus. The sXc900 system gave a non-uniformity of the SAR distribution of less than 30% for cells in a monolayer. It is very difficult to achieve homogeneity of SAR (Kuster and Scönborn 2000), but with good air flow across the samples, the temperature becomes distributed throughout the sample and no localised 'hot-spots' were detected in the sXc waveguide system (Schuderer et al. 2004a and b).

The field and environment within the exposure systems was controlled through a combination of field sensors, temperature sensors for the air environment and an optimised airflow system. Temperature was measured using an integrated TP100 temperature probe, which had been extensively tested within the experimental setup (Schuderer et al. 2004a and b). The exposure apparatus has been demonstrated to lead to temperature changes of less than 0.1°C within the 3.1 mL of culture medium in a 35 mm Petri dish (Schuderer et al. 2004a and b). The exposure systems were controlled by a signal unit, which enabled different GSM modulated and non-modulated signals to be set by the experimenter. Other exposure parameters that could be set were the duration of the exposure, a single on cycle or a combination of on/off cycles and the average SAR. Field strength and temperature, as well as all commands were logged to encrypted files. The assignment of either waveguide to be the sham or active was made randomly by the controlling computer and the coding for this was held at IT'IS and was only broken when the assay data were complete.

2.2 RF exposure signals

Three RF exposure signals were used in all the investigations:

- GSM Basic. This is the signal emitted when a person is talking into a mobile phone. The carrier frequency is combined with an amplitude modulation which has a repetition frequency of 217 Hz. In addition to this GSM-217 Hz TDMA frame, every 26th frame is idle, which acts to add an 8 Hz modulation component to the signal.

- GSM Talk. The signal switches between GSM Basic and GSM DTX (discontinuous transmission mode), which is the signal emitted whilst a person is listening. Transmission is reduced to 12 active frames compared to 100 for GSM Basic. A DTX frame structure results in 2, 8 and 217 Hz modulation components. The computer randomly changes the duration of each mode in order to simulate a person having a conversation.

- Continuous wave (CW). This signal is comprised of just the carrier frequency. It has the same thermal load as the other signals but no modulation component. The CW signal can be applied as a reference signal.

Figure 2.5 shows the pulse structure of the GSM signals. Each frame has a period of 4.61 ms and contains a 576 µs burst of information. GSM-217 Hz is composed of a repetition of these frames (shown in grey), where every 26th frame is blanked (shown in white) for the GSM Basic signal. The GSM DTX mode is active during periods of silence and transmission is reduced.

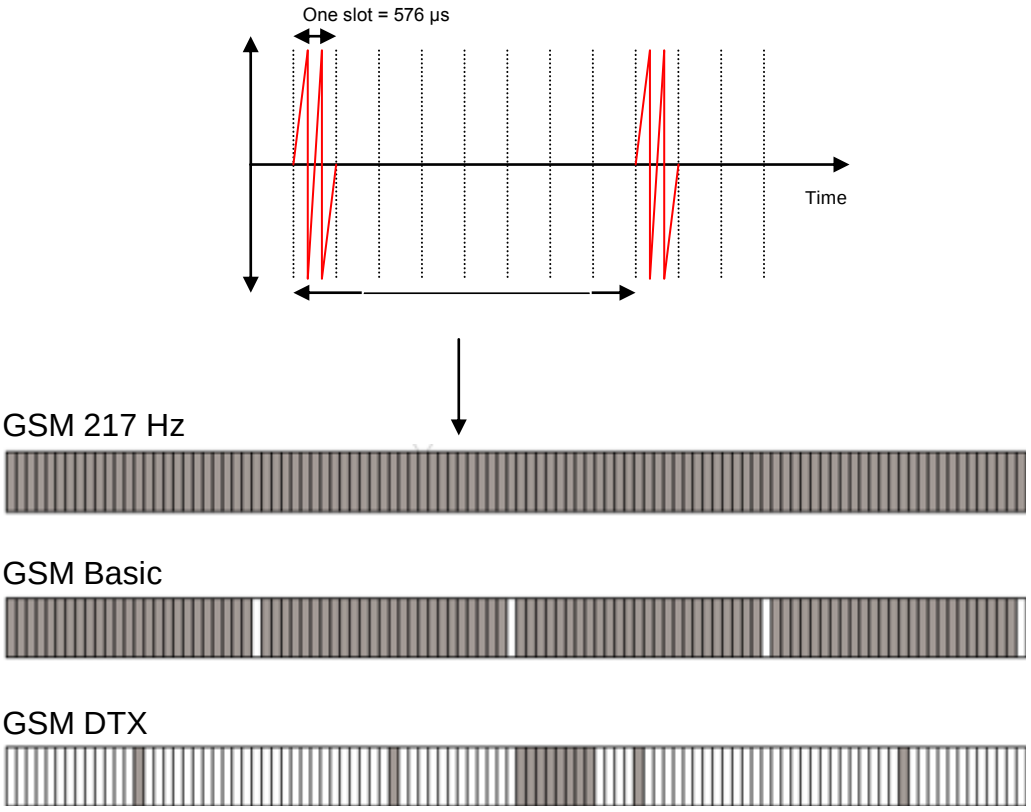

Figure 2.5. Pulse structure of the GSM signals.

2.3 RF exposure regimes

The exposure regimes and experimental conditions used in each experiment are given below:

2.3.1 Human G_0 lymphocytes in whole blood

- 935 MHz GSM Basic signal with an SAR of 1 W/kg
- 935 MHz GSM Basic signal with an SAR of 2 W/kg
- 1800 MHz GSM Basic signal with an SAR of 2 W/kg
- 935 MHz CW signal with an SAR of 1 W/kg
- 935 MHz GSM Talk signal with an SAR of 1 W/kg
- 935 MHz GSM Talk intermittent (10 min RF on, 5 min RF off) signal with an SAR of 1 W/kg

Table 2.1. Table showing the experimental conditions used with every RF signal and repeated using a total of 4 donors. For every code chromosomal aberrations, micronuclei and sister chromatid exchanges were assayed.
S = Sham; X = 1 Gy x-rays; F = Field

Experimental condition	Code
24 h sham exposure only	S
24 h RF field exposure only	F
1 Gy x-rays before a 24 h sham exposure	X + S
1 Gy x-rays before a 24 h RF field exposure	X + F
24 h sham exposure before 1 Gy x-rays	S + X
24 h RF field exposure before 1 Gy x-rays	F + X

2.3.2 PHA stimulated whole blood cultures

- 1800 MHz GSM Basic intermittent signal (5 min RF on, 10 min RF off) with an SAR of 2 W/kg
- 1800 MHz CW intermittent signal (5 min RF on, 10 min RF off) with an SAR of 2 W/kg

Table 2.2. Table showing the experimental conditions used with each RF signal and repeated using a total of 4 donors.
S = Sham; X = 1 Gy x-rays; F = Field

Experimental condition	Code	Assay
48 h sham exposure only	S	Chromosomal aberrations
48 h RF field exposure only	F	

2.3.3 Proliferating and differentiated neuroblastoma cells

- 935 MHz GSM Basic signal with an SAR of 2 W/kg
- 935 MHz GSM Talk signal with an SAR of 2 W/kg
- 935 MHz CW signal with an SAR of 2 W/kg

Table 2.3. Table showing the experimental conditions used with every RF signal and repeated 3 times for each apoptosis assay with proliferating and differentiated N2a cells.
S = Sham; RF = RF field

Experimental condition	Code	Assays
24 h sham exposure only	S	Annexin V Caspase activation *In situ* end labelling
24 h RF field exposure only	RF	

2.4 RF exposure protocol for unstimulated G_0 lymphocytes, cell culture, slide preparation and scoring

Preliminary tests revealed that keeping whole blood at 37°C in Petri dishes for 24 h had no detrimental effect on subsequent lymphocyte culture. Therefore heparinised venous blood was taken with informed consent and the ethical approval from the Health Protection Agency's (HPA) Radiation Protection Division Voluntary Studies Advisory Committee and the Oxfordshire Clinical Research Ethics Committee (reference CO2.201) ethics procedures, from four normal, healthy, non-smoking donors. Two of the donors were females aged 42 and 47 y and two were males ages 59 and 61 y. The same four donors were used throughout the study, but only blood from one donor could be exposed at any one time because of the limited capacity of the RF waveguide chambers. Therefore, blood from the four donors was exposed to a given signal over successive weeks.

Prior to a 24 h RF field or sham exposure, the whole blood was aliquoted into 35 mm Petri dishes (Nunc). The blood was exposed in the Petri dishes to 1.0

Gy of 250 kVp x-rays, with 11 mA, 1.08 mm copper HVL, at a dose rate of ~1.0 Gy / minute, either before or after the RF or sham exposure; zero ionising radiation dose control samples were also included. Physical dosimetry was carried out in the same geometry as the specimens using a Farmer dosemeter, which had been calibrated at the National Physics Laboratory (Chamber Reference: E08010365/2. Electrometer Reference E08010365/1). During the experimental exposures the dose was also monitored by an ionisation chamber placed next to the specimen holder, as shown in Figure 2.6. The x-irradiations were given at room temperature (~20°C) and the blood samples remained at this temperature for a period of approximately 10 min whilst they were transported between the x-ray and RF exposure facilities. Prior to the blood being exposed or sham exposed for 24 h to an RF field, the Petri dishes were placed in the waveguides for 1 h to allow the temperature to stabilise at 37°C and the cells to settle out of the surface plus meniscus layer. After the RF exposure the Petri dishes were immediately removed from the waveguides and taken to the x-ray facility, during which time the blood cooled to room temperature in about 15 min.

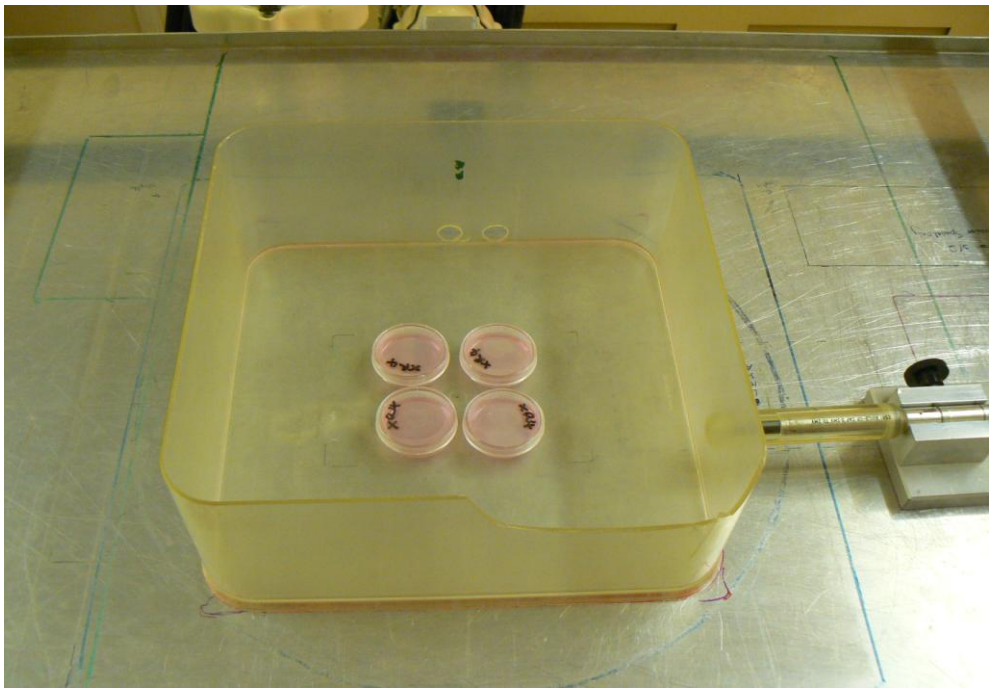

Figure 2.6. Petri dishes containing cells in the x-ray exposure facility. The x-ray tube was directly underneath the samples so the beam passed vertically through the samples. An ionisation chamber can be seen in the right of the picture.

After the RF exposure and x-irradiation of G_0 lymphocytes, together with appropriate sham and unirradiated controls, the whole blood was mixed with pre-warmed Minimal Essential Medium (Invitrogen) supplemented with 10% heat inactivated foetal bovine serum (FBS) (Invitrogen), 1% PHA (Invitrogen), 100 units/mL penicillin plus 100 µg/mL streptomycin (Invitrogen) and 2 mM L-glutamine (Invitrogen). The batch numbers of all the culture reagents were kept consistent throughout. Aliquots of 5 mL of the above were placed in culture vessels to give four replicate cultures for the chromosome aberration (CA), micronucleus (MN) and sister chromatid exchange (SCE) assays. 5-bromodeoyuridine (Sigma) was added to each CA and SCE culture at a final concentration of 10 µg/mL. All the cells were then cultured in an incubator at 37 ± 0.1°C with a humidified atmosphere of 5 ± 0.2% CO_2 in air. Colcemid (Sigma) at 0.2 µg/mL was added 3 h before termination of the CA and SCE cultures. At the end of the culture period, 48 h for the CA assay and 72 h for the SCE assay, metaphases were harvested by a standard hypotonic treatment in 0.075 M potassium chloride for 7 min at 37°C followed by three changes of 3:1 methanol:acetic acid fixative. Fixed cells were dropped onto clean microscope slides and air dried. The slides were stained by the fluorescence plus Giemsa (FPG) technique (Perry and Wolff 1974) to ensure that first and second division metaphases were scored for CA and SCE respectively. For the MN assay the cell cultures had cytochalasin B (Sigma) added at 24 h, giving a final concentration of 6 µg/mL, to block cytokinesis. Cells were harvested after a total of 72 h in culture by treatment with 0.075 M potassium chloride at 4°C followed by fixation in methanol:acetic acid (3:1) with 1% formaldehyde. There followed two further changes of 3:1 methanol:acetic acid fixative. Fixed cells were dropped onto clean microscope slides, air dried and stained with 5% Giemsa. These culture, fixation and staining procedures follow standard protocols commonly employed in radiation cytogenetics laboratories and recommended in a widely accepted manual published by the International Atomic Energy Agency (IAEA 2001).

All the microscope slides were coded for ‘blind’ analysis. 500 metaphases per donor per data point were scored for chromosome and chromatid aberrations for the CA assay. Examples of chromosome and chromatid aberrations are shown in Figures 2.7 and 2.8 respectively. Non-centromeric switches were

scored in 50 metaphases per donor per data point for the SCE assay and 500 binucleated cells with well-preserved cytoplasm were scored per donor per data point for the MN assay. Figures 2.9 and 2.10 show an example of bi-nucleate cells, one with a micronucleus, and a 2nd division metaphase with several SCE respectively. Preliminary tests irradiating whole blood in Petri dishes allowed the power and sample size, which in this context is the number of donors, required to detect an enhancing effect of RF fields on x-ray induced aberrations to be calculated. A power of 0.8 or more was achieved by a sample size of 3 or 4 donors with aberrations scored in 500 cells (CA and MN assays) and 50 cells (SCE assay).

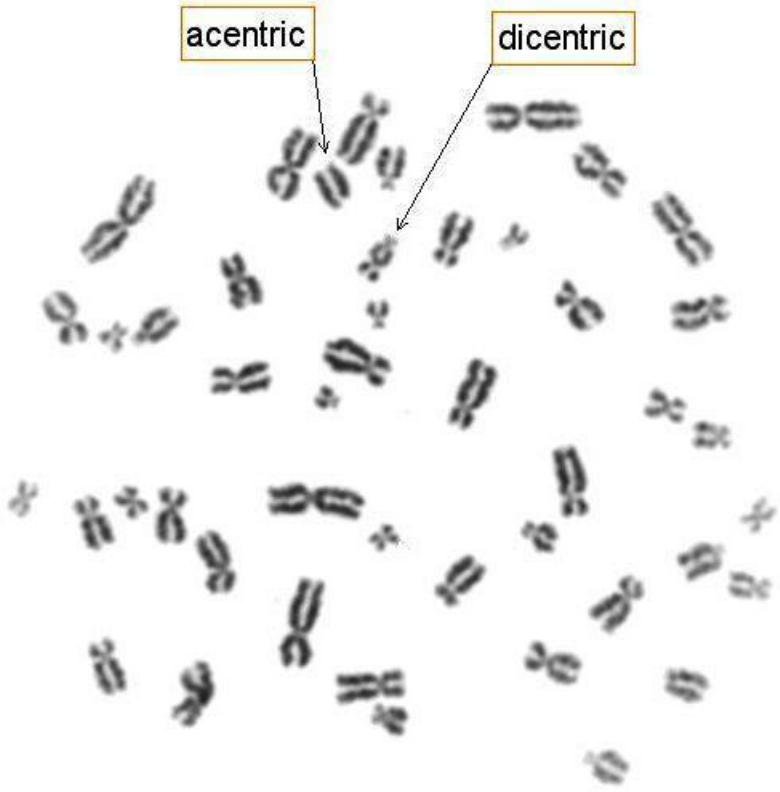

Figure 2.7. A lymphocyte metaphase spread containing a dicentric and its accompanying acentric fragment.

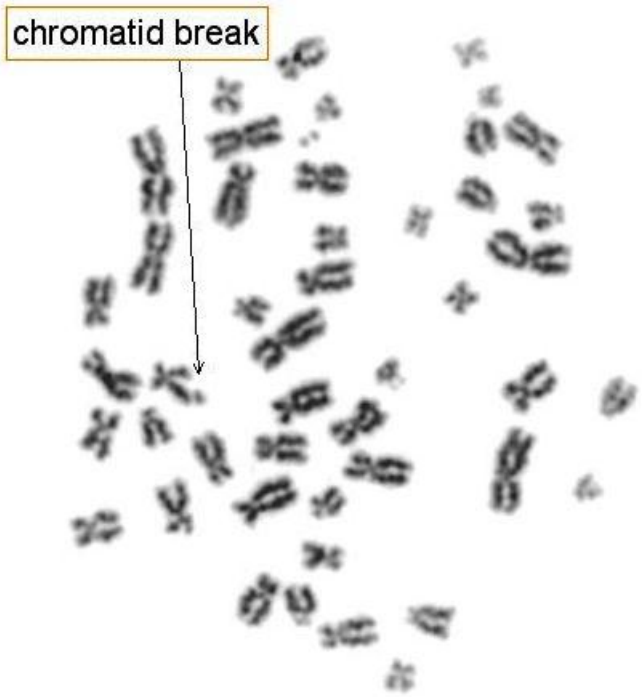

Figure 2.8. A lymphocyte metaphase spread containing a chromatid break.

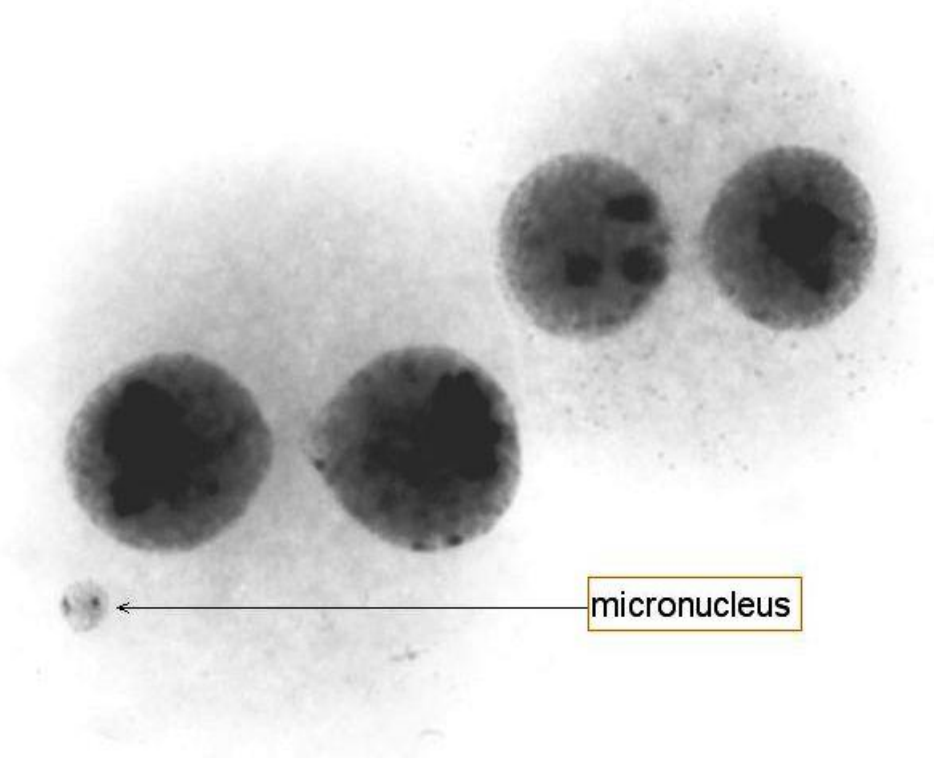

Figure 2.9. Two bi-nucleate lymphocytes, one containing a micronucleus.

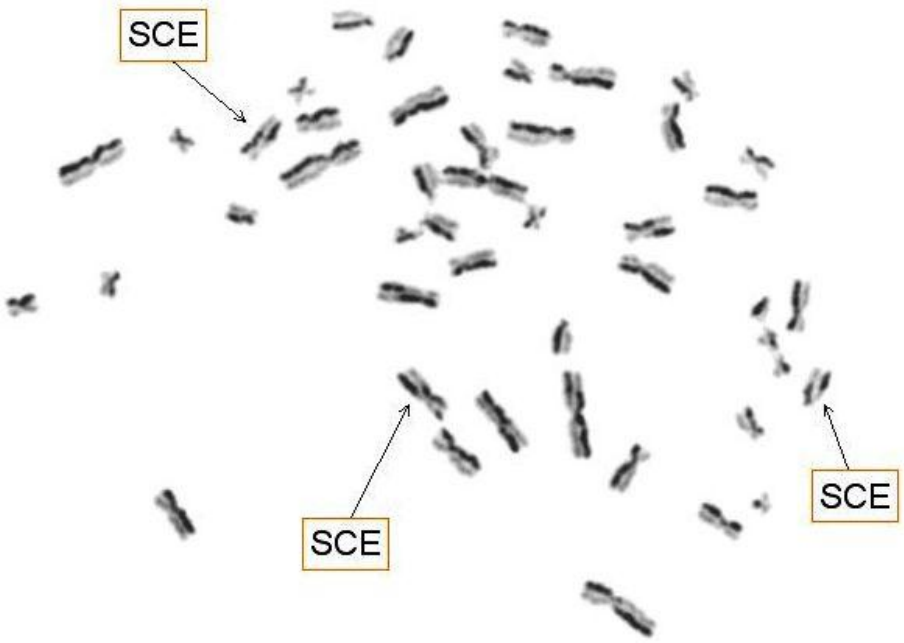

Figure 2.10. A lymphocyte 2nd division metaphase spread containing sister chromatid exchanges, (not all are indicated).

Any effect on the *in vitro* cell cycling speed, caused by the exposure regimes, was determined by re-scoring the slides from the SCE and MN assays. The relative numbers of cells in their 1st, 2nd and 3rd division in 200 cells per donor per point were recorded from the SCE assay slides. Cells with 1 to 4 nuclei in a total of 500 cells per donor per point were determined from the MN assay slides. These data then permit the Nuclear Division Indices (NDI) to be calculated; see section 3.8.4. It is clear the $NDI_{(SCE)}$ and $NDI_{(MN)}$ are not measuring *in vitro* cell cycling speed in exactly the same way. The SCE data are based on cells that have completed at least one cycle and are observed only in metaphase. By contrast the MN data include mononuclear cells that are still in their first interphase plus multinuclear cells that are in any part of the interphases in the following 3 cycles.

2.5 Cell culture, RF exposure protocol for PHA stimulated whole blood cultures, slide preparation and scoring

Using the same four donors, PHA stimulated whole blood cultures were exposed to RF fields as described in section 2.3.2. A 'giant' whole blood culture, equivalent to 8 standard whole blood cultures, using MEM medium supplemented with 10% HI-FBS, 1% PHA, 100 units/mL penicillin and 100 µg/mL streptomycin, 2 mM L-glutamine and 10 µg/mL 5-bromodeoyuridine was aliquoted into 35 mm Petri dishes. Four replicate dishes, each containing 3.1 mL of culture, were placed into each waveguide and exposed or sham exposed to the 1800 MHz intermittent RF field for a total of 48 h. The exposure was stopped briefly (~5 min) after 45 h to add Colcemid at 0.2 µg/mL and then resumed for a further 3 h. All the cultures were then processed for the CA assay described above.

2.6 Neuroblastoma cell culture and RF exposure protocol

Murine neuroblastoma Neuro2a (N2a) cells (Augusti-Tocco and Sato 1969) were obtained from Dr. J. Uney, Bristol University. Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen) supplemented with 10% HI-FBS, 2 mM L-glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin (Invitrogen) in 75 cm^3 tissue culture flasks (Sarstedt) in a 5 ± 0.2% CO_2 humidified atmosphere at 37 ± 0.1°C. The flasks were split every 2 to 3 days when the cells had reached ~80% confluence by a standard trypsinisation technique. Firstly, growth medium was removed from the flask and the cells were washed in pre-warmed (37°C) phosphate buffered saline (PBS). The cells were then incubated at room temperature for ~1 min in a 0.25% trypsin solution (Invitrogen). When the cells became detached pre-warmed DMEM containing 10% HI-FBS was added to the flask in a ratio of at least 5:1 with the trypsin solution. The cell suspension was transferred to a sterile centrifuge tube and spun at 300 g for 10 min. Fresh growth medium was added to the cell pellet, which was divided between 5 new culture flasks. This normal maintenance

culturing continued until cells were required for the experimental studies; alternatively cells were frozen for longer term storage.

When proliferating N2a cells were to be used in the experimental studies they were seeded into 35 mm Petri dishes at a density of 10^5 cells in 3.1 mL of growth medium, 24 h before any treatment. Figure 2.11 shows proliferating N2a cells that are generally round in shape and without axon-like processes.

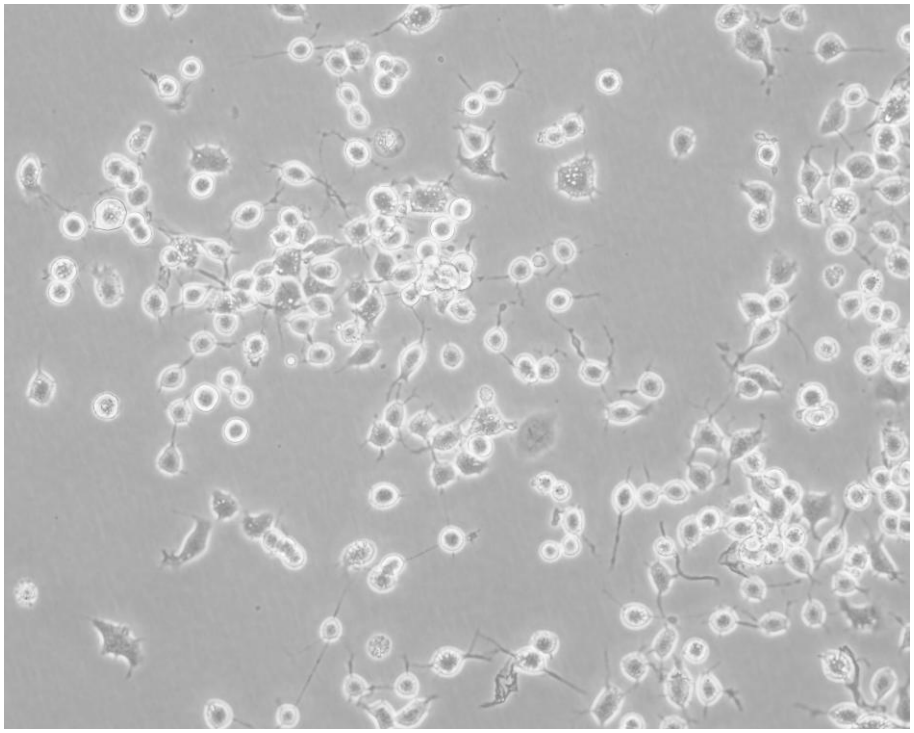

Figure 2.11. Proliferating N2a cells 2 days after plating into Petri dishes. A few cells have short processes.

Alternatively, N2a cells were induced to enter a post-mitotic state and differentiate by serum withdrawal (Seeds et al. 1970). Briefly, cells were maintained at confluence for at least 3 days before seeding them at a density of 2×10^5 cells in a 35 mm Petri dish with 3.1 mL of growth medium containing only 1% HI-FBS, 24 h before being placed into the RF exposure system. Differentiated N2a cells can be seen in Figure 3.12 with extended axon-like processes.

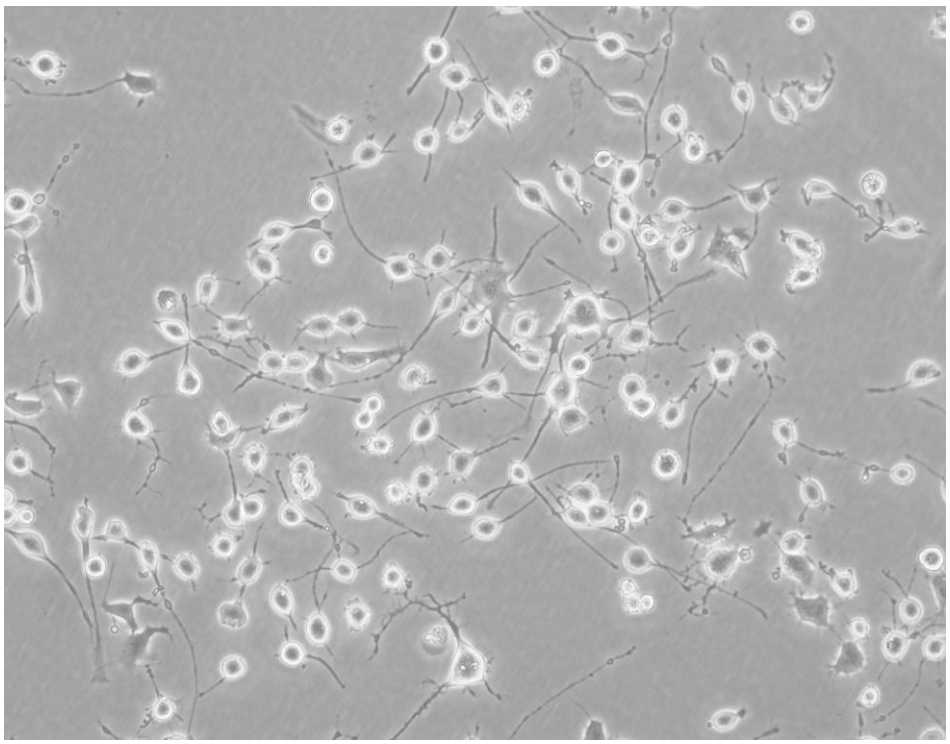

Figure 3.12. Differentiated N2a cells 2 days after plating into Petri dishes. Many cells have long processes.

One hour before the RF field exposure, dishes containing the proliferating or differentiated N2a cells were placed inside the waveguides to allow the temperature to stabilise at 37°C. Cells were then exposed or sham exposed for 24 h to the RF field. Cells irradiated with 4 Gy of 250 kVp 1.2 mm Cu HVL x-rays, at a dose rate of 1 Gy/min, were used as a positive control together with a zero dose control. Four Gy of x-rays was chosen, because preliminary tests showed this dose to give significant levels of apoptosis compared to 0 Gy control samples for both differentiated and proliferating N2a cells. These tests also allowed the required sample size, which in this context is the number of repeat experiments and the power of the assay to detect a difference to be calculated. A sample size of 3 repeat experiments could achieve a power of 0.8 when a total of 1000 cells were scored for each sample using the Annexin V and caspase assays and 3000 cells per sample using the in *situ end* labelling (Apo Direct) assay. The x-irradiations were carried out at room temperature (~20°C) and the samples were also maintained at room temperature whilst being conveyed (~5 min) between the x-ray facility and the laboratory. Following all treatments the dishes were held in an incubator and assessed at several time points for the presence of cells undergoing apoptosis.

2.7 Assessment of apoptosis

Three independent assays for apoptosis were employed using commercially available kits. These were the Annexin V-FITC (fluorescein isothiocyanate) apoptosis detection kit (BD Pharmingen), CaspaTag pan-caspase *in situ* assay kit (Chemicon International) and the *in situ* end labelling (Apo Direct) kit (BD Pharmingen).

2.7.1 Annexin V binding assay

The Annexin V binding assay detects phospholipid phosphatidylserine (PS) that has been translocated from the inner to the outer leaflet of the plasma membrane during the apoptotic process. Annexin V, labelled with a fluorochrome, is a protein with a high binding affinity for PS and apoptotic cells can thus be detected under a fluorescent microscope. The kit was used according to the manufacturer's protocol, but modified for use with adherent cells as described by van Engeland et al. (1996). In brief, the cells were washed twice in cold (4°C) PBS. The medium and the PBS were transferred to a centrifuge tube and spun to recover any non-adherent cells. Both the adherent and the recovered non-adherent cells were directly labelled with FITC-Annexin V, along with propridium iodide (PI), in a specific binding buffer. Following incubation at room temperature in the dark for 20 min, cold binding buffer was added to the cells. The bulb of a plastic pipette was used to carefully scrape cells off the dish, which were then transferred to a centrifuge tube. The dish was washed again with cold binding buffer to remove all the cells. After centrifugation the cell pellet was pipetted onto a cold slide and viewed under a fluorescence microscope. The number of apoptotic cells, which appeared Annexin V-FITC positive and PI negative, were recorded in a total of 1000 cells per data point. Cells were assayed at 0, 2, 4, 8, 24 and 48 h post exposure, with a corresponding control. Each experiment was repeated twice with fresh exposures of cells.

2.7.2 Measurement of caspase activation.

The caspase activation assay uses a carboxyfluorescein-labelled fluoromethyl ketone peptide inhibitor (FAM-VAD-FMK) that binds covalently to caspases active during apoptosis and prevents further enzymic activity. The bound peptide is retained in the cell and fluoresces green under a UV microscope. Following RF exposure/sham and x-ray exposure cells were assessed for pan-caspase activity; again according to the manufacturer's protocol but modified for adherent cells. Firstly, the cells were washed twice in DMEM medium and both the culture and the wash media were transferred to a centrifuge tube to collect any cells that had detached from the growth surface. The recovered and the adherent cells were then directly incubated with the FITC-labelled FAM-VAD-FMK inhibitor for 70 min at 37°C in an atmosphere of 5% CO_2 in air. Cells were incubated for a further 5 min after the addition of Hoechst stain. The cells were then washed with cold buffer and the bulb of a plastic pipette was used to carefully scrape the adherent cells off the surface of the dish, which were then transferred to a centrifuge tube. After rinsing the dish again the cells were centrifuged and the pellet mounted on a cold microscope slide with a drop of wash buffer containing PI. Caspase positive cells undergoing apoptosis were stained FITC positive/PI negative and the number of such cells, in a total of 1000 per data point, was recorded. Figure 3.13 shows a typical microscope image of N2a cells stained by the caspase activation assay. The cell stained green is apoptotic, the blue cells are normal and the cell stained red is dead. Cells were assayed at 0, 4, 8, 24 and 48 h post exposure, with a corresponding control. Each experiment was repeated two more times with fresh exposures of cells.

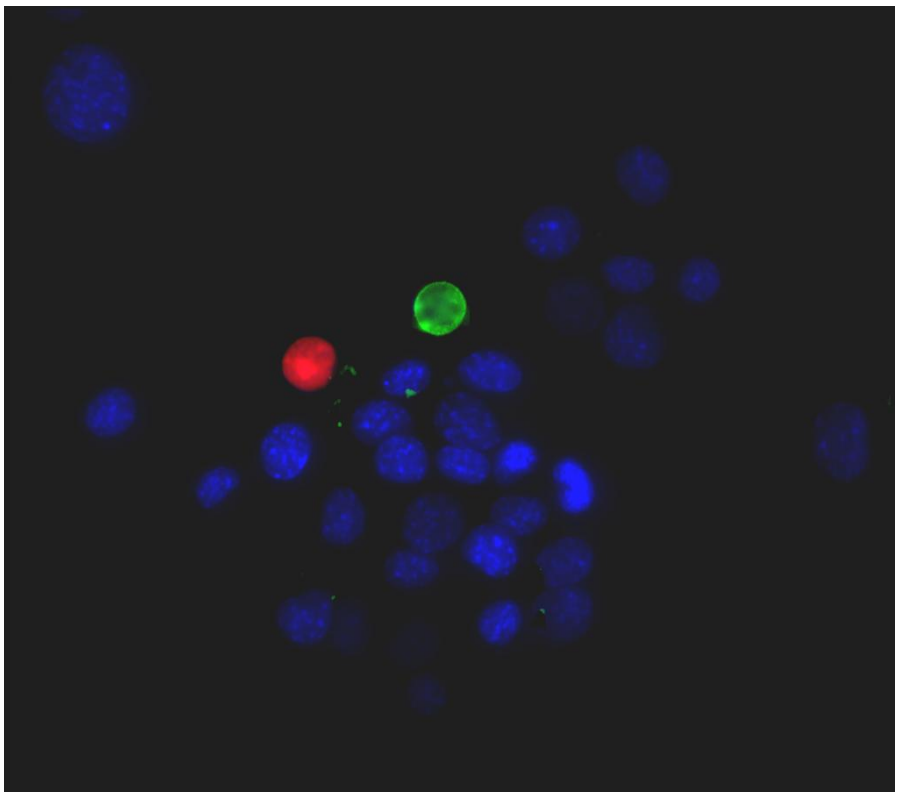

Figure 3.13. A microscope image of N2a cells stained by the caspase activation assay. The cell stained green is apoptotic, the blue cells are normal and the cell stained red is dead.

2.7.3 *In situ* end labelling (Apo Direct) assay

A later step in the apoptotic pathway is DNA fragmentation by endonucleases. The Apo Direct assay uses terminal deoxynucleotidyl transferase (TdT), which catalyses the labelling of DNA breaks with FITC labelled deoxyuridine triphosphates (dUTP), so that apoptotic cells fluoresce under a UV microscope. Following exposure and post incubation, cells that had detached from the base of the Petri dish were collected in the culture medium and combined with the adherent cells harvested by trypsinisation, as described previously for the sub-culture of N2a cells. Using the manufacture's protocol, cells were then fixed with 1% paraformaldehyde for 1 h on ice. After washing in PBS the cells were incubated in PBS with 0.2% Tween 20 at room temperature for 5 min. Following another wash in PBS the cells were suspended in 70% ice cold ethanol and kept at -20°C for at least 48 h. Cells from three replicate dishes were pooled for staining and analysis. After centrifugation and removal of the 70% ethanol, cells were washed twice in buffer. The fixed cells were then incubated with terminal

TdT in the presence of FITC-dUTP for 75 min at 37°C to detect DNA breaks. At the end of the incubation cells were washed twice in rinse buffer and the cell pellet was added to a drop of Vectashield antifade solution (Vector) containing 100 ng/mL Hoechst stain on a microscope slide. Viewed under a fluorescent microscope the apoptotic cells appeared green (FITC positive) and the rest blue. 3000 cells per data point were scored at times of 0, 24 and 48 h post exposure, together with a control. Each experiment was repeated twice with fresh exposures of cells.

2.8 Statistical analysis

Decoded data were subjected to statistical analysis and all statistical parameters were calculated using Microsoft Excel® and Minitab® 15.

2.8.1 Conformity to Poisson

For the CA, MN and SCE assays the distribution of damage among the scored cells from each donor were tested for conformity with the Poisson distribution, by calculating the dispersion index σ^2/y (ratio of variance to mean) and its normalised test statistic U. Over-dispersion is indicated by a value of $\sigma^2/y > 1.0$ and $U > 1.96$ (Edwards et al. 1979). Any over-dispersion in a data set was taken into account by an appropriate increase in the Poisson standard error (SE) (IAEA 2001). The SE was increased by multiplying the number of aberrations by the average value of σ^2/y, before calculating the Poisson error in the usual manner. As shown below

$$SE = \frac{\sqrt{AO}}{N}$$

where A is the number of aberrations seen, O is the average σ^2/y and N is the number of cells scored.

2.8.2 Chi-squared test for homogeneity

For the cytogenetic studies and for each end point assayed the data from the individual donors were pooled following a Chi-squared test for homogeneity that was carried out for each of the exposure regimes. In addition this test was carried out to ensure the apoptosis data from the three experimental repeats could be pooled.

The Chi-squared test for homogeneity was used to test the consistency of the results between blood donors/repeated apoptosis assays. No significant differences between donors/experiments allowed data to be pooled and make the analysis easier. To test for homogeneity the hypothesis of no difference between the samples was examined by calculating Chi-squared values (χ^2) using the following equation,

$$\chi^2 = \sum_i \frac{O_i - E_i}{E_i}$$

where O is the observed number and E the expected number of aberrations or apoptotic cells, for *i* measurements. E was calculated by finding the mean of the observed values. The null hypothesis predicts no significant difference ($\chi^2 <$ DF) between the observed and the expected value. Also, with random variation, large deviations from zero should not occur very often. The χ^2 value was compared to the Chi-squared distribution for a given number of degrees of freedom (DF), calculated from $n-1$ where n is the sample size, to give a probability value (p). Deviations from the expected were considered significant at a p value of less than 0.05 and inhomogeneity was taken into account by an appropriate increase to the SE on the mean of each pooled data point. In such cases the SE was enhanced by multiplying the total aberrations by the ratio of the Chi-squared value / degrees of freedom (χ^2 / DF),before calculating the Poisson SE in the usual way.

2.8.3 Student's t-test

The Student's t-test was used to determine the significance of any difference between the means of exposed and sham exposed cells. Data analysed in this way were the pooled chromosome aberrations and the chromatid damage in cycling cells, as well as the pooled apoptosis data at each time point.

The t-test value for the data from cycling cells was calculated using the following equation,

$$t = \frac{m_{sham} - m_{\exp osed}}{\sqrt{\left(\frac{s_s^2}{n_s} + \frac{s_e^2}{n_e}\right)}}$$

where m is the mean number of aberrations in the sham or exposed cells, s is the standard deviation of the sham (s) and exposed (e) samples and n the number of samples (donors). The standard deviation (SD) was calculated using the formula,

$$s = \sqrt{\frac{\sum_i (x_i - m)^2}{(n-1)}}$$

where x is the observation, m is the mean and n is the number of samples (donors). The degrees of freedom for the t-test was calculated by

$(n_{sham} - 1) + (n_{\exp osed} - 1)$

This version of the t-test assumes the samples are unpaired and the variances are different. It is the appropriate t-test to use when sample sizes are small. It was chosen for the data in these studies because the samples are independent and small in size. For all t-tests the population is also assumed to be normally distributed. A calculated t-test statistic follows the Student's distribution when there is no significant difference between the two means, for a given value of degrees of freedom. A difference in the two means was considered significant when the p value on the t-test statistic was less than 0.05%.

T-tests were also performed on the pooled apoptosis data. However the data from the *in situ* end labelling assay (Apo Direct) was not as normally distributed as the data from the other two assays; it had a narrower distribution than expected. Therefore, SE was used instead of SD to take account of the variation in the mean. This was done for all the data from the three apoptosis assays so as to be as consistent as possible.

2.8.4 Nuclear division index (NDI)

The NDI was calculated using the following equation (Eastmond and Tucker 1989),

$$NDI = \frac{N_1 + 2N_2 + 3N_3 + 4N_4}{n}$$

where n is the total number of cells and N_1, N_2, N_3 and N_4 are respectively the number of cells with one, two three and four nuclei. The same equation can be applied to the FPG stained material for cells in 1st, 2nd and 3rd division.

Uncertainties in the NDI is not addressed in the Eastmond and Tucker paper and indeed do not seem to be published anywhere. It is therefore considered here and I am grateful to Dr. Richard Haylock and Dr. Elizabeth Ainsbury for producing the method.

Standard error analysis cannot be used to calculate the uncertainties on the NDI because the values of N_1 to N_4 are correlated. Therefore, a measure of how the variables are dependent on each other, the covariance, needs to be taken into account. Assuming the NDI values N_1 to N_4 form a multinomial distribution the variance of each value of N_1 to N_4 can be calculated using the equation

$$\mathrm{var}(N_i) = np_i(1 - p_i)$$

and the covariance of each value by the equation

$$\mathrm{cov}(N_i, N_j) = -np_i p_j$$

i and j = 1, 2, 3 or 4 for N_1, N_2, N_3 and N_4, where n is the total number of cells scored and p_i and p_j are the probabilities of N_i and N_j, which are equal to N_i and N_j divided by n.

The total variance of NDI was then calculated by using the following equation,

$$\mathrm{var}(NDI) = \sum_{i=1}^{4} N_i^2 \,\mathrm{var}(N_i) + 2\sum_{i=1}^{4}\sum_{j=i+1}^{4} N_i N_j \,\mathrm{cov}(N_i N_j)$$

2.8.5 Analysis of variance (ANOVA)

To make comparisons and test for significant differences between more than two sample means a general linear model ANOVA test was used for both the data from the cytogenetic and apoptosis studies. The technique examines the variation within a whole group of sample means and consists of a comparison between two estimates of the overall variance. One estimate is based on the variance of the sample means about the total mean and the second is based on the variance of individual measurements. The ratio of the two, known as the F statistic, was calculated and a probability of obtaining such a ratio if the null hypothesis of no difference (F=1) is true, was determined.

ANOVA testing allows simultaneous comparisons of a number of factors and has an important advantage over multiple t-tests' because it avoids the error associated with performing many t-tests. As the number of multiple t-tests increases the greater is the probability of a test giving a false result. ANOVA can be used providing the data points are independent and their distribution is normal. A general linear model ANOVA was used because this allows a different number of replicates per treatment to be analysed i.e. unbalanced data. This method was used to evaluate the importance of several factors such as RF field status (on or off), x-ray status (on or off) and order of exposure (x-rays then RF or *vice versa*).

2.8.6 Power

In any statistical decision making process the possible errors are described as either type I (α, or false positive) or type II (β or false negative). The probability

of making a type I error, rejecting a true null hypothesis, is the significance level (e.g. $\alpha = 0.05$ or 5%) and $1 - \alpha$ is the confidence level (e.g. 95%). A type II error is the probability of not rejecting a false null hypothesis. The probability of committing a type II error is β; $1 - \beta$ is the power, which is the probability of avoiding such an error. Therefore, the power of a test provides an indication of the statistical strength of a data set and tells us, in terms of a probability value, the ability of a test to detect a significant difference given that it does exist.

Power is affected by three factors: the sample size, which in the context of this work means the number of blood donors or the number of repeat experiments per data point; the significance level and the assigned level of interest in the difference between the control and experimental samples. For the cytogenetic data in this study the required significance level was 0.05 and the sample size for a power of 0.8 was calculated using Minitab®15 to ensure any enhancing effect of RF fields on aberrations induced by x-rays would have been detected. These values demonstrate that the data are robust enough to have detected a 20% or larger difference between sham and exposed samples with 95% confidence.

3. Chromosomal studies with human lymphocytes using GSM Basic signals

3.1 Introduction

Published studies investigating the possible genotoxicity of RF fields using standard *in vitro* assays for chromosomal breakage have been reviewed and the vast majority of studies have concluded that, when heating is not a significant factor, RF fields are not genotoxic. However, a small number of studies have suggested a positive effect, thereby leaving open the question of whether in some circumstances RF fields are indeed genotoxic. It is possible that effects of RF fields may act, and therefore only become apparent, in conjunction with other well established genotoxic agents. Thus, RF fields may act in an indirect dose modifying manner, for instance by affecting the ability of cells to repair or influence mis-repair or non-repair of the primary lesions produced in their DNA. Using standard cytogenetic assays, such as those involving chromosome aberrations, micronuclei and sister chromatid exchanges, this would be expressed as an observed frequency of chromosomal alterations above that expected from a proven mutagen alone. Maes et al (1996) showed such an enhancing effect of RF fields on chromosomal damage induced by the chemotherapeutic agent mitomycin C in human lymphocytes. Initially, results from the same group suggested a weak synergistic effect of RF fields (Maes et al 1997); however these results have not been repeated in their subsequent work (Maes et al 2000 and 2001).

Using the well characterised sXc exposure system, this study investigates whether RF fields related to mobile phone communication can potentiate the genotoxic effects of another mutagen *in vitro*. The mutagen chosen was x-rays, for which the genotoxic effects are well understood in a number of standard cytogenetic assays. The primary lesions of interest produced by ionising radiation are DNA SSBs and DSBs and the residue of unrepaired or misrepaired lesions, particularly of DSBs, is responsible for visible chromosomal alterations. It is possible that an RF field exposure to cells already containing

strand breaks may alter the fidelity of their repair, or prior exposure to an RF field may alter the repair capacity in cells that subsequently incur strand breaks. Therefore, cells were exposed to x-rays either before or after the application of an RF field. All the RF exposures have been to an 1800 or 935 MHz GSM Basic signal delivering an SAR of 1 or 2 W/kg, as these are appropriate to much mobile communication. The study has used the human lymphocyte as the cell of choice from four blood donors. G_0 lymphocytes were exposed continuously to RF fields for 24 h and the temperature was kept very close to 37°C, as DNA repair processes involve the interaction of several intracellular enzymes.

Three cytogenetic tests which assay genotoxicity were performed using aliquots of blood from the same exposed sample. The chromosome aberration (CA), micronucleus (MN) and the sister chromatid exchange (SCE) assays. The MN and SCE techniques also provided data that could be used to measure the nuclear division index (NDI) or speed of *in vitro* cellular proliferation. In addition, PHA stimulated lymphocytes were also exposed for 48 h to sham and intermittent RF fields only, i.e. without x-rays, to investigate any genotoxic effects on cycling cells. A full description of the materials and methods is given in chapter 2.

3.2 Results and discussion

These data come from a joint project between the UK Health Protection Agency (HPA) and the Italian Ente per le Nuove Tecnologie, l'Energia a l'Ambiente (ENEA) and some results have been published (Stronati et al. 2006). In parallel with my studies at 1800 and 935 MHz GSM Basic, the ENEA laboratory also conducted the alkaline comet assay together with CA and MN assays, but not SCE, using G_0 lymphocytes only. However the results shown here summarise my data alone.

An average temperature rise of 0.05°C with a range of 0.02 to 0.09°C occurred during the RF exposure, which was based on data collected continuously during each 24 h exposure in all studies. The temperature rise is sufficiently low so that

if any effect were to be observed it is reasonable to presume that it was due to non-thermal processes.

Shown in Table 3.1 are the results of calculating the actual power of the dicentric, micronuclei and SCE assays to detect an enhancing effect of RF fields for the three GSM Basic signals used. On average a power of 0.8 was achieved with a sample size of four in all three assays. Therefore, the statistical strength of the data is sufficient to have detected a difference between RF and sham exposed samples when combined with x-rays.

Table 3.1. The actual power and required sample size of the combined exposures to detect an enhancing effect of RF fields on dicentrics, micronuclei and SCE induced by x-rays.
S = Sham; X = 1 Gy x-ray; F = RF field

Assay and experimental conditions compared	935 MHz GSM Basic 1 W/kg		935 MHz GSM Basic 2 W/kg		1800 MHz GSM Basic 2 W/kg	
	Actual power for sample size of 4	Required sample size for a power of 0.8	Actual power for sample size of 4	Required sample size for a power of 0.8	Actual power for sample size of 4	Required sample size for a power of 0.8
Dicentric: X + S, X + F	1.00	3	1.00	3	0.84	4
Dicentric: S + X, F + X	1.00	2	0.84	4	1.00	3
MN: X + S, X + F	0.97	3	0.55	7	0.76	5
MN: S + X, F + X	0.67	6	1.00	2	—	—
SCE: X + S, X + F	1.00	3	1.00	3	1.00	3
SCE: S + X, F + X	1.00	2	1.00	2	1.00	2

3.2.1 Chromosome aberrations

The results of the chromosome aberration assay are presented in Tables 3.2 to 3.4 for the following RF signals: 935 MHz GSM Basic (1 W/kg), 935 MHz GSM Basic (2 W/kg) and 1800 MHz GSM Basic (2 W/kg) respectively. Tables 3.2 to 3.4 show that dicentrics, centric rings and excess acentrics are only present in the exposure regimes that included x-rays. Exposure to the RF field alone produced numbers of aberrations consistent with the sham only and these were in accordance with the expected background frequency from the laboratory's historical control database. Using this assay, there was no evidence that RF fields alone are genotoxic. Dicentric distributions are show in Tables 3.2 to 3.4 and, as expected, (Edwards et al. 1979) they conform to the Poisson distribution. This is shown in column 9, where on average a ratio of variance to mean (σ^2/y) ~ 1.0 and column 11 showing only a few (5 shown in red out of a total of 48) U values > 1.96. For presentation in Figure 3.1 data have been combined by pooling the replicate results for the four donors for each of the three RF signals used.

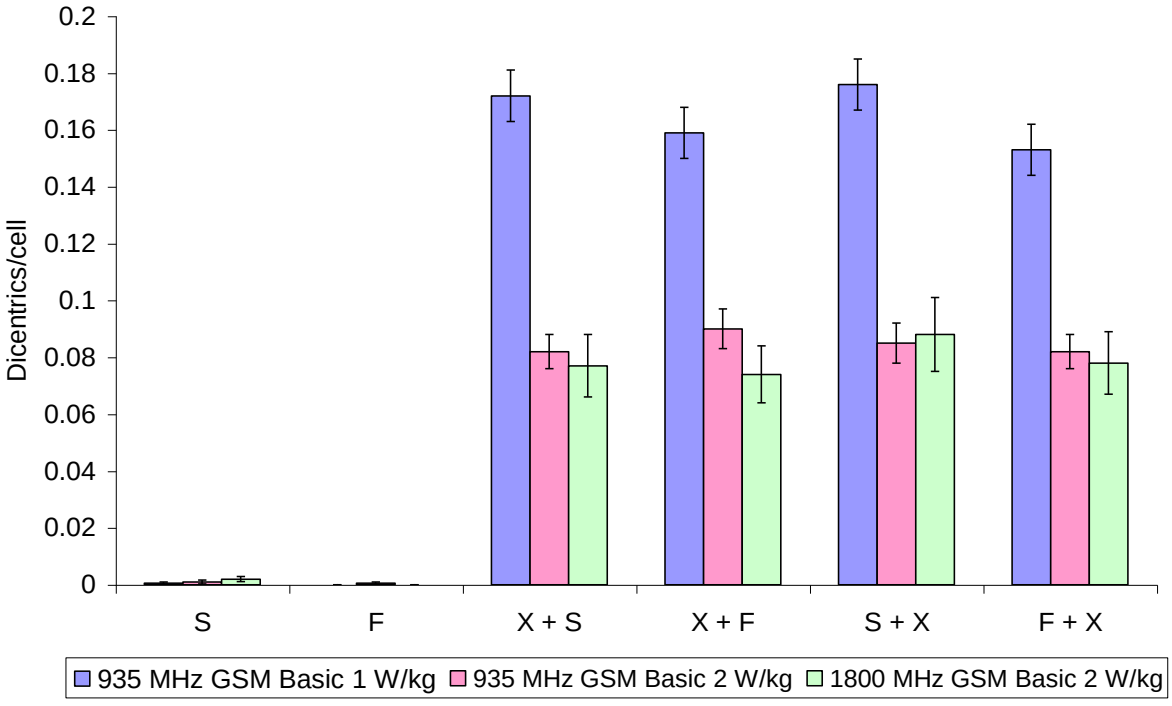

Figure 3.1. The pooled donors' data of dicentric chromosomal aberration yields ± SE for the 3 RF exposure regimes.
S = sham; X = 1 Gy x-rays; F = RF field

The pooled data and the results of the chi-squared test for homogeneity, on 3 degrees of freedom, are shown in Table 3.5. Inhomogeneity in the pooled data, as indicated by a p value of < 0.05, was taken into account by increasing the standard error on the data at the appropriate experimental conditions, as described in Chapter 2 section 2.8.2. It is clear from Figure 3.1 that the sets of 4 positive columns (X+S; X+F; S+X; F+X) for any given GSM Basic signal are statistically indistinguishable. RF field exposure either before or after x-rays resulted in dicentric frequencies which were no different from those produced by x-rays with a sham exposure.

Other unstable aberrations, centric rings and excess acentrics, are also shown in Tables 3.2 to 3.4 and in Appendix 1 the distribution of these aberrations amongst the cells are recorded in Tables A.1 to A.3 and A.4 to A.6 respectively. In addition, the data for chromatid type aberrations found for the different exposure regimes in 4 donors using the three GSM Basic signals are recorded in Appendix 1 in Tables A.7 to A.9. The different types of chromatid aberrations are recorded separately, but as the numbers are small all the statistical analysis has been performed on the sum of chromatid gaps, breaks, iso-tid gaps and exchanges. Centric rings and total chromatid aberrations conform to the Poisson distribution, but the data for excess acentrics show signs of over-dispersion in the samples exposed to x-rays. Approximately 30%, (again shown in red in Tables A.4 to A.6), had U values > 1.96. On average the x-ray exposed samples had a ratio of variance to mean (σ^2/y) of 1.09, which agrees with previous data produced in the HPA laboratory (Lloyd et al. 1986b). This factor, 1.09, was used to enhance the individual Poisson SE. Just as for dicentrics, the replicate results for the four donors have been combined to give pooled data for centric rings, excess acentrics and chromatid aberrations for every exposure regime and each GSM Basic signal. The pooled data are shown in Table 3.6 for centric rings, Table 3.7 for excess acentrics and Table 3.8 for total chromatid aberrations, together with the results of the chi-squared test for homogeneity. No evidence for inhomogeneity was found in the centric ring, excess acentric nor the chromatid data. The results of these other unstable aberrations and the chromatid damage lead to an identical conclusion that the RF fields used in this study have not affected the chromosomal aberrations induced by x-rays.

A general linear model ANOVA was performed on the chromosome and chromatid data and the results are shown in Table 3.9. This shows the RF field was not an important factor, nor was the order of the x-ray exposure on the frequency of any aberration. For dicentrics, centric rings and excess acentrics, x-rays was a significant factor ($p < 0.001$), but not for chromatid aberrations. It is known that chromatid aberrations are induced following irradiation of cells in G_2/S phase of the cell cycle (IAEA 2001), as the blood lymphocytes in this study were irradiated in G_0 no effect of x-rays is to be expected. The ANOVA test also revealed that signal was a significant factor ($p < 0.001$) for all the chromosomal aberrations. However, this is the result of the higher number of aberrations seen in the data set for the 935 MHz GSM Basic signal at an SAR of 1 W/kg than the other signals with an SAR of 2 W/kg. Although there is no statistically significant difference, ($p = 0.28$), for any exposure regime involving x-rays with the 935 MHz GSM Basic signal at 1 W/kg, Figure 3.1 shows the dicentric yield for all these exposure regimes with this signal is approximately double that of the other two data sets; an average dicentric yield of 0.165 compared to 0.082. Tables 3.6 to 3.8 show the combined yields of centric rings, excess acentrics and chromatid aberrations respectively which are also at least double for the 935 MHz GSM Basic signal (1 W/kg) compared to the other signals. The result cannot be a due to any effect of the RF field, because it occurs in all the x-ray exposure regimes; nor can it be attributed to any one donor as all 4 give high dicentric yields.

Preliminary experiments using the 4 donors had shown whole blood, irradiated in Petri dishes, either before or after being held for 24 hours at 37ºC in a tissue culture incubator, gave dicentric yields consistent with the laboratory's x-ray calibration curve (Lloyd et al. 1986b) of 0.10 ± 0.01 dicentrics/cell for a dose of 1 Gy. The Chi-squared test on the GSM Basic (1 W/kg) data set, (Table 3.5), showed no evidence of inhomogeneity between the donors, as no p value was less than 0.05. Therefore, if an error had occurred during the x-ray exposure of these samples it would need to have been four mistakes for the days each donor was used. Following checks at the irradiation facility it was confirmed that the blood had been exposed to 1 Gy of x-rays. In addition, that the fact the yields of chromatid aberrations were also higher is odd and would suggest that it is not radiation linked. A check was made on the temperature, humidity and

CO_2 levels in the incubator housing the sXc equipment and everything was found to be correct. The tissue culture incubator was also inspected and found to be running correctly. In addition, the batch numbers of the medium, serum and other culture reagents were consistent throughout the study using all the GSM Basic signals. Fresh slides were made from remaining fixed cell suspensions for some of the cultures giving the highest dicentric yields and these were again assessed by myself and a number of the original slides were also rescored by an experienced colleague. Both sets of scoring confirmed the high dicentric yield. Having considered all the 'known factors' that could possibly affect the dicentric yield, the reason for the higher than expected aberration yields in the data set for the 935 MHz GSM Basic (1 W/kg) signal remains unresolved. However, it is clear the RF field had no effect on the dicentric yield, as the exposed and sham samples were not significantly different.

Table 3.2. Number of chromosome aberrations scored for the different exposure regimes in 4 donors using a 935 MHz GSM Basic signal at an SAR of 1 W/kg. The dicentric yields and distributions amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of dicentrics	Number of centric rings	Number of excess acentrics	Dicentrics per cell ± SE	Distribution of dicentrics				σ^2 / y ± SE	U
							0	1	2	3		
1, S	500	497	1	0	2	0.002 ± 0.002	499	1			—	—
1, F	500	497	0	0	3	—	500				—	—
1, X + S	500	365	91	5	66	0.182 ± 0.019	419	71	10		1.04 ± 0.06	0.63
1, X + F	500	395	79	3	41	0.158 ± 0.018	431	60	8	1	1.12 ± 0.06	1.95
1, S + X	500	375	89	4	53	0.178 ± 0.019	418	75	7		0.98 ± 0.06	-0.3
1, F + X	500	402	59	2	44	0.118 ± 0.015	445	51	4		1.02 ± 0.06	0.31
2, S	500	499	0	0	1	—	500				—	—
2, F	500	499	0	0	1	—	500				—	—
2, X + S	500	387	86	2	46	0.172 ± 0.019	421	72	7		0.99 ± 0.06	-0.11
2, X + F	500	364	86	2	64	0.172 ± 0.019	418	78	4		0.92 ± 0.06	-1.23
2, S + X	500	363	97	5	68	0.194 ± 0.020	412	79	9		0.99 ± 0.06	-0.1
2, F + X	500	367	92	3	58	0.184 ± 0.019	416	76	8		0.99 ± 0.06	-0.13
3, S	500	499	0	0	1	—	500				—	—
3, F	500	500	0	0	0	—	500				—	—
3, X + S	500	388	81	3	47	0.162 ± 0.018	426	68	5	1	1.04 ± 0.06	0.6
3, X + F	500	408	60	6	44	0.120 ± 0.015	445	50	5		1.05 ± 0.06	0.78
3, S + X	500	388	79	3	56	0.158 ± 0.018	428	65	7		1.02 ± 0.06	0.34
3, F + X	500	392	72	6	44	0.144 ± 0.017	433	62	5		1.00 ± 0.06	-0.05
4, S	500	499	0	0	1	—	500				—	—
4, F	500	499	0	0	1	—	500				—	—
4, X + S	500	363	85	13	73	0.170 ± 0.018	423	69	8		1.02 ± 0.06	0.32
4, X + F	500	337	92	2	85	0.184 ± 0.019	414	80	6		0.95 ± 0.06	-0.82
4, S + X	500	378	86	3	53	0.172 ± 0.019	422	70	8		1.02 ± 0.06	0.26
4, F + X	500	376	82	6	58	0.164 ± 0.018	425	68	7		1.00 ± 0.06	0.14

Table 3.3. Number of chromosome aberrations scored for the different exposure regimes in 4 donors using a 935 MHz GSM Basic signal at an SAR of 2 W/kg. The dicentric yields and distributions amongst the cells are also shown. Figures in red indicate U values > 1.96 (see section 2.8.1 for explanation).
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of dicentrics	Number of centric rings	Number of excess acentrics	Dicentrics per cell ± SE	Distribution of dicentrics				σ^2 / y ± SE	U
							0	1	2	3		
1, S	500	499	1	0	0	0.002 ± 0.002	499	1			—	—
1, F	500	497	1	0	2	0.002 ± 0.002	499	1			—	—
1, X + S	500	444	33	0	29	0.066 ± 0.012	468	31	1		1.00 ± 0.06	-0.05
1, X + F	500	456	34	3	17	0.068 ± 0.012	470	26	4		1.17 ± 0.06	2.72
1, S + X	500	456	31	0	28	0.062 ± 0.011	470	29	1		1.00 ± 0.06	0.07
1, F + X	500	438	37	0	29	0.074 ± 0.012	463	37			0.93 ± 0.06	-1.16
2, S	500	498	1	0	1	0.002 ± 0.002	499	1			—	—
2, F	500	499	0	0	1	—	500				—	—
2, X + S	500	439	40	1	26	0.08 ± 0.013	461	38	1		0.97 ± 0.06	-0.45
2, X + F	500	439	41	1	24	0.082 ± 0.013	460	39	1		0.97 ± 0.06	-0.5
2, S + X	500	434	42	1	32	0.084 ± 0.013	460	38	2		1.01 ± 0.06	0.02
2, F + X	500	427	44	0	34	0.088 ± 0.013	458	41	0	1	1.05 ± 0.06	0.81
3, S	500	498	0	0	2	—	500				—	—
3, F	500	499	0	0	1	—	500				—	—
3, X + S	500	426	38	1	40	0.076 ± 0.012	463	36	1		0.98 ± 0.06	-0.34
3, X + F	500	417	56	2	43	0.112 ± 0.015	450	45	4	1	1.14 ± 0.06	2.24
3, S + X	500	404	54	2	48	0.108 ± 0.015	447	52	1		0.93 ± 0.06	-1.1
3, F + X	500	438	36	2	29	0.072 ± 0.012	465	34	1		0.99 ± 0.06	-0.23
4, S	500	500	0	0	0	—	500				—	—
4, F	500	500	0	0	0	—	500				—	—
4, X + S	500	414	53	2	44	0.106 ± 0.015	449	49	2		0.97 ± 0.06	-0.46
4, X + F	500	411	48	2	53	0.096 ± 0.014	454	44	2		0.99 ± 0.06	-0.17
4, S + X	500	430	43	1	34	0.086 ± 0.013	459	39	2		1.01 ± 0.06	0.14
4, F + X	500	436	47	0	21	0.094 ± 0.014	455	43	2		0.99 ± 0.06	-0.11

Table 3.4. Number of chromosome aberrations scored for the different exposure regimes in 4 donors using a 1800 MHz GSM Basic signal at an SAR of 2 W/kg. The dicentric yields and distributions amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of dicentrics	Number of centric rings	Number of excess acentrics	Dicentrics per cell ± SE	Distribution of dicentrics				σ^2 / y ± SE	U
							0	1	2	3		
1, S	500	498	1	0	2	0.002 ± 0.002	499	1			—	—
1, F	500	499	0	0	1	—	499				—	—
1, X + S	500	439	37	1	32	0.074 ± 0.012	465	33	2		1.04 ± 0.06	0.58
1, X + F	500	445	31	1	40	0.062 ± 0.011	472	25	3		1.13 ± 0.06	2.15
1, S + X	500	438	37	1	31	0.074 ± 0.012	466	31	3		1.09 ± 0.06	1.45
1, F + X	500	439	33	2	34	0.066 ± 0.011	467	33			0.94 ± 0.06	-1.03
2, S	500	500	0	0	0	—	500				—	—
2, F	500	499	0	0	2	—	500				—	—
2, X + S	500	420	46	2	45	0.092 ± 0.014	458	38	4		1.08 ± 0.06	1.34
2, X + F	500	435	35	1	36	0.070 ± 0.012	468	29	3		1.10 ± 0.06	1.66
2, S + X	500	429	42	3	38	0.084 ± 0.013	461	36	3		1.06 ± 0.06	0.97
2, F + X	500	435	47	1	28	0.094 ± 0.014	457	39	4		1.08 ± 0.06	1.25
3, S	500	499	1	0	0	0.002 ± 0.002	499	1			—	—
3, F	500	500	0	0	0	—	500				—	—
3, X + S	500	453	24	2	27	0.048 ± 0.010	476	24			0.95 ± 0.06	-0.74
3, X + F	500	451	30	0	26	0.060 ± 0.011	473	24	3		1.14 ± 0.06	2.28
3, S + X	500	448	33	2	21	0.066 ± 0.011	469	29	2		1.06 ± 0.06	0.92
3, F + X	500	453	26	1	22	0.052 ± 0.010	475	24	1		1.03 ± 0.06	0.43
4, S	500	499	1	0	0	0.002 ± 0.002	499	1			—	—
4, F	500	499	0	0	1	—	500				—	—
4, X + S	500	427	47	6	31	0.094 ± 0.014	458	37	5		1.12 ± 0.06	1.93
4, X + F	500	432	52	1	25	0.104 ± 0.104	453	43	3	1	1.13 ± 0.06	2.06
4, S + X	500	423	63	4	21	0.126 ± 0.016	441	55	4		1.00 ± 0.06	0.05
4, F + X	500	434	50	2	22	0.100 ± 0.014	454	42	4		1.06 ± 0.06	0.99

Table 3.5. The mean yields per cell ± SE of pooled dicentric data for 3 RF exposure regimes. χ^2 and p values on 3 degrees of freedom obtained by the chi-squared test for homogeneity are also shown. Figures in red indicate p values < 0.05 (see section 2.8.2 for discussion)
S = sham; X = 1 Gy x-rays; F = RF field

Experimental condition	935 MHz GSM Basic 1 W/kg					935 MHz GSM Basic 2 W/kg					1800 MHz GSM Basic 2 W/kg				
	Total number cells scored	Total number of dicentrics	Dicentrics per cell ±SE	χ^2	p	Total number cells scored	Total number of dicentrics	Dicentrics per cell ±SE	χ^2	p	Total number cells scored	Total number of dicentrics	Dicentrics per cell ±SE	χ^2	p
S	2000	1	0.0005 ± 0.0005	3.00	0.392	2000	2	0.0010 ± 0.0007	2.00	0.572	2000	3	0.0015 ± 0.0009	1.00	0.801
F	2000	0	—	—	—	2000	1	0.0005 ± 0.0005	3.00	0.392	2000	0	—	—	—
X + S	2000	343	0.1715 ± 0.0093	0.59	0.898	2000	164	0.0820 ± 0.0064	5.32	0.150	2000	154	0.0770 ± 0.0107	8.86	0.031
X + F	2000	317	0.1585 ± 0.0089	7.30	0.063	2000	179	0.0895 ± 0.0067	5.96	0.114	2000	148	0.0740 ± 0.0102	8.49	0.037
S + X	2000	351	0.1755 ± 0.0094	1.90	0.593	2000	170	0.0850 ± 0.0065	6.24	0.101	2000	175	0.0875 ± 0.0133	12.22	0.007
F +X	2000	305	0.1525 ± 0.0087	7.83	0.050	2000	164	0.0820 ± 0.0064	2.10	0.552	2000	156	0.0780 ± 0.0114	10.00	0.019

Table 3.6. The mean yields per cell ± SE of pooled centric ring data for 3 RF exposure regimes. χ^2 and p values on 3 degrees of freedom obtained by the chi-squared test for homogeneity are also shown.
S = sham; X = 1 Gy x-rays; F = RF field

Experimental condition	935 MHz GSM Basic 1 W/kg					935 MHz GSM Basic 2 W/kg					1800 MHz GSM Basic 2 W/kg				
	Total number cells scored	Total number of centric rings	Centric rings per cell ±SE	χ^2	p	Total number cells scored	Total number of centric rings	Centric rings per cell ±SE	χ^2	p	Total number cells scored	Total number of centric rings	Centric rings per cell ±SE	χ^2	p
S	2000	0	—	—	—	2000	0	—	—	—	2000	0	—	—	—
F	2000	0	—	—	—	2000	0	—	—	—	2000	0	—	—	—
X + S	2000	23	0.0115 ± 0.0050	13.00	0.005	2000	4	0.0020 ± 0.0010	2.00	0.572	2000	11	0.0055 ± 0.0017	5.36	0.147
X + F	2000	13	0.0065 ± 0.0018	3.31	0.347	2000	8	0.0040 ± 0.0014	1.00	0.801	2000	3	0.0015 ± 0.0009	1.00	0.801
S +X	2000	15	0.0075 ± 0.0019	0.73	0.865	2000	4	0.0020 ± 0.0010	2.00	0.572	2000	10	0.0050 ± 0.0016	2.00	0.572
F + X	2000	17	0.0085 ± 0.0021	3.00	0.392	2000	2	0.0010 ± 0.0007	6.00	0.112	2000	6	0.0030 ± 0.0012	0.67	0.881

Table 3.7. The mean yields per cell ± SE of pooled excess acentric data for 3 RF exposure regimes. χ^2 and p values on 3 degrees of freedom obtained by the chi-squared test for homogeneity are also shown.
S = sham; X = 1 Gy x-rays; F = RF field

Experimental condition	935 MHz GSM Basic 1 W/kg					935 MHz GSM Basic 2 W/kg					1800 MHz GSM Basic 2 W/kg				
	Total number cells scored	Total number of excess acentrics	Excess acentrics per cell ±SE	χ^2	p	Total number cells scored	Total number of excess acentrics	Excess acentrics per cell ±SE	χ^2	p	Total number cells scored	Total number of excess acentrics	Excess acentrics per cell ±SE	χ^2	p
S	2000	5	0.0025 ± 0.0011	0.60	0.896	2000	3	0.0015 ± 0.0009	3.67	0.300	2000	2	0.0010 ± 0.0007	6.00	0.112
F	2000	5	0.0025 ± 0.0011	3.80	0.284	2000	4	0.0020 ± 0.0010	2.00	0.572	2000	4	0.0020 ± 0.0010	2.00	0.572
X + S	2000	242	0.1210 ± 0.0081	6.43	0.092	2000	139	0.0695 ± 0.0065	6.41	0.093	2000	135	0.0675 ± 0.0061	5.41	0.144
X + F	2000	201	0.1005 ± 0.0074	6.30	0.098	2000	137	0.0685 ± 0.0174	24.26	<0.001	2000	117	0.0585 ± 0.0056	5.29	0.152
S +X	2000	226	0.1130 ± 0.0078	3.56	0.313	2000	129	0.0645 ± 0.0124	13.17	0.004	2000	111	0.0555 ± 0.0055	7.45	0.059
F + X	2000	201	0.1005 ± 0.0074	3.20	0.362	2000	113	0.0565 ± 0.0055	3.07	0.380	2000	106	0.0530 ± 0.0054	3.74	0.291

Table 3.8. The mean yields per cell ± SE of pooled chromatid (c'tid) aberration data for 3 RF exposure regimes. χ^2 and p values on 3 degrees of freedom obtained by the chi-squared test for homogeneity are also shown.
S = sham; X = 1 Gy x-rays; F = RF field

Condition	935 MHz GSM Basic 1 W/kg					935 MHz GSM Basic 2 W/kg					1800 MHz GSM Basic 2 W/kg				
	Total number cells scored	Total c'tid damage	C'tid damage per cell ±SE	χ^2	p	Total number cells scored	Total c'tid damage	C'tid damage per cell ±SE	χ^2	p	Total number cells scored	Total c'tid damage	C'tid damage per cell ±SE	χ^2	p
S	2000	21	0.0105 ± 0.0023	0.52	0.914	2000	28	0.0140 ± 0.0026	2.00	0.572	2000	16	0.0080 ± 0.0020	6.50	0.090
F	2000	37	0.0185 ± 0.0072	16.73	<0.001	2000	13	0.0065 ± 0.0018	0.23	0.972	2000	20	0.0100 ± 0.0022	7.60	0.055
X + S	2000	50	0.0250 ± 0.0066	10.48	0.015	2000	25	0.0125 ± 0.0043	8.76	0.033	2000	23	0.0115 ± 0.0043	9.87	0.020
X + F	2000	60	0.0300 ± 0.0102	20.67	<0.001	2000	21	0.0105 ± 0.0023	2.43	0.488	2000	15	0.00875 ± 0.0019	5.53	0.137
S +X	2000	50	0.0250 ± 0.0035	7.44	0.059	2000	30	0.0150 ± 0.0027	3.60	0.308	2000	19	0.0095 ± 0.0036	8.16	0.043
F + X	2000	46	0.0230 ± 0.0034	5.13	0.162	2000	26	0.0130 ± 0.0051	11.85	0.008	2000	21	0.0105 ± 0.0048	13.10	0.004

Table 3.9. F and p values for the factors, field, x-rays, order and signal, obtained from general linear model ANOVA testing for dicentrics, centric rings, excess acentrics and chromatid aberrations using the 3 GSM Basic signals.

Factor	Dicentrics		Centric rings		Excess acentrics		Chromatid aberrations	
	F	P	F	p	F	p	F	p
Field	0.77	0.384	1.62	0.207	0.60	0.442	0.01	0.939
X-rays	229.61	< 0.001	21.90	< 0.001	165.79	< 0.001	3.24	0.076
Order	0.03	0.854	0.48	0.491	2.04	0.158	0.00	0.950
Signal	38.63	< 0.001	10.24	< 0.001	22.81	< 0.001	12.63	< 0.001

3.2.2 Micronucleus assay

The results of the micronucleus assay are presented in Tables 3.10 to 3.12 for the RF signals, 935 MHz GSM Basic (1 W/kg), 935 MHz GSM Basic (2 W/kg), 1800 MHz GSM Basic (2 W/kg) respectively. The pooled results from replicate donors, for each of the three RF signal used, are presented in Table 3.13 and in Figure 3.2. As shown in Tables 3.10 to 3.12, many of the individual data points exhibit significant over-dispersion (shown in red) with respect to the Poisson distribution and the average σ^2/y was 1.2. This is a known feature of the micronucleus assay (Prosser et al. 1988) and was reflected in the assignment of statistical uncertainties to the data. The number of micronuclei in the unexposed, sham only, regimes is consistent with the background levels found by the laboratory in a previous study (Prosser et al. 1988) and the exposures to RF field alone also produced similar micronucleus yields. Thus, the same conclusion can be drawn from the MN assay results as from the CA data; that is RF fields alone are not genotoxic.

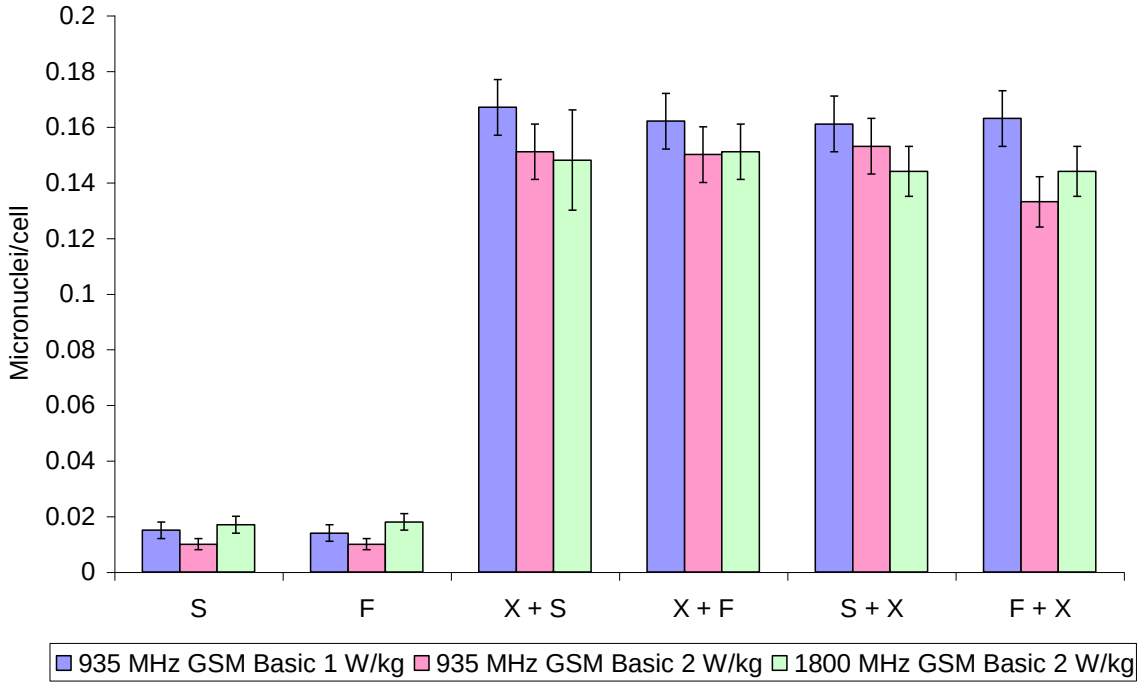

Figure 3.2. The pooled donors' data of micronucleus yields ± SE for the 3 RF exposure regimes.
S = sham; X = 1 Gy x-ray; F = RF field

Again, only the exposure regimes to include x-rays (X+S; X+F; S+X; F+X) have produced micronucleus levels in excess of background and for a given signal these four exposure regimes are not significantly different, as shown by Figure 3.2. The results of an ANOVA test, shown in Table 3.14, also confirmed that the RF field and order of exposure were not important factors for the induction of micronuclei, whereas x-ray exposure was significant with a p value of less than 0.001. Just as for dicentrics, centric rings, excess acentrics and chromatid damage ANOVA revealed there were significant differences in the micronucleus yields between the signals ($p = 0.021$) and as for the chromosomal data this appears to be the result of the higher micronucleus yields for the 935 MHz GSM Basic (1 W/kg) signal. The micronucleus yields for exposure regimes that include x-rays are greater than the other signals by ~10%, as shown in Figure 3.2. Aliquots of the same blood were processed for all the assays, but it appears the longer culture time of 72 h used in the MN assay has resulted in a smaller difference in yields between the signals as opposed to a factor of two for the CA assay. Overall the results of the MN assay are in agreement with the CA assay that GSM Basic fields, used in this study, when combined with x-rays do not moderate the genotoxicity of the ionising radiation.

Table 3.10. The number of micronuclei scored for the different exposure regimes in 4 donors using a 935 MHz GSM Basic signal at an SAR of 1 W/kg. The micronucleus yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of micronuclei	Number of anaphase bridges	Micronuclei per cell ± SE	Distribution of micronuclei								σ^2 / y ± SE	U
						0	1	2	3	4	5	6	7		
1, S	500	489	11	0	0.022 ± 0.007	489	11							0.98 ± 0.06	-0.33
1, F	500	494	6	0	0.012 ± 0.005	494	6							0.99 ± 0.06	-0.17
1, X + S	500	429	85	3	0.170 ± 0.020	429	59	10	2					1.21 ± 0.06	3.32
1, X + F	500	425	85	4	0.170 ± 0.020	428	59	13						1.14 ± 0.06	2.19
1, S + X	500	433	71	3	0.142 ± 0.018	434	61	5						1.00 ± 0.06	0.01
1, F + X	500	422	83	5	0.166 ± 0.020	423	71	6						0.98 ± 0.06	-0.31
2, S	500	494	7	0	0.014 ± 0.006	494	5	1						1.27 ± 0.06	4.68
2, F	500	492	9	1	0.018 ± 0.007	492	7	1						1.21 ± 0.06	3.46
2, X + S	500	435	75	3	0.150 ± 0.019	438	52	8	1	1				1.31 ± 0.06	4.87
2, X + F	500	432	72	2	0.144 ± 0.019	433	62	5						1.00 ± 0.06	-0.05
2, S + X	500	440	75	3	0.150 ± 0.019	442	48	7	2	0	0	0	1	1.76 ± 0.06	12.09
2, F + X	500	444	67	1	0.134 ± 0.018	445	45	8	2					1.23 ± 0.06	4.56
3, S	500	496	5	0	0.010 ± 0.005	496	3	1						1.39 ± 0.06	6.94
3, F	500	492	8	0	0.016 ± 0.006	492	8							0.99 ± 0.06	-0.24
3, X + S	500	419	89	1	0.178 ± 0.021	420	71	9						1.03 ± 0.06	0.42
3, X + F	500	415	98	6	0.196 ± 0.022	415	75	8	1	1				1.15 ± 0.06	2.43
3, S + X	500	422	93	0	0.186 ± 0.021	422	64	13	1					1.16 ± 0.06	2.55
3, F + X	500	417	93	2	0.186 ± 0.021	418	71	11						1.05 ± 0.06	0.84
4, S	500	494	7	0	0.014 ± 0.006	494	5	1						1.27 ± 0.06	4.68
4, F	500	496	4	0	0.008 ± 0.004	496	4							0.99 ± 0.06	-0.11
4, X + S	500	431	85	2	0.170 ± 0.020	432	55	9	4					1.33 ± 0.06	5.19
4, X + F	500	445	68	2	0.136 ± 0.018	445	46	6	2	1				1.40 ± 0.06	6.31
4, S + X	500	428	82	1	0.164 ± 0.020	429	62	7	2					1.16 ± 0.06	2.47
4, F + X	500	427	82	2	0.164 ± 0.020	428	62	10						1.08 ± 0.06	1.3

Table 3.11. The number of micronuclei scored for the different exposure regimes in 4 donors using a 935 MHz GSM Basic signal at an SAR of 2 W/kg. The micronucleus yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of micronuclei	Number of anaphase bridges	Micronuclei per cell ± SE	Distribution of micronuclei					σ^2 / y ± SE	U
						0	1	2	3	4		
1, S	500	491	9	0	0.018 ± 0.006	491	9				0.98 ± 0.06	-0.27
1, F	500	494	6	0	0.012 ± 0.005	494	6				0.99 ± 0.06	-0.17
1, X + S	500	433	81	3	0.162 ± 0.020	434	53	11	2		1.26 ± 0.06	4.14
1, X + F	500	436	73	2	0.146 ± 0.019	436	56	7	1		1.13 ± 0.06	2.07
1, S + X	500	439	68	2	0.136 ± 0.018	440	53	6	1		1.13 ± 0.06	2.08
1, F + X	500	447	61	2	0.122 ± 0.017	447	45	8			1.14 ± 0.06	2.27
2, S	500	497	3	0	0.006 ± 0.004	497	3				1.00 ± 0.05	-0.08
2, F	500	494	6	0	0.012 ± 0.005	494	6				0.99 ± 0.06	-0.17
2, X + S	500	443	61	3	0.122 ± 0.017	445	50	4	1		1.11 ± 0.06	1.75
2, X + F	500	436	67	2	0.134 ± 0.018	438	58	3	1		1.05 ± 0.06	0.75
2, S + X	500	437	71	5	0.142 ± 0.018	438	53	9			1.11 ± 0.06	1.81
2, F + X	500	436	73	2	0.146 ± 0.019	436	58	4	1	1	1.21 ± 0.06	3.38
3, S	500	496	4	0	0.008 ± 0.004	496	4				0.99 ± 0.06	-0.11
3, F	500	497	3	0	0.006 ± 0.004	497	3				1.00 ± 0.05	-0.78
3, X + S	500	427	75	5	0.150 ± 0.019	429	67	4			0.96 ± 0.06	-0.66
3, X + F	500	428	84	2	0.168 ± 0.020	430	59	8	3		1.24 ± 0.06	3.8
3, S + X	500	421	87	5	0.174 ± 0.020	424	67	7	2		1.13 ± 0.06	2.02
3, F + X	500	446	69	3	0.138 ± 0.018	438	57	3	2		1.13 ± 0.06	1.99
4, S	500	497	3	0	0.006 ± 0.004	497	3				1.00 ± 0.05	-0.08
4, F	500	496	4	0	0.008 ± 0.004	496	4				0.99 ± 0.06	-0.11
4, X + S	500	429	85	5	0.170 ± 0.020	430	58	9	3		1.26 ± 0.06	4.07
4, X + F	500	438	75	4	0.150 ± 0.019	440	50	6	3	1	1.41 ± 0.06	6.57
4, S + X	500	430	79	5	0.158 ± 0.019	432	61	4	2	1	1.25 ± 0.06	3.97
4, F + X	500	444	63	2	0.126 ± 0.017	446	46	7	1		1.19 ± 0.06	3.09

Table 3.12. The number of micronuclei scored for the different exposure regimes in 4 donors using a 1800 MHz GSM Basic signal at an SAR of 1 W/kg. The micronucleus yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of micronuclei	Number of anaphase bridges	Micronuclei per cell ± SE	Distribution of micronuclei									σ^2 / y ± SE	U
						0	1	2	3	4	5	6	7	8		
1, S	500	492	8	0	0.016 ± 0.006	492	8								0.99 ± 0.06	-0.24
1, F	500	489	12	0	0.024 ± 0.008	489	10	1							1.15 ± 0.06	2.39
1, X + S	500	431	82	6	0.164 ± 0.020	432	60	7	0	0	0	0	0	1	1.69 ± 0.06	11.01
1, X + F	500	434	81	3	0.162 ± 0.020	434	51	15							1.21 ± 0.06	3.35
1, S + X	500	440	66	6	0.132 ± 0.018	442	50	8							1.11 ± 0.06	1.79
1, F + X	500	437	69	4	0.138 ± 0.018	438	56	5	1						1.10 ± 0.06	1.53
2, S	500	494	7	0	0.014 ± 0.006	494	5	1							1.27 ± 0.06	4.68
2, F	500	495	6	0	0.012 ± 0.005	495	4	1							1.32 ± 0.06	5.61
2, X + S	500	440	64	6	0.128 ± 0.018	443	51	5	1						1.12 ± 0.06	1.98
2, X + F	500	439	67	2	0.134 ± 0.018	441	52	6	1						1.14 ± 0.06	2.18
2, S + X	500	431	77	4	0.154 ± 0.019	432	59	9							1.08 ± 0.06	1.3
2, F + X	500	442	64	3	0.128 ± 0.018	444	50	4	2						1.89 ± 0.06	2.98
3, S	500	495	5	0	0.010 ± 0.005	495	5								0.99 ± 0.06	-0.14
3, F	500	494	7	0	0.014 ± 0.006	494	5	1							1.27 ± 0.06	4.68
3, X + S	500	444	57	3	0.114 ± 0.017	446	51	3							1.00 ± 0.06	-1.08
3, X + F	500	437	72	4	0.144 ± 0.019	440	49	10	1						1.22 ± 0.06	3.49
3, S + X	500	438	64	3	0.128 ± 0.018	439	58	3							0.97 ± 0.06	-0.51
3, F + X	500	439	64	4	0.128 ± 0.018	443	51	5	1						1.12 ± 0.06	1.98
4, S	500	489	13	0	0.026 ± 0.008	489	9	2							1.28 ± 0.06	4.6
4, F	500	491	10	0	0.020 ± 0.007	491	8	1							1.18 ± 0.06	-0.13
4, X + S	500	424	93	4	0.186 ± 0.021	425	60	13	1	1					1.29 ± 0.06	4.67
4, X + F	500	428	81	3	0.162 ± 0.020	429	62	8	1						1.11 ± 0.06	1.78
4, S + X	500	425	80	3	0.160 ± 0.020	425	71	3	1						0.99 ± 0.06	1.77
4, F + X	500	421	90	1	0.180 ± 0.021	421	70	7	2						1.11 ± 0.06	3.04

Table 3.13. The mean yields per cell ± SE of pooled data from the micronucleus assay for 3 RG exposure regimes. χ^2 and p values on 3 degrees of freedom obtained by the chi-squared test for homogeneity are also shown.
S = sham; X = 1 Gy x-ray; F = RF field; MN = micronuclei

Experimental condition	935 MHz GSM Basic 1 W/kg					935 MHz GSM Basic 2 W/kg					1800 MHz GSM Basic 2 W/kg				
	Total number cells scored	Total number of MN	MN per cell ±SE	χ^2	p	Total number cells scored	Total number of MN	MN per cell ±SE	χ^2	p	Total number cells scored	Total number of MN	MN per cell ±SE	χ^2	p
S	2000	30	0.0150 ± 0.0030	2.53	0.469	2000	19	0.0095 ± 0.0024	5.21	0.157	2000	33	0.0165 ± 0.0031	3.41	0.239
F	2000	27	0.0135 ± 0.0028	2.19	0.535	2000	19	0.0095 ± 0.0024	1.42	0.701	2000	35	0.0175 ± 0.0032	2.60	0.457
X + S	2000	334	0.1670 ± 0.0010	1.28	0.734	2000	302	0.1510 ± 0.0095	4.38	0.223	2000	296	0.1480 ± 0.0180	11.00	0.012
X + F	2000	323	0.1615 ± 0.098	6.87	0.076	2000	299	0.1495 ± 0.0095	1.99	0.574	2000	301	0.1505 ± 0.0095	1.92	0.588
S +X	2000	321	0.1605 ± 0.098	3.47	0.324	2000	305	0.1525 ± 0.0096	2.87	0.412	2000	287	0.1435 ± 0.0093	2.63	0.452
F + X	2000	325	0.1625 ± 0.0099	4.24	0.236	2000	266	0.1330 ± 0.0089	1.37	0.713	2000	287	0.1435 ± 0.0093	6.42	0.093

Table 3.14. F and p values for the factors, field, x-rays, order and signal, obtained from general linear model ANOVA testing for the micronuclei, using the 3 GSM Basic signals.

Factor	Micronuclei	
	F	p
Field	0.44	0.511
X-rays	856.89	< 0.001
Order	1.32	0.254
Signal	4.09	0.021

3.2.3 Sister chromatid exchange assay

Tables 3.15 to 3.17 show the results of the SCE assay for the RF signals 935 MHz GSM Basic (1 W/kg), 935 MHz GSM Basic (2 W/kg) and 1800 MHz GSM Basic (2 W/kg), respectively. The SCE distributions, on average show slight under-dispersion with $\sigma^2/y \sim 0.9$ when compared to the Poisson distribution, however this feature of the SCE assay has been observed before in the HPA laboratory (Moquet et al. 1987 and 1989). As the under-dispersion was not significant ($U > -1.96$), the standard errors shown have been calculated using the Poisson distribution. Table 3.18 and Figure 3.3 shows the pooled SCE assay results for each signal.

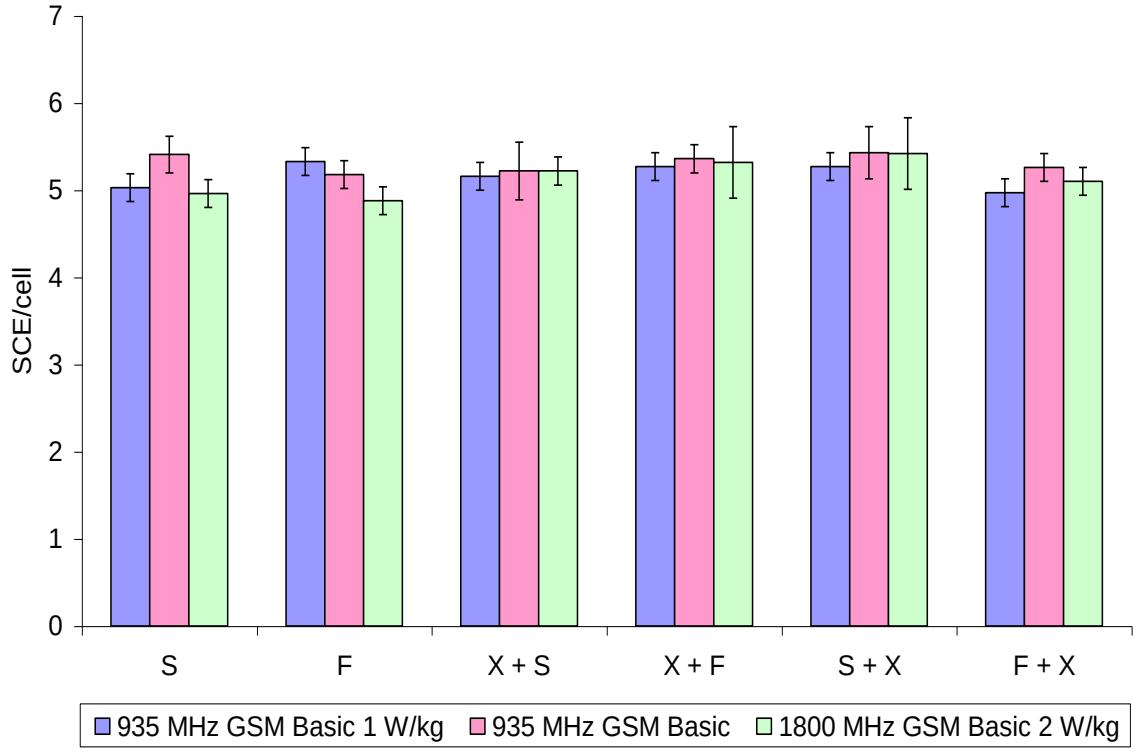

Figure 3.3. The pooled donors' data of SCE yields ± SE for the 3 RF exposure regimes.
S = sham; X = 1 Gy x-ray; F = RF field

It is clear that the SCE frequency was not dependent on RF fields or x-ray exposure for all exposure conditions and signal types resulted in about 5 SCE/cell; the normal control level for this laboratory (Moquet et al. 1989). This

was confirmed by the results of ANOVA testing, shown in Table 3.19, where no factor tested was significant for SCE induction. It is acknowledged that x-irradiation is inefficient at inducing SCEs in G_0 lymphocytes (Littlefield et al. 1979), therefore the rationale for including this assay was primarily to explore for any genotoxicity of the combination of RF fields and x-rays. No genotoxicity of RF fields or enhancement was observed with any of the exposure regimes.

Table 3.15. Number of SCEs scored for the different exposure regimes in 4 donors using a 935 MHz GSM Basic signal at an SAR of 1 W/kg. The SCE yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of SCEs	SCEs per cell ± SE	Distribution of SCEs																	σ^2 / y ± SE	U
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17		
1, S	50	252	5.04 ± 0.32	0	1	2	3	16	12	5	7	2	1	1								0.62 ± 0.20	-1.87
1, F	50	257	5.14 ± 0.32	0	1	3	4	11	15	5	5	2	2	2								0.77 ± 0.20	-1.14
1, X + S	50	245	4.90 ± 0.31	0	2	6	5	8	11	5	7	5	0	1								0.90 ± 0.20	-0.49
1, X + F	50	254	5.08 ± 0.32	0	2	3	6	9	13	5	6	2	2	1	1							0.93 ± 0.20	-0.34
1, S + X	50	246	4.92 ± 0.31	0	2	5	6	9	9	4	10	4	1									0.85 ± 0.20	-0.73
1, F + X	50	249	4.98 ± 0.32	0	2	3	6	11	9	12	1	2	2	1	1							0.91 ± 0.20	-0.47
2, S	50	240	4.80 ± 0.31	0	2	3	6	14	9	8	4	2	0	1	1							0.84 ± 0.20	-0.78
2, F	50	266	5.32 ± 0.33	0	0	2	7	11	11	6	6	2	3	1	1							0.80 ± 0.20	-0.99
2, X + S	50	265	5.30 ± 0.33	0	0	5	2	12	7	12	5	4	3									0.68 ± 0.20	-1.59
2, X + F	50	268	5.36 ± 0 33	0	0	1	9	10	6	9	8	5	1	1								0.67 ± 0.20	-1.65
2, S + X	50	271	5.42 ± 0.33	0	1	2	8	8	10	4	7	5	3	2								0.90 ± 0.20	-0.47
2, F + X	50	248	4.96 ± 0.31	0	2	3	7	14	6	5	5	7	0	0	0	1						0.98 ± 0.20	-0.10
3, S	50	265	5.30 ± 0.33	0	1	5	6	9	6	7	6	7	2	0	0	1						1.02 ± 0.20	0.09
3, F	50	277	5.54 ± 0.33	0	2	4	3	11	8	4	9	1	3	4	0	1						1.12 ± 0.20	0.93
3, X + S	50	278	5.56 ± 0.33	0	0	2	4	8	14	10	5	3	1	1	2							0.72 ± 0.20	-1.39
3, X + F	50	261	5.22 ± 0.32	0	1	2	8	10	9	6	7	3	2	2								0.85 ± 0.20	-0.76
3, S + X	50	268	5.36 ± 0.33	0	0	2	6	12	11	7	3	4	2	3								0.8 ± 0.20	-1.00
3, F + X	50	253	5.06 ± 0.32	0	3	3	9	10	8	3	5	6	1	0	1	0	0	0	0	1		1.50 ± 0.20	2.46
4, S	50	249	4.98 ± 0.32	0	2	3	9	11	9	5	6	2	1	0	1	0	0	0	0	0	1	1.43 ± 0.20	2.13
4, F	50	266	5.32 ± 0.33	0	1	6	4	13	1	7	11	3	1	1	2							1.09 ± 0.20	0.46
4, X + S	50	244	4.88 ± 0.31	0	2	7	7	8	9	5	6	3	0	1	0	2						1.29 ± 0.20	1.46
4, X + F	50	271	5.42 ± 0.33	0	2	5	5	9	8	3	8	3	3	2	1	1						1.29 ± 0.20	1.43
4, F + S	50	268	5.38 ± 0.33	0	3	1	11	8	4	7	6	5	2	0	1	1	1					1.37 ± 0.20	1.83
4, F + X	50	244	4.88 ± 0.31	0	1	3	9	10	8	8	7	3	1									0.70 ± 0.20	-1.49

Table 3.16. Number of SCEs scored for the different exposure regimes in 4 donors using a 935 MHz GSM Basic signal at an SAR of 2 W/kg. The SCE yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of SCEs	SCEs per cell ± SE	Distribution of SCEs															σ^2 / y ± SE	U
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1, S	50	256	5.12 ± 0.32	0	1	2	9	8	11	7	4	5	3						0.77 ± 0.20	-1.14
1, F	50	243	4.86 ± 0.31	0	1	7	11	8	5	5	3	7	1	1	0	1			1.26 ± 0.20	1.29
1, X + S	50	258	5.16 ± 0.32	0	1	2	6	11	13	8	2	2	3	1	1				0.83 ± 0.20	-0.83
1, X + F	50	245	4.90 ± 0.31	0	2	5	8	11	10	2	3	4	3	1	0	0	1		1.28 ± 0.20	1.37
1, S + X	50	302	6.04 ± 0.35	0	0	1	2	7	15	8	5	5	5	0	1	1			0.70 ± 0.20	-1.48
1, F + X	50	260	5.20 ± 0.32	0	0	2	7	10	12	11	1	4	1	1	1				0.71 ± 0.20	-1.42
2, S	50	283	5.66 ± 0.34	0	0	1	4	13	8	11	3	3	5	1	1				0.75 ± 0.20	-1.25
2, F	50	282	5.64 ± 0.34	0	0	2	5	11	12	6	4	2	4	2	1	1			0.97 ± 0.20	-0.16
2, X + S	50	252	5.04 ± 0.32	0	1	5	8	6	9	6	9	5	1						0.82 ± 0.20	-0.9
2, X + F	50	282	5.64 ± 0.34	0	0	3	3	10	11	8	5	6	1	2	0	1			0.82 ± 0.20	-0.91
2, S + X	50	291	5.82 ± 0.34	0	0	1	7	9	9	7	7	1	7	0	1	0	0	1	1.00 ± 0.20	0.003
2, F + X	50	267	5.34 ± 0.33	0	2	2	4	12	7	12	4	2	2	2	0	1			0.95 ± 0.20	-0.24
3, S	50	301	6.02 ± 0.35	0	0	3	4	10	8	7	4	5	5	0	2	1	0	1	1.19 ± 0.20	0.94
3, F	50	267	5.34 ± 0.33	0	1	4	6	6	11	8	4	6	3	1					0.87 ± 0.20	-0.65
3, X + S	50	306	6.12 ± 0.35	0	1	4	4	3	12	7	2	7	5	2	2	0	0	1	1.23 ± 0.20	1.15
3, X + F	50	292	5.84 ± 0.34	0	0	1	9	8	9	8	4	3	2	2	2	1	0	1	1.23 ± 0.20	1.15
3, S + X	50	251	5.02 ± 0.32	0	1	4	4	17	5	7	8	1	0	2	1				0.89 ± 0.20	-0.54
3, F + X	50	259	5.18 ± 0.32	0	2	4	8	6	8	10	5	1	3	2	1				1.09 ± 0.20	0.46
4, S	50	241	4.82 ± 0.31	0	1	4	8	10	9	8	6	3	1						0.71 ± 0.20	-1.44
4, F	50	243	4.86 ± 0.31	0	1	5	7	11	10	7	4	2	1	1	0	1			0.97 ± 0.20	-0.17
4, X + S	50	228	4.56 ± 0.30	0	0	6	8	10	14	4	7	0	1						0.60 ± 0.20	-1.98
4, X + F	50	253	5.06 ± 0.32	0	1	7	6	8	9	5	7	2	3	1	1				1.09 ± 0.20	0.46
4, F + S	50	241	4.82 ± 0.31	0	0	3	13	11	8	4	6	1	3	0	1				0.89 ± 0.20	-0.56
4, F + X	50	266	5.32 ± 0.33	0	0	5	6	10	10	7	3	2	3	3	0	1			1.09 ± 0.20	0.42

Table 3.17. Number of SCEs scored for the different exposure regimes in 4 donors using a 1800 MHz GSM Basic signal at an SAR of 2 W/kg. The SCE yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of SCEs	SCEs per cell ± SE	Distribution of SCEs													σ^2 / y ± SE	U
				0	1	2	3	4	5	6	7	8	9	10	11	12		
1, S	50	238	4.76 ± 0.31	0	2	3	6	9	12	13	2	3					0.59 ± 0.20	-2.04
1, F	50	260	5.20 ± 0.32	0	1	3	11	10	7	4	5	2	3	2	1	1	1.26 ± 0.20	1.27
1, X + S	50	247	4.94 ± 0.31	0	0	3	12	6	13	6	5	2	2	0	1		0.80 ± 0.20	-1.01
1, X + F	50	309	6.18 ± 0.35	0	0	2	2	9	8	13	3	3	4	3	2	1	0.92 ± 0.20	-0.41
1, S + X	50	306	6.12 ± 0.35	0	0	6	1	6	9	3	10	8	3	2	1	1	1.00 ± 0.20	-0.01
1, F + X	50	293	5.86 ± 0.34	0	0	4	2	10	9	4	8	7	4	0	2		0.88 ± 0.20	-0.61
2, S	50	232	4.64 ± 0.30	0	1	8	8	3	18	4	4	0	3	1			0.92 ± 0.20	-0.39
2, F	50	220	4.40 ± 0.30	0	2	4	5	17	13	5	2	0	2				0.61 ± 0.20	-1.92
2, X + S	50	260	5.20 ± 0.32	0	0	4	7	7	9	12	5	5	0	0	1		0.71 ± 0.20	-1.42
2, X + F	50	244	4.88 ± 0.31	0	3	3	8	14	6	3	4	5	2	1	1		1.16 ± 0.20	0.79
2, S + X	50	213	4.26 ± 0.29	0	2	5	12	12	5	6	8						0.71 ± 0.20	-1.45
2, F + X	50	244	4.88 ± 0.31	0	3	2	9	7	10	8	9	0	0	1	1		0.89 ± 0.20	-0.54
3, S	50	277	5.54 ± 0.33	0	1	0	6	8	12	12	1	5	3	2			0.71 ± 0.20	-1.44
3, F	50	248	4.96 ± 0.31	0	1	6	6	10	12	4	3	4	2	1	0	1	1.08 ± 0.20	0.39
3, X + S	50	263	5.26 ± 0.32	0	1	3	8	5	13	7	6	2	4	0	1		0.88 ± 0.20	-0.62
3, X + F	50	290	5.80 ± 0.34	0	1	5	0	6	10	6	12	7	2	0	1		0.77 ± 0.20	-1.12
3, S + X	50	274	5.48 ± 0.33	0	0	1	8	9	9	9	6	4	1	3			0.74 ± 0.20	-1.29
3, F + X	50	244	4.88 ± 0.31	0	3	4	2	8	17	8	2	6					0.71 ± 0.20	-1.45
4, S	50	245	4.90 ± 0.31	0	1	4	10	12	6	6	5	1	3	0	2		1.08 ± 0.20	0.38
4, F	50	248	4.96 ± 0.31	0	3	6	3	8	14	6	3	1	3	3			1.12 ± 0.20	0.59
4, X + S	50	274	5.48 ± 0.33	0	0	1	4	13	8	13	4	2	4	1			0.61 ± 0.20	-1.96
4, X + F	50	221	4.42 ± 0.30	0	1	6	13	10	7	6	3	1	2	0	1		0.97 ± 0.20	-0.15
4, F + S	50	291	5.82 ± 0.34	0	2	3	4	6	8	4	11	6	4	1	0	1	1.01 ± 0.20	0.04
4, F + X	50	238	4.76 ± 0.31	0	3	7	8	6	6	8	5	4	2	1			1.15 ± 0.20	0.72

Table 3.18. The mean yields per cell ± SE of pooled data from the SCE assay for 3 RF exposure regimes. χ^2 and p values on 5 degrees of freedom obtained by the chi-squared test for homogeneity are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Experimental condition	935 MHz GSM Basic 1 W/kg					935 MHz GSM Basic 2 W/kg					1800 MHz GSM Basic 2 W/kg				
	Total number cells scored	Total number of SCEs	SCEs per cell ±SE	χ^2	p	Total number cells scored	Total number of SCEs	SCEs per cell ±SE	χ^2	p	Total number cells scored	Total number of SCEs	SCEs per cell ±SE	χ^2	p
S	200	1006	5.03 ± 0.16	1.28	0.735	200	1081	5.41 ± 0.16	8.02	0.046	200	992	4.96 ± 0.16	4.86	0.182
F	200	1066	5.33 ± 0.16	0.75	0.860	200	1035	5.18 ± 0.16	4.27	0.234	200	976	4.88 ± 0.16	3.54	0.315
X + S	200	1032	5.16 ± 0.16	3.16	0.368	200	1044	5.22 ± 0.33	12.28	0.006	200	1044	5.22 ± 0.16	1.42	0.701
X + F	200	1054	5.27 ± 0.16	0.66	0.883	200	1072	5.36 ± 0.16	5.69	0.127	200	1064	5.32 ± 0.41	18.55	<0.001
S +X	200	1053	5.27 ± 0.16	1.53	0.675	200	1085	5.43 ± 0.30	9.81	0.020	200	1084	5.42 ± 0.41	18.44	<0.001
F + X	200	994	4.97 ± 0.16	0.16	0.983	200	1052	5.26 ± 0.16	0.19	0.979	200	1019	5.10 ± 0.16	7.75	0.051

Table 3.19. F and p values for the factors, field, x-rays, order and signal, obtained from general linear model ANOVA testing for dicentrics, centric rings, excess acentrics and chromatid aberrations using the 3 GSM Basic signals

Factor	SCE	
	F	p
Field	0.24	0.625
X-rays	0.78	0.379
Order	0.02	0.877
Signal	0.97	0.385

3.2.4 Cell cycling

The MN and SCE cell cultures were initiated with synchronized primary G_0 lymphocytes, but during the 72 h before fixation they establish a degree of asynchrony (Purrott et al. 1981). Hence by 72 h some PHA stimulated cells would have reached their first metaphase whilst the progeny of others would have progressed further to reach their second, third or even fourth division. The relative numbers of cells at these different stages gives a measure of the average speed of cell cycling; the nuclear division index (NDI) (Eastmond and Tucker 1989). Exposure to mutagenic agents can alter the rate of progress through their cell cycle, perhaps by cells being held at cycle check points, and this would be reflected by the NDI.

Tables 3.20 to 3.22 and Tables 3.23 to 3.25 show the results of the NDI_{MN} and NDI_{SCE} respectively, for each of the three RF signals used in the study. The NDI_{MN} and NDI_{SCE} results for each donor have been pooled and are shown in Figures 3.4 and 3.5 respectively.

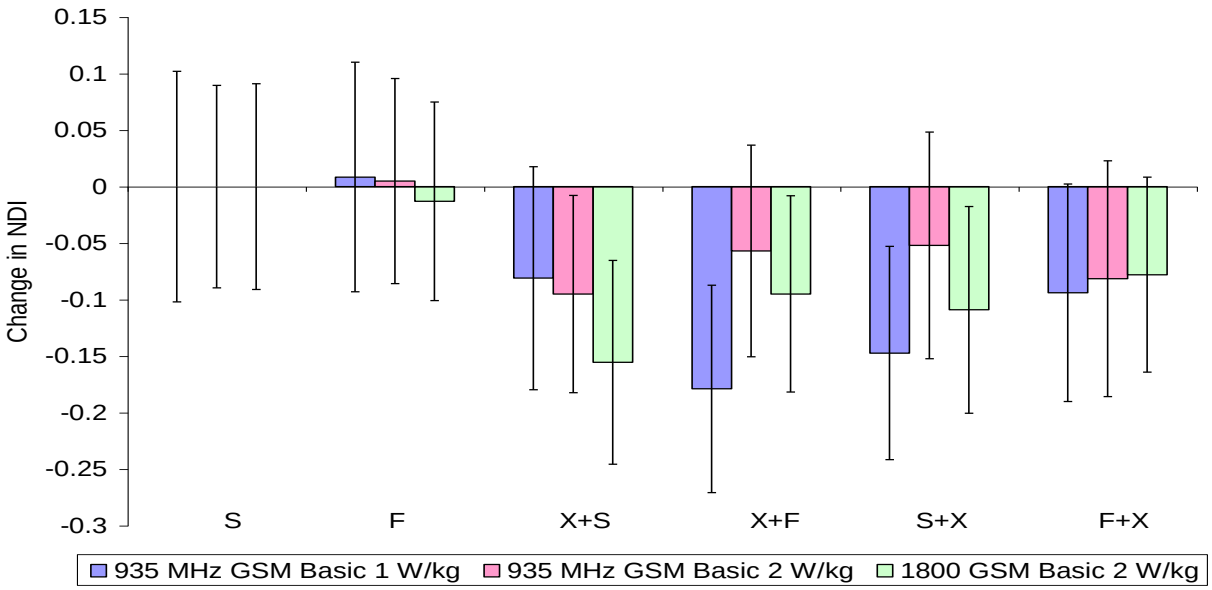

Figure 3.4. Normalised changes in the nuclear division index derived from slides prepared for the cytokinesis-blocked MN assay for the 3 RF exposure regimes. The individual donor's data have been pooled also the data for each donor were compared with that person's sham value. Pooling has required normalisation to the sham so that values above and below zero indicate cycle time accelerated and slowed respectively.
S = sham; X = 1 Gy x-ray; F = RF field

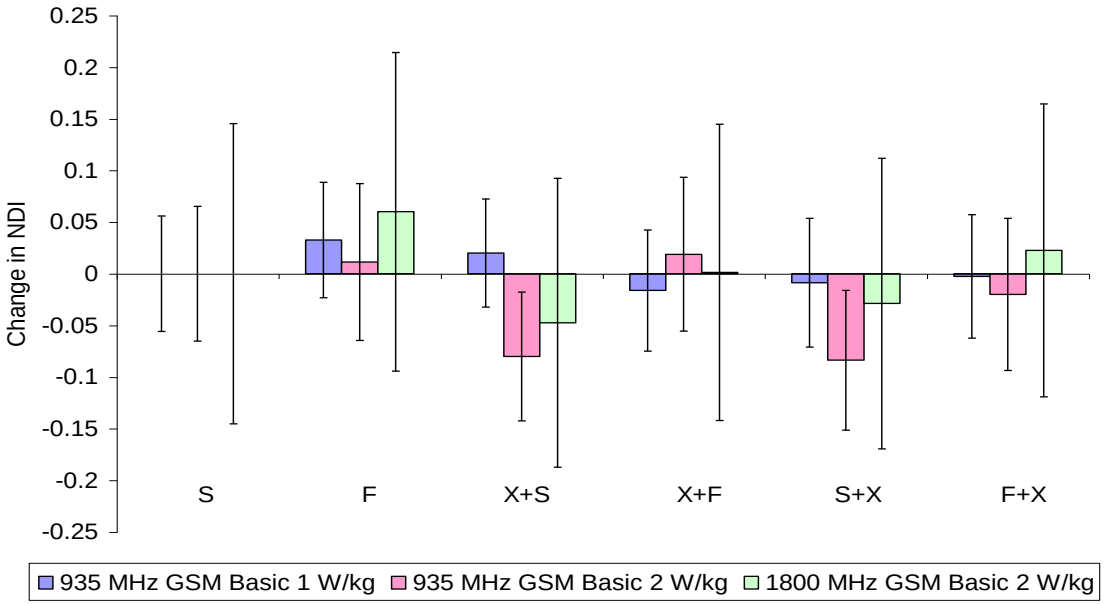

Figure 3.5. Normalised changes in the nuclear division index derived from slides prepared for the SCE assay for the 3 RF exposure regimes. The individual donor's data have been pooled also the data for each donor were compared to that person's sham value. Pooling has required normalisation to the sham so that values above or below zero indicate cell cycle times accelerated or slowed respectively.
S = sham; X = 1 Gy x-rays; F = RF field

ANOVA testing, shown in Table 3.26, revealed that donor number to be an important factor for the NDI with $p < 0.001$. As there are inherent differences in the speed of cell cycling between individuals the pooling of NDI values included a normalising procedure whereby the sham value for each donor was subtracted from that person's other data points. The differences between the sham and the other exposure regimes were averaged over the donors and standard errors on these mean differences were calculated by adding individual standard errors in quadrature. In Figures 3.4 and 3.5, positive values indicate faster proliferation with respect to the pooled shams and negative values indicate slower growth. Figure 3.4 clearly shows the values for the x-irradiated samples to be negative and in Figure 3.5 they are either negative or the error bars include zero. This is the expected mitotic delaying effect of the ionising radiation (Purrott et al. 1980), although ANOVA revealed x-rays were only an important factor for NDI_{MN}, ($p = 0.001$). As Table 3.24 shows RF field was not an important factor for cell cycling, but signal type was a significant factor, ($p <$

0.001) for both NDI_{MN} and NDI_{SCE}. However, the MN and SCE assays using the three RF signals were carried out at different times and it is possible that small changes in PHA concentration, incubator temperature, as well donor variability could have contributed to this. Overall no effect of RF alone or in combination with x-rays was seen on the speed of cell cycling.

Table 3.20. The nuclear division index derived from slides prepared for the cytokinesis-blocked MN assay fro the different exposure regimes in 4 donors using a 935 MHz GSM Basic signal at an SAR of 1 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Number of cells in 500 with1, 2, 3 or 4 nuclei				NDI	± SE
	1	2	3	4		
1, S	221	209	41	29	1.756	0.128
1, F	201	214	42	43	1.854	0.103
1, X + S	233	205	35	27	1.712	0.125
1, X + F	231	247	13	9	1.600	0.118
1, S + X	242	204	31	23	1.670	0.124
1, F + X	213	241	28	18	1.702	0.143
2, S	184	214	66	36	1.908	0.139
2, F	155	229	68	48	2.018	0.126
2, X + S	167	255	59	19	1.860	0.116
2, X + F	199	228	47	26	1.800	0.128
2, S + X	177	234	53	36	1.896	0.122
2, F + X	197	216	48	39	1.858	0.116
3, S	148	289	37	26	1.882	0.126
3, F	156	301	22	21	1.816	0.141
3, X + S	189	266	24	21	1.754	0.082
3, X + F	231	235	22	12	1.630	0.115
3, S + X	245	225	15	15	1.600	0.125
3, F + X	188	280	19	13	1.714	0.121
4, S	188	242	44	26	1.816	0.120
4, F	221	229	25	25	1.708	0.114
4, X + S	227	218	27	28	1.712	0.136
4, X + F	250	209	24	17	1.616	0.110
4, S + X	242	217	23	18	1.634	0.120
4, F + X	227	216	31	26	1.712	0.131

Table 3.21. The nuclear division index derived from slides prepared for the cytokinesis-blocked MN assay fro the different exposure regimes in 4 donors using a 935 MHz GSM Basic signal at an SAR of 2 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Number of cells in 500 with 1, 2, 3 or 4 nuclei				NDI	± SE
	1	2	3	4		
1, S	233	207	37	23	1.70	0.13
1, F	231	220	30	19	1.67	0.10
1, X + S	262	199	23	16	1.59	0.12
1, X + F	273	184	26	17	1.57	0.12
1, S + X	295	158	29	18	1.54	0.12
1, F + X	250	212	23	15	1.61	0.14
2, S	191	251	40	18	1.77	0.14
2, F	202	231	44	23	1.78	0.13
2, X + S	258	218	16	8	1.55	0.12
2, X + F	204	256	28	12	1.70	0.13
2, S + X	195	248	40	17	1.76	0.12
2, F + X	219	215	48	18	1.73	0.12
3, S	307	188	4	1	1.40	0.13
3, F	306	184	8	2	1.41	0.14
3, X + S	328	165	5	2	1.36	0.08
3, X + F	318	177	3	2	1.38	0.11
3, S + X	309	181	8	2	1.41	0.12
3, F + X	336	155	6	3	1.35	0.12
4, S	291	196	8	5	1.45	0.12
4, F	289	189	15	7	1.48	0.11
4, X + S	286	198	13	3	1.47	0.14
4, X + F	296	188	13	3	1.45	0.11
4, S + X	316	170	7	7	1.41	0.12
4, F + X	360	129	8	3	1.31	0.13

Table 3.22. The nuclear division index derived from slides prepared for the cytokinesis-blocked MN assay fro the different exposure regimes in 4 donors using a 1800 MHz GSM Basic signal at an SAR of 2 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Number of cells in 500 with 1, 2, 3 or 4 nuclei				NDI	± SE
	1	2	3	4		
1, S	191	250	26	33	1.80	0.13
1, F	204	236	25	35	1.78	0.10
1, X + S	251	215	18	16	1.60	0.12
1, X + F	218	237	16	29	1.71	0.12
1, S + X	252	207	22	19	1.62	0.12
1, F + X	222	225	26	27	1.72	0.14
2, S	244	172	44	40	1.76	0.14
2, F	216	194	54	36	1.82	0.13
2, X + S	267	179	35	19	1.61	0.12
2, X + F	216	219	45	20	1.74	0.13
2, S + X	263	172	40	25	1.65	0.12
2, F + X	233	184	46	37	1.77	0.12
3, S	188	265	29	18	1.75	0.13
3, F	194	281	17	8	1.68	0.14
3, X + S	254	218	20	8	1.56	0.08
3, X + F	261	206	23	10	1.56	0.11
3, S + X	276	201	15	8	1.51	0.12
3, F + X	262	213	20	5	1.54	0.12
4, S	252	226	11	11	1.56	0.12
4, F	255	225	12	8	1.55	0.11
4, X + S	287	193	12	8	1.48	0.14
4, X + F	275	208	11	6	1.48	0.11
4, S + X	205	266	22	7	1.66	0.12
4, F + X	257	224	11	8	1.54	0.13

Table 3.23. The nuclear division index derived from slides prepared for the SCE assay for the different exposure regimes in 4 donors using a 935 MHz GSM Basic signal at an SAR of 1 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Division status of 200 cells			NDI	± SE
	M1	M2	M3		
1, S	49	69	82	2.17	0.23
1, F	52	55	93	2.21	0.18
1, X + S	57	64	79	2.11	0.27
1, X + F	55	61	84	2.15	0.17
1, S + X	61	58	81	2.10	0.13
1, F + X	62	54	84	2.11	0.09
2, S	58	64	78	2.10	0.21
2, F	63	55	82	2.10	0.23
2, X + S	53	64	83	2.15	0.21
2, X + F	52	66	82	2.15	0.21
2, S + X	51	61	88	2.19	0.21
2, F + X	54	58	88	2.17	0.20
3, S	98	60	42	1.72	0.26
3, F	90	61	49	1.80	0.26
3, X + S	94	69	37	1.72	0.25
3, X + F	92	68	40	1.74	0.23
3, S + X	100	55	45	1.73	0.21
3, F + X	101	60	39	1.69	0.23
4, S	80	76	44	1.82	0.22
4, F	81	70	49	1.84	0.25
4, X + S	75	68	57	1.91	0.22
4, X + F	95	69	36	1.71	0.19
4, S + X	96	56	48	1.76	0.17
4, F + X	85	65	50	1.83	0.25

Table 3.24. The nuclear division index derived from slides prepared for the SCE assay for the different exposure regimes in 4 donors using a 935 MHz GSM Basic signal at an SAR of 2 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Division status of 200 cells			NDI	± SE
	M1	M2	M3		
1, S	56	51	93	2.19	0.23
1, F	47	40	113	2.33	0.18
1, X + S	72	58	70	1.99	0.27
1, X + F	48	59	93	2.23	0.17
1, S + X	61	53	86	2.13	0.13
1, F + X	66	39	95	2.15	0.09
2, S	42	55	103	2.31	0.21
2, F	44	53	103	2.30	0.23
2, X + S	55	65	80	2.13	0.21
2, X + F	50	54	96	2.23	0.21
2, S + X	63	50	87	2.12	0.21
2, F + X	47	48	105	2.29	0.20
3, S	72	74	54	1.91	0.26
3, F	73	86	41	1.84	0.26
3, X + S	71	91	38	1.84	0.25
3, X + F	87	68	45	1.79	0.23
3, S + X	97	70	33	1.68	0.21
3, F + X	92	66	42	1.75	0.23
4, S	60	56	84	2.12	0.22
4, F	61	58	81	2.10	0.25
4, X + S	49	52	99	2.25	0.22
4, X + F	40	50	110	2.35	0.19
4, S + X	47	54	99	2.26	0.17
4, F + X	43	63	94	2.26	0.25

Table 3.25. The nuclear division index derived from slides prepared for the SCE assay for the different exposure regimes in 4 donors using a 1800 MHz GSM Basic signal at an SAR of 2 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Division status of 200 cells			NDI	± SE
	M1	M2	M3		
1, S	22	40	138	2.58	0.23
1, F	18	25	157	2.70	0.18
1, X + S	29	40	131	2.51	0.27
1, X + F	22	32	146	2.62	0.17
1, S + X	35	35	130	2.48	0.13
1, F + X	19	38	143	2.62	0.09
2, S	22	38	140	2.59	0.21
2, F	19	37	144	2.63	0.23
2, X + S	23	52	125	2.51	0.21
2, X + F	18	50	132	2.57	0.21
2, S + X	24	42	134	2.55	0.21
2, F + X	19	35	146	2.64	0.20
3, S	28	62	110	2.41	0.26
3, F	28	40	132	2.52	0.26
3, X + S	39	52	109	2.35	0.25
3, X + F	33	60	107	2.37	0.23
3, S + X	27	64	109	2.41	0.21
3, F + X	31	55	114	2.42	0.23
4, S	30	60	110	2.40	0.22
4, F	27	70	103	2.38	0.25
4, X + S	28	60	112	2.42	0.22
4, X + F	24	67	109	2.43	0.19
4, S + X	21	72	107	2.43	0.17
4, F + X	26	68	106	2.40	0.25

Table 3.26. F and p values for the factors, field, x-rays, order, signal and donor, obtained from the general linear model ANOVA tests on the NDI data.

Factor	NDI_{mn}		NDI_{sce}	
	F	p	F	p
Field	0.03	0.874	2.26	0.138
X-rays	11.77	0.001	1.52	0.222
Order	1.08	0.304	0.01	0.924
Signal	52.01	< 0.001	168.59	< 0.001
Donor	31.98	< 0.001	39.18	< 0.001

3.2.5 PHA stimulated whole blood cultures

So far this chapter has described the results of a series of experiments where whole heparinised blood has been exposed to regimes of x-rays and RF and then lymphocytes were stimulated by PHA and cultured to the assay end-points. During the limited time that the 1800 MHz exposure system was available a small supplementary study was performed in which cells in cycle were exposed to RF. No x-rays were used in this study. The measured increase in temperature of the cells due to the RF field was 0.07°C, with a range of 0.03 to 0.09°C.

Table 3.27 shows the number of chromosome and chromatid aberrations scored in the 4 donors from PHA stimulated cells exposed or sham exposed for 48 h to an 1800 MHz GSM Basic or CW intermittent signal (5 min RF on, 10 min RF off), delivering an SAR of 2 W/kg. The results of Student's t-test on the mean number of chromosomal aberrations only, chromatid damage only and the mean of the combined aberrations are also shown. As with the previous study, when the experimental conditions did not include x-rays (S and F), the numbers of chromosomal aberrations are small and consistent with normal background levels. The numbers of chromatid aberrations are also small, with chromatid gaps predominating. For both intermittent RF signals the pooled total number of chromatid aberrations is lower than the corresponding sham value. However, as shown in Table 3.25, Student's t-tests comparing sham and exposed revealed no significant differences at the 5% level.

Table 3.27. Number of chromosome and chromatid aberrations scored in 4 donors from PHA stimulated cells exposed or sham exposed for 48 hours to an 1800 MHz GSM Basic and a CW intermittent signal at an SAR of 2 W/kg. The t-test statistic (t) and p values on 6 degrees of freedom (DF) are also shown. S = sham; F = RF field.

Exposure conditions	Donor, Sham or Field	Cells scored	Chromosome damage						Chromatid damage							Chromosome plus chromatid damage	
			Dicentrics	Centric rings	Excess acentrics	Total	t	p	Tid gap	Tid break	Iso tid gap	Tid exchange	Total	t	p	t	p
1800 MHz GSM Basic 2 W/kg	1 S	500	0	0	1	1			2	1	0	1	4				
	2 S	500	1	0	1	2			3	1	0	0	4				
	3 S	500	1	0	0	1			1	3	0	0	4				
	4 S	500	0	0	0	0			9	2	0	0	11				
	Totals	**2000**	**2**	**0**	**2**	**4**	1.57	0.084	**15**	**7**	**0**	**1**	**23**	0.85	0.214	1.37	0.110
	1 F	500	0	0	2	2			2	0	0	0	2				
	2 F	500	0	0	1	1			4	0	0	0	4				
	3 F	500	0	0	0	0			5	2	0	0	7				
	4 F	500	0	0	1	1			3	0	0	0	3				
	Totals	**2000**	**0**	**0**	**4**	**4**			**14**	**2**	**0**	**0**	**16**				
1800 MHz CW 2 W/kg	1 S	500	0	0	0	0			3	1	0	0	4				
	2 S	500	0	0	0	0			3	0	0	1	4				
	3 S	500	0	0	1	1			3	1	0	0	4				
	4 S	500	0	0	0	0			3	1	0	0	4				
	Totals	**2000**	**0**	**0**	**1**	**1**	0.66	0.268	**12**	**3**	**0**	**1**	**16**	1.13	0.150	1.69	0.071
	1 F	500	0	0	0	0			3	0	0	0	3				
	2 F	500	0	0	1	1			0	0	0	0	0				
	3 F	500	0	0	1	1			3	1	0	0	4				
	4 F	500	0	0	1	1			1	0	0	0	1				
	Totals	**2000**	**0**	**0**	**3**	**3**			**7**	**1**	**0**	**0**	**8**				

3.3 Conclusion

This study has used several standard *in vitro* tests for chromosomal damage in G_0 human lymphocytes exposed *in vitro* to a combination of x-rays and RF fields. It has examined whether a 24 h continuous exposure to a 935 MHz GSM Basic signal delivering SARs of 1 or 2 W/kg or an 1800 MHz GSM Basic signal with an SAR of 2 W/kg is genotoxic *per se*, or whether it can influence the genotoxicity of the well established clastogentic agent; x-radiation. Within the experimental parameters of the study in all instances no genotoxic or dose modifying effect from the RF signal was observed compared with appropriate sham controls. In addition, PHA stimulated lymphocytes exposed to an intermittent 1800 MHz CW or GSM Basic signal both delivering an SAR of 2 W/kg together with appropriate sham controls showed no evidence of a direct genotoxic effect.

4. Chromosomal studies with human lymphocytes using CW and GSM Talk signals

4.1 Introduction

The aim of this study was to investigate further the possible genotoxic effect of 935 MHz RF field exposure alone and in combination with x-rays on human lymphocytes using standard cytogenetic end-points. To investigate the possibility that genotoxic effects following an *in vitro* exposure may depend on modulation or an intermittent field exposure, three signal types were used. A CW unmodulated signal, a continuous GSM Talk modulated signal and an intermittent GSM Talk signal. The intermittent GSM Talk signal was alternately switched on for 10 min and then off for 5 min over a total 24 h exposure period. RF fields of 1 W/kg were chosen to allow comparison with the results of the previous study. This SAR is realistic with regard to the actual energy absorption in localised tissue when a person uses a mobile phone, the upper limit being 2 W/kg (ICNIRP 1996). The three RF signal types were chosen in order to extend the work described in Chapter 3 with a signal more representative of holding a mobile phone conversation. All the culturing, slide preparation and scoring materials and methods are described in Chapter 2, which also contains a description of the RF signals.

4.2 Results and discussion

The results are presented in the same format as for those in the earlier work in Chapter 3. Also, similar to the previous study, an average temperature rise of 0.06°C with a range of 0.05 to 0.09°C occurred during the RF exposure.

Show in Table 4.1 are the results of calculating the actual power of the dicentric, micronuclei and SCE assays, for the 3 signals, to detect an elevated level of aberrations induced by x-rays in samples also exposed to RF fields. On

average a power of 0.8 was achieved with a sample size of four in all three assays. Therefore, the statistical strength of the data is sufficient to have detected an enhancing effect of RF fields

Table 4.1. The actual power and required sample size of the combined exposures to detect an enhancing effect of RF fields on dicentrics, micronuclei and SCE induced by x-rays.
S = Sham; X = 1 Gy x-ray; F = RF field

Assay and experimental conditions compared	935 MHz CW 1 W/kg		935 MHz GSM Talk 1 W/kg		935 MHz GSM Talk intermittent 1 W/kg	
	Actual power for sample size of 4	Required sample size for a power of 0.8	Actual power for sample size of 4	Required sample size for a power of 0.8	Actual power for sample size of 4	Required sample size for a power of 0.8
Dicentric X + S, X + F	0.99	3	0.99	3	0.88	4
Dicentric S + X, F + X	0.76	5	1.00	3	0.76	5
MN: X + S, X + F	1.00	3	1.00	3	0.92	4
MN: S + X, F + X	1.00	2	1.00	3	1.00	3
SCE: X + S, X + F	0.55	7	0.94	4	1.00	3
SCE: S + X, F + X	0.40	9	1.00	3	1.00	2

The results of the CA assay are presented in Tables 4.2 to 4.4, the MN assay in Tables 4.9 to 4.11 and the SCE assay in Tables 4.13 to 4.15 for each of the three signals studied: 935 MHz CW (SAR 1 W/kg), 935 MHz GSM Talk (1 W/kg) and 935 MHz GSM Talk intermittent (1 W/kg). The results of pooling data from the 4 donors for dicentrics, centric rings, excess acentrics, and chromatid aberrations are shown in Tables 4.5 to 4.8 and Tables 4.12 and 4.16 show the combined data for micronuclei and SCEs respectively. Figures 4.1 to 4.3 show the pooled results from replicate donors for the three different assays. As with the previous study, (Chapter 3), the overall distribution of the aberrations conform to a Poisson distribution for dicentrics (Tables 4.2 to 4.4), centric rings

(Tables A.10 to A.12) and chromatid aberrations (Tables A.16 to A.18). The micronuclei (Tables 4.9 to 4.11) were significantly over-dispersed, (average σ^2/y = 1.2), and the excess acentrics (Tables A.13 to A.15) also showed signs of over-dispersion, (average σ^2/y = 1.09), while the SCE distributions (Tables 4.13 to 4.15) were slightly under-dispersed. As in the previous study, described in Chapter 3, the value of σ^2/y, on average, was less than 1.00 but this was not significant (U > -1.96).

Again, it is apparent for Tables 4.2 to 4.4, Tables 4.9 to 4.11 and Figures 4.1 and 4.2 that an elevated level of dicentrics and micronuclei is only present for the exposure regimes that included x-rays. Indeed, ANOVA testing, as shown in Table 4.17, reveals that x-rays were a significant factor for dicentric, centric ring, excess acentric and micronucleus yields, with a p value of less than 0.001 in all cases.

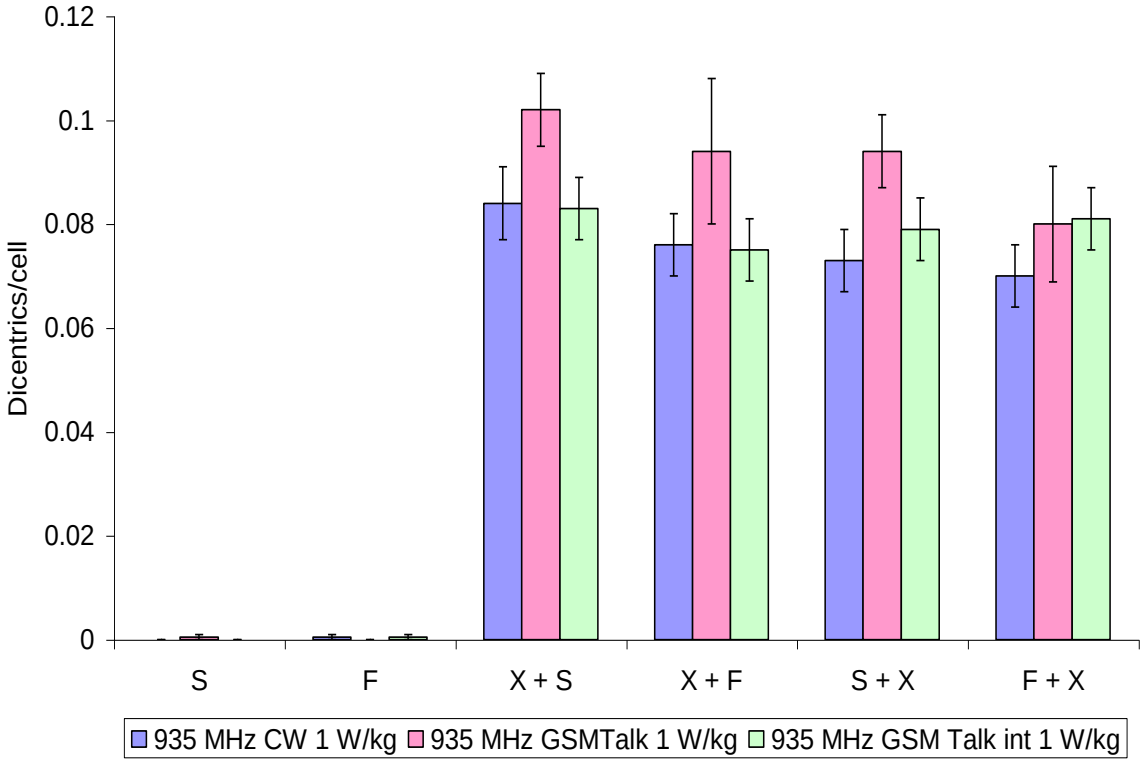

Figure 4.1. The pooled donors' data of dicentric chromosomal aberration yields ± SE for the 3 RF exposure regimes.
S = sham; X = 1 Gy x-rays; F = RF field

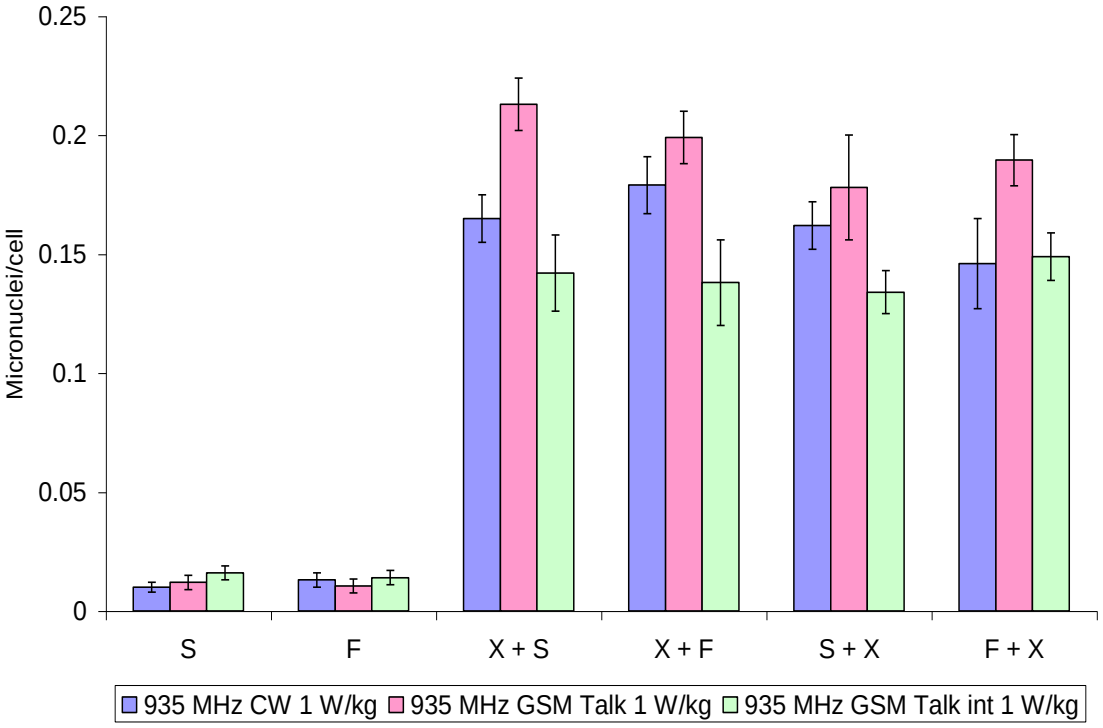

Figure 4.2. The pooled donors' data of micronucleus yields ± SE for the 3 RF exposure regimes.
S = sham; X = 1 Gy x-ray; F = RF field

Unlike the previous study, ANOVA revealed the frequency of chromatid aberrations and SCEs to be dependent on x-ray dose, with p = 0.002 and 0.001 respectively. One of the assumptions made for ANOVA testing is that the distribution of the data is approximately normal. It is known that x-rays do not induce chromatid aberrations and SCEs in lymphocytes irradiated in G_0, therefore the distributions of the chromatid and SCE data were checked by comparing the standard deviation (SD) of the data with the expected Poisson SD, calculated from the average number of aberrations divided by the square root of the number of data points. Unlike the dicentric and micronucleus results the SD of the chromatid and the SCE data were much lower than the expected Poisson SD, indicating the actual distribution of these data to be narrower than a normal distribution. Therefore the ANOVA result may not be valid for the chromatid damage and SCE assays as one of the conditions of the test was not met.

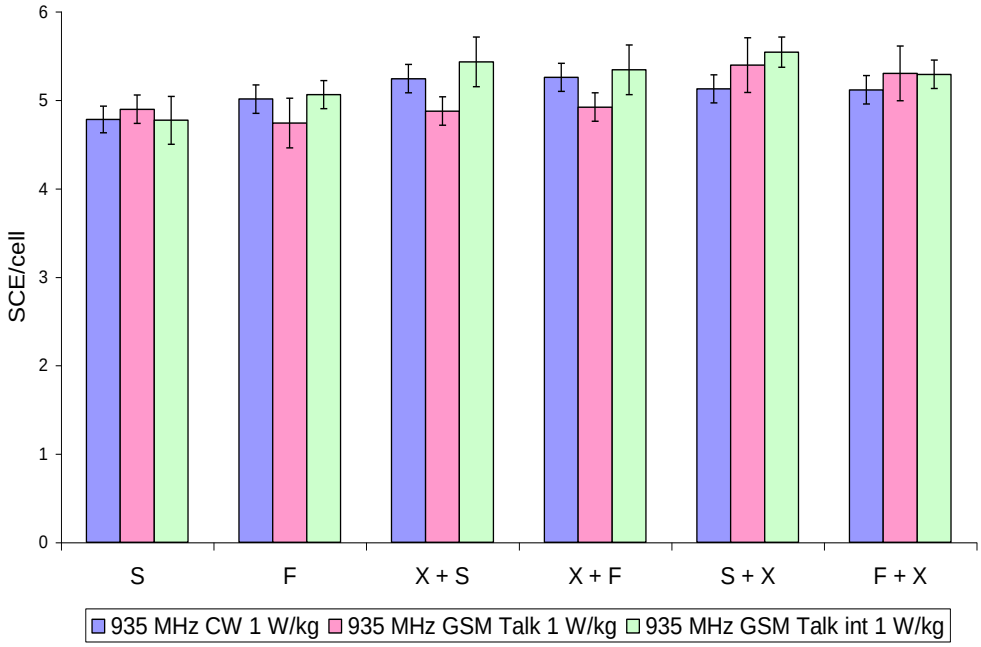

Figure 4.3. The pooled donors' data of SCE yields ± SE for the 3 RF exposure regimes.
S = sham; X = 1 Gy x-ray; F = RF field

As shown in Tables 4.13 to 4.15 and Figure 4.3 the frequencies of SCEs are ~5/cell and this is consistent throughout with the normal control levels found in this laboratory and with the levels found in the previous study using GSM Basic signals. No enhancement of SCE frequencies was observed with any of the exposure regimes or any signal type. As previously, exposure to an RF field alone (F) produced dicentric and micronucleus frequencies consistent with the sham (S), which were also consistent with the laboratory's historical control database. For a given signal the RF field exposure either before or after x-rays resulted in dicentric and micronucleus frequencies that were not statistically different from x-rays alone. As Table 4.17 shows, ANOVA revealed no effect of the field for any end-point nor was order of exposure (RF exposure followed by x-irradiation or *vice versa*) a significant factor. However, ANOVA testing did suggest the type of signal used was an important factor for dicentrics ($p = 0.007$), excess acentrics ($p = 0.038$) and micronuclei induction ($p < 0.001$). As shown in Figures 4.1 and 4.2 there were some variation in the overall dicentric and micronucleus yields between the signals. On average the results for the continuous GSM Talk signal were approximately 20% higher for dicentrics and

about 35% higher for micronuclei compared to the other two signals. However, the variations in yield in any x-ray group for a given signal were not significant.

Overall the yields of dicentrics and micronuclei were also in agreement with the frequency of these aberrations observed in the studies with 935 MHz GSM Basic and 1800 MHz GSM Basic both with an SAR of 2 W/kg; further suggesting all the dicentric yields in the earlier study using a 935 MHz GSM Basic (1 W/kg) signal were unusually high.

Results of the NDI_{MN} are presented in Tables 4.18 to 4.20 and the NDI_{SCE} in Tables 4.21 to 4.23 for each signal type. The combined NDI_{MN} and NDI_{SCE} are shown in Figure 4.4 and 4.5 respectively. Again, as shown in Table 4.24, ANOVA testing revealed that for both NDI_{MN} and NDI_{SCE} donor was an important factor ($p < 0.001$). As described in Chapter 3 section 3.2.4, the difference in the speed of cell cycling between people was taken into account when calculating the change in NDI. Again, Figures 4.4 and 4.5 show a tendency for the values of the irradiated samples to be negative indicating slower growth with respect to the shams. Although not statistically significant, this represents the expected mitotic delay produced by ionising radiation (Purrott et al. 1980). Inspection of the NDI data from this and the previous study, showed a tendency for the speed of cell cycling for donors 1 and 2 to be faster than persons 3 and 4. Overall, no effect of RF alone or in combination with x-rays was seen on the speed of cell cycling.

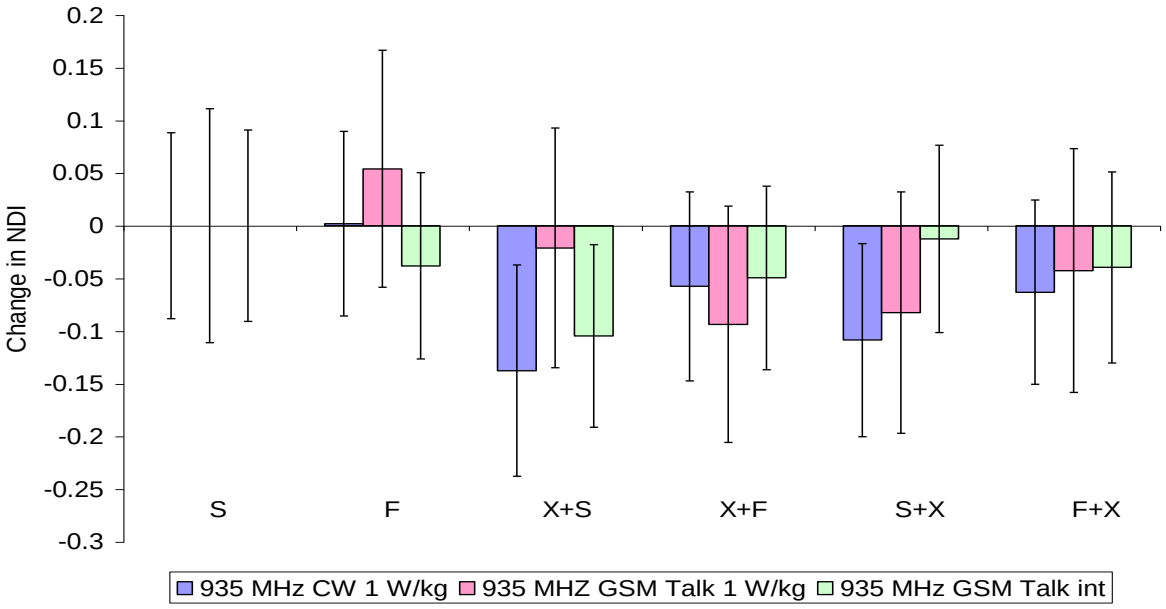

Figure 4.4. Normalised changes in the nuclear division index derived from slides prepared for the cytokinesis-blocked MN assay for the 3 RF exposure regimes. The individual donor's data have been pooled also the data for each donor were compared with that person's sham value. Pooling has required normalisation to the sham so that values above and below zero indicate cycle time accelerated and slowed respectively.
S = sham; X = 1 Gy x-ray; F = RF field

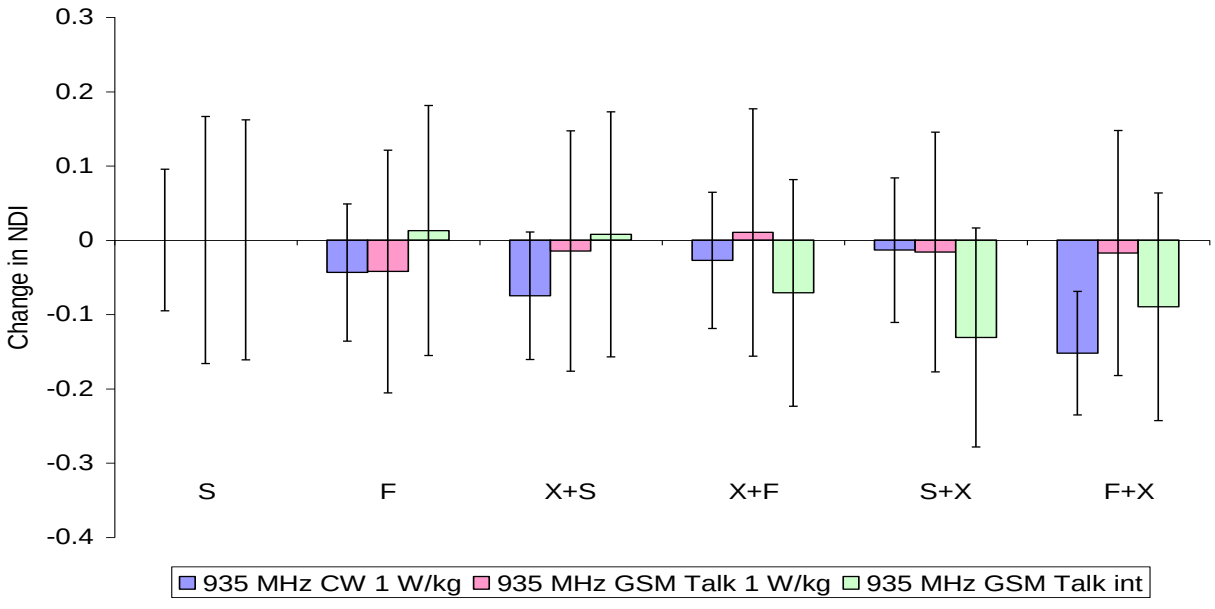

Figure 4.5. Normalised changes in the nuclear division index derived from slides prepared for the SCE assay for the 3 RF exposure regimes. The individual donor's data have been pooled also the data for each donor were compared to that person's sham value. Pooling has required normalisation to the sham so that values above or below zero indicate cell cycle times accelerated or slowed respectively.
S = sham; X = 1 Gy x-rays; F = RF field

Table 4.2. Number of chromosome aberrations scored for the different exposure regimes in 4 donors using a 935 MHz CW signal at an SAR of 1 W/kg. The dicentric yields and distributions amongst the cells are also shown. Figures in red indicate a U value > 1.96 (see section 2.8.1 for explanation)
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of dicentrics	Number of centric rings	Number of excess acentrics	Dicentrics per cell ± SE	Distribution of dicentrics				σ^2 / y ± SE	U
							0	1	2	3		
1, S	500	498	0	0	2	—	500				—	—
1, F	500	497	0	0	3	—	500				—	—
1, X + S	500	445	37	1	25	0.074 ± 0.012	466	32	1	1	1.15 ± 0.06	2.31
1, X + F	500	435	43	3	24	0.086 ± 0.013	459	39	2		1.01 ± 0.06	1.45
1, S + X	500	448	32	0	26	0.064 ± 0.011	468	32			0.94 ± 0.06	-0.99
1, F + X	500	452	38	1	16	0.076 ± 0.012	464	34	2		1.03 ± 0.06	0.5
2, S	500	500	0	0	0	—	500				—	—
2, F	500	500	0	0	0	—	500				—	—
2, X + S	500	431	37	2	40	0.074 ± 0.012	466	31	3		1.09 ± 0.06	1.45
2, X + F	500	452	37	1	18	0.074 ± 0.012	467	29	4		1.15 ± 0.06	2.31
2, S + X	500	444	36	0	21	0.072 ± 0.012	465	34	1		0.99 ± 0.06	-0.23
2, F + X	500	436	34	1	35	0.068 ± 0.012	468	30	2		1.05 ± 0.06	0.83
3, S	500	500	0	0	0	—	500				—	—
3, F	500	498	1	0	1	0.002 ± 0.002	499	1			—	—
3, X + S	500	437	41	4	29	0.082 ± 0.013	463	33	4		1.12 ± 0.06	1.86
3, X + F	500	448	36	0	21	0.072 ± 0.012	467	30	3		1.10 ± 0.06	1.55
3, S + X	500	428	40	2	44	0.080 ± 0.013	461	38	1		0.97 ± 0.06	-0.45
3, F + X	500	444	35	0	24	0.070 ± 0.012	466	33	1		0.99 ± 0.06	-0.17
4, S	500	500	0	0	0	—	500				—	—
4, F	500	500	0	0	0	—	500				—	—
4, X + S	500	426	53	1	31	0.106 ± 0.015	450	47	3		1.01 ± 0.06	1.47
4, X + F	500	447	35	2	24	0.070 ± 0.012	467	32	0	1	1.10 ± 0.06	1.66
4, S + X	500	441	37	2	29	0.074 ± 0.012	464	35	1		0.98 ± 0.06	-0.29
4, F + X	500	468	33	2	34	0.066 ± 0.012	468	31	1		1.00 ± 0.06	-0.05

Table 4.3. Number of chromosome aberrations scored for the different exposure regimes in 4 donors using a 935 MHz GSM Talk signal at an SAR of 1 W/kg. The dicentric yields and distributions amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of dicentrics	Number of centric rings	Number of excess acentrics	Dicentrics per cell ± SE	Distribution of dicentrics				σ^2 / y ± SE	U
							0	1	2	3		
1, S	500	498	0	0	2	—	500				—	—
1, F	500	500	0	0	0	—	500				—	—
1, X + S	500	419	55	1	41	0.110 ± 0.015	448	49	3		1.00 ± 0.06	1.74
1, X + F	500	406	59	7	45	0.118 ± 0.015	447	47	6		1.09 ± 0.06	1.4
1, S + X	500	432	45	0	32	0.090 ± 0.013	458	39	3		1.05 ± 0.06	0.73
1, F + X	500	435	38	1	38	0.076 ± 0.012	464	34	2		1.03 ± 0.06	0.05
2, S	500	500	0	0	0	—	500				—	—
2, F	500	500	0	0	0	—	500				—	—
2, X + S	500	442	34	0	29	0.068 ± 0.012	467	32	1		0.99 ± 0.06	-0.12
2, X + F	500	446	35	0	27	0.07 ± 0.01	468	29	3		1.10 ± 0.06	1.66
2, S + X	500	448	36	0	21	0.072 ± 0.012	465	34	1		0.99 ± 0.06	-0.23
2, F + X	500	430	41	3	34	0.082 ± 0.013	460	39	1		0.97 ± 0.06	-0.5
3, S	500	499	1	1	1	0.002 ± 0.002	499	1			—	—
3, F	500	500	0	0	0	—	500				—	—
3, X + S	500	412	59	2	45	0.118 ± 0.015	446	49	5		1.05 ± 0.06	0.85
3, X + F	500	449	35	1	22	0.070 ± 0.012	469	27	4		1.16 ± 0.06	2.58
3, S + X	500	434	46	2	26	0.092 ± 0.014	455	44	1		0.95 ± 0.06	-0.74
3, F + X	500	441	34	0	35	0.068 ± 0.012	469	28	3		1.11 ± 0.06	1.78
4, S	500	499	0	0	1	—	500				—	—
4, F	500	500	0	0	0	—	500				—	—
4, X + S	500	428	55	3	29	0.110 ± 0.015	448	49	3		1.00 ± 0.06	0.02
4, X + F	500	418	59	3	37	0.118 ± 0.015	445	51	4		1.02 ± 0.06	0.31
4, S + X	500	415	60	2	37	0.120 ± 0.015	443	54	3		0.98 ± 0.06	-0.29
4, F + X	500	427	47	3	32	0.094 ± 0.014	458	38	3	1	1.16 ± 0.06	2.61

Table 4.4. Number of chromosome aberrations scored for the different exposure regimes in 4 donors using a 935 MHz GSM Talk intermittent signal at an SAR of 1 W/kg. The dicentric yields and distributions amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of dicentrics	Number of centric rings	Number of excess acentrics	Dicentrics per cell ± SE	Distribution of dicentrics				σ^2 / y ± SE	U
							0	1	2	3		
1, S	500	500	0	0	0	—	500				—	—
1, F	500	500	0	0	0	—	500				—	—
1, X + S	500	417	51	3	36	0.102 ± 0.014	450	49	1		0.94 ± 0.06	-0.97
1, X + F	500	435	41	1	27	0.082 ± 0.013	461	37	2		1.02 ± 0.06	0.28
1, S + X	500	443	36	2	24	0.072 ± 0.012	464	36			0.93 ± 0.06	-1.12
1, F + X	500	447	37	2	23	0.074 ± 0.012	465	33	2		1.04 ± 0.06	0.58
2, S	500	499	0	0	1	—	500				—	—
2, F	500	499	1	0	0	0.002 ± 0.002	499	1			—	—
2, X + S	500	442	35	1	27	0.070 ± 0.012	467	31	2		1.05 ± 0.06	0.74
2, X + F	500	435	35	2	30	0.070 ± 0.012	465	35			0.93 ± 0.06	-1.09
2, S + X	500	452	31	1	20	0.062 ± 0.011	470	29	1		1.00 ± 0.06	0.07
2, F + X	500	439	39	1	27	0.078 ± 0.013	461	39			0.92 ± 0.06	-1.22
3, S	500	500	0	0	0	—	500				—	—
3, F	500	499	0	0	1	—	500				—	—
3, X + S	500	442	35	2	24	0.070 ± 0.012	466	33	1		0.99 ± 0.06	-0.17
3, X + F	500	435	42	3	31	0.084 ± 0.013	463	32	5		1.16 ± 0.06	2.5
3, S + X	500	435	39	2	31	0.078 ± 0.013	465	31	4		1.13 ± 0.06	2.07
3, F + X	500	432	39	2	33	0.078 ± 0.012	464	33	3		1.08 ± 0.06	1.25
4, S	500	500	0	0	0	—	500				—	—
4, F	500	500	0	0	0	—	500				—	—
4, X + S	500	430	45	1	27	0.090 ± 0.013	456	43	1		0.96 ± 0.06	-0.7
4, X + F	500	436	41	3	36	0.082 ± 0.013	463	34	2	1	1.16 ± 0.06	2.63
4, S + X	500	436	51	0	18	0.102 ± 0.014	454	41	5		1.10 ± 0.06	1.54
4, F + X	500	436	47	3	24	0.094 ± 0.014	456	42	1	1	1.08 ± 0.06	1.25

Table 4.5. The mean yields per cell ± SE of pooled dicentric data for 3 RF exposure regimes. χ^2 and p values on 3 degrees of freedom obtained by the chi-squared test for homogeneity are also shown. Figures in red indicate p values < 0.05 (see section 2.8.2 for discussion)
S = sham; X = 1 Gy x-rays; F = RF field

Condition	935 MHz CW 1 W/kg					935 MHz GSM Talk 1 W/kg					935 MHz GSM Talk intermittent 1 W/kg				
	Total number cells scored	Total number of dicentrics	Dicentrics per cell ±SE	χ^2	p	Total number cells scored	Total number of dicentrics	Dicentrics per cell ±SE	χ^2	p	Total number cells scored	Total number of dicentrics	Dicentrics per cell ±SE	χ^2	p
S	2000	0	—	—	—	2000	1	0.0005 ± 0.0005	3.00	0.392	2000	0	—	—	—
F	2000	1	0.0005 ± 0.0005	3.00	0.392	2000	0	—	—	—	2000	1	0.0005 ± 0.0005	3.00	0.392
X + S	2000	168	0.0840 ± 0.0065	4.10	0.251	2000	203	0.1015 ± 0.0071	7.58	0.056	2000	166	0.0830 ± 0.006	4.51	0.212
X + F	2000	151	0.0755 ± 0.0061	1.03	0.795	2000	188	0.0940 ± 0.0139	12.26	0.007	2000	159	0.0795 ± 0.0063	0.77	0.856
S +X	2000	145	0.0725 ± 0.0060	3.34	0.825	2000	187	0.0935 ± 0.0068	6.30	0.621	2000	157	0.0785 ± 0.0063	5.52	0.137
F + X	2000	140	0.0700 ± 0.0059	0.40	0.940	2000	160	0.0800 ± 0.0111	2.25	0.027	2000	162	0.0810 ± 0.0064	1.46	0.692

Table 4.6. The mean yields per cell ± SE of pooled centric ring data for 3 RF exposure regimes. χ^2 and p values on 3 degrees of freedom obtained by the chi-squared test for homogeneity are also shown.
S = sham; X = 1 Gy x-rays; F = RF field

Experimental condition	935 MHz CW 1 W/kg					935 MHz GSM Talk 1 W/kg					935 MHz GSM Talk intermittent 1 W/kg				
	Total number cells scored	Total number of centric rings	Centric rings per cell ±SE	χ^2	p	Total number cells scored	Total number of centric rings	Centric rings per cell ±SE	χ^2	p	Total number cells scored	Total number of centric rings	Centric rings per cell ±SE	χ^2	p
S	2000	0	—	—	—	2000	1	0.0005 ± 0.0005	3.00	0.392	2000	0	—	—	—
F	2000	0	—	—	—	2000	0	—	—	—	2000	0	—	—	—
X + S	2000	8	0.0040 ± 0.0014	3.00	0.392	2000	6	0.0030 ± 0.0012	3.33	0.343	2000	7	0.0035 ± 0.0013	1.57	0.666
X + F	2000	6	0.0030 ± 0.0012	3.33	0.343	2000	11	0.0055 ± 0.0031	10.45	0.015	2000	9	0.0045 ± 0.0015	1.22	0.748
S +X	2000	4	0.0020 ± 0.0010	4.00	0.261	2000	4	0.0020 ± 0.0010	5.40	0.145	2000	5	0.0025 ± 0.0011	2.20	0.532
F + X	2000	4	0.0020 ± 0.0010	2.00	0.572	2000	7	0.0035 ± 0.0013	3.33	0.343	2000	8	0.0040 ± 0.0014	1.00	0.801

Table 4.7. The mean yields per cell ± SE of pooled excess acentric data for 3 RF exposure regimes. χ^2 and p values on 3 degrees of freedom obtained by the chi-squared test for homogeneity are also shown.
S = sham; X = 1 Gy x-rays; F = RF field

Experimental condition	935 MHz CW 1 W/kg					935 MHz GSM Talk 1 W/kg					935 MHz GSM Talk intermittent 1 W/kg				
	Total number cells scored	Total number of excess acentrics	Excess acentrics per cell ±SE	χ^2	p	Total number cells scored	Total number of excess acentrics	Excess acentrics per cell ±SE	χ^2	p	Total number cells scored	Total number of excess acentrics	Excess acentrics per cell ±SE	χ^2	p
S	2000	2	0.0010 ± 0.0007	6.00	0.112	2000	4	0.0020 ± 0.0010	2.00	0.572	2000	1	0.0005 ± 0.0005	3.00	0.392
F	2000	4	0.0020 ± 0.0010	6.00	0.112	2000	0	—	—	—	2000	1	0.0005 ± 0.0005	3.00	0.392
X + S	2000	125	0.0625 ± 0.0058	3.86	0.277	2000	144	0.0720 ± 0.0063	5.67	0.129	2000	114	0.0570 ± 0.0058	2.84	0.417
X + F	2000	87	0.0435 ± 0.0049	1.14	0.768	2000	131	0.0665 ± 0.0107	9.67	0.022	2000	124	0.0620 ± 0.0058	1.35	0.716
S +X	2000	120	0.0600 ± 0.0103	9.80	0.020	2000	116	0.0580 ± 0.0058	3.05	0.383	2000	93	0.0465 ± 0.0050	4.25	0.236
F + X	2000	109	0.0545 ± 0.0094	8.91	0.031	2000	139	0.0695 ± 0.0062	0.28	0.964	2000	107	0.0535 ± 0.0054	2.27	0.518

Table 4.8. The mean yields per cell ± SE of pooled chromatid aberration data for 3 RF exposure regimes. χ^2 and p values on 3 degrees of freedom obtained by the chi-squared test for homogeneity are also shown.
S = sham; X = 1 Gy x-rays; F = RF field

Experimental condition	935 MHz CW 1 W/kg					935 MHz GSM Talk 1 W/kg					935 MHz GSM Talk intermittent 1 W/kg				
	Total number cells scored	Total chromatid damage	Chromatid damage per cell ±SE	χ^2	p	Total number cells scored	Total chromatid damage	Chromatid damage per cell ±SE	χ^2	p	Total number cells scored	Total chromatid damage	Chromatid damage per cell ±SE	χ^2	p
S	2000	5	0.0025 ± 0.0011	3.80	0.284	2000	8	0.0040 ± 0.0014	4.00	0.261	2000	5	0.0025 ± 0.0011	0.60	0.896
F	2000	6	0.0030 ± 0.0012	3.33	0.343	2000	8	0.0040 ± 0.0024	9.00	0.029	2000	10	0.0050 ± 0.0016	5.20	0.158
X + S	2000	8	0.0040 ± 0.0014	1.00	0.801	2000	16	0.0080 ± 0.0033	8.29	0.040	2000	25	0.0125 ± 0.0068	22.36	<0.001
X + F	2000	9	0.0045 ± 0.0026	9.22	0.026	2000	21	0.0105 ± 0.0023	7.00	0.072	2000	23	0.0115 ± 0.0024	6.39	0.094
S +X	2000	17	0.0085 ± 0.0021	0.65	0.886	2000	12	0.0060 ± 0.0017	5.20	0.158	2000	19	0.0095 ± 0.0022	5.21	0.157
F + X	2000	15	0.0075 ± 0.0019	2.33	0.506	2000	17	0.0085 ± 0.0021	4.41	0.220	2000	17	0.0085 ± 0.0021	2.06	0.560

Table 4.9. The number of micronuclei scored for the different exposure regimes in 4 donors using a 935 MHz CW signal at an SAR of 1 W/kg. The micronucleus yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of micronuclei	Number of anaphase bridges	Micronuclei per cell ± SE	Distribution of micronuclei						σ^2 / y ± SE	U
						0	1	2	3	4	5		
1, S	500	495	5	0	0.010 ± 0.005	495	5					0.99 ± 0.06	-0.14
1, F	500	494	7	0	0.014 ± 0.006	494	5	1				1.27 ± 0.06	4.68
1, X + S	500	434	76	2	0.152 ± 0.019	435	54	11				1.14 ± 0.06	2.22
1, X + F	500	432	79	3	0.142 ± 0.020	432	58	9	1			1.15 ± 0.06	2.35
1, S + X	500	438	70	0	0.140 ± 0.018	438	54	8				1.09 ± 0.06	1.44
1, F + X	500	443	64	2	0.128 ± 0.018	443	50	7				1.09 ± 0.06	1.48
2, S	500	493	8	0	0.016 ± 0.006	493	6	1				1.24 ± 0.06	3.99
2, F	500	493	7	0	0.014 ± 0.006	493	7					0.99 ± 0.06	-0.21
2, X + S	500	443	65	3	0.130 ± 0.018	446	44	9	1			1.24 ± 0.06	3.85
2, X + F	500	437	74	5	0.148 ± 0.019	440	51	6	2	0	1	1.45 ± 0.06	7.17
2, S + X	500	430	77	5	0.154 ± 0.019	432	59	6	2			1.16 ± 0.06	2.54
2, F + X	500	445	57	3	0.114 ± 0.017	445	53	2				0.96 ± 0.06	-0.67
3, S	500	496	4	0	0.008 ± 0.004	496	4					0.99 ± 0.05	-0.18
3, F	500	494	6	0	0.012 ± 0.005	494	6					0.99 ± 0.06	-0.17
3, X + S	500	424	91	5	0.182 ± 0.021	424	62	13	1			1.17 ± 0.06	2.73
3, X + F	500	419	91	6	0.182 ± 0.021	422	69	5	4			1.19 ± 0.06	3.08
3, S + X	500	428	81	5	0.162 ± 0.020	429	63	7	0	1		1.16 ± 0.06	2.56
3, F + X	500	435	73	5	0.146 ± 0.019	437	53	10				1.13 ± 0.06	2.07
4, S	500	497	3	0	0.006 ± 0.004	497	3					1.00 ± 0.05	-0.08
4, F	500	494	6	0	0.012 ± 0.005	494	6					0.99 ± 0.06	-0.17
4, X + S	500	424	97	3	0.194 ± 0.022	425	57	14	4			1.35 ± 0.06	5.47
4, X + F	500	407	114	4	0.228 ± 0.023	408	74	15	2	1		1.25 ± 0.06	3.94
4, S + X	500	415	96	4	0.192 ± 0.022	415	74	11				1.04 ± 0.06	0.62
4, F + X	500	423	97	0	0.198 ± 0.022	423	60	15	1	1		1.30 ± 0.06	4.82

Table 4.10. The number of micronuclei scored for the different exposure regimes in 4 donors using a 935 MHz GSM Talk signal at an SAR of 1 W/kg. The micronucleus yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of micronuclei	Number of anaphase bridges	Micronuclei per cell ± SE	Distribution of micronuclei						σ^2 / y ± SE	U
						0	1	2	3	4	5		
1, S	500	496	4	0	0.008 ± 0.004	496	4					0.99 ± 0.05	-0.18
1, F	500	493	7	0	0.014 ± 0.006	493	7					0.99 ± 0.06	-0.21
1, X + S	500	416	107	2	0.214 ± 0.023	417	62	18	3			1.29 ± 0.06	4.65
1, X + F	500	419	100	2	0.200 ± 0.022	421	63	12	3	1		1.34 ± 0.06	5.44
1, S + X	500	417	98	2	0.196 ± 0.022	418	67	14	1			1.15 ± 0.06	2.43
1, F + X	500	422	92	4	0.184 ± 0.021	423	62	15				1.14 ± 0.06	2.29
2, S	500	494	6	0	0.012 ± 0.005	494	6					0.99 ± 0.06	-0.17
2, F	500	496	4	0	0.008 ± 0.004	496	4					0.99 ± 0.05	-0.18
2, X + S	500	419	95	4	0.190 ± 0.021	421	64	14	1			1.17 ± 0.06	2.7
2, X + F	500	426	86	2	0.172 ± 0.020	427	60	13				1.13 ± 0.06	2.11
2, S + X	500	430	75	2	0.150 ± 0.019	430	66	3	1			1.01 ± 0.06	0.19
2, F + X	500	427	86	3	0.172 ± 0.020	429	60	9	1	0	1	1.34 ± 0.06	5.44
3, S	500	495	6	0	0.012 ± 0.005	495	4	1				1.32 ± 0.06	5.61
3, F	500	495	5	0	0.010 ± 0.005	495	5					0.99 ± 0.06	-0.14
3, X + S	500	410	107	5	0.214 ± 0.023	410	76	12	1	1		1.18 ± 0.06	2.87
3, X + F	500	418	102	3	0.204 ± 0.022	420	68	7	2	1	2	1.56 ± 0.06	8.95
3, S + X	500	435	70	2	0.140 ± 0.018	436	58	6				1.03 ± 0.06	0.53
3, F + X	500	428	83	3	0.166 ± 0.020	429	60	10	1			1.15 ± 0.06	2.38
4, S	500	492	8	0	0.016 ± 0.006	492	8					0.99 ± 0.06	-0.24
4, F	500	495	5	0	0.010 ± 0.005	495	5					0.99 ± 0.06	-0.14
4, X + S	500	407	116	3	0.232 ± 0.024	408	74	14	2	2		1.32 ± 0.06	5.11
4, X + F	500	404	109	7	0.218 ± 0.023	409	76	12	3			1.17 ± 0.06	2.69
4, S + X	500	411	112	1	0.224 ± 0.023	411	69	17	3			1.24 ± 0.06	3.85
4, F + X	500	402	118	8	0.236 ± 0.024	407	70	21	2			1.22 ± 0.06	3.55

Table 4.11. The number of micronuclei scored for the different exposure regimes in 4 donors using a 935 MHz GSM Talk intermittent signal at an SAR of 1 W/kg. The micronucleus yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number normal	Number of micronuclei	Number of anaphase bridges	Micronuclei per cell ± SE	Distribution of micronuclei						σ^2 / y ± SE	U
						0	1	2	3	4	5		
1, S	500	490	10	0	0.020 ± 0.007	490	10					0.98 ± 0.06	-0.3
1, F	500	493	7	0	0.014 ± 0.006	493	7					0.99 ± 0.06	-0.21
1, X + S	500	448	58	3	0.116 ± 0.017	449	47	2	1	1		1.27 ± 0.06	4.24
1, X + F	500	443	64	2	0.128 ± 0.018	443	51	5	1			1.12 ± 0.06	1.98
1, S + X	500	444	64	2	0.128 ± 0.018	445	48	5	2			1.22 ± 0.06	3.47
1, F + X	500	436	78	3	0.156 ± 0.019	437	52	8	2	1		1.36 ± 0.06	5.72
2, S	500	493	7	0	0.014 ± 0.006	493	7					0.99 ± 0.06	-0.21
2, F	500	495	5	0	0.010 ± 0.005	495	5					0.99 ± 0.06	-0.14
2, X + S	500	446	65	1	0.130 ± 0.018	447	45	5	2	1		1.40 ± 0.06	6.3
2, X + F	500	444	60	0	0.120 ± 0.017	444	52	4				1.02 ± 0.06	0.24
2, S + X	500	444	65	2	0.130 ± 0.018	444	48	7	1			1.18 ± 0.06	2.87
2, F + X	500	438	65	4	0.130 ± 0.018	440	55	5				1.03 ± 0.06	0.41
3, S	500	495	6	0	0.012 ± 0.005	495	4	1				1.32 ± 0.06	5.61
3, F	500	494	6	0	0.012 ± 0.005	494	6					0.99 ± 0.06	-0.17
3, X + S	500	439	69	2	0.138 ± 0.018	440	52	7	1			1.15 ± 0.06	2.45
3, X + F	500	450	59	2	0.118 ± 0.017	450	43	6	0	1		1.29 ± 0.06	4.64
3, S + X	500	441	68	1	0.136 ± 0.018	441	54	3	1	0	1	1.34 ± 0.06	5.37
3, F + X	500	433	76	1	0.152 ± 0.019	433	58	9				1.09 ± 0.06	1.38
4, S	500	491	9	0	0.018 ± 0.007	491	9					0.98 ± 0.06	-0.27
4, F	500	492	9	0	0.018 ± 0.007	492	7	1				1.21 ± 0.06	3.46
4, X + S	500	419	92	4	0.184 ± 0.021	419	71	9	1			1.08 ± 0.06	1.26
4, X + F	500	420	93	4	0.186 ± 0.021	420	67	13				1.10 ± 0.06	1.52
4, S + X	500	439	70	1	0.140 ± 0.018	439	52	9				1.12 ± 0.06	1.9
4, F + X	500	433	79	3	0.158 ± 0.020	433	57	8	2			1.20 ± 0.06	3.16

Table 4.12. The mean yields per cell ± SE of pooled data from the micronucleus assay for 3 RF exposure regimes. χ^2 and p values on 3 degrees of freedom obtained by the chi-squared test for homogeneity are also shown.
S = sham; X = 1 Gy x-ray; F = RF field; MN = micronuclei.

Experimental condition	935 MHz CW 1 W/kg					935 MHz GSM Talk 1 W/kg					935 MHz GSM Talk intermittent 1 W/kg				
	Total number cells scored	Total number of MN	MN per cell ±SE	χ^2	p	Total number cells scored	Total number of MN	MN per cell ±SE	χ^2	p	Total number cells scored	Total number of MN	MN per cell ±SE	χ^2	p
S	2000	20	0.0100 ± 0.0024	2.80	0.423	2000	24	0.0120 ± 0.0027	1.33	0.721	2000	32	0.0160 ± 0.0031	1.25	0.741
F	2000	26	0.0130 ± 0.0028	0.15	0.985	2000	21	0.0105 ± 0.0025	0.90	0.824	2000	27	0.0135 ± 0.0028	1.3	0.730
X + S	2000	329	0.1645 ± 0.0099	7.67	0.053	2000	425	0.2125 ± 0.0113	2.10	0.553	2000	284	0.1420 ± 0.0161	9.15	0.027
X + F	2000	358	0.1790 ± 0.0195	10.65	0.014	2000	397	0.1985 ± 0.0109	2.81	0.422	2000	276	0.1380 ± 0.0178	11.33	0.010
S +X	2000	324	0.1620 ± 0.0099	4.47	0.215	2000	355	0.1775 ± 0.0216	13.15	0.004	2000	267	0.1335 ± 0.0089	0.34	0.952
F + X	2000	291	0.1455 ± 0.0191	12.55	0.006	2000	379	0.1895 ± 0.0107	8.05	0.068	2000	298	0.1490 ± 0.0095	1.68	0.642

Table 4.13. Number of SCEs scored for the different exposure regimes in 4 donors using a 935 MHz GSM CW signal at an SAR of 1 W/kg. The SCE yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of SCEs	SCEs per cell ± SE	Distribution of SCEs																σ^2 / y ± SE	U
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1, S	50	228	4.50 ± 0.30	0	1	3	8	18	9	3	4	2	2							0.68 ± 0.20	-1.58
1, F	50	252	5.04 ± 0.32	0	1	2	3	13	14	10	2	4	0	1						0.56 ± 0.20	-2.19
1, X + S	50	259	5.18 ± 0.32	0	3	6	5	6	9	6	4	7	2	1	0	1				1.25 ± 0.20	1.24
1, X + F	50	255	5.10 ± 0.32	0	0	3	5	16	8	9	3	4	1	0	0	0	0	1		0.87 ± 0.20	-0.66
1, S + X	50	252	5.04 ± 0.32	0	0	6	11	7	7	6	7	2	1	1	1	0	1			1.21 ± 0.20	1.02
1, F + X	50	240	4.80 ± 0.31	0	1	5	10	11	8	4	3	5	1	1	1					1.05 ± 0.20	0.27
2, S	50	239	4.78 ± 0.31	0	0	5	9	10	11	7	3	3	1	0	1					0.80 ± 0.20	-1.01
2, F	50	242	4.84 ± 0.31	0	1	2	10	8	12	10	4	1	1	1						0.66 ± 0.20	-1.68
2, X + S	50	297	5.94 ± 0.35	0	0	4	1	11	9	7	6	3	3	4	2					0.98 ± 0.20	-0.11
2, X + F	50	295	5.90 ± 0.34	0	0	2	5	4	11	12	5	6	2	1	2					0.75 ± 0.20	-1.25
2, S + X	50	255	5.10 ± 0.32	0	0	4	5	15	8	7	2	6	2	1						0.79 ± 0.20	-1.06
2, F + X	50	257	5.14 ± 0.32	0	2	4	8	3	12	8	6	4	1	2						0.94 ± 0.20	-0.31
3, S	50	245	4.90 ± 0.31	0	0	7	7	15	4	8	2	3	1	0	1	2				1.24 ± 0.20	1.21
3, F	50	270	5.40 ± 0.33	0	0	3	6	13	9	5	6	3	1	2	1	0	1			1.05 ± 0.20	0.25
3, X + S	50	256	5.12 ± 0.32	0	1	4	7	12	7	6	8	1	2	0	0	1	1			1.13 ± 0.20	0.64
3, X + F	50	256	5.12 ± 0.32	0	3	5	2	6	13	10	4	4	2	1						0.90 ± 0.20	-0.51
3, S + X	50	248	4.96 ± 0.32	0	0	4	5	15	6	10	6	3	1							0.61 ± 0.20	-1.94
3, F + X	50	266	5.32 ± 0.33	0	1	3	3	14	11	8	3	1	1	3	1	1				1.03 ± 0.20	0.16
4, S	50	244	4.88 ± 0.31	0	2	7	7	10	7	5	3	4	1	4						1.26 ± 0.20	1.29
4, F	50	238	4.76 ± 0.31	0	1	5	7	10	14	7	2	2	1	0	0	0	0	0	1	1.05 ± 0.20	0.25
4, X + S	50	236	4.72 ± 0.31	0	5	4	9	8	5	6	6	6	0	0	0	0	1			1.32 ± 0.20	1.56
4, X + F	50	245	4.90 ± 0.31	0	0	5	9	14	5	6	6	2	1	0	1	0	1			1.08 ± 0.20	0.38
4, S + X	50	270	5.40 ± 0.33	0	0	5	7	3	11	6	10	6	2							0.76 ± 0.20	-1.21
4, F + X	50	260	5.20 ± 0.32	0	0	3	7	14	6	5	8	3	3	1						0.80 ± 0.20	-0.99

Table 4.14. Number of SCEs scored for the different exposure regimes in 4 donors using a 935 MHz GSM Talk signal at an SAR of 1 W/kg. The SCE yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of SCEs	SCEs per cell ± SE	Distribution of SCEs														σ^2 / y ± SE	U
				0	1	2	3	4	5	6	7	8	9	10	11	12	13		
1, S	50	248	4.96 ± 0.31	0	1	5	8	9	10	5	4	4	3	1				0.95 ± 0.20	-0.22
1, F	50	208	4.16 ± 0.29	0	2	7	16	9	7	2	3	0	3	0	0	0	1	1.27 ± 0.20	1.34
1, X + S	50	240	4.80 ± 0.31	0	2	3	9	9	11	6	6	1	2	1				0.84 ± 0.20	-0.78
1, X + F	50	247	4.94 ± 0.31	0	3	5	2	15	8	3	8	4	1	0	0	0	1	1.10 ± 0.20	0.51
1, S + X	50	300	6.00 ± 0.35	0	2	2	2	3	8	14	10	2	5	1	1			0.76 ± 0.20	-1.18
1, F + X	50	242	4.84 ± 0.31	0	0	5	6	16	7	4	7	3	2					0.74 ± 0.20	-1.31
2, S	50	239	4.78 ± 0.31	0	0	5	10	5	14	8	4	3	1					0.66 ± 0.20	-1.69
2, F	50	266	5.32 ± 0.33	0	0	1	9	9	11	8	5	4	1	1	0	1		0.78 ± 0.20	-1.1
2, X + S	50	240	4.80 ± 0.31	0	0	6	7	12	11	5	2	5	0	2				0.85 ± 0.20	-0.74
2, X + F	50	226	4.52 ± 0.30	0	2	6	7	10	15	4	3	0	0	2	1			0.97 ± 0.20	-0.16
2, S + X	50	247	4.94 ± 0.31	0	3	10	4	7	6	4	8	3	3	0	1	1		1.47 ± 0.20	2.35
2, F + X	50	239	4.78 ± 0.31	0	0	6	4	10	16	5	8	0	1					0.56 ± 0.20	-2.2
3, S	50	243	4.86 ± 0.31	0	2	3	10	9	8	4	9	3	2					0.87 ± 0.20	-0.67
3, F	50	255	5.10 ± 0.32	0	2	3	9	6	12	6	5	2	2	2	1			1.05 ± 0.20	0.25
3, X + S	50	240	4.80 ± 0.31	0	3	2	10	8	10	10	2	1	2	1	1			0.99 ± 0.20	-0.07
3, X + F	50	262	5.24 ± 0.32	0	1	2	8	8	8	10	6	4	3					0.74 ± 0.20	-1.31
3, S + X	50	293	5.86 ± 0.34	0	2	2	2	6	9	10	9	4	4	1	1			0.82 ± 0.20	-0.92
3, F + X	50	304	6.08 ± 0.35	0	3	1	6	9	4	6	2	6	8	2	1	2		1.38 ± 0.20	1.89
4, S	50	249	4.98 ± 0.32	0	3	3	9	8	4	9	8	3	3					0.96 ± 0.20	-1.84
4, F	50	219	4.48 ± 0.30	0	1	8	9	9	9	7	4	3						0.76 ± 0.20	-1.18
4, X + S	50	255	5.10 ± 0.32	0	3	3	6	9	10	6	4	3	6					1.01 ± 0.20	0.05
4, X + F	50	249	4.98 ± 0.32	0	2	3	7	7	14	5	7	3	1	1				0.80 ± 0.20	-0.99
4, S + X	50	239	4.78 ± 0.31	0	0	4	6	14	12	6	4	3	1					0.58 ± 0.20	-2.07
4, F + X	50	275	5.50 ± 0.33	0	1	5	6	5	9	10	4	2	5	1	2			1.11 ± 0.20	0.53

Table 4.15. Number of SCEs scored for the different exposure regimes in 4 donors using a 935 MHz GSM Talk intermittent signal at an SAR of 1 W/kg. The SCE yields and distribution amongst the cells are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of SCEs	SCEs per cell ± SE	Distribution of SCEs																σ^2 / y ± SE	U
				0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
1, S	50	271	5.42 ± 0.33	0	1	4	7	5	12	7	6	3	1	1	2	1				1.12 ± 0.20	0.57
1, F	50	260	5.20 ± 0.32	0	3	3	8	6	11	6	3	5	2	2	0	0	1			1.26 ± 0.20	1.27
1, X + S	50	302	6.04 ± 0.35	0	4	0	7	3	5	11	8	2	3	3	3	1				1.29 ± 0.20	1.44
1, X + F	50	259	5.18 ± 0.32	0	3	2	6	14	8	4	3	4	2	3	0	0	1			1.28 ± 0.20	1.40
1, S + X	50	290	5.80 ± 0.34	0	2	2	5	8	7	6	7	5	7	0	0	0	0	1		1.12 ± 0.20	0.59
1, F + X	50	282	5.64 ± 0.34	0	4	4	6	1	8	6	8	8	2	1	1	0	1			1.23 ± 0.20	1.71
2, S	50	237	4.60 ± 0.30	0	8	7	3	7	5	8	3	3	2	3	0	1				1.76 ± 0.20	3.79
2, F	50	260	5.20 ± 0.32	0	2	0	7	12	11	5	7	3	1	1	0	1				0.86 ± 0.20	-0.68
2, X + S	50	249	4.98 ± 0.32	0	5	5	2	8	11	6	7	3	1	1	0	0	1			1.26 ± 0.20	1.28
2, X + F	50	266	5.32 ± 0.33	0	0	3	9	9	8	7	2	9	1	2						0.88 ± 0.20	-0.61
2, S + X	50	249	4.98 ± 0.32	0	0	8	3	7	15	6	8	1	0	1	1					0.82 ± 0.20	-0.92
2, F + X	50	238	4.76 ± 0.31	0	0	3	12	13	8	7	1	2	2	1	1					0.89 ± 0.20	-0.56
3, S	50	240	4.80 ± 0.31	0	0	7	8	8	14	2	7	1	0	2	1					0.96 ± 0.20	-0.19
3, F	50	263	5.26 ± 0.32	0	0	6	7	10	7	7	6	2	2	1	0	1	0	0	1	1.33 ± 0.20	1.65
3, X + S	50	246	4.92 ± 0.31	0	3	5	6	11	7	6	3	5	2	1	1					1.18 ± 0.20	0.88
3, X + F	50	305	6.10 ± 0.35	0	1	0	5	8	5	8	11	5	4	2	1					0.77 ± 0.20	-1.14
3, S + X	50	284	5.68 ± 0.34	0	2	0	7	4	11	10	8	3	3	0	1	1				0.87 ± 0.20	-0.63
3, F + X	50	274	5.48 ± 0.33	0	2	3	4	10	8	11	5	2	0	2	0	2	1			1.25 ± 0.20	1.22
4, S	50	206	4.12 ± 0.29	0	0	9	11	12	6	9	2	0	1							0.65 ± 0.20	-1.74
4, F	50	228	4.56 ± 0.30	0	3	5	8	6	15	6	4	1	1	0	1					0.91 ± 0.20	-0.42
4, X + S	50	289	5.78 ± 0.34	0	3	3	4	6	8	7	5	7	4	0	2	0	1			1.26 ± 0.20	1.24
4, X + F	50	238	4.76 ± 0.31	0	3	5	8	11	7	4	5	4	1	0	2					1.21 ± 0.20	1.02
4, S + X	50	285	5.70 ± 0.34	0	0	3	5	12	7	7	6	5	2	1	0	0	0	1	1	1.25 ± 0.20	1.23
4, F + X	50	253	5.06 ± 0.32	0	3	9	7	5	4	5	6	5	3	1	2					1.54 ± 0.20	2.66

Table 4.16. The mean yields per cell ± SE of pooled data from the SCE assay for 3 RF exposure regimes. χ^2 and p values on 5 degrees of freedom obtained by the chi-squared test for homogeneity are also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Experimental condition	935 MHz CW 1 W/kg					935 MHz GSM Talk 1 W/kg					935 MHz GSM Talk intermittent 1 W/kg				
	Total number cells scored	Total number of SCEs	SCEs per cell ±SE	χ^2	p	Total number cells scored	Total number of SCEs	SCEs per cell ±SE	χ^2	p	Total number cells scored	Total number of SCEs	SCEs per cell ±SE	χ^2	p
S	200	956	4.78 ± 0.15	0.76	0.859	200	979	4.90 ± 0.16	0.26	0.967	200	954	4.77 ± 0.27	9.19	0.031
F	200	1002	5.01 ± 0.16	2.44	0.486	200	948	4.74 ± 0.28	9.83	0.020	200	1011	5.06 ± 0.16	3.26	0.354
X + S	200	1048	5.24 ± 0.16	7.43	0.059	200	975	4.88 ± 0.16	0.69	0.875	200	1086	5.43 ± 0.28	8.81	0.032
X + F	200	1051	5.26 ± 0.16	5.56	0.135	200	984	4.92 ± 0.16	2.71	0.439	200	1068	5.34 ± 0.28	8.80	0.032
S +X	200	1025	5.13 ± 0.16	1.08	0.782	200	1079	5.40 ± 0.31	10.82	0.013	200	1108	5.54 ± 0.17	3.85	0.278
F + X	200	1023	5.12 ± 0.16	1.46	0.692	200	1060	5.30 ± 0.31	10.66	0.014	200	1011	5.06 ± 0.16	3.26	0.205

Table 4.17. F and p values for the factors, field, x-rays, order and signal, obtained from general linear model ANOVA testing for six cytogenetic end points.

Factor	Dicentrics		Centric rings		Excess acentrics		Chromatid aberrations		Micronuclei		SCE	
	F	P	F	p	F	p	F	p	F	p	F	p
Field	1.58	0.213	1.17	0.284	0.12	0.729	0.14	0.706	0.02	0.901	0.01	0.926
X-rays	505.58	< 0.001	16.83	< 0.001	283.09	< 0.001	10.21	0.002	428.93	< 0.001	12.22	0.001
Order	3.95	0.051	3.94	0.051	1.05	0.308	0.22	0.644	3.31	0.073	0.85	0.359
Signal	5.30	0.007	0.57	0.567	3.44	0.038	2.27	0.112	12.17	< 0.001	1.63	0.203

Table 4.18. The nuclear division index derived from slides prepared for the cytokinesis-blocked MN assay fro the different exposure regimes in 4 donors using a 935 MHz CW signal at an SAR of 1 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Number of cells in 500 with 1, 2, 3 or 4 nuclei				NDI	± SE
	1	2	3	4		
1, S	195	237	40	28	1.80	0.13
1, F	211	244	28	17	1.70	0.10
1, X + S	204	266	14	16	1.68	0.12
1, X + F	189	255	28	28	1.79	0.12
1, S + X	254	204	26	16	1.61	0.12
1, F + X	230	217	37	16	1.68	0.14
2, S	202	224	46	28	1.80	0.14
2, F	186	242	55	17	1.81	0.13
2, X + S	182	252	43	23	1.81	0.12
2, X + F	206	216	51	27	1.80	0.13
2, S + X	213	221	50	16	1.74	0.12
2, F + X	204	231	41	24	1.77	0.12
3, S	193	262	25	20	1.74	0.13
3, F	224	250	14	12	1.63	0.14
3, X + S	373	117	6	4	1.28	0.08
3, X + F	290	186	13	11	1.49	0.11
3, S + X	304	168	13	15	1.48	0.12
3, F + X	262	207	16	15	1.57	0.12
4, S	247	226	14	13	1.59	0.12
4, F	193	243	33	31	1.80	0.11
4, X + S	237	238	12	13	1.60	0.14
4, X + F	233	236	17	14	1.62	0.11
4, S + X	225	230	28	17	1.67	0.12
4, F + X	237	213	31	19	1.66	0.13

Table 4.19. The nuclear division index derived from slides prepared for the cytokinesis-blocked MN assay fro the different exposure regimes in 4 donors using a 935 MHz GSM Talk signal at an SAR of 1 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Number of cells in 500 with 1, 2, 3 or 4 nuclei				NDI	± SE
	1	2	3	4		
1, S	164	230	44	62	2.01	0.13
1, F	136	249	54	61	2.08	0.10
1, X + S	169	239	48	44	1.93	0.12
1, X + F	189	226	43	42	1.88	0.12
1, S + X	200	212	47	41	1.86	0.12
1, F + X	158	239	47	56	2.00	0.14
2, S	186	209	69	36	1.91	0.14
2, F	155	238	66	41	1.99	0.13
2, X + S	179	217	69	35	1.92	0.12
2, X + F	197	223	52	28	1.82	0.13
2, S + X	204	202	57	37	1.85	0.12
2, F + X	186	231	55	28	1.85	0.12
3, S	217	242	25	16	1.68	0.13
3, F	191	260	29	20	1.76	0.14
3, X + S	182	267	35	16	1.77	0.08
3, X + F	213	246	32	9	1.67	0.11
3, S + X	202	249	37	12	1.72	0.12
3, F + X	201	262	21	16	1.70	0.12
4, S	316	153	24	7	1.44	0.12
4, F	321	146	27	6	1.44	0.11
4, X + S	357	123	16	4	1.33	0.14
4, X + F	371	113	13	3	1.30	0.11
4, S + X	382	97	19	2	1.28	0.12
4, F + X	369	107	21	3	1.32	0.13

Table 4.20. The nuclear division index derived from slides prepared for the cytokinesis-blocked MN assay fro the different exposure regimes in 4 donors using a 935 MHz GSM Talk intermittent signal at an SAR of 1 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Number of cells in 500 with 1, 2, 3 or 4 nuclei				NDI	± SE
	1	2	3	4		
1, S	263	200	22	15	1.58	0.13
1, F	267	214	13	6	1.52	0.10
1, X + S	277	199	13	11	1.52	0.12
1, X + F	273	204	11	12	1.52	0.12
1, S + X	254	206	21	19	1.61	0.12
1, F + X	278	187	21	14	1.54	0.14
2, S	185	251	44	20	1.80	0.14
2, F	206	237	36	21	1.74	0.13
2, X + S	230	227	26	17	1.66	0.12
2, X + F	201	244	38	17	1.74	0.13
2, S + X	208	220	50	22	1.77	0.12
2, F + X	225	230	31	14	1.67	0.12
3, S	199	254	39	8	1.71	0.13
3, F	188	275	26	11	1.72	0.14
3, X + S	238	246	12	4	1.56	0.08
3, X + F	216	244	30	10	1.67	0.11
3, S + X	204	257	25	14	1.70	0.12
3, F + X	215	230	38	17	1.71	0.12
4, S	255	208	26	11	1.59	0.12
4, F	265	209	16	10	1.54	0.11
4, X + S	280	191	20	9	1.52	0.14
4, X + F	261	212	22	5	1.54	0.11
4, S + X	266	205	20	9	1.54	0.12
4, F + X	260	197	30	13	1.59	0.13

Table 4.21. The nuclear division index derived from slides prepared for the SCE assay for the different exposure regimes in 4 donors using a 935 MHz CW signal at an SAR of 1 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Division status of 200 cells			NDI	± SE
	M1	M2	M3		
1, S	30	53	117	2.44	0.23
1, F	37	67	96	2.30	0.18
1, X + S	29	67	104	2.38	0.27
1, X + F	30	68	102	2.36	0.17
1, S + X	25	54	121	2.48	0.13
1, F + X	32	66	102	2.35	0.09
2, S	16	63	121	2.53	0.21
2, F	15	53	132	2.59	0.23
2, X + S	22	80	98	2.38	0.21
2, X + F	21	58	121	2.50	0.21
2, S + X	17	59	124	2.54	0.21
2, F + X	20	72	108	2.44	0.20
3, S	51	67	82	2.16	0.26
3, F	45	71	84	2.20	0.26
3, X + S	57	73	70	2.07	0.25
3, X + F	56	69	75	2.10	0.23
3, S + X	59	75	66	2.04	0.21
3, F + X	77	68	55	1.89	0.23
4, S	48	68	84	2.18	0.22
4, F	60	71	69	2.05	0.25
4, X + S	44	77	79	2.18	0.22
4, X + F	46	62	92	2.23	0.19
4, S + X	49	64	87	2.19	0.17
4, F + X	67	65	68	2.01	0.25

Table 4.22. The nuclear division index derived from slides prepared for the SCE assay for the different exposure regimes in 4 donors using a 935 MHz GSM Talk signal at an SAR of 1 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Division status of 200 cells			NDI	± SE
	M1	M2	M3		
1, S	18	42	140	2.61	0.23
1, F	15	38	147	2.66	0.18
1, X + S	17	43	140	2.62	0.27
1, X + F	20	44	136	2.58	0.17
1, S + X	14	43	143	2.65	0.13
1, F + X	22	46	132	2.55	0.09
2, S	12	40	148	2.68	0.21
2, F	16	40	144	2.64	0.23
2, X + S	9	44	147	2.69	0.21
2, X + F	7	33	160	2.77	0.21
2, S + X	10	42	148	2.69	0.21
2, F + X	11	30	159	2.74	0.20
3, S	17	43	140	2.62	0.26
3, F	24	55	121	2.49	0.26
3, X + S	22	48	130	2.54	0.25
3, X + F	28	50	122	2.47	0.23
3, S + X	26	59	115	2.45	0.21
3, F + X	20	44	136	2.58	0.23
4, S	31	47	122	2.46	0.22
4, F	27	49	124	2.49	0.25
4, X + S	27	55	118	2.46	0.22
4, X + F	21	41	138	2.59	0.19
4, S + X	24	49	127	2.52	0.17
4, F + X	26	54	120	2.47	0.25

Table 4.23. The nuclear division index derived from slides prepared for the SCE assay for the different exposure regimes in 4 donors using a 935 MHz GSM Talk intermittent signal at an SAR of 1 W/kg.
S = sham; X = 1 Gy x-ray; F = RF field

Donor and condition	Division status of 200 cells			NDI	± SE
	M1	M2	M3		
1, S	26	42	132	2.53	0.23
1, F	27	57	116	2.45	0.18
1, X + S	20	30	150	2.65	0.27
1, X + F	35	51	114	2.40	0.17
1, S + X	41	58	101	2.30	0.13
1, F + X	38	75	87	2.25	0.09
2, S	17	54	129	2.56	0.21
2, F	12	49	139	2.64	0.23
2, X + S	14	53	133	2.60	0.21
2, X + F	17	53	130	2.57	0.21
2, S + X	18	51	131	2.57	0.21
2, F + X	28	49	123	2.48	0.20
3, S	16	36	148	2.66	0.26
3, F	19	36	145	2.63	0.26
3, X + S	24	37	139	2.58	0.25
3, X + F	27	41	132	2.53	0.23
3, S + X	27	46	127	2.50	0.21
3, F + X	16	48	136	2.60	0.23
4, S	20	50	130	2.55	0.22
4, F	18	38	144	2.63	0.25
4, X + S	28	44	128	2.50	0.22
4, X + F	19	58	123	2.52	0.19
4, S + X	34	52	114	2.40	0.17
4, F + X	19	40	141	2.61	0.25

Table 4.24. F and p values for the factors, field, x-rays, order, signal and donor, obtained from the general linear model ANOVA tests on the NDI data.

Factor	NDI_{mn}		NDI_{sce}	
	F	p	F	p
Field	0.24	0.626	0.53	0.468
X-rays	2.55	0.115	3.02	0.087
Order	0.25	0.618	1.57	0.216
Signal	3.55	0.040	48.26	< 0.001
Donor	15.41	< 0.001	12.11	< 0.001

4.3 Conclusion

The study has extended earlier work that used a continuous GSM Basic signal to a signal more representative of holding a mobile phone conversation. It has used this signal both continuously and intermittently and also examined the underlying carrier frequency. Within the limitations of the study, namely exposure of G_0 lymphocytes over 24 h to 935 MHz RF signals at an SAR of 1 W/kg, no evidence was found of direct or co-genotoxicity of the RF fields when used alone or in combination with x-rays using standard cytogenetic assays for chromosome aberrations, micronuclei and sister chromatid exchanges.

5. Apoptosis studies with murine neuroblastoma cells exposed to 935 MHz RF signals

5.1 Introduction

Two key areas of public concern relate to the possible effects of mobile phone use on brain function and on the development of brain cancer. Despite conflicting findings, these concerns have been raised by epidemiological studies suggesting that long term use of a mobile phone associates with the occurrence of acoustic neuroma (Lonn et al. 2004, Mild et al. 2007 and Hours et al. 2007). However, it was also stated that an over-estimation of mobile phone use may have confounded the long term findings of these studies

As insufficient energy is delivered by RF fields to cause direct DNA damage (NRPB 2003), RF fields are unlikely to be initiators of tumours. However less certainty is associated with potential promoting effects. Such effects would likely be associated with proliferation of abnormal cells as a consequence of perturbations to the normal apoptotic process. Brain injury, for example as a result of cerebral ischemia, is associated with neuronal apoptosis (Linnik et al. 1995) and, additionally, any excessive loss of neurones due to apoptosis is likely to have an adverse effect on brain function. It is known that apoptosis is also involved in the process of carcinogenesis (Zörnig et al. 2001, Wechsler-Reya and Scott 2001).

Assays to detect the distinct events in the apoptotic pathway have been used to study the induction of apoptosis by RF fields. Most published work considers effects on haemopoietic cells, peripheral blood lymphocytes in particular (Lagroye et al. 2002, Capri et al. 2004a, 2004b and Belyaev et al. 2005) or lymphoblastoid cells (Hook et al. 2004) where no consistent association between exposure to low levels of RF and apoptosis have been reported. More recently, additional cell types have been studied where it was noted that the induction of various effects was dependent on the cell type studied (Caraglia et al. 2005 and Joubert et al. 2008). It is also likely that the signalling and induction

of apoptosis differs between proliferating undifferentiated and quiescent differentiated cells.

A cell line derived from brain tissue, neuroblastoma, has been used. Because of the variability in apoptotic response being dependent on cell type, this is perhaps more relevant than using blood cells (Augusti-Tocco and Sato 1969), has been used. Given the involvement of apoptosis in brain disease and the sensitivity of the process to cellular insult, the aim of the study was to investigate whether RF fields, characteristic of mobile phones, can induce apoptosis in murine neuroblastoma cells in both proliferating and differentiated states. As described in the materials and methods section 2.6, (Chapter 2), the RF exposed cells, together with shams and 4 Gy x-ray positive controls were assayed over a time course of 0 to 48 h post exposure. Three 935 MHz RF signals were used; GSM Basic, GSM Talk and a CW signal to expose cells continuously for 24 h. Three different assays to detect apoptosis were used and each experiment was repeated 3 times.

5.2 Results

An average temperature rise of 0.06^{o}C with a range of 0.03 to 0.10^{o}C occurred, which is based on data collected continuously during each exposure. Again this is low enough to prevent thermal induction of apoptosis so that any positive effect seen to result from the RF would be due to non-thermal processes.

5.2.1 Annexin V binding assay

The individual results for the Annexin V binding assay for apoptosis, using proliferating and differentiated N2a cells, are presented in Tables 5.1 and 5.2 respectively. The results of the chi-squared test for homogeneity between the samples for a given time point from each exposure regime are also shown. For each exposure condition, the data were combined by pooling the replicate results at each time point. Any significant inhomogeneity ($p < 0.05$, printed in red in Tables 5.1 and 5.2) shown by the chi-squared test was taken into account

by an appropriate increase in the SE of the combined data. Table 5.3 presents the combined data from each of the three experiments using the four exposure conditions for both proliferating and differentiated cells. Also shown are the results of the Student's t-tests, used to determine the significance of any difference between the exposed and sham control cells. The data are plotted in Figures 5.1 and 5.2, for proliferating and differentiated N2a cells respectively.

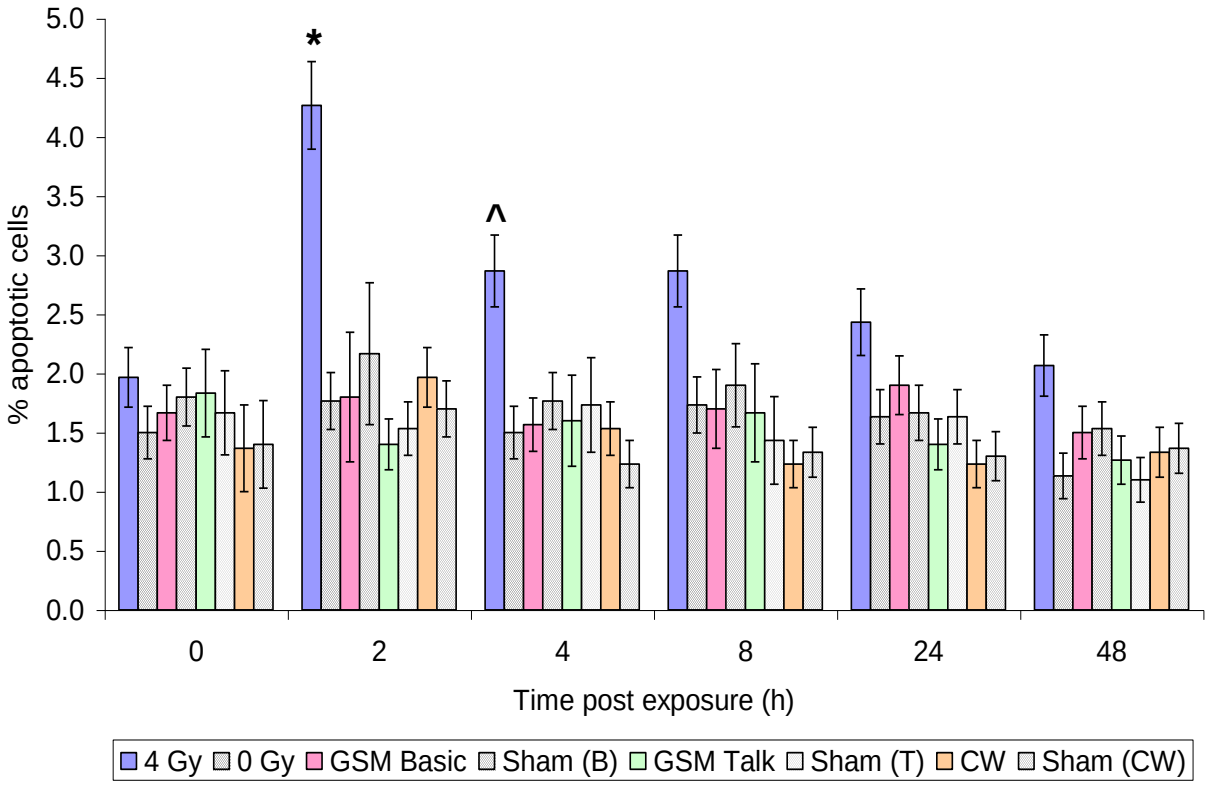

Figure 5.1. The combined percentage ± SE of apoptotic cells in proliferating N2a cells measured using the Annexin V binding assay after a 24 hour exposure to 935 MHz GSM Basic, GSM Talk or CW signals (2 W/kg) or sham exposed, together with positive controls exposed to 4 Gy x-rays and unirradiated controls.
Sham (B) = Sham control for GSM Basic; Sham (T) = Sham control for GSM Talk; Sham (CW) = Sham control for CW.
***** or **^** shown above an exposed data point indicates $p<0.05$ or $p=0.05$ respectively for the pair of exposed/sham data points.

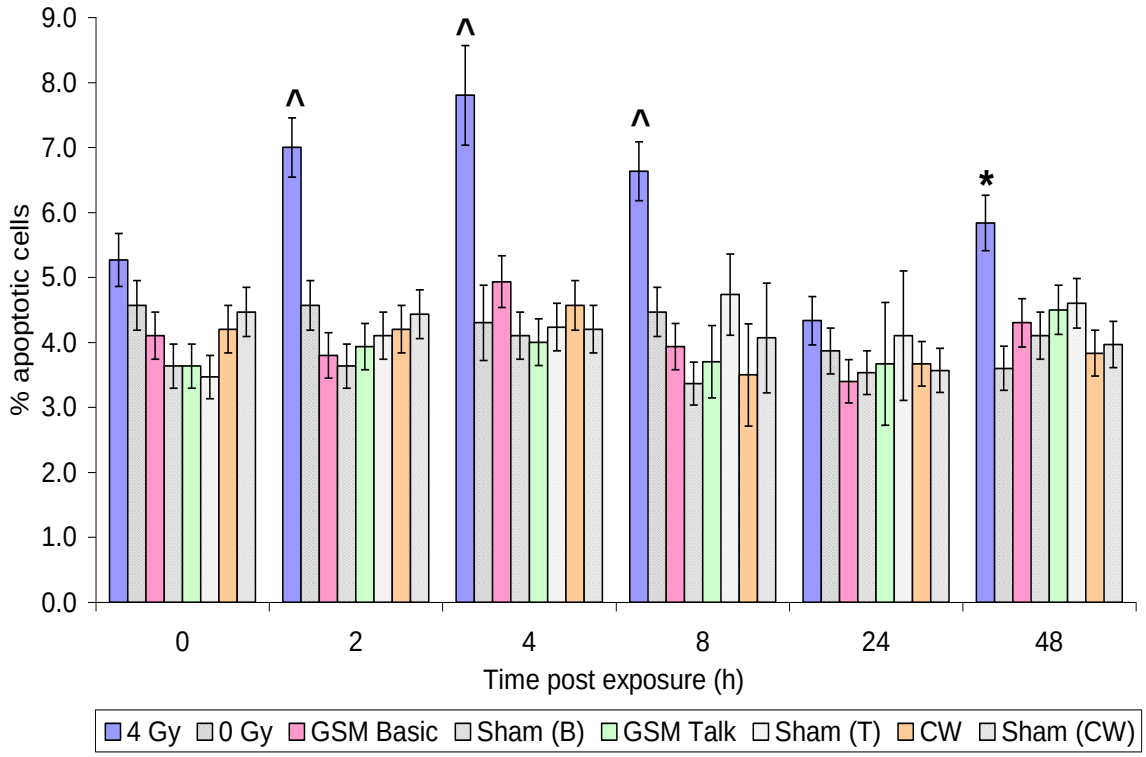

Figure 5.2. The combined percentage ± SE of apoptotic cells in differentiated N2a cells measured using the Annexin V binding assay after a 24 hour exposure to 935 MHz GSM Basic, GSM Talk or CW signals (2 W/kg) or sham exposed, together with positive controls exposed to 4 Gy x-rays and unirradiated controls.
Sham (B) = Sham control for GSM Basic; Sham (T) = Sham control for GSM Talk; Sham (CW) = Sham control for CW.
***** or **^** shown above an exposed data point indicates $p<0.05$ or $p=0.05$ respectively for the pair of exposed/sham data points.

With proliferating cells (Figure 5.1) the positive controls showed an x-ray induced increase in apoptotic cells which was significantly different from the unirradiated control at 2 h post exposure ($p = 0.032$). The level of apoptosis remained higher than that in the zero dose controls between 4 and 48 h post exposure, but as column 8 in Table 5.3 shows, the p values from the Student's t-test were just above the 5% significance level, indicating that with the exception of the values shown in red the increases were not significant at the 5% level. The results with x-irradiated differentiated cells were similar where the level of apoptosis increased between 2 and 8 h post exposure compared to the unirradiated controls although this was not quite significant, as Figure 5.2

shows. The level of apoptosis then returned to control levels at 24 h, but rose again at 48 h post exposure and this rise was just significant ($p = 0.045$). However, both proliferating and differentiated cells exposed to RF fields in all three modes showed no obvious peak in apoptosis levels and a no time point was there a significant difference between an exposed and an individually matched sham exposed control.

Table 5.1. Measurement of apoptosis levels using the Annexin V binding assay in proliferating N2a cells given a 24 h exposure to 935 MHz GSM Basic, GSM Talk, or CW signals (2 W/kg) and sham exposures together with positive controls exposed to 4 Gy x-rays and unirradiated controls. Each data point shows the total ± SE of apoptotic cells from assaying 1000 cells in each of three experiments. The values obtained by the chi-squared test for homogeneity (χ^2), together with p values on 5 degrees of freedom, are also shown.

	Time	1				2				3				χ^2	p
	(hours)	0 Gy	± SE	4 Gy	± SE	0 Gy	± SE	4 Gy	± SE	0 Gy	± SE	4 Gy	± SE		
X-rays	0	18	4.20	24	4.84	16	3.97	21	4.53	11	3.30	14	3.72	4.41	0.492
	2	14	3.72	44	6.49	20	4.43	41	6.27	19	4.32	43	6.41	1.28	0.937
	4	19	4.32	31	5.48	16	3.97	33	5.65	10	3.15	22	4.64	5.20	0.393
	8	18	4.20	33	5.65	16	3.97	27	5.13	18	4.20	26	5.03	1.15	0.949
	24	19	4.32	31	5.48	17	4.09	24	4.84	13	3.58	18	4.20	4.62	0.464
	48	12	3.44	21	4.53	12	3.44	25	4.94	10	3.15	16	3.97	2.20	0.820
	Time	1				2				3					
	(hours)	sham	± SE	RF	± SE	sham	± SE	RF	± SE	sham	± SE	RF	± SE		
GSM Basic	0	21	4.53	19	4.32	17	4.09	17	4.09	16	3.97	14	3.72	1.54	0.909
	2	37	5.97	28	5.22	17	4.09	13	3.58	11	3.30	13	3.58	25.44	< 0.001
	4	22	4.64	15	3.84	18	4.20	15	3.84	13	3.58	17	4.09	2.47	0.781
	6	27	5.13	22	4.64	10	3.15	13	3.58	20	4.43	16	3.97	10.15	0.071
	24	19	4.32	23	4.74	11	3.30	13	3.58	20	4.43	21	4.53	5.87	0.319
	48	20	4.43	18	4.20	13	3.58	15	3.84	13	3.58	12	3.44	3.33	0.649
GSM Talk	0	21	4.53	27	5.13	9	2.99	13	3.58	20	4.43	15	3.84	11.57	0.041
	2	18	4.20	11	3.30	12	3.44	11	3.30	16	3.97	20	4.43	5.07	0.407
	4	24	4.84	26	5.03	12	3.44	9	2.99	16	3.97	13	3.58	14.18	0.014
	8	21	4.53	27	5.13	8	2.82	13	3.58	14	3.72	10	3.15	15.79	0.007
	24	18	4.20	15	3.84	10	3.15	14	3.72	21	4.53	13	3.58	4.10	0.535
	48	11	3.30	11	3.30	11	3.30	10	3.15	11	3.30	17	4.09	2.26	0.812
CW	0	21	4.53	23	4.74	10	3.15	8	2.82	11	3.30	10	3.15	14.99	0.010
	2	19	4.32	20	4.43	12	3.44	17	4.09	20	4.43	22	4.64	2.88	0.719
	4	11	3.30	16	3.97	11	3.30	12	3.44	15	3.84	18	4.20	2.08	0.838
	8	15	3.84	13	3.58	11	3.30	10	3.15	14	3.72	14	3.72	1.35	0.929
	24	8	2.82	10	3.15	20	4.43	17	4.09	11	3.30	10	3.15	8.65	0.124
	48	14	3.72	11	3.30	15	3.84	12	3.44	12	3.44	17	4.09	1.89	0.864

Table 5.2. Measurement of apoptosis levels using the Annexin V binding assay in differentiated N2a cells given a 24 h exposure to 935 MHz GSM Basic, GSM Talk, or CW signals (2 W/kg) and sham exposures together with positive controls exposed to 4 Gy x-rays and unirradiated controls. Each data point shows the total ± SE of apoptotic cells from assaying 1000 cells in each of three experiments. The values obtained by the chi-squared test for homogeneity (χ^2), together with p values on 5 degrees of freedom, are also shown.

	Time	1				2				3				χ^2	p
	(hours)	0 Gy	± SE	4 Gy	± SE	0 Gy	± SE	4 Gy	± SE	0 Gy	± SE	4 Gy	± SE		
X-rays	0	40	6.20	51	6.96	42	6.34	47	6.69	55	7.21	60	7.51	4.59	0.468
	2	34	5.73	61	7.57	45	6.56	63	7.68	58	7.39	77	8.43	8.59	0.127
	4	31	5.48	62	7.63	42	6.34	84	8.77	56	7.27	88	8.96	12.33	0.031
	8	36	5.89	51	6.96	53	7.08	77	8.43	45	6.56	71	8.12	8.83	0.116
	24	30	5.39	32	5.57	46	6.62	51	6.96	40	6.20	47	6.69	8.01	0.156
	48	26	5.03	47	6.69	43	6.41	69	8.01	39	6.12	59	7.45	8.55	0.128
	Time	1				2				3					
	(hours)	sham	± SE	RF	± SE	sham	± SE	RF	± SE	sham	± SE	RF	± SE		
GSM Basic	0	28	5.22	31	5.48	43	6.41	44	6.49	38	6.05	48	6.76	7.06	0.216
	2	35	5.81	27	5.13	30	5.39	45	6.56	44	6.49	42	6.34	7.67	0.176
	4	44	6.49	50	6.89	40	6.20	43	6.41	39	6.12	55	7.21	1.81	0.874
	6	35	5.81	25	4.94	31	5.48	45	6.56	35	5.81	48	6.76	8.27	0.142
	24	28	5.22	32	5.57	35	5.81	35	5.81	43	6.41	35	5.81	3.37	0.642
	48	37	5.97	32	5.57	45	6.56	55	7.21	41	6.27	42	6.34	6.97	0.223
GSM Talk	0	26	5.03	31	5.48	41	6.27	41	6.27	37	5.97	37	5.97	4.88	0.431
	2	29	5.31	31	5.48	50	6.89	47	6.69	44	6.49	40	6.20	8.98	0.110
	4	42	6.34	37	5.97	42	6.34	39	6.12	43	6.41	44	6.49	0.67	0.985
	8	31	5.48	30	5.39	53	7.08	34	5.73	58	7.39	47	6.69	12.99	0.023
	24	27	5.13	26	5.03	65	7.80	57	7.33	31	5.48	27	5.13	38.20	< 0.001
	48	40	6.20	44	6.49	59	7.45	52	7.02	39	6.12	39	6.12	7.43	0.190
CW	0	31	5.48	41	6.27	44	6.49	38	6.05	59	7.45	47	6.69	9.79	0.081
	2	34	5.73	38	6.05	49	6.83	33	5.65	50	6.89	55	7.21	9.96	0.076
	4	41	6.27	51	6.96	34	5.73	45	6.56	51	6.96	41	6.27	4.59	0.469
	8	26	5.03	16	3.97	42	6.34	38	6.05	54	7.15	51	6.96	27.59	< 0.001
	24	31	5.48	29	5.31	33	5.65	35	5.81	43	6.41	46	6.62	6.37	0.272
	48	33	5.65	26	5.03	40	6.20	45	6.56	46	6.62	44	6.49	8.10	0.151

Table 5.3. Measurement of apoptosis levels using the Annexin V binding assay in proliferating and differentiated N2a cells given a 24 h exposure to 935 MHz GSM Basic, GSM Talk or a CW signal (2W/kg) and sham exposures, together with positive controls exposed to 4 Gy x-rays and unirradiated controls. Each data point shows the combined total ± SE of apoptotic cells from assaying 1000 cells in each of three experiments. The t-test statistic (t) and p values for each pair of exposed/sham data points are also shown.

		Proliferating N2a cells						Differentiated N2a cells					
	Time (hours)	0 Gy	± SE	4 Gy	± SE	t	p	0 Gy	± SE	4 Gy	± SE	t	p
X-rays	0	45	6.66	59	7.60	2.40	0.126	137	11.43	158	12.23	2.17	0.137
	2	53	7.22	128	11.07	9.83	0.032	137	11.42	210	13.69	6.22	0.051
	4	45	6.66	86	9.14	6.28	0.050	129	17.41	234	23.05	6.3	0.050
	8	52	7.15	86	9.14	5.08	0.062	134	11.31	199	13.62	6.36	0.050
	24	49	6.94	73	8.43	3.81	0.082	116	10.55	130	11.15	1.58	0.180
	48	34	5.80	62	7.79	4.99	0.063	108	10.19	175	12.82	7.09	0.045
	Time (hours)	sham	± SE	RF	± SE	t	p	sham	± SE	RF	± SE	t	p
GSM Basic	0	54	7.28	50	7.02	0.69	0.309	109	10.24	123	10.86	1.62	0.176
	2	65	17.97	54	16.43	0.78	0.289	109	10.24	114	10.47	0.59	0.330
	4	53	7.21	47	6.80	1.05	0.242	123	10.86	148	11.86	2.69	0.113
	6	57	10.61	51	10.05	0.71	0.303	101	9.88	118	10.64	2.03	0.146
	24	50	7.01	57	7.47	1.18	0.223	106	10.10	102	9.93	0.49	0.355
	48	46	6.73	45	6.65	0.18	0.442	123	10.86	129	11.10	0.67	0.312
GSM Talk	0	50	10.66	55	11.16	0.56	0.337	104	10.01	109	10.25	0.6	0.327
	2	46	6.73	42	6.43	0.74	0.296	123	10.85	118	10.64	0.57	0.335
	4	52	12.00	48	11.52	0.42	0.374	127	11.02	120	10.73	0.79	0.288
	8	43	11.12	50	12.39	0.72	0.302	142	18.69	111	16.63	2.15	0.139
	24	49	6.93	42	6.43	1.28	0.211	123	29.89	110	28.32	0.55	0.341
	48	33	5.72	38	6.13	1.03	0.245	138	11.46	135	11.35	0.32	0.401
CW	0	42	11.12	41	10.99	0.11	0.465	134	11.30	126	10.99	0.88	0.270
	2	51	7.08	59	7.61	1.33	0.205	133	11.27	126	10.98	0.77	0.291
	4	37	6.04	46	6.73	1.72	0.167	126	10.98	137	11.44	1.2	0.221
	8	40	6.28	37	6.05	0.60	0.329	122	25.38	105	23.59	0.85	0.276
	24	39	6.20	37	6.05	0.40	0.379	107	10.15	110	10.28	0.36	0.390
	48	41	6.36	40	6.28	0.19	0.439	119	10.69	115	10.51	0.46	0.362

5.2.2 Caspase activation assay

For proliferating N2a cells the caspase activation assay results are shown in Table 5.4 for individual dishes, together with the results of the chi-squared test for homogeneity. The combined data from three experiments are presented in Figure 5.3 and Table 5.6, which also includes the results of the Student's t-tests. The caspase activation assay data using differentiated N2a cells are shown in Table 5.5 (individual dishes), Table 5.6 and Figure 5.4 (combined data). Again the SE on the combined data were appropriately increased where there was evidence of inhomogeneity.

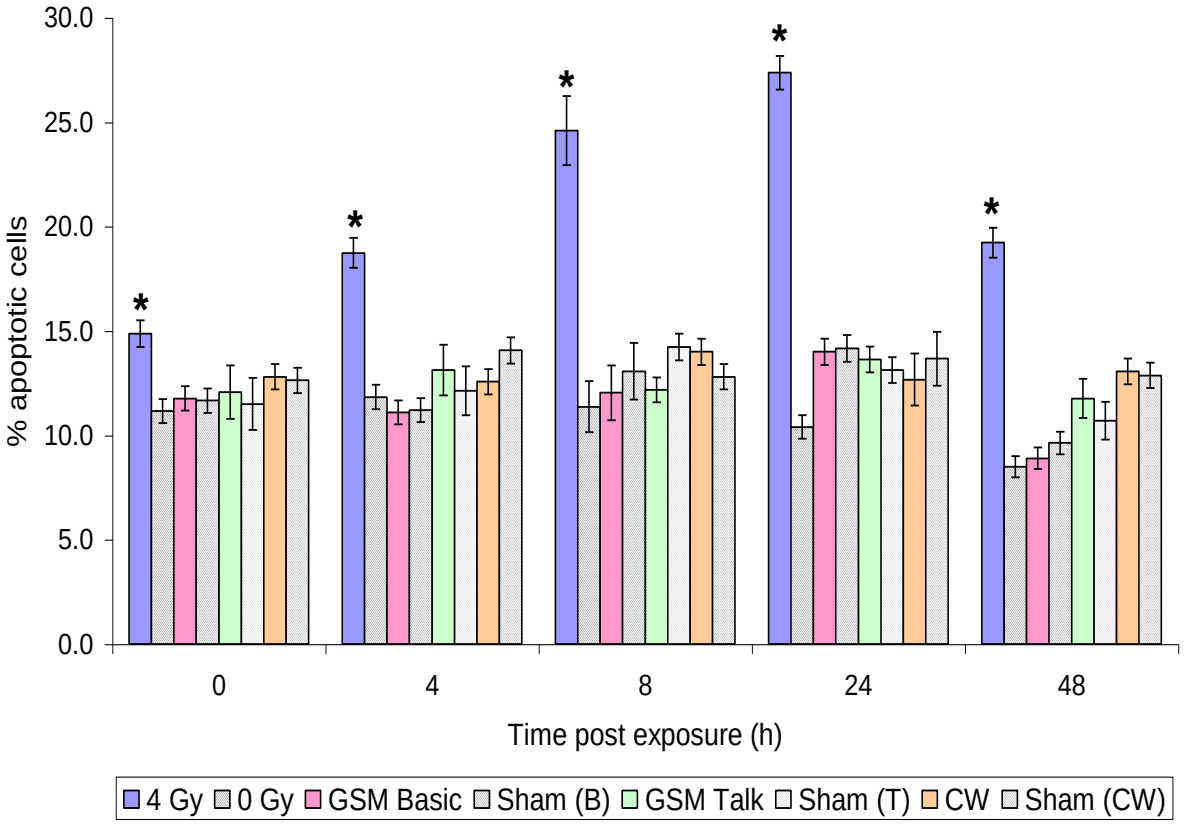

Figure 5.3. The combined percentage ± SE of apoptotic cells in proliferating N2a cells measured using the caspase activation assay after a 24 hour exposure to 935 MHz GSM Basic, GSM Talk or CW signals (2 W/kg) or sham exposed, together with positive controls exposed to 4 Gy x-rays and unirradiated controls.
Sham (B) = Sham control for GSM Basic; Sham (T) = Sham control for GSM Talk; Sham (CW) = Sham control for CW.
***** shown above an exposed data point indicates $p<0.05$ for the pair of exposed/sham data points.

As shown in Figure 5.3, there was a steady and significant rise in apoptosis levels from 0 h post exposure up to 24 h, in the x-irradiated positive controls using proliferating cells. At 48 h the level of apoptosis was lower than at 24 h but was still significantly above that seen with the unirradiated samples. Using differentiated cells a significant difference in the level of x-ray induced apoptosis occurred at 8 h post exposure, ($p = 0.038$), as shown in Figure 5.4, compared to the unirradiated control samples. The level remained elevated at 48 h post exposure, but the difference between the samples given 4 Gy of x-rays and the zero dose controls was not significant.

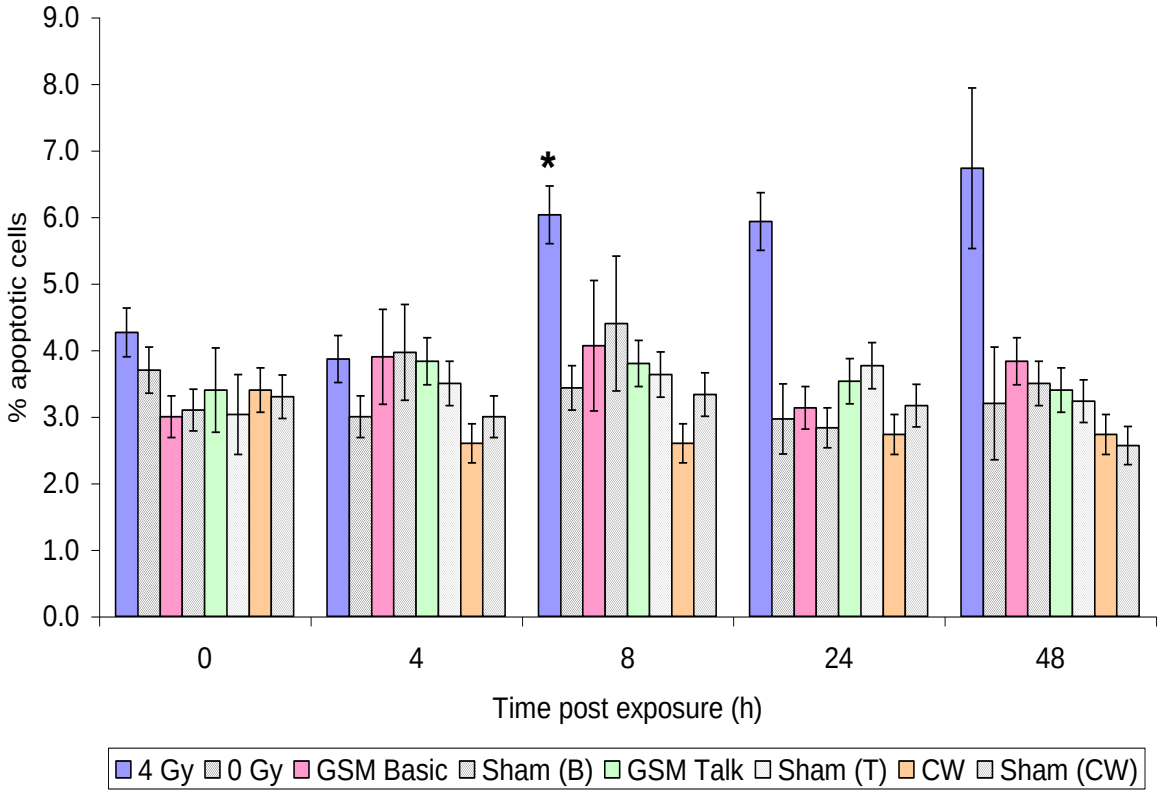

Figure 5.4. The combined percentage ± SE of apoptotic cells in differentiated N2a cells measured using the caspase activation assay after a 24 hour exposure to 935 MHz GSM Basic, GSM Talk or CW signals (2 W/kg) or sham exposed, together with positive controls exposed to 4 Gy x-rays and unirradiated controls.
Sham (B) = Sham control for GSM Basic; Sham (T) = Sham control for GSM Talk; Sham (CW) = Sham control for CW.
***** shown above an exposed data point indicates $p<0.05$ for the pair of exposed/sham data points.

For all RF signals and at all time points, both proliferating and differentiated cells showed no significant difference in apoptosis levels from its matched sham exposed control. Although it was not quite significant at the 5% level, the Student's t-test revealed one point that had a p value of 0.076, for the sham and RF GSM Talk exposed proliferating cells at 8 h (Table 5.6, column 8). Here the exposed cells showed a decrease in the level of apoptosis compared to the sham, which was consistent in all three replicates. This result seemed to be due to high sham values compared to other time points, rather than a low exposed value. Therefore the point was repeated (Table 5.4) and no significant difference was found in the level of apoptosis between the sham and the exposed cells. The combined data from the 3 repeat samples were 313 ± 43.57 and 341 ± 45.18 for the sham and GSM Talk exposures respectively, with a p value of 0.231. This, together with the lack of significant changes in apoptosis for this exposure signal and time point in the other assays, tends to suggest that it was a chance finding.

Table 5.4. Measurement of apoptosis levels using the caspase activation assay in proliferating N2a cells given a 24 h exposure to 935 MHz GSM Basic, GSM Talk, or CW signals (2 W/kg) and sham exposures together with positive controls exposed to 4 Gy x-rays and unirradiated controls. Each data point shows the total ± SE of apoptotic cells from assaying 1000 cells in each of three experiments. The values obtained by the chi-squared test for homogeneity (χ^2), together with p values on 5 degrees of freedom, are also shown.

	Time	1				2				3				χ^2	p
	(hours)	0 Gy	± SE	4 Gy	± SE	0 Gy	± SE	4 Gy	± SE	0 Gy	± SE	4 Gy	± SE		
X-rays	0	128	10.56	167	11.79	115	10.09	150	11.29	92	9.14	129	10.60	10.83	0.055
	4	121	10.31	206	12.79	118	10.20	193	12.48	116	10.13	163	11.68	5.30	0.380
	8	132	10.70	287	14.30	121	10.31	244	13.58	88	8.96	207	12.81	22.26	< 0.001
	24	90	9.05	239	13.49	104	9.65	281	14.21	118	10.20	301	14.51	11.09	0.050
	48	75	8.33	217	13.03	96	9.32	173	11.96	84	8.77	187	12.33	7.87	0.164
	Time	1				2				3					
	(hours)	sham	± SE	RF	± SE	sham	± SE	RF	± SE	sham	± SE	RF	± SE		
GSM Basic	0	101	9.53	126	10.49	136	10.84	99	9.44	113	10.01	128	10.56	9.88	0.079
	4	115	10.09	132	10.70	115	10.09	112	9.97	106	9.73	89	9.00	8.82	0.116
	6	94	9.23	96	9.32	159	11.56	130	10.63	139	10.94	135	10.81	24.45	< 0.001
	24	138	10.91	141	11.01	147	11.20	128	10.56	140	10.97	151	11.32	2.22	0.819
	48	96	9.32	88	8.96	101	9.53	83	8.72	92	9.14	96	9.32	1.39	0.926
GSM Talk	0	121	10.31	154	11.41	101	9.53	124	10.42	123	10.39	84	8.77	23.02	< 0.001
	4	136	10.84	158	11.53	98	9.40	135	10.81	130	10.63	101	9.53	19.40	0.002
	8	140	10.97	124	10.42	144	11.10	129	10.60	143	11.07	112	9.97	1.32	0.933
	8 repeat	120	10.28	98	9.40	93	9.18	103	9.61	100	9.49	140	10.97	13.02	0.023
	24	127	10.53	157	11.50	130	10.63	144	11.10	137	10.87	108	9.82	9.85	0.079
	48	106	9.73	86	8.87	105	9.69	131	10.67	110	9.89	136	10.84	13.02	0.023
CW	0	146	11.17	122	10.35	121	10.31	120	10.28	112	9.97	142	11.04	7.23	0.204
	4	137	10.87	116	10.13	125	10.46	130	10.63	160	11.59	131	10.67	5.62	0.345
	8	117	10.16	139	10.94	149	11.26	131	10.67	118	10.20	150	11.29	6.47	0.263
	24	156	11.47	127	10.53	161	11.62	124	10.42	93	9.18	129	10.60	21.12	< 0.001
	48	133	10.74	114	10.05	128	10.56	148	11.23	125	10.46	130	10.63	4.68	0.456

Table 5.5. Measurement of apoptosis levels using the caspase activation assay in differentiated N2a cells given a 24 h exposure to 935 MHz GSM Basic, GSM Talk, or CW signals (2 W/kg) and sham exposures together with positive controls exposed to 4 Gy x-rays and unirradiated controls. Each data point shows the total ± SE of apoptotic cells from assaying 1000 cells in each of three experiments. The values obtained by the chi-squared test for homogeneity (χ^2), together with p values on 5 degrees of freedom, are also shown.

	Time	1				2				3				χ^2	p
	(hours)	0 Gy	± SE	4 Gy	± SE	0 Gy	± SE	4 Gy	± SE	0 Gy	± SE	4 Gy	± SE		
X-rays	0	32	5.57	36	5.89	42	6.34	51	6.96	37	5.97	41	6.27	4.09	0.537
	4	25	4.94	35	5.81	29	5.31	34	5.73	36	5.89	47	6.69	4.77	0.444
	8	37	5.97	72	8.17	28	5.22	59	7.45	38	6.05	50	6.89	5.82	0.324
	24	41	6.27	77	8.43	23	4.74	52	7.02	25	4.94	49	6.83	14.53	0.013
	48	48	6.76	99	9.44	26	5.03	56	7.27	22	4.64	47	6.69	35.19	< 0.001
	Time	1				2				3					
	(hours)	sham	± SE	RF	± SE	sham	± SE	RF	± SE	sham	± SE	RF	± SE		
GSM Basic	0	42	6.34	29	5.31	24	4.84	29	5.31	27	5.13	32	5.57	6.20	0.287
	4	53	7.08	57	7.33	37	5.97	33	5.65	29	5.31	27	5.13	20.45	0.001
	6	67	7.91	57	7.33	38	6.05	45	6.56	27	5.13	20	4.43	36.93	< 0.001
	24	35	5.81	31	5.48	25	4.94	36	5.89	25	4.94	27	5.13	3.65	0.601
	48	38	6.05	28	5.22	31	5.48	38	6.05	36	5.89	49	6.83	6.50	0.261
GSM Talk	0	36	5.89	33	5.65	18	4.20	21	4.53	37	5.97	48	6.76	18.30	0.003
	4	39	6.12	46	6.62	43	6.41	32	5.57	23	4.74	37	5.97	9.03	0.108
	8	43	6.41	42	6.34	31	5.48	38	6.05	35	5.81	34	5.73	2.90	0.716
	24	33	5.65	30	5.39	38	6.05	38	6.05	42	6.34	38	6.05	2.29	0.808
	48	27	5.13	35	5.81	40	6.20	35	5.81	30	5.39	32	5.57	3.04	0.693
CW	0	34	5.73	35	5.81	30	5.39	23	4.74	35	5.81	44	6.49	6.95	0.224
	4	35	5.81	21	4.53	27	5.13	34	5.73	28	5.22	23	4.74	5.04	0.412
	8	27	5.13	20	4.43	42	6.34	37	5.97	31	5.48	21	4.53	10.62	0.059
	24	29	5.31	18	4.20	38	6.05	29	5.31	28	5.22	35	5.81	7.35	0.196
	48	23	4.74	27	5.13	27	5.13	35	5.81	27	5.13	20	4.43	4.54	0.475

Table 5.6. Measurement of apoptosis levels using the caspase activation assay in proliferating and differentiated N2a cells given a 24 h exposure to 935 MHz GSM Basic, GSM Talk or a CW signal (2W/kg) and sham exposures, together with positive controls exposed to 4 Gy x-rays and unirradiated controls. Each data point shows the combined total ± SE of apoptotic cells from assaying 1000 cells in each of three experiments. The t-test statistic (t) and p values for each pair of exposed/sham data points are also shown.

		Proliferating N2a cells						Differentiated N2a cells					
	Time (hours)	0 Gy	± SE	4 Gy	± SE	t	p	0 Gy	± SE	4 Gy	± SE	t	p
X-rays	0	335	17.25	446	19.46	7.40	0.043	111	10.34	128	11.07	1.94	0.151
	4	355	17.69	562	21.35	12.93	0.025	90	9.34	116	10.55	3.20	0.097
	8	341	36.61	738	49.63	11.15	0.028	103	9.97	181	13.03	8.23	0.038
	24	312	16.71	821	24.38	29.83	0.011	89	15.78	178	12.96	5.70	0.055
	48	255	15.27	577	21.56	21.11	0.015	96	25.49	202	36.2	4.15	0.075
	Time (hours)	sham	± SE	RF	± SE	t	p	sham	± SE	RF	± SE	t	p
GSM Basic	0	350	17.56	353	17.63	0.21	0.434	93	9.48	90	9.35	0.39	0.382
	4	336	17.27	333	17.17	0.21	0.433	119	21.57	117	21.37	0.11	0.464
	6	392	40.66	361	39.34	0.95	0.258	132	30.46	122	29.35	0.41	0.376
	24	425	19.10	420	19.00	0.32	0.401	85	9.09	94	9.54	1.18	0.223
	48	289	16.16	267	15.59	1.70	0.170	105	10.07	115	10.51	1.19	0.222
GSM Talk	0	345	37.56	362	38.21	0.55	0.340	91	17.92	102	18.93	0.73	0.299
	4	364	35.18	394	36.37	1.03	0.246	105	10.05	115	10.51	1.19	0.222
	8	427	19.13	365	17.90	4.10	0.076	109	10.24	114	10.47	0.59	0.330
	24	394	18.49	409	18.76	0.99	0.252	113	10.43	106	10.11	0.83	0.279
	48	321	27.24	353	28.35	1.41	0.196	97	9.69	102	9.93	0.62	0.322
CW	0	379	18.18	384	18.29	0.34	0.397	99	9.78	102	9.92	0.37	0.386
	4	422	19.02	377	18.15	2.96	0.104	90	9.34	78	8.71	1.63	0.175
	8	384	18.28	420	19.00	2.37	0.127	100	9.83	78	8.71	2.90	0.106
	24	410	38.58	380	37.53	0.97	0.256	95	9.59	82	8.92	1.72	0.168
	48	386	18.34	392	18.44	0.40	0.379	77	8.67	82	8.93	0.70	0.306

5.2.3 *In situ* end labelling (Apo Direct) assay

Presented in Tables 5.7 and 5.8 are the individual results of the *in situ* end labelling (Apo Direct) assay using proliferating and differentiated N2a cells respectively. The results of the chi-squared test for homogeneity are also shown. Both proliferating and differentiated cells showed less variation in the numbers of apoptotic cells within the three repeat experiments for every RF or x-ray exposure compared to the Annexin V binding and caspase activation assays. These two assays used cells from one Petri dish per time point per experiment, whereas the *in situ* end labelling assay required several dishes to be combined. This may have resulted in less individual variation in the data points or the *in situ* end labelling assay is more reliable at detecting apoptosis under the experimental conditions used in this study. However, as expected when cells become more asynchronous in their response, the variation in the numbers of apoptotic cells between the three repeat experiments for each exposure regime did increase with time.

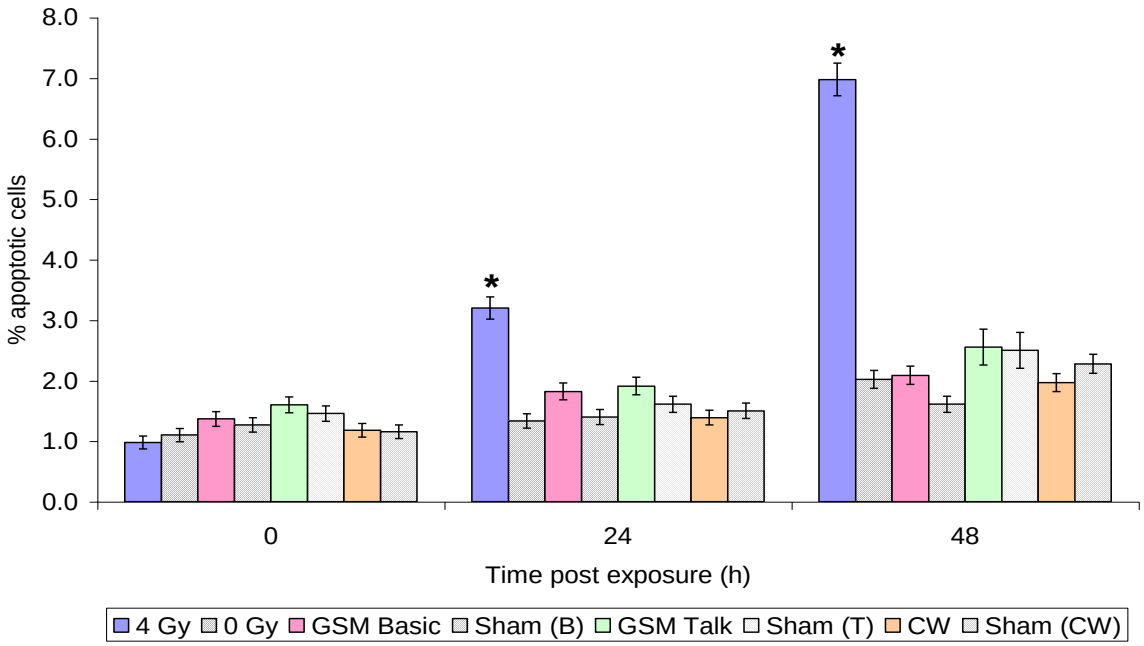

Figure 5.5. The combined percentage ± SE of apoptotic cells in proliferating N2a cells measured using the Apo direct *in situ* end labelling assay after a 24 hour exposure to 935 MHz GSM Basic, GSM Talk or CW signals (2 W/kg) or sham exposed, together with positive controls exposed to 4 Gy x-rays and unirradiated controls.
Sham (B) = Sham control for GSM Basic; Sham (T) = Sham control for GSM Talk; Sham (CW) = Sham control for CW.
***** shown above an exposed data point indicates $p<0.05$ for the pair of exposed/sham data points.

Table 5.9 shows the combined data from three experiments for both proliferating and differentiated cells, along with the results of the Student's t-test. The combined percentage of apoptotic cells using proliferating and differentiated for the 4 exposure regimes are also shown in Figures 5.5 and 5.6 respectively.

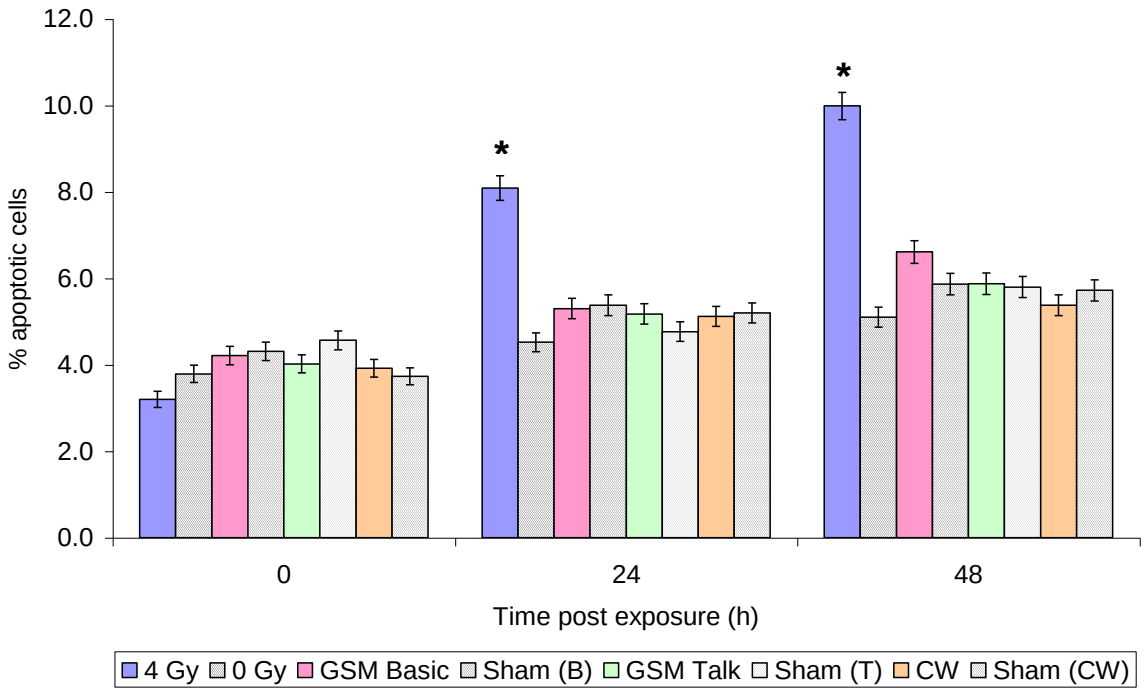

Figure 5.6. The combined percentage ± SE of apoptotic cells in differentiated N2a cells measured using the Apo direct *in situ* end labelling assay after a 24 hour exposure to 935 MHz GSM Basic, GSM Talk or CW signals (2 W/kg) or sham exposed, together with positive controls exposed to 4 Gy x-rays and unirradiated controls.
Sham (B) = Sham control for GSM Basic; Sham (T) = Sham control for GSM Talk; Sham (CW) = Sham control for CW.
***** shown above an exposed data point indicates $p<0.05$ for the pair of exposed/sham data points.

The x-irradiated positive controls using both proliferating and differentiated cells showed a significant difference in the level of apoptosis at 24 and 48 h post exposure compared to their unirradiated controls. However, no significant differences were seen in proliferating or differentiated cells exposed to any RF signal at any time point post exposure.

Table 5.7. Measurement of apoptosis levels using the Apo direct *in situ* end labelling assay in proliferating N2a cells given a 24 h exposure to 935 MHz GSM Basic, GSM Talk, or CW signals (2 W/kg) and sham exposures together with positive controls exposed to 4 Gy x-rays and unirradiated controls. Each data point shows the total ± SE of apoptotic cells from assaying 3000 cells in each of three experiments. The values obtained by the chi-squared test for homogeneity (χ^2), together with p values on 5 degrees of freedom, are also shown.

	Time	1				2				3				χ^2	p
	(hours)	0 Gy	± SE	4 Gy	± SE	0 Gy	± SE	4 Gy	± SE	0 Gy	± SE	4 Gy	± SE		
X-rays	0	35	5.88	31	5.54	34	5.80	31	5.54	30	5.45	26	5.08	0.99	0.803
	24	36	5.96	94	9.54	44	6.58	98	9.74	40	6.28	96	9.64	0.88	0.971
	48	57	7.48	198	13.6	61	7.73	222	14.34	64	7.91	208	13.91	1.80	0.877
	Time	1				2				3					
	(hours)	sham	± SE	RF	± SE	sham	± SE	RF	± SE	sham	± SE	RF	± SE		
GSM Basic	0	41	6.36	37	6.05	32	5.63	42	6.44	41	6.36	44	6.58	2.06	0.841
	24	46	6.73	53	7.22	38	6.13	53	7.22	42	6.44	58	7.54	1.07	0.957
	48	50	7.01	66	8.03	43	6.51	60	7.67	52	7.15	62	7.79	1.22	0.943
GSM Talk	0	44	6.58	50	7.01	46	6.73	42	6.44	41	6.36	52	7.15	1.46	0.918
	24	52	7.15	59	7.61	49	6.94	50	7.01	44	6.58	63	7.85	2.22	0.818
	48	101	9.88	84	9.04	59	7.61	66	8.03	65	7.97	80	8.82	16.09	0.007
CW	0	35	5.88	33	5.71	36	5.96	31	5.54	33	5.71	42	6.44	2.08	0.838
	24	42	6.44	38	6.13	44	6.58	44	6.58	49	6.94	43	6.51	1.07	0.956
	48	64	7.91	58	7.54	68	8.15	57	7.48	73	8.44	62	7.79	0.83	0.975

Table 5.8. Measurement of apoptosis levels using the Apo direct *in situ* end labelling assay in differentiated N2a cells given a 24 h exposure to 935 MHz GSM Basic, GSM Talk, or CW signals (2 W/kg) and sham exposures together with positive controls exposed to 4 Gy x-rays and unirradiated controls. Each data point shows the total ± SE of apoptotic cells from assaying 3000 cells in each of three experiments. The values obtained by the chi-squared test for homogeneity (χ^2), together with p values on 5 degrees of freedom, are also shown.

	Time	1				2				3				χ^2	p
	(hours)	0 Gy	SE	4 Gy	SE	0 Gy	SE	4 Gy	SE	0 Gy	SE	4 Gy	SE		
X-rays	0	113	10.43	100	9.83	118	10.65	96	9.64	110	10.29	92	9.44	0.62	0.987
	24	138	11.47	233	14.66	133	11.27	259	15.38	136	11.39	236	14.75	1.76	0.881
	48	161	12.34	309	16.65	140	11.55	306	16.58	158	12.23	284	16.03	2.93	0.711
	Time	1				2				3					
	(hours)	sham	SE	RF	SE	sham	SE	RF	SE	sham	SE	RF	SE		
GSM Basic	0	132	11.23	127	11.03	131	11.19	135	11.35	125	10.94	117	10.60	1.51	0.912
	24	176	12.87	162	12.38	157	12.20	144	11.71	151	11.97	171	12.70	4.49	0.481
	48	160	12.31	188	13.27	198	13.60	204	13.79	170	12.66	203	13.76	5.22	0.390
GSM Talk	0	130	11.15	121	10.78	132	11.23	103	9.97	149	11.90	138	11.47	6.67	0.246
	24	141	11.59	157	12.20	137	11.43	158	12.23	151	11.97	151	11.97	0.91	0.969
	48	170	12.66	171	12.70	192	13.41	177	12.91	160	12.31	181	13.04	3.37	0.643
CW	0	115	10.52	125	10.94	110	10.29	120	10.73	111	10.34	108	10.20	1.42	0.922
	24	150	11.94	166	12.52	151	11.97	147	11.82	167	12.56	148	11.86	2.65	0.753
	48	163	12.42	170	12.66	174	12.80	158	12.23	178	12.94	156	12.16	1.41	0.923

Table 5.9. Measurement of apoptosis levels using the Apo direct *in situ* end labelling assay in proliferating and differentiated N2a cells given a 24 h exposure to 935 MHz GSM Basic, GSM Talk or a CW signal (2W/kg) and sham exposures, together with positive controls exposed to 4 Gy x-rays and unirradiated controls. Each data point shows the combined total ± SE of apoptotic cells from assaying 3000 cells in each of three experiments. The t-test statistic (t) and p values for each pair of exposed/sham data points are also shown.

		Proliferating N2a cells						Differentiated N2a cells					
	Time (hours)	0 Gy	± SE	4 Gy	± SE	t	p	0 Gy	± SE	4 Gy	± SE	t	p
X-rays	0	99	9.90	88	9.34	1.00	0.249	341	18.11	288	16.69	3.73	0.083
	24	120	10.87	288	16.70	9.42	0.034	407	19.71	728	25.87	17.10	0.019
	48	182	13.35	628	24.17	17.58	0.018	459	20.86	899	28.44	21.60	0.015
	Time (hours)	sham	± SE	RF	± SE	t	p	sham	± SE	RF	± SE	t	p
GSM Basic	0	114	10.61	123	11.02	0.71	0.303	388	19.26	379	19.05	0.58	0.334
	24	126	11.15	164	12.69	2.67	0.114	484	21.40	477	21.25	0.40	0.378
	48	145	11.94	188	13.56	2.83	0.108	528	22.29	595	23.57	3.58	0.087
GSM Talk	0	131	11.36	144	11.91	0.96	0.257	411	19.80	362	18.63	3.12	0.099
	24	145	11.94	172	12.99	1.84	0.159	429	20.21	466	21.02	2.20	0.136
	48	225	26.49	230	26.80	0.16	0.449	522	22.17	529	22.32	0.39	0.383
CW	0	104	10.13	106	10.24	0.17	0.447	336	17.99	353	18.41	1.14	0.229
	24	135	11.53	125	11.10	0.77	0.291	468	21.06	461	20.91	0.41	0.377
	48	205	14.15	177	13.17	1.81	0.161	515	22.03	484	21.39	1.75	0.165

5.3 Discussion

Overall the exposure of mouse neuroblastoma cells, either proliferating or in a differentiated state, to a GSM Basic, Talk or CW signal of 935 MHz for 24 h at an SAR of 2 W/kg did not induce significant differences in the levels of apoptosis compared to sham exposed / control samples, as measured by any of the three assays between 0 and 48 h post exposure. The total number of experiments carried out for RF exposure was 84 (Basic, Talk, CW exposures; Annexin V, caspase and Apo Direct assays). Using 5% as the level of significance, statistically approximately 4 results (5%) could be expected to be falsely revealed as positive in 84 experiments. The fact that no positive result was revealed by these analyses indicated that the weight of evidence is in favour of there being no effect of RF exposures.

Using the Annexin V binding assay the numbers of apoptotic cells seen with the differentiated cultures are approximately double that of the proliferating cell cultures in both the sham and RF field exposed dishes. Reducing the serum in the culture medium stresses the neuroblastoma cells; hence the greater number of apoptotic cells. As with the Annexin V assay, more apoptotic cells were also observed in the differentiated, compared to the proliferating cells using the *in situ* end labelling assay. In contrast, using the caspase activation assay, in both RF and sham exposed dishes the numbers of apoptotic cells were consistently higher for proliferating calls than for those differentiated by serum withdrawal. This observation is the opposite of that seen using the Annexin V and *in situ* end labelling assays, but it is possible that the reduction in serum concentration causes more cells to enter competing cellular pathways resulting in cell death such as necrosis. In contrast Höytö et al. (2008) observed higher levels of apoptosis in serum deprived murine fibroblasts (L929 cells) compared to cells grown in medium with 10% serum. However apoptosis was only measured by caspase-3 activity, whereas the present study measured pan-caspase activation.

The present work has shown that a 4 Gy x-ray exposure of murine N2a cells, in both proliferating and differentiated states, produced a peak in apoptosis levels,

which occurred at different times post exposure depending on the assay used to assess apoptosis. The Annexin V assay showed a significant peak ($p = 0.036$) at 2 h for proliferating cells (Figure 5.1). Although the differentiated cells showed a peak in the percentage of apoptotic cells at 4 h (Figure 5.2), this was not significantly different from the levels seen at 0, 2 or 8 h post exposure. However, it was significantly different from to the levels of apoptosis at 24 h ($p = 0.047$). With the caspase activation assay, the significant peak ($p = 0.024$) in apoptosis arose at 24 h (Figure 5.3) for proliferating cells, whereas for differentiated cells there was a broad peak between 8 and 48 h (Figure 5.4). Here there was no significant differences in the levels of apoptosis between 8 and 48 h, but at all these time points the difference was significant ($p = 0.047$) when compared to the level at 4 h. By contrast, the peaks in apoptosis levels for both proliferating (Figure 5.5) and differentiated (Figure 5.6) cells arose at 48 h post exposure, ($p = 0.016$ and 0.041 respectively), for the *in situ* end labelling assays. The relative timings of these peaks is entirely as expected because the Annexin V binding assay detects an early membrane change event and the *in situ* end labelling assay detects DNA fragmentation, which is typical of late-stage apoptotic cells, whereas the caspase activation assay detects the cascade of proteolytic caspases, an early to intermediate feature of the apoptotic process (Elmore 2007).

The x-ray results also show the three apoptosis assays are sensitive enough to pick up an effect in both proliferating and differentiated N2a cells. During an RF field exposure it is possible that small temperature gradients and / or hot spots could have been induced in the cell cultures and these could have affected the results, as changes in temperature would be an indicator of unequal energy deposition within the dish. However, previous detailed analysis (Schuderer et al. 2004b) of the exposure apparatus in the geometry used in these experiments found no hot spots in the medium, and at all times the temperature gradients were found to be small, much lower than those required to induce convection.

5.4 Conclusion

Exposure of N2a mouse neuroblastoma cells, either in a proliferating or a differentiated state, to a GSM Basic, GSM Talk or CW signal of 935 MHz for 24 h at an SAR of 2 W/kg did not induce differences in the levels of apoptosis compared to sham exposed / control samples; as measured by the Annexin V, caspase activation and the Apo Direct assays between 0 and 48 h post exposure.

6. General discussion

The widespread use of mobile phone technology has stimulated debate about the possible health effects resulting from exposure to RF fields. A number of physical, biological and chemical agents are known to initiate or promote cancer. Therefore the primary concern is whether RF fields also cause cancer, especially brain tumours. Public anxiety has been fuelled by alarmist reports in the press with such headlines as

> "Mobile phone craze danger to our children" Sunday Mirror 16/1/2005
>
> "Using mobiles 'more deadly than smoking'" Manchester Evening News 15/1/2005
>
> "Cancer risk in mobile phones: Official" The Sun 16/10/2008

There is no plausible biophysical mechanism for cancer induction by RF fields and the epidemiological evidence for a link between mobile phone use and cancer is weak. Therefore the results of *in vitro* cellular studies are important to evaluate the risk of low level RF field exposure. Strong evidence that RF fields are incapable of producing the types of cellular lesions known to be associated with carcinogenesis would strengthen the view that the weak epidemiological findings may be illusory. Alternatively, if cellular studies provided evidence that RF field exposure could produce such lesions the epidemiological studies that found a link between mobile phone use and tumours would be more credible. The study of *in vitro* genotoxic effects is a standard method for assessing carcinogenic potential, as many known cancer causing agents produce DNA damage. Also, an assessment of any change to important cellular processes such as apoptosis, known to play a role in carcinogenesis, could provide evidence to confirm or refute a link between mobile phones and cancer.

An examination of the published literature (Chapter 1) regarding *in vitro* genotoxic effects following exposure to low level RF fields suggest they are not directly genotoxic, but may operate in an indirect manner. There are fewer published studies on RF field exposure and apoptosis. Many of the investigations have only assessed apoptosis at one or two time points and not used cells derived from brain tissue. The main focus of the *in vitro* studies presented here was firstly to evaluate the ability of low level RF fields from several GSM modulated signals and an unmodulated continuous wave to

enhance the DNA damage caused by x-rays in human lymphocytes. The appropriate controls used in the study design also allowed the effects of the RF field alone to be compared with sham controls. Secondly, the aim was to assess the capability of 935 MHz RF signals to induce apoptosis in the murine neuroblastoma cell line N2a, by following the level of apoptotic cells over a time course of 0 to 48 h post exposure.

6.1 Investigations into the possibility that RF fields are directly genotoxic

In chapters 3 and 4 the possible genotoxic effects of RF fields were assessed using well characterised sXc exposure systems. The systems, designed and produced by IT'IS, were engineered and used in such a way as to control important factors, such as the homogeneity of SAR and the temperature within the sample, as far as practically possible. Several standard cytogenetic assays for chromosome aberrations, micronuclei and sister chromatid exchanges were used to assess genotoxicity in G_0 human lymphocytes exposed *in vitro* to RF fields. The study examined whether a 24 h continuous exposure to a 935 MHz GSM Basic signal delivering an SAR of 1 or 2 W/kg, an 1800 MHz GSM Basic signal (2 W/kg), a 935 MHz GSM Talk signal (1 W/kg) and the underlying CW carrier frequency of 935 MHz at an SAR of 1 W/kg are genotoxic *per se*. The effect of an intermittent GSM Talk signal, 10 min RF on and 5 min RF off, for a total of 24 h delivering an SAR of 1 W/kg was also investigated. In all instances no genotoxic effect from the RF signal was observed compared to appropriate sham exposed controls. PHA stimulated lymphocytes exposed for a total of 48 h to an intermittent (5 min RF on, 10 min RF off) 1800 MHz GSM Basic or CW signal both delivering an SAR of 2 W/kg, together with appropriate sham exposures showed no evidence of a direct genotoxic effect.

The results of the present chromosomal investigations are in agreement with many other studies, which have reported no direct genotoxic effect in human lymphocytes, following exposure to a range of low level RF fields that were modulated or unmodulated, continuous or intermittent, at several different

SARs, ranging from 0 to 10 W/Kg. These investigations also employed one or more of the standard cytogenetic assays used in the present study and include Maes et al. (1995, 1997, 2000, 2001), Antonopoulos et al. (1997), Vijayalaxmi et al. (1997b, 2001 a and b, 2006b) McNamee et al. (2002 a and b, 2003), Zeni et al. (2003, 2005, 2008) and Scarfi et al. (2006). In addition the results of the present study are in agreement with other investigations that have used other cell types or DNA strand breaks to assess genotoxicity, such as Malyapa et al. (1997b, 1998), Li et al. (2001), Hook et al. (2004), Sakuma et al. 2006), Speit et al. (2007) and Zhijian et al. (2009). In this group of studies, only one investigated the genotoxicity of a 935 MHz GSM signal (Maes et al. 1997), although GSM 900 MHz signals were used in the studies by Maes et al. (2001), Zeni et al. (2003) and Scarfi et al. (2006). In one of the studies, Zhijian et al. (2009), an 1800 MHz GSM intermittent signal was used to expose human leukocytes. In addition, Speit et al. (2007) examined human fibroblasts and Chinese hamster cells exposed to 1800 MHz GSM intermittent signals and found no effect of the RF fields. In common with the present study SARs of 2 W/kg or lower have been used in the majority of the studies that have also found no effect of RF fields, although few have investigated as many signal types in one study, with the exception of Hook et al. (2004).

The present study has been designed to allow control of the temperature of the samples during the RF exposures, because the RF energy they absorbed can cause the temperature to rise. Temperature has also been controlled in many of the other studies that have found no effect of RF fields, although in some studies, such as Maes et al. 1997, the temperature of the samples was not recorded.

Conflicting results have been published by Maes et al. (1993), Zotti-Martinelli et al. (2000, 2005), Tice et al. (2002), d'Ambrosio et al. (2002) and Diem et al. (2005). These studies have not used GSM signals or a frequency used in the present study, with the exception of Tice et al. (2002) and Diem et al. (2005) There is the possibility that localised heating may have been a confounding factor in some studies (Maes *et al.* 1993, Tice et al. 2002) or the temperature measured in control samples may not reflect the temperature of the exposed samples (Zotti-Martelli et al. 2000 and 2005). Temperatures of > 39°C, *in vivo*

and *in vitro*, can produce a number of effects in mammalian cells such as, altering viability and cell proliferation, the induction of DNA strand breaks and inhibiting DNA damage repair (Weissenborn and Obe 1991, Vijayalaxmi and Obe 2004). Lloyd et al. (1986a) found no induction of chromosomal aberrations and SCEs in human G_0 lymphocytes held at 40°C for 15 min.

d'Ambrosio et al. (2002) reported that, when compared to controls, micronucleus levels were unaffected after exposure to a CW signal, but a significant increase was observed in cells exposed to the PW signal. The authors have suggested that human lymphocytes show a different response which depends on the phase modulation of the RF field. The present work, however, found no effect of modulation on micronucleus frequencies, but unlike d'Ambrosio et al. (2002) the lymphocytes were in G_0 and not PHA stimulated at the time of exposure. However, Zeni et al. (2003 and 2008) exposed G_0 and stimulated lymphocytes to intermittent CW and GSM modulated signals and found micronucleus levels to be unaffected.

Despite using a number of different RF signals the present study has only investigated the possible genotoxicity of two frequencies of electromagnetic radiation in the "radio" band. Many of the positive studies, as well as those finding no effect of RF, have used other frequencies and signals. If biological effects only occur in certain 'frequency windows' (Belyaev 2009) a larger number of frequencies need to be studied. As no effect of RF was observed at SARs of 1 and 2 W/kg the present study could also be extended to include lower SARs. The assays used in this study produce low background aberration frequencies, therefore to increase the possibility of detecting a difference between the RF and sham exposed samples more cells need to be scored and more blood donors used. This is also relevant to the small study of PHA stimulated lymphocytes.

The present work also found no significant changes in chromosome and chromatid damage in PHA stimulated lymphocytes following intermittent modulated and unmodulated RF signals. In addition, continuous and intermittent GSM Talk signals did not produce an increase in chromosome aberrations, micronuclei or SCE in lymphocytes exposed in G_o. The present study examining

intermittent RF exposure was a direct result of the findings of Diem et al. (2005), although Diem et al. (2005) used a different end point to measure genotoxicity and different cell types. The study by Diem et al. used a well characterised exposure system: an sXc 1800 system similar to the one used in the present study. This study cannot be criticised on the grounds of poor temperature control and thus considerable research effort worldwide has been put into investigating the possible genotoxicity of intermittent RF exposure. The work by Diem et al. is more fully discussed in the following Section 6.1.1.

6.1.1 Genotoxicity of intermittent RF exposure and alleged scientific misconduct.

The study by Diem et al. (2005) raised the idea that an intermittent exposure is more genotoxic than a continuous exposure. Human skin fibroblasts (ES-1) and rat granulosa cells (GFSH-R17) exposed to 1800 MHz GSM signals showed significant increases in comet tail factor (CTF); a surrogate marker of DNA strand breaks. Exposures that had been intermittent or GSM Talk produced a stronger effect than the continuous CW signal. The work was a part of a large EU funded REFLEX **(**Risk Evaluation of Potential Environmental Hazards from Low Energy Electromagnetic Field Exposure Using Sensitive *in vitro* Methods) project and a similar effect was found using the MN assay (REFLEX Final Report 2004). The same research group at the Medical University of Vienna (MUW) also found that intermittent ELF-EMF field exposure (50 Hz, 1 to 1000 μT) caused DNA damage whilst a continuous exposure to the same field did not (REFLEX Final Report 2004; Ivancsits et al. 2003 and 2005). Independent scoring of micronucleus slides prepared at Vienna was undertaken by two laboratories outside the REFLEX project for fibroblasts exposed to an intermittent ELF-EMF field (50 Hz, 1 μT, 15 h total exposure - 5 min on / 10 min off) and a GSM Basic RF field (1950 MHz, 2 W/kg, continuous 15 h exposure), together with appropriate control slides. The RF exposed cells showed about a 5 fold increase in micronuclei and the ELF-EMF exposed cells showed a 6 to10 fold increase compared with the sham exposed control cells. However, there were differences in the actual number of micronuclei scored by each laboratory and these were attributed to the different staining techniques employed.

Within the REFLEX project a second research group at the Free University Berlin, using the human promyelocytic cell line HL-60 exposed for 24 h to an 1800 MHz CW field, reported a significant difference compared to sham exposed cells at SARs of 1.3, 1.6 and 2 W/kg when assessed by the comet and the MN assays; although at 0.2, 1 and 3 W/kg no significant difference was observed with either assay. In addition HL-60 cells exposed for 24 h to several 1800 MHz signals, including an intermittent (5 min on / 10 min off) CW and a continuous Talk signal both delivering an SAR of 1.3 W/kg, showed a significant increase in the frequency of micronuclei compared to the sham exposed controls; the comet assay also produced similar results. However, this work, carried out as a part of the REFLEX project and described in the REFLEX Final Report (2004) has never been published in the peer reviewed literature.

Prior to the study by Diem et al. (2005) few *in vitro* investigations into the possible genotoxic effects of RF fields used intermittent signals, with the exception of Vijayalaxmi et al. (1997b) and Zeni et al. (2003). In both of these studies human lymphocytes were used, either in the G_0 state and assessed by the CA and MN assays (Vijayalaxmi et al. 1997b) or G_0 and PHA stimulated cells assayed for micronucleus induction (Zeni et al. 2003). Both studies found no significant difference compared to sham exposed control samples for any exposure regime. The work of Ivancsits et al. (2005) suggested the genotoxic effect of ELF-EMF was cell type specific and if the same were true for RF-EMF this could explain why no genotoxic effects were seen using lymphocytes. The findings of Diem et al. (2005) and Ivancsits et al. (2003 and 2005) provoked much disquiet and scientific debate regarding confounding factors (Vijayalaxmi et al. 2006), the exposure set up and statistical concerns (Summary Report: FGF workshop 2008). The implication of this work was that intermittency of signal was the crucial factor so that virtually all previous *in vitro* experiments, as described in the examination of the literature in chapter 1, had been carried out with continuous exposures and therefore, of course, failed to show an effect. As a result, a number of independent replication studies or investigations using intermittent ELF and RF fields were undertaken. A large replication and extension study on the genotoxic effects of RF fields using the same exposure equipment was undertaken by Speit et al. (2007). Incidentally, it was this group that had rescored the original micronucleus slides prepared by the MUW

laboratory for the REFLEX project and confirmed the higher frequency of micronuclei in RF exposed samples.

Speit et al. (2007) exposed human fibroblast ES-1 cells and Chinese hamster V79 cells to 1800 MHz RF CW or a GSM modulated signal, both delivering an SAR of 2 Wkg. Exposure to the CW signal was intermittent, 5 min RF on / 10 min RF off and continuous to the GSM Basic signal; total exposure time varied between 1 and 24 h. The ES-1 cells were assessed using the comet, chromosome aberration and micronucleus assays, while the comet and micronucleus assays were employed with the V-79 cells. No evidence for a genotoxic effect was found for intermittent or continuous exposure in any assay with either cell type. In addition the ELF work of Ivancsits et al. (2003) could not be independently reproduced by Scarfi et al. (2005). A study using G_0 and lymphocytes at different stages of their cell cycle exposed to an intermittent 1950 MHz UMTS (Universal Mobile Telecommunication System) signal also reported no increase in micronucleus frequencies or cell cycle kinetics (Zeni et al. 2008). Zhijian et al. (2009) have reported no effect on DNA strand breaks in human leukocytes exposed to an intermittent 1800 GSM signal.

However another recent investigation by Schwarz et al. (2008), from the same laboratory at MUW as Diem and Ivancsits, also used a 1950 MHz UMTS signal. The study extended the work of Diem et al. (2005) using lower SAR levels and the alkaline comet assay and CTF as a surrogate marker of DNA strand breaks. Human fibroblasts, type ES1, were exposed to a 1950 MHz UMTS signal. Cells were either continuously exposed for 24 hours to a range of SARs (0.05 to 2.0 W/kg) or the continuous exposure time was varied from 4 to 48 hours (SAR 0.1 W/kg). The influence of four different intermittent patterns ranging from 5 min RF on / 10 min RF off to 10 min RF on / 20 min RF off were also compared to a continuous exposure. The total time of each exposure was 16 hours and an SAR of 0.1 W/kg was used. A statistically significant increase in the CTF was reported for all SARs used with the 24 hour continuous exposure and after 8 hours exposure to the UTMS signal with an SAR of 0.1 W/kg. Similar results were obtained with the micronucleus assay. The four intermittent exposures all produced similar percentage values for CTF, which were higher than for the continuous exposure. In addition, three fibroblast and three short term lymphocyte cultures from different donors were exposed for 16 h to an

intermittent signal, (5 min RF on / 10 min RF off), with an SAR of 0.1 W/kg. No effect of a 1950 MHz UMTS exposure was seen with the stimulated lymphocytes, as Zeni at al (2008) had also reported for micronuclei, whereas all the cultured fibroblasts showed a significant increase in CTF compared to sham exposed controls. These results were repeated using the micronucleus assay.

Shortly after the publication of this study comments by Lerchel (2008) appeared, which questioned “the validity and origin of the data”. His main concern centred on the very low standard deviations for the CTFs quoted in the paper, which did not exceed 5%, whereas in a paper by Diem et al. (2002) standard deviations of ~25% were reported. In reply, Rüdiger (2008) dismissed the concern over low standard deviations by citing other published findings of his group as well as two studies by other authors. Additionally, he referred to the double blind conditions under which the study was carried out. In the conclusion he states “points being made (*by Lerchel*) do neither give reason to doubt the validity of the data nor to modify the conclusions”. The controversy regarding the study by Schwarz at al (2008), as well as the investigation by Diem et al. (2005), did not end there and on 23rd May 2008 the Medical University of Vienna released a press statement in which it was stated the data had been reviewed and it was suspected the results may have been fabricated, as one junior researcher had based work carried out for the review on producing preconceived results. The Rector of the University asked the authors to withdraw the two papers. However, when the senior researcher discovered that the Chairperson of the University’s Committee on Scientific Ethics had links to the mobile phone industry he refused to withdraw the papers. He also alleged the junior researcher denied any wrong doing, although it later emerged she had known the sXc sham / field encoding since about 2005 (MUW press release July 2008). Wolf (2008) published a paper demonstrating how an operator could easily determine the exposure conditions of the sXc equipment. Kuster (2008), in his comments on Wolf’s publication, promised to modify the systems software to prevent the information in Wolf (2008) being used to indicate which wave-guide was switched on. However, as he also points out, the sXc system is not totally fool-proof and “Good Laboratory Practice and scientific integrity cannot be replaced by coded controls on laboratory equipment”.

Meanwhile, a temporary replacement for the head of the MUW ethics committee was found so as to ensure there was no impression of bias in the case and the senior researcher agreed to withdraw the Schwarz et al. (2008) paper, but only on the grounds that the blind coding could no longer be assured (MUW press release July 2008). He maintained the work for the 2005 paper was done in 2003 and therefore not tainted (Vogel 2008). An MUW press release (September 2008) reported the task for its council for ethics in science was "… to determine all further publications in which this author was involved while using same experimental design…" and recommend their withdrawal.

Some months later Editorials appeared in the Journals that had published Schwarz el al. 2008 (International Archives of Occupational and Environmental Health, Drexler and Schaller 2009) and Diem et al. 2005 (Mutation Research, Baan 2009). Both journals had the respective papers reviewed again and in both cases found the criticism of the statistics alone, although suggestive, could not prove, indisputably, the serious allegation of data fraud. However, Drexler and Schaller (2009) went on to express the doubts of the Editors on the reliability of the results in the Schwarz et al. (2008) paper, because the blind coding may have been compromised and apologised for publishing the paper. Mutation Research published a letter from the corresponding author for the Diem et al. 2005 paper, which categorically stated the doubts regarding the blind coding were unfounded for this publication (Rüdiger 2009). In his letter Rüdiger (2009) states the work had been completed in 2003, before there was evidence the laboratory worker knew how to decode the exposure system, (September 2005). Also, the work had been performed in another institute and the exposure system had been programmed by a worker in the host laboratory. Consequently, the publication of Diem et al. (2005) was not withdrawn by the Editors of Mutation Research (Baan 2009).

All in all, the fact remains that no peer reviewed study, as well as the present work, which was done before any hint of impropriety had emerged, have been able to replicate or confirm the results of Diem et al. (2005). Therefore, given the suspicions surrounding that study the question regarding an intermittent RF field exposure being more genotoxic than a continuous field can now no longer be regarded as an important or indeed real effect.

6.2 Investigations into the possibility of co-genotoxicity of RF fields and another mutagen

In chapters 3 and 4, the ability of RF fields to influence the genotoxicity of the well established clastogenic agent, x-radiation, was examined. No evidence was found to suggest co-genotoxicity between RF fields and 1 Gy x-rays, regardless of whether the cells were exposed to x-rays then the RF field or *vice versa*. Again, G_0 human lymphocytes were exposed *in vitro* to several RF fields: a 24 h continuous exposure to a 935 MHz GSM Basic signal delivering an SAR of 1 or 2 W/kg, an 1800 MHz GSM Basic signal (2 W/kg), a 935 MHz GSM Talk signal (1 W/kg), the underlying CW carrier frequency of 935 MHz at an SAR of 1 W/kg and an intermittent GSM Talk signal, 10 min RF on and 5 min RF off, for a total of 24 h delivering an SAR of 1 W/kg. The chromosome aberration, micronucleus and sister chromatid exchange assays were again used to determine any dose modifying effect of RF fields compared to sham exposed controls.

Again, temperature was an important factor to control. Weissenborn and Obe (1991) found chromosome aberrations in human G_0 lymphocytes to be unaffected following hyperthermia treatment of 40°C for 30 and 60 min. However, when the same hyperthermia treatment was given after x-irradiation chromosome aberration frequencies were found to be higher than in irradiated samples only. In addition, similar results were observed for lymphocytes in different stages of the cell cycle. The results of Weissenborn and Obe (1991), suggest the repair of radiation-induced DNA strand breaks are inhibited by increased temperature. Therefore, the temperature of the samples was well controlled in the present study.

Fewer studies have reported on co-genotoxicity of RF fields with either physical or chemical mutagens than on the genotoxicity of RF fields alone. As in the present investigation, the majority of these studies have used human G_0 lymphocytes. A series of four studies by Maes et al. (1996, 1997, 2000 and 2001) investigated the interaction of RF fields of different frequencies or GSM modulations and mitomycin C (MMC). Unlike the present study a synergistic

action of RF fields was observed by Maes et al. (1996 and 1997), although thermal effects cannot be ruled out in either of the two studies. Maes et al. 2000 and 2001, irradiated cells with x-rays (up to 1 Gy) after the RF exposure alternatively cells were cultured with / without MMC. Analysis of chromosome aberrations showed no enhancing effects of RF fields on x-ray induced damage, which agrees with the present study. However, when MMC treatment followed RF exposure some differences in SCE levels were observed when compared to control samples, but the results did not show a consistent pattern (Maes et al. 2000), or no evidence for any synergistic effect was seen (Maes 2001). Unlike the results of Maes et al. (1996, 1997, 2000 and 2001), which have gone from suggesting a synergistic effect of RF fields to no effect, the present study consistently showed no dose modifying effect with a number of GSM modulated signals. In addition the present study is in agreement with Zhijian et al. (2009), in that no synergistic effect of an intermittent RF exposure in combination with x-rays was observed.

In contrast to the present study, Baohong et al. (2005 and 2007) reported an enhancing effect of RF fields, although the co-exposure was to chemicals (2005) or UVC (2007) and not x-rays. In common with the present study, human G_0 lymphocytes were exposed to an RF field in an sXc waveguide system, to a GSM signal at 1800 MHz; although the SAR was higher at 3 W/kg. Unlike the present study, only one assay to assess genotoxicity was used on a limited number of blood donors. Therefore, the results lack conformation by a second assay and a larger study group. In addition, the two studies by Baohong et al. (2005 and 2007) produced some inconsistent results e.g. the response was independent of the chemical concentration, only two of the four chemicals tested showed a dose modifying effect and the results were not consistent over all the UVC exposure groups.

Although a different RF signal was used, the recent study by Manti et al. (2008), who also investigated possible enhancing effects of RF fields and x-rays, is more difficult to reconcile with the present work. In that study human G_0 lymphocytes exposed to 4 Gy x-rays were subsequently placed in an RF field of a 1.95 GHz UTMS signal, (SAR 0.5 and 2 W/kg) for 24 h. The combined exposure of x-rays and RF field did not increase the fraction of aberrant cells,

however a small but significant increase in chromosome exchanges of 11% was observed in the 2 W/kg samples when compared to the sham exposed controls, although at an SAR of 0.5 W/kg no enhancement was seen as two of the four donors had aberration frequencies below that of their 0 W/kg sample. A 28% decrease in the number of fragments at 2 W/kg compared to 0 W/kg was also noted, unlike the present study where no significant difference was found in the number of excess acentrics in the whole genome for the combined exposure groups. The authors speculate that RF fields could change the visible exchange frequency or the occurrence of fragments by altering the correct rejoining of x-ray induced double strand breaks by DNA repair enzymes. Alternatively, highly damaged cells could reach metaphase if apoptosis was inhibited by RF fields. It should be noted the number of chromosome exchanges reported in this study were the total of simple as well as complex interchanges, i.e. those arising from the interaction of 3 or more breaks on 2 or more chromosomes (Edwards and Savage 1999). Cells carrying complex rearrangements on average contain more aberrations per cell therefore a small change in the number of such cells could have a large effect on the exchange frequency. Also, the number of complex rearrangements increases with dose with 1 Gy x-rays giving rise to few complex rearrangements compared to 4 Gy (Finnon et al. 1995). In the present investigation, using 1 Gy x-rays, few heavily damaged cells were observed which may go some way to explaining the contradictory results of the two studies.

In the present study, the statistical strength of the CA and MN data was such that an enhancing effect of RF fields of about 20% would have been detected. This suggests any possible effect of RF fields would be small, however to improve the statistical strength of the data further, to allow for a much smaller enhancing effect to be detected, more cells per person would need to be scored and the number of donors increased. Given the results of Baohong (2005) and Manti (2008) the current study on co-genotoxicity could be extended to include chemical mutagens and higher doses of x-rays. In addition other frequencies relevant to mobile phone communication could be studied, as well as lower SARs.

6.3 RF fields and cell proliferation

As described in chapters 3 and 4, the results of the present study also found no significant alteration in cell proliferation, measured by the NDI, with any of the RF fields used. A number of the studies, using human lymphocytes to study the possible genotoxic effects of RF fields have also used cell proliferation or cell cycle analysis to determine any cytotoxic effects of RF fields. As in the present investigation, the majority of these studies (Maes et al. 1993, 1996, 1997, 2001; Antonopoulos et al. 1997; Vijayalaxmi et al. 1997, 2001 and b; d'Ambrosio et al. 2002; Zeni et al. 2003, 2005, 2008 and Scarf et al. 2006) found no alteration in cell proliferation or cell cycle kinetics. In contrast some studies have observed transient or inconsistently positive effects.

Tice et al. (2002) found an SAR dependent decrease in the proliferation index, i.e. a slowing in the speed of cell cycling, which was significant at 10 W/kg, for a 3 h exposure to a voice modulated TDMA RF field. However the high SAR of 10 W/kg used may have produced "hot spots" in the medium which caused localised heating of the cells. Capri et al. (2004b) have also shown a slight decrease in the proliferation of human lymphocytes, measured by [^{3}H] thymidine incorporation, exposed to a GSM modulated 900 MHz RF signal; although no significant change was seen when an unmodulated CW field was used. However the decrease in proliferation was only observed with the lowest concentration of mitogen used and cell cycle, measured by flow cytometry was found to be unaffected by the RF fields.

In the present study the use of NDI as a measure of cell proliferation was only able to give an indication that the effect of RF was not large. In order to produce statistically stronger data more cells would need to be scored from more donors in any future study. Alternatively, the use of flow cytometry would allow proliferation data to be based on a far greater number of cells. The biggest confounder in such assays is that the intrinsic speed of cell cycling is very variable from person to person. Therefore, the importance of having robust control cycling data for each person, to adjust the values measured from their irradiated material, cannot be over emphasised.

6.4 Investigations into the potential of RF fields to induce apoptosis

In chapter 5 the ability of RF fields to induce apoptosis was determined. Mouse neuroblastoma cells, either in a proliferating or a differentiated state, exposed continuously for 24 h to 935 MHz GSM Basic, Talk or the unmodulated CW signal all at an SAR of 2 W/kg, showed no induced differences in the levels of apoptosis compared to sham exposed control samples. Apoptosis was measured by three different assays: Annexin V, caspase activation and *in situ* end labelling between 0 and 48 h post exposure.

The result of the present apoptosis study is in agreement with several recent studies that have also used neuroblastoma cells and failed to demonstrate changes in apoptosis levels after exposure to 900 MHz GSM RF fields (Gurisik et al. 2006, Merola et al. 2006 and Joubert et al. 2006). Also, in common with the present study, apoptosis was mainly detected by caspase activation and *in situ* end labelling. Using another brain-derived cell line, human glioblastoma, Hirose et al. (2006) used the Annexin V assay after 2.14 GHz w-CDMA or CW RF field exposures, but found no change in apoptosis levels between exposed and sham exposed cells. Although Höytö et al. (2008) used a different murine cell line to the present study, (fibroblasts), and a different RF frequency, no significant increase in caspase-3 activity was found. As in the present study the cells were either proliferating or were serum deprived. In addition, studies with human lymphoma or leukaemia cell lines exposed to GSM RF fields have also failed to show changes in apoptosis levels when assessed by the Annexin V assay (Hook et al. 2004, Lantow et al. 2006 and Lagroye et al. 2002). More recently Palumbo et al. (2008) found a small but significant increase in caspase 3 activity in Jurkat T cells 6 h post exposure to a 900 GSM RF signal and this was confirmed in proliferating human lymphocytes, but not in cells exposed in G_0. However, this result was not found in the present study, where apoptosis levels were unaffected by RF fields in proliferating and quiescent neuroblastoma cells. When apoptosis levels were determined by the Annexin V and PARP assays by Palumbo et al. (2008) no significant differences between exposed and sham exposed any cell type was observed.

In contrast to the present study, however, the positive result of Palumbo et al. (2008) tends to support the findings of Marinelli et al. (2004). In that study human T lymphoblastoid leukaemia cells exposed to a 900 MHz CW signal for 2 h showed an increase in cells undergoing apoptosis, possibly the result of early activation of the p53 independent and dependent pathways. Although Hirose et al. (2006) concluded from their study that RF fields did not induce p53 dependent apoptosis. Marinelli et al. (2004) also found that when the RF exposure time was increased (24 and 48 h) the level of apoptosis reduced, but was still significantly higher than in unexposed cells. In addition, Caraglia et al. (2005) have also shown a positive effect of a short term exposure to a 1.95 GHz RF signal on human oropharyngeal epidermal carcinoma cells, when apoptosis levels were measured by the Annexin V assay 1 to 3 h post exposure. A time dependent increase and a time dependent decrease in apoptosis levels and HSP90 were found respectively.

The majority of studies using cell lines to investigate the effect of RF fields on apoptosis, including the present study, have found no significant differences between exposed and sham exposed control samples, whereas positive effects are either transitory or inconsistent. This is also the case in studies using primary cells exposed *in vitro*. Two studies by Capri et al. (2004 a and b) used GSM modulated signals, as in the present study, but at frequencies of 1800 and 900 MHz. Although human lymphocytes were the cell type used in this study, not neuroblastoma cells, no effect of RF fields on dRib induced apoptosis was measured by the Annexin V assay. The spontaneous levels of apoptosis were also unchanged. Also, the present study is in agreement with Belyaev et al. (2005) who have also reported no significant changes in the apoptotic response of non-cycling G_0 lymphocytes exposed to a 915 MHz RF field. Although only circumstantial evidence, these results are also supported by many of the studies that have used human lymphocytes to investigate the possible induction of DNA strand breaks after RF exposure as the fragmentation of DNA in apoptotic cells would have been obvious using the comet assay.

The present study used a neuronal cell line, but the lack of a positive effect is in agreement with studies using primary brain-derived cells, such rat cortical neurones (Joubert et al. 2007) and rat astrocytes (Lagroye et al. 2002) that

have also found no change in apoptosis levels following exposure to 900 MHz GSM fields. More recently however, Joubert et al. (2008) have reported a significant difference in the apoptosis frequency between cells exposed to a 900 MHz CW signal and sham exposed rat primary neurones assayed by DAPI staining and TUNEL, although localised RF thermal effects within the cultures during the exposure could not be ruled out. In the present study the temperature of the samples was well controlled, as temperatures greater than 40°C have been shown to induce apoptosis (Sakaguchi et al. 1995).

Most of the studies, both positive and those showing no effect of RF fields on the level of apoptosis have used GSM signals, at a frequency of 900 MHz. However, in some studies reporting an effect higher frequencies, (e.g. 1950 MHz), and lower SARs, (e.g. 0.003 W/kg), have been used than in the present work. Therefore further studies at low SARs and the higher frequencies would be needed to fully confirm or refute the results of these positive studies. A number of post exposure time points were used in the present study to assess apoptosis. However, a study to include different time points would further investigate any possible time window of increased apoptosis levels. Also, in an effort to detect a very small change in apoptosis levels more replicate experiments would be needed and more cells scored. FACS analysis would increase the number of cells assessed. In addition it would be interesting to combine the present study with an assessment of gene expression levels for genes important in apoptosis. Although some studies have found changes in the expression of apoptosis genes following RF exposure (Ivaschuk et al. 1997; Buttliglione et al. 2007; Nikolova et al. 2005) the results were not consistent or they were transient. Also, some studies have not used well characterised exposure equipment (Pacini et al. 2002; Zhao et al. 2007).

6.5 The precautionary principle

Many of the studies conducted since 2000 are the result of research needs identified by international and national bodies that have considered the evidence for possible health risks after exposure to RF fields. At that time, while no definite health effect associated with mobile phone use *per se* was identified a precautionary approach to mobile phone use was recommended (WHO 2000, IEGMP 2000). This approach to risk management is used in situations of scientific uncertainty and is known as the precautionary principle. The Commission for the European Communities in a communication document (EU COM 2000-1) states that it should only be used if the risk is scientifically plausible and that any measures taken should be proportional to the risk, while scientific uncertainty is to be addressed by appropriate research. Although use of the precautionary principle has been described as

> "bureaucrat-speak for 'cover your ass'". The Times 15/1/2005

it could reassure the public by demonstrating that decision makers are doing everything sensible that can be done. While this type of risk management requires scientific research it should also be recognised that science can never provide one hundred percent certainty. Therefore, it seems impossible to avoid the small number of studies that produce a positive effect being reported by frightening and alarming headlines in the press, such as

> "Mobile phones: New cancer fear" The Sun 21/2/2008
>
> "Mobile phone cancer risk 'higher for children'" The Telegraph 19/4/2008

while the larger number of studies that find no significant effect do not capture the headlines in the same way.

6. Conclusion

Within the experimental parameters of the study, in all instances, no genotoxic or dose modifying effect of RF fields was observed compared to appropriate sham exposed controls. Also, no induced differences in the levels of apoptosis compared to sham exposed control samples were observed. The data presented here serve to strengthen the case that RF fields associated with mobile phones are not genotoxic, by acting directly or indirectly and do not cause apoptosis in brain cells.

Although not all published studies relate specifically to RF fields produced by mobile phones they provide relevant information to an assessment of genotoxicity or the induction of apoptosis. There are many variables used in all such studies, which include frequency of the signal, the modulation, the SAR, the exposure time, the instrumentation, the cells studied and the assays themselves. Early studies have tended to lack experimental and exposure detail and/or have inadequate temperature control. However, the majority of studies demonstrate RF fields do not produce DNA strand breaks and, given the grave doubts cast over the work of Diem et al. (2005) and the lack of independent verification, the question of intermittent exposures inducing DNA damage is no longer open. Evidence from studies using a broad range of frequencies and modulations at SARs that do not cause elevated temperatures do not induce chromosomal aberrations, micronuclei or SCEs. There is some evidence suggesting RF exposure does not act synergistically with known chemical or physical mutagens, but recent work (e.g. Manti et al. 2008) is sufficiently intriguing not to exclude the possibility. Similarly, most studies looking at the possible induction of apoptosis by a number of RF signals in different cell types tend to suggest there is no or only a small transient effect after RF fields exposure. At present the potential health effects, if any, of such small and ephemeral changes in apoptosis and gene expression following low level RF exposure are unknown.

Clearly RF fields are not strongly genotoxic or potent inducers of apoptosis otherwise it would be evident from the standard tests for chromosomal

breakage and apoptosis, which have demonstrated the genotoxic action and the induction of apoptosis by ionising radiation. The general consensus view is that RF fields are not genotoxic or induce apoptosis, but sufficient numbers of reports expressing a contrary view exist for the question to remain open. Inevitably reports of a positive effect receive greater media and pressure group attention and serve to exacerbate public concern. Until the relevance of positive experimental findings is clearer, preferably backed by plausible modes for mechanisms, the societal view is to sanction the continued use of mobile phones, enjoying the benefits they clearly confer, but from a public health regulatory point of view to adopt the precautionary principle. However, high quality scientific research on RF field effects, using well defined exposure criteria and dosimetry, is essential for international or national bodies, such as the WHO, and the HPA, to provide non-scientists such as politicians and the public with objective information on the risk of mobile phone use.

Appendix 1

Table A.1. The number of centric rings scored in the CA assay for the different exposure regimes using a 935 GSM Basic signal at an SAR of 1 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of centric rings	Distribution of centric rings		σ^2 / y ± SE	U
			0	1		
1, S	500	0	500		—	—
1, F	500	0	500		—	—
1, X + S	500	5	495	5	0.99 ± 0.06	-0.14
1, X + F	500	3	497	3	1.00 ± 0.05	-0.08
1, S + X	500	4	496	4	0.99 ± 0.05	-0.11
1, F + X	500	2	498	2	0.99 ± 0.04	-0.04
2, S	500	0	500		—	—
2, F	500	0	500		—	—
2, X + S	500	2	498	2	0.99 ± 0.04	-0.04
2, X + F	500	2	498	2	0.99 ± 0.04	-0.04
2, S + X	500	5	495	5	0.99 ± 0.06	-0.14
2, F + X	500	3	497	3	1.00 ± 0.05	-0.08
3, S	500	0	500		—	—
3, F	500	0	500		—	—
3, X + S	500	3	497	3	1.00 ± 0.05	-0.08
3, X + F	500	6	494	6	0.99 ± 0.06	-0.17
3, S + X	500	3	497	3	1.00 ± 0.05	-0.08
3, F + X	500	6	494	6	0.99 ± 0.06	-0.17
4, S	500	0	500		—	—
4, F	500	0	500		—	—
4, X + S	500	13	487	13	0.98 ± 0.06	-0.40
4, X + F	500	2	498	2	0.99 ± 0.04	-0.04
4, S + X	500	3	497	3	1.00 ± 0.05	-0.08
4, F + X	500	6	494	6	0.99 ± 0.06	-0.17

Table A.2. The number of centric rings scored in the CA assay for the different exposure regimes using a 935 MHz GSM Basic signal at an SAR of 2 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of centric rings	Distribution of centric rings		σ^2 / y ± SE	U
			0	1		
1, S	500	0	500		—	—
1, F	500	0	500		—	—
1, X + S	500	0	500		—	—
1, X + F	500	3	497	3	1.00 ± 0.05	-0.08
1, S + X	500	0	500		—	—
1, F + X	500	0	500		—	—
2, S	500	0	500		—	—
2, F	500	0	500		—	—
2, X + S	500	1	499	1	—	—
2, X + F	500	1	499	1	—	—
2, S + X	500	1	499	1	—	—
2, F + X	500	0	500		—	—
3, S	500	0	500		—	—
3, F	500	0	500		—	—
3, X + S	500	1	499	1	—	—
3, X + F	500	2	498	2	0.99 ± 0.04	-0.04
3, S + X	500	2	498	2	0.99 ± 0.04	-0.04
3, F + X	500	2	498	2	0.99 ± 0.04	-0.04
4, S	500	0	500		—	—
4, F	500	0	500		—	—
4, X + S	500	2	498	2	0.99 ± 0.04	-0.04
4, X + F	500	2	498	2	0.99 ± 0.04	-0.04
4, S + X	500	1	499	1	—	—
4, F + X	500	0	500		—	—

Table A.3. The number of centric rings scored in the CA assay for the different exposure regimes using an 1800 MHz GSM Basic signal at an SAR of 2 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of centric rings	Distribution of centric rings		σ^2 / y ± SE	U
			0	1		
1, S	500	0	500		—	—
1, F	500	0	500		—	—
1, X + S	500	1	499	1	—	—
1, X + F	500	1	499	1	—	—
1, S + X	500	1	499	1	—	—
1, F + X	500	2	498	2	0.99 ± 0.04	-0.04
2, S	500	0	500		—	—
2, F	500	0	500		—	—
2, X + S	500	2	498	2	0.99 ± 0.04	-0.04
2, X + F	500	1	499	1	—	—
2, S + X	500	3	497	3	1.00 ± 0.05	-0.08
2, F + X	500	1	499	1	—	—
3, S	500	0	500		—	—
3, F	500	0	500		—	—
3, X + S	500	2	498	2	0.99 ± 0.04	-0.04
3, X + F	500	0	500		—	—
3, S + X	500	2	498	2	0.99 ± 0.04	-0.04
3, F + X	500	1	499	1	—	—
4, S	500	0	500		—	—
4, F	500	0	500		—	—
4, X + S	500	6	494	6	0.99 ± 0.06	-0.17
4, X + F	500	1	499	1	—	—
4, S + X	500	4	496	4	0.99 ± 0.05	-0.11
4, F + X	500	2	498	2	0.99 ± 0.04	-0.04

Table A.4. The number of excess acentrics scored in the CA assay for the different exposure regimes using a 935 MHz GSM Basic signal at 1 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of excess acentrics	Distribution of excess acentrics					σ^2 / y ± SE	U
			0	1	2	3	4		
1, S	500	2	498	2				1.00 ± 0.04	-0.04
1, F	500	3	497	3				1.00 ± 0.05	-0.08
1, X + S	500	66	438	58	4			0.99 ± 0.06	-0.14
1, X + F	500	41	461	37	2			1.02 ± 0.06	0.28
1, S + X	500	53	454	40	5	1		1.20 ± 0.06	3.16
1, F + X	500	44	456	44				0.91 ± 0.06	-1.38
2, S	500	1	499	1				—	—
2, F	500	1	499	1				—	—
2, X + S	500	46	462	30	8			1.26 ± 0.06	4.13
2, X + F	500	64	442	52	6			1.06 ± 0.06	0.98
2, S + X	500	68	441	51	7	1		1.16 ± 0.06	2.55
2, F + X	500	58	445	52	3			0.99 ± 0.06	-0.17
3, S	500	1	499	1				—	—
3, F	500	0	500					—	—
3, X + S	500	47	456	41	3			1.04 ± 0.06	0.57
3, X + F	500	44	460	36	4			1.10 ± 0.06	1.53
3, S + X	500	56	450	46	3	0	1	1.21 ± 0.06	3.38
3, F + X	500	44	459	38	3			1.05 ± 0.06	0.81
4, S	500	1	499	1				—	—
4, F	500	1	499	1				—	—
4, X + S	500	73	437	55	6	2		1.19 ± 0.06	2.95
4, X + F	500	85	424	68	7	1		1.07 ± 0.06	1.07
4, S + X	500	53	451	45	4			1.05 ± 0.06	0.75
4, F + X	500	58	449	45	5	1		1.16 ± 0.06	2.58

Table A.5. The number of excess acentrics scored in the CA assay for the different exposure regimes using a 935 MHz GSM Basic signal at 2 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of excess acentrics	Distribution of excess acentrics			σ^2 / y ± SE	U
			0	1	2		
1, S	500	0	500			—	—
1, F	500	2	498	2		1.00 ± 0.04	-0.04
1, X + S	500	29	472	27	1	1.01 ± 0.06	0.21
1, X + F	500	17	485	13	2	1.20 ± 0.06	3.32
1, S + X	500	28	473	26	1	1.02 ± 0.06	0.28
1, F + X	500	29	473	25	2	1.08 ± 0.06	1.32
2, S	500	1	499	1		—	—
2, F	500	1	499	1		—	—
2, X + S	500	26	476	22	2	1.10 ± 0.06	1.68
2, X + F	500	24	478	20	2	1.12 ± 0.06	1.95
2, S + X	500	32	471	26	3	1.23 ± 0.06	2.02
2, F + X	500	34	467	32	1	0.99 ± 0.06	-0.12
3, S	500	2	498	2		1.00 ± 0.04	-0.04
3, F	500	1	499	1		—	—
3, X + S	500	40	462	36	2	1.02 ± 0.06	0.35
3, X + F	500	43	460	37	3	1.06 ± 0.06	0.89
3, S + X	500	48	456	40	4	1.07 ± 0.06	1.16
3, F + X	500	29	472	27	1	1.01 ± 0.06	0.21
4, S	500	0	500			—	—
4, F	500	0	500			—	—
4, X + S	500	44	460	36	4	1.10 ± 0.06	1.53
4, X + F	500	53	452	43	5	1.09 ± 0.06	1.35
4, S + X	500	34	466	34		0.93 ± 0.06	-1.06
4, F + X	500	21	480	19	1	1.06 ± 0.06	0.90

Table A.6. The number of excess acentrics scored in the CA assay for the different exposure regimes using an 1800 MHz GSM Basic signal at 2 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of excess acentrics	Distribution of excess acentrics				σ^2 / y ± SE	U
			0	1	2	3		
1, S	500	2	498	2			1.00 ± 0.04	-0.04
1, F	500	1	499	1			—	—
1, X + S	500	32	471	26	3		1.13 ± 0.06	2.02
1, X + F	500	40	470	22	6	2	1.52 ± 0.06	8.37
1, S + X	500	31	469	31			0.94 ± 0.06	-0.97
1, F + X	500	34	470	26	4		1.17 ± 0.06	2.72
2, S	500	0	500				—	—
2, F	500	2	498	2			1.00 ± 0.04	-0.04
2, X + S	500	45	461	33	6		1.18 ± 0.06	2.86
2, X + F	500	36	467	30	3		1.10 ± 0.06	1.55
2, S + X	500	38	467	28	5		1.19 ± 0.06	3.03
2, F + X	500	28	473	26	1		1.02 ± 0.06	0.28
3, S	500	0	500				—	—
3, F	500	0	500				—	—
3, X + S	500	27	475	23	2		1.10 ± 0.06	1.55
3, X + F	500	26	476	22	2		1.10 ± 0.06	1.68
3, S + X	500	21	479	21			0.96 ± 0.06	-0.65
3, F + X	500	22	478	22			0.96 ± 0.06	-0.68
4, S	500	0	500				—	—
4, F	500	1	499	1			—	—
4, X + S	500	31	473	24	2	1	1.26 ± 0.06	4.23
4, X + F	500	25	475	25			0.95 ± 0.06	-0.78
4, S + X	500	21	479	21			0.96 ± 0.06	-0.65
4, F + X	500	22	479	20	1		1.05 ± 0.06	0.79

Table A.7. The number of chromatid aberrations scored in the CA assay for the different exposure regimes using a 935 MHz GSM Basic signal at an SAR of 1 W/kg. The distribution of total chromatid damage amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Chromatid gap	Chromatid break	Iso chromatid gap	Chromatid exchange	Total	Distribution of chromatid damage			σ^2 / y ± SE	U
							0	1	2		
1, S	500	5	1	0	0	6	494	6		0.99 ± 0.06	-0.17
1, F	500	5	1	0	0	6	494	6		0.99 ± 0.06	-0.17
1, X + S	500	15	1	0	0	16	484	16		0.97 ± 0.06	-0.49
1, X + F	500	4	3	0	0	7	493	7		0.99 ± 0.06	-0.21
1, S + X	500	8	3	1	0	12	488	12		0.98 ± 0.06	-0.36
1, F + X	500	8	4	1	0	13	487	13		0.98 ± 0.06	-0.40
2, S	500	2	1	1	0	4	496	4		0.99 ± 0.05	-0.11
2, F	500	19	1	0	0	20	480	20		0.96 ± 0.06	-0.62
2, X + S	500	16	4	0	0	20	480	20		0.96 ± 0.06	-0.62
2, X + F	500	9	6	0	1	16	485	14	1	1.10 ± 0.06	1.55
2, S + X	500	13	1	1	0	15	485	15		0.97 ± 0.06	-0.46
2, F + X	500	12	2	0	1	15	485	15		0.97 ± 0.06	-0.46
3, S	500	5	0	0	0	5	495	5		0.99 ± 0.06	-0.14
3, F	500	5	1	0	0	6	494	6		0.99 ± 0.06	-0.17
3, X + S	500	3	2	1	2	8	493	6	1	1.24 ± 0.06	3.99
3, X + F	500	25	3	0	1	29	472	27	1	1.01 ± 0.06	0.21
3, S + X	500	12	5	1	0	18	483	16	1	1.08 ± 0.06	1.26
3, F + X	500	2	3	0	0	5	495	5		0.99 ± 0.06	-0.14
4, S	500	4	1	0	1	6	494	6		0.99 ± 0.06	-0.17
4, F	500	3	2	0	0	5	495	5		0.99 ± 0.06	-0.14
4, X + S	500	4	2	0	0	6	495	4	1	1.32 ± 0.06	5.61
4, X + F	500	6	2	0	0	8	492	8		0.99 ± 0.06	-0.24
4, S + X	500	3	2	0	0	5	495	5		0.99 ± 0.06	-0.14
4, F + X	500	11	2	0	0	13	487	13		0.98 ± 0.06	-0.40

Table A.8. The number of chromatid aberrations scored in the CA assay for the different exposure regimes using a 935 MHz GSM Basic signal at an SAR of 2 W/kg. The distribution of total chromatid damage amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Chromatid gap	Chromatid break	Iso chromatid gap	Chromatid exchange	Total	Distribution of chromatid damage			σ^2 / y ± SE	U
							0	1	2		
1, S	500	8	0	0	0	8	492	8		0.99 ± 0.06	-0.24
1, F	500	3	0	0	0	3	497	3		1.00 ± 0.05	-0.08
1, X + S	500	0	0	0	0	0	500	0		—	—
1, X + F	500	3	0	0	0	3	497	3		1.00 ± 0.05	-0.08
1, S + X	500	2	2	0	1	5	495	5		0.99 ± 0.06	-0.14
1, F + X	500	3	2	0	0	5	495	5		0.99 ± 0.06	-0.14
2, S	500	7	0	0	0	7	493	7		0.99 ± 0.06	-0.21
2, F	500	3	0	0	0	3	497	3		1.00 ± 0.05	-0.08
2, X + S	500	9	0	0	0	9	491	9		0.98 ± 0.06	-0.27
2, X + F	500	3	2	0	0	5	495	5		0.99 ± 0.06	-0.14
2, S + X	500	7	2	0	0	9	492	7	1	1.21 ± 0.06	3.46
2, F + X	500	11	3	0	0	14	486	14		0.97 ± 0.06	-0.43
3, S	500	5	2	1	1	9	491	9		0.98 ± 0.06	-0.27
3, F	500	3	1	0	0	4	496	4		0.99 ± 0.05	-0.11
3, X + S	500	3	6	0	0	9	491	9		0.98 ± 0.06	-0.27
3, X + F	500	4	4	0	0	8	492	8		0.99 ± 0.06	-0.24
3, S + X	500	6	4	0	1	11	490	9	1	1.16 ± 0.06	2.69
3, F + X	500	3	0	0	0	3	497	3		1.00 ± 0.05	-0.08
4, S	500	3	1	0	0	4	496	4		0.99 ± 0.05	-0.11
4, F	500	3	0	0	0	3	497	3		1.00 ± 0.05	-0.08
4, X + S	500	3	4	0	0	7	493	7		0.99 ± 0.06	-0.21
4, X + F	500	3	2	0	0	5	495	5		0.99 ± 0.06	-0.14
4, S + X	500	3	2	0	0	5	495	5		0.99 ± 0.06	-0.14
4, F + X	500	4	0	0	0	4	497	2	1	1.50 ± 0.05	9.03

Table A.9. The number of chromatid aberrations scored in the CA assay for the different exposure regimes using an 1800 MHz GSM Basic signal at an SAR of 2 W/kg. The distribution of total chromatid damage amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Chromatid gap	Chromatid break	Iso chromatid gap	Chromatid exchange	Total	Distribution of chromatid damage			σ^2 / y ± SE	U
							0	1	2		
1, S	500	1	0	0	0	1	499	1		—	—
1, F	500	2	1	0	0	3	497	3		1.00 ± 0.05	-0.08
1, X + S	500	3	1	0	0	4	496	4		0.99 ± 0.05	-0.11
1, X + F	500	2	3	0	1	6	494	6		0.99 ± 0.06	-0.17
1, S + X	500	2	2	0	0	2	498	2		0.99 ± 0.04	-0.04
1, F + X	500	2	1	0	0	3	497	3		1.00 ± 0.05	-0.08
2, S	500	3	0	0	0	3	497	3		1.00 ± 0.05	-0.08
2, F	500	8	2	0	0	10	490	10		0.98 ± 0.06	-0.30
2, X + S	500	7	5	0	0	12	489	10	1	1.15 ± 0.06	2.39
2, X + F	500	4	2	0	0	6	494	6		0.99 ± 0.06	-0.17
2, S + X	500	6	3	1	0	10	490	10		0.98 ± 0.06	-0.30
2, F + X	500	3	3	0	1	7	494	5	1	1.27 ± 0.06	4.68
3, S	500	4	3	1	0	8	492	8		0.99 ± 0.06	-0.24
3, F	500	4	1	0	0	5	495	5		0.99 ± 0.06	-0.14
3, X + S	500	2	3	0	0	5	495	5		0.99 ± 0.06	-0.14
3, X + F	500	1	1	0	0	2	498	2		0.99 ± 0.04	-0.04
3, S + X	500	1	2	0	0	3	497	3		1.00 ± 0.05	-0.08
3, F + X	500	8	0	0	3	11	489	11		0.98 ± 0.06	-0.33
4, S	500	3	1	0	0	4	496	4		0.99 ± 0.05	-0.11
4, F	500	1	1	0	0	2	498	2		0.99 ± 0.04	-0.04
4, X + S	500	1	1	0	0	2	498	2		0.99 ± 0.04	-0.04
4, X + F	500	0	1	0	0	1	499	1		—	—
4, S + X	500	2	2	0	0	4	496	4		0.99 ± 0.05	-0.11
4, F + X	500	0	0	0	0	0	500	0		—	—

Table A.10. The number of centric rings scored in the CA assay for the different exposure regimes using a 935 MHz CW signal at an SAR of 1 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of centric rings	Distribution of centric rings		σ^2 / y ± SE	U
			0	1		
1, S	500	0	500		—	—
1, F	500	0	500		—	—
1, X + S	500	1	499	1	—	—
1, X + F	500	3	497	3	1.00 ± 0.05	-0.08
1, S + X	500	0	500		—	—
1, F + X	500	1	499	1	—	—
2, S	500	0	500		—	—
2, F	500	0	500		—	—
2, X + S	500	2	498	2	0.99 ± 0.04	-0.04
2, X + F	500	1	499	1	—	—
2, S + X	500	0	500		—	—
2, F + X	500	1	499	1	—	—
3, S	500	0	500		—	—
3, F	500	0	500		—	—
3, X + S	500	4	496	4	0.99 ± 0.05	-0.11
3, X + F	500	0	500		—	—
3, S + X	500	2	498	2	0.99 ± 0.04	-0.04
3, F + X	500	0	500		—	—
4, S	500	0	500		—	—
4, F	500	0	500		—	—
4, X + S	500	1	499	1	—	—
4, X + F	500	2	498	2	0.99 ± 0.04	-0.04
4, S + X	500	2	498	2	0.99 ± 0.04	-0.04
4, F + X	500	2	498	2	0.99 ± 0.04	-0.04

Table A.11. The number of centric rings scored in the CA assay for the different exposure regimes using a 935 MHz GSM Talk signal at an SAR of 1 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of centric rings	Distribution of centric rings		σ^2 / y ± SE	U
			0	1		
1, S	500	0	500		—	—
1, F	500	0	500		—	—
1, X + S	500	1	499	1	—	—
1, X + F	500	7	493	7	0.99 ± 0.06	-0.21
1, S + X	500	0	500		—	—
1, F + X	500	1	499	1	—	—
2, S	500	0	500		—	—
2, F	500	0	500		—	—
2, X + S	500	0	500		—	—
2, X + F	500	0	500		—	—
2, S + X	500	0	500		—	—
2, F + X	500	3	497	3	1.00 ± 0.05	-0.08
3, S	500	1	499	1	—	—
3, F	500	0	500		—	—
3, X + S	500	2	498	2	0.99 ± 0.04	-0.04
3, X + F	500	1	499	1	—	—
3, S + X	500	2	498	2	0.99 ± 0.04	-0.04
3, F + X	500	0	500		—	—
4, S	500	0	500		—	—
4, F	500	0	500		—	—
4, X + S	500	3	497	3	1.00 ± 0.05	-0.08
4, X + F	500	3	497	3	1.00 ± 0.05	-0.08
4, S + X	500	3	497	3	1.00 ± 0.05	-0.08
4, F + X	500	2	498	2	0.99 ± 0.04	-0.04

Table A.12. The number of centric rings scored in the CA assay for the different exposure regimes using a 935 MHz GSM Talk intermittent signal at an SAR of 1 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of centric rings	Distribution of centric rings		σ^2 / y ± SE	U
			0	1		
1, S	500	0	500		—	—
1, F	500	0	500		—	—
1, X + S	500	3	497	3	1.00 ± 0.05	-0.08
1, X + F	500	1	499	1	—	—
1, S + X	500	2	498	2	0.99 ± 0.04	-0.04
1, F + X	500	2	498	2	0.99 ± 0.04	-0.04
2, S	500	0	500		—	—
2, F	500	0	500		—	—
2, X + S	500	1	499	1	—	—
2, X + F	500	2	498	2	0.99 ± 0.04	-0.04
2, S + X	500	1	499	1	—	—
2, F + X	500	1	499	1	—	—
3, S	500	0	500		—	—
3, F	500	0	500		—	—
3, X + S	500	2	498	2	0.99 ± 0.04	-0.04
3, X + F	500	3	497	3	1.00 ± 0.05	-0.08
3, S + X	500	2	498	2	0.99 ± 0.04	-0.04
3, F + X	500	2	498	2	0.99 ± 0.04	-0.04
4, S	500	0	500		—	—
4, F	500	0	500		—	—
4, X + S	500	1	499	1	—	—
4, X + F	500	3	497	3	1.00 ± 0.05	-0.08
4, S + X	500	0	500		—	—
4, F + X	500	3	497	3	1.00 ± 0.05	-0.08

Table A.13. The number of excess acentrics scored in the CA assay for the different exposure regimes using a 935 MHz CW signal at 1 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of excess acentrics	Distribution of excess acentrics				σ^2 / y ± SE	U
			0	1	2	3		
1, S	500	2	498	2			1.00 ± 0.04	-0.04
1, F	500	3	497	3			1.00 ± 0.05	-0.08
1, X + S	500	25	478	19	3		1.19 ± 0.06	3.10
1, X + F	500	24	477	22	1		1.04 ± 0.06	0.60
1, S + X	500	26	475	24	1		1.03 ± 0.06	0.43
1, F + X	500	16	485	14	1		1.1 ± 0.06	1.55
2, S	500	0	500				—	—
2, F	500	0	500				—	—
2, X + S	500	40	462	36	2		1.02 ± 0.06	0.35
2, X + F	500	18	482	18			0.97 ± 0.06	-0.55
2, S + X	500	21	479	21			0.96 ± 0.06	-0.65
2, F + X	500	35	468	29	3		1.10 ± 0.06	1.66
3, S	500	0	500				—	—
3, F	500	1	499	1			—	—
3, X + S	500	29	472	27	1		1.01 ± 0.06	2.09
3, X + F	500	21	479	21			0.96 ± 0.06	-0.65
3, S + X	500	44	464	30	4	2	1.37 ± 0.06	5.90
3, F + X	500	24	478	20	2		1.12 ± 0.06	1.95
4, S	500	0	500				—	—
4, F	500	0	500				—	—
4, X + S	500	31	473	23	4		1.20 ± 0.06	3.19
4, X + F	500	24	479	18	3		1.20 ± 0.06	3.30
4, S + X	500	29	474	23	3		1.15 ± 0.06	2.43
4, F + X	500	34	468	30	2		1.05 ± 0.06	0.83

Table A.14. The number of excess acentrics scored in the CA assay for the different exposure regimes using a 935 MHz GSM Talk signal at an SAR of 1 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of excess acentrics	Distribution of excess acentrics				σ^2 / y ± SE	U
			0	1	2	3		
1, S	500	2	498	2			1.00 ± 0.04	-0.04
1, F	500	0	500				—	—
1, X + S	500	41	464	32	3	1	1.21 ± 0.06	3.41
1, X + F	500	45	458	39	3		1.05 ± 0.06	0.73
1, S + X	500	32	470	28	2		1.06 ± 0.06	1.01
1, F + X	500	38	466	30	4		1.14 ± 0.06	2.19
2, S	500	0	500				—	—
2, F	500	0	500				—	—
2, X + S	500	29	474	23	3		1.15 ± 0.06	2.43
2, X + F	500	27	474	25	1		1.02 ± 0.06	0.36
2, S + X	500	21	479	21			0.96 ± 0.06	-0.65
2, F + X	500	34	468	30	2		1.05 ± 0.06	0.83
3, S	500	1	499	1			—	—
3, F	500	0	500				—	—
3, X + S	500	45	462	32	5	1	1.27 ± 0.06	4.28
3, X + F	500	22	479	20	1		1.05 ± 0.06	0.79
3, S + X	500	26	475	24	1		1.03 ± 0.06	0.43
3, F + X	500	35	471	24	4	1	1.33 ± 0.06	5.33
4, S	500	1	499	1			—	—
4, F	500	0	500				—	—
4, X + S	500	29	473	25	2		1.08 ± 0.06	1.32
4, X + F	500	37	470	25	3	2	1.42 ± 0.06	6.65
4, S + X	500	37	468	27	5		1.20 ± 0.06	3.18
4, F + X	500	32	470	28	2		1.06 ± 0.06	1.01

Table A.15. The number of excess acentrics scored in the CA assay for the different exposure regimes using a 935 MHz GSM Talk intermittent signal at an SAR of 1 W/kg. Their distribution amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Number of excess acentrics	Distribution of excess acentrics				σ^2 / y ± SE	U
			0	1	2	3		
1, S	500	0	500				—	—
1, F	500	0	500				—	—
1, X + S	500	36	467	30	3		1.10 ± 0.06	1.55
1, X + F	500	27	473	27			0.95 ± 0.06	-0.84
1, S + X	500	24	477	22	1		1.04 ± 0.06	0.60
1, F + X	500	23	479	19	2		1.13 ± 0.06	2.10
2, S	500	1	499	1			—	—
2, F	500	0	500				—	—
2, X + S	500	27	475	23	2		1.10 ± 0.06	1.55
2, X + F	500	30	470	30			0.94 ± 0.06	-0.93
2, S + X	500	20	480	20			0.96 ± 0.06	-0.62
2, F + X	500	27	474	25	1		1.02 ± 0.06	0.36
3, S	500	0	500				—	—
3, F	500	1	499	1			—	—
3, X + S	500	24	476	24			0.95 ± 0.06	-0.74
3, X + F	500	31	471	27	2		1.07 ± 0.06	1.11
3, S + X	500	31	471	27	2		1.07 ± 0.06	1.11
3, F + X	500	33	467	33			0.94 ± 0.06	-1.03
4, S	500	0	500				—	—
4, F	500	0	500				—	—
4, X + S	500	27	474	25	1		1.02 ± 0.06	0.36
4, X + F	500	36	471	23	5	1	1.38 ± 0.06	6.01
4, S + X	500	18	482	18			0.97 ± 0.06	-0.55
4, F + X	500	24	476	24			0.95 ± 0.06	-0.74

Table A.16. The number of chromatid aberrations scored in the CA assay for the different exposure regimes using a 935 MHz CW signal at an SAR of 1 W/kg. The distribution of total chromatid damage amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Chromatid gap	Chromatid break	Iso chromatid gap	Chromatid exchange	Total	Distribution of chromatid damage		σ^2 / y ± SE	U
							0	1		
1, S	500	1	1	0	0	3	497	3	1.00 ± 0.05	-0.08
1, F	500	1	2	0	0	3	497	3	1.00 ± 0.05	-0.08
1, X + S	500	1	1	0	0	2	498	2	0.99 ± 0.04	-0.04
1, X + F	500	0	0	0	0	0	500	0	—	—
1, S + X	500	1	3	0	0	4	496	4	0.99 ± 0.05	-0.11
1, F + X	500	1	1	0	1	3	497	3	1.00 ± 0.05	-0.08
2, S	500	0	0	0	0	1	499	1	—	—
2, F	500	0	0	0	1	0	500	0	—	—
2, X + S	500	1	2	0	0	3	497	3	1.00 ± 0.05	-0.08
2, X + F	500	2	0	0	0	2	498	2	0.99 ± 0.04	-0.04
2, S + X	500	1	2	0	0	3	497	3	1.00 ± 0.05	-0.08
2, F + X	500	1	0	0	1	2	498	2	0.99 ± 0.04	-0.04
3, S	500	0	0	0	0	0	500	0	—	—
3, F	500	0	1	0	0	1	499	1	—	—
3, X + S	500	0	1	0	0	1	499	1	—	—
3, X + F	500	4	1	1	0	6	494	6	0.99 ± 0.06	-0.17
3, S + X	500	3	2	0	0	5	495	5	0.99 ± 0.06	-0.14
3, F + X	500	4	1	0	1	6	494	6	0.99 ± 0.06	-0.17
4, S	500	0	1	0	0	1	499	1	—	—
4, F	500	2	0	0	0	2	498	2	0.99 ± 0.04	-0.04
4, X + S	500	0	2	0	0	2	498	2	0.99 ± 0.04	-0.04
4, X + F	500	1	0	0	0	1	499	1	—	—
4, S + X	500	2	2	0	1	5	495	5	0.99 ± 0.06	-0.14
4, F + X	500	3	1	0	0	4	496	4	0.99 ± 0.05	-0.11

Table A.17. The number of chromatid aberrations scored in the CA assay for the different exposure regimes using a 935 MHz GSM Talk signal at an SAR of 1 W/kg. The distribution of total chromatid damage amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Chromatid gap	Chromatid break	Iso chromatid gap	Chromatid exchange	Total	Distribution of chromatid damage		σ^2 / y ± SE	U
							0	1		
1, S	500	2	0	0	0	2	498	2	0.99 ± 0.04	-0.04
1, F	500	0	0	0	0	0	500	0	—	—
1, X + S	500	1	1	0	0	2	498	2	0.99 ± 0.04	-0.04
1, X + F	500	7	1	0	0	0	500	0	—	—
1, S + X	500	1	1	0	0	2	498	2	0.99 ± 0.04	-0.04
1, F + X	500	1	3	0	0	4	496	4	0.99 ± 0.05	-0.11
2, S	500	1	1	0	0	2	498	2	0.99 ± 0.04	-0.04
2, F	500	3	0	0	0	3	497	3	1.00 ± 0.05	-0.08
2, X + S	500	3	2	0	0	5	495	5	0.99 ± 0.06	-0.14
2, X + F	500	4	2	0	0	6	494	6	0.99 ± 0.06	-0.17
2, S + X	500	2	1	0	0	3	497	3	1.00 ± 0.05	-0.08
2, F + X	500	5	0	0	0	5	495	5	0.99 ± 0.06	-0.14
3, S	500	2	2	0	0	4	496	4	0.99 ± 0.05	-0.11
3, F	500	4	1	0	0	5	495	5	0.99 ± 0.06	-0.14
3, X + S	500	2	0	0	0	0	500	0	—	—
3, X + F	500	4	1	0	0	5	495	5	0.99 ± 0.06	-0.14
3, S + X	500	5	2	0	0	7	493	7	0.99 ± 0.06	-0.21
3, F + X	500	1	3	0	3	7	493	7	0.99 ± 0.06	-0.21
4, S	500	0	0	0	0	0	500	0	—	—
4, F	500	0	0	0	0	0	500	0	—	—
4, X + S	500	5	2	0	0	7	493	7	0.99 ± 0.06	-0.21
4, X + F	500	0	2	0	0	2	498	2	0.99 ± 0.04	-0.04
4, S + X	500	0	0	0	0	0	500	0	—	—
4, F + X	500	0	1	0	0	1	499	1	—	—

Table A.18. The number of chromatid aberrations scored in the CA assay for the different exposure regimes using a 935 MHz GSM Talk intermittent signal at an SAR of 1 W/kg. The distribution of total chromatid damage amongst the cells is also shown.
S = sham; X = 1 Gy x-ray; F = RF field

Donor, experimental condition	Number of cells scored	Chromatid gap	Chromatid break	Iso chromatid gap	Chromatid exchange	Total	Distribution of chromatid damage			σ^2 / y ± SE	U
							0	1	2		
1, S	500	0	1	0	0	1	499	1		—	—
1, F	500	2	1	0	0	3	498	1	1	1.66 ± 0.05	12.85
1, X + S	500	10	3	1	1	15	485	15		0.97 ± 0.06	-0.46
1, X + F	500	5	2	0	0	7	493	7		0.99 ± 0.06	-0.21
1, S + X	500	2	5	0	0	7	493	7		0.99 ± 0.06	-0.21
1, F + X	500	3	2	1	0	6	494	6		0.99 ± 0.06	-0.17
2, S	500	1	0	0	0	1	499	1		—	—
2, F	500	3	1	0	1	5	495	5		0.99 ± 0.06	-0.14
2, X + S	500	3	0	0	0	3	498	1	1	1.66 ± 0.05	12.85
2, X + F	500	8	2	0	0	10	490	10		0.98 ± 0.06	-0.30
2, S + X	500	2	2	0	0	4	496	4		0.99 ± 0.05	-0.11
2, F + X	500	5	0	0	0	5	495	5		0.99 ± 0.06	-0.14
3, S	500	2	0	0	0	2	498	2		0.99 ± 0.04	-0.04
3, F	500	2	0	0	0	2	498	2		0.99 ± 0.04	-0.04
3, X + S	500	4	0	0	0	1	499	1		—	—
3, X + F	500	1	3	0	0	4	496	4		0.99 ± 0.05	-0.11
3, S + X	500	6	1	0	0	7	493	7		0.99 ± 0.06	-0.21
3, F + X	500	1	11	0	0	2	498	2		0.99 ± 0.04	-0.04
4, S	500	1	0	0	0	1	499	1		—	—
4, F	500	0	0	0	0	0	500	0		—	—
4, X + S	500	2	1	0	0	3	497	3		1.00 ± 0.05	-0.08
4, X + F	500	1	1	0	0	2	498	2		0.99 ± 0.04	-0.04
4, S + X	500	1	0	0	0	1	499	1		—	—
4, F + X	500	3	1	0	0	4	496	4		0.99 ± 0.05	-0.11

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935 MHz cellular phone radiation. An *in vitro* study of genotoxicity in human lymphocytes

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Abstract

Purpose: The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with x-rays was investigated *in vitro* using several assays on human lymphocytes. The chosen specific absorption rate (SAR) values are near the upper limit of actual energy absorption in localized tissue when persons use some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen.

Methods: Blood specimens from 14 donors were exposed continuously for 24 h to a Global System for Mobile Communications (GSM) basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of *in vitro* cell cycling.

Results: By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did not modify any measured effects of the x-radiation.

Conclusions: This study has used several standard *in vitro* tests for chromosomal and DNA damage in Go human lymphocytes exposed *in vitro* to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic *per se* or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed.

Keywords: *Lymphocyte, genotoxicity, radiofrequency, cellular phone*

Introduction

The scientific literature contains a number of publications describing studies that have investigated whether radiofrequency radiation (RFR), such as that produced by cellular telephones, is genotoxic (extensively reviewed by Vijayalaxmi & Obe 2004). The majority of studies have concluded that, in the absence of significant heating, it is not genotoxic, but a minority of reports do suggest an effect of RFR on cells exposed *in vitro* and assayed by several standard tests for chromosomal or DNA breakage. The general consensus view is that RFR *per se* is not genotoxic but sufficient numbers of reports expressing a contrary view exist for the question still to remain open. A recent report (Diem et al. 2005), for example, has suggested genotoxicity *in vitro* is confined only to certain cell types and only when exposure to RFR is intermittent.

The occasional reports of genotoxicity may be indicating that whilst RFR is not directly genotoxic it might be operating indirectly in a more subtle epigenetic manner. One plausible possibility has been raised that RFR may potentiate the effect of other undisputed environmental mutagens, perhaps by compromising the fidelity of repair of initial

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DNA damage. An enhanced tendency to mis- or non-repair will be expressed as a greater frequency of visible chromosomal alterations or strand breakage in the various experimental assays.

Maes et al. (1996) showed such an enhancing effect of RFR on chromosomal damage and strand breakage induced by the mutagenic chemical mitomycin C in human lymphocytes. This work attracted considerable attention but the group reported further work that failed to confirm the magnitude of their original finding. They reported (Maes et al. 1997) a much weaker synergistic effect; then an inconsistent synergistic effect (Maes et al. 2000) and finally no synergism (Maes et al. 2001). The present paper further explores the idea of RFR acting in combination with a proven mutagen. The chosen mutagen was x-rays for which the genotoxic effects with several assays are well documented.

Materials and methods

A series of experiments was performed jointly by two laboratories; the Italian National Agency for New Technologies, Energy and the Environment (ENEA) and the UK Health Protection Agency (HPA). Heparinized whole blood was taken with informed consent and in accordance with both institutions' ethics procedures from normal, healthy, non-smoker donors. Four subjects, 2 male aged 59 and 61 y and 2 female; 42 and 47 y, were used at HPA and 10 donors, all male; 30–45 y, at ENEA. In all studies each laboratory used 1.0 Gy of 250 kVp x-rays at a dose rate of 1.0 Gy/min. These irradiations were carried out in 35 mm Petri dishes at room temperature (20°C) and the specimens were also maintained at this temperature whilst being conveyed for ~5 min between the x-ray and RFR facilities. Each x-irradiated blood sample from each donor was accompanied by a parallel sample that received zero dose. For the RFR exposures the blood still in the Petri dishes was exposed for 24 h either before or after x-rays. RFR exposures were carried out at 37°C in identical waveguide apparatuses (Information Technologies in Society Foundation [IT'IS], Zurich) installed in tissue culture incubators with humidified atmospheres of 5% CO_2 in air. The exposures were to a GSM basic 935 MHz signal. Full details of the apparatus, the signal profile, the exposure geometry and dosimetry is described by Schuderer et al. (2004). The measured increase in temperature of the cells, due to the RFR was around 0.05°C which is considered sufficiently low that any effect observed could be attributed to non-thermal processes.

Both laboratories performed their respective genotoxicity assays on blood exposed at a Specific Absorption Rate (SAR) of 1 W/Kg and, additionally, one laboratory obtained data at 2 W/Kg. All RFR exposures were accompanied with parallel sham-exposures in an identical waveguide within the incubator. On each occasion the assignment of either waveguide to be the sham or active was made randomly by the controlling computer and the coding for this was held at IT'IS until broken when the assay data were complete. Following the x-ray, RFR, and control exposures the blood samples were aliquoted and processed for chromosomal and comet assays. Microscope slides for all assays were additionally coded for 'blind' analysis.

Both laboratories performed the chromosome aberration (CA) and micronucleus (MN) assays. HPA additionally measured the sister chromatid exchanges (SCE) whilst ENEA undertook the alkaline ($pH \geq 13$) comet assay for DNA strand breakage. The MN and SCE techniques also provided data that could be used to measure the speed of *in vitro* cellular proliferation: the nuclear division indices (NDI_{MN} or NDI_{SCE}).

The comet assay was performed on unstimulated cells essentially by a standard technique with minor modifications (Singh et al. 1988, Stronati et al. 2004). Slides were analysed by a computerized image analysis system and to evaluate the amount of DNA damage three endpoints; computer generated tail moment (tail length × fraction of total DNA in the tail), tail DNA fraction and tail length were used. For each donor/data point mean values were calculated from 100 cells (50 from each of two replicate slides). Slides were examined, at ×200 magnification and comets were analysed by a computerized image analysis system (Delta Sistemi, Rome, Italy).

For the remaining assays whole blood was cultured for 48 or 72 h, as appropriate, in an incubator at 37°C with an atmosphere of 5% CO_2 in air, using Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen Paisley, UK) supplemented with 10% foetal calf serum (Invitrogen), 2% phytohaemagglutinin (Invitrogen) (PHA), and 1.5% penicillin-streptomycin (Invitrogen). For the CA and SCE assays 5-bromodeoxyuridine (Sigma) was also included at a final concentration of 10 μg/ml and Colcemid (Sigma Poole, UK) at 0.2 μg/ml was added 3 h before termination with hypotonic potassium chloride solution and fixation in methanol/acetic acid (International Atomic Energy Agency [IAEA] 2001). Air-dried preparations were stained with fluorescence plus Giemsa to ensure that first and second division metaphases were scored for CA and SCE respectively. For MN, cytochalasin B (Sigma) was added (6 μg/ml) at 44 h to block cytokinesis and air dried preparations stained in Giemsa (Fenech 2000).

For the CA assay chromosome and chromatid-type aberrations were recorded, but not gaps. At ENEA, where 10 donors were used, 200 metaphases per donor per data point were scored whilst at HPA, with 4 donors, the value was 500 metaphases. For the SCE assay non-centromeric switches were scored in 50 metaphases per donor per point; 1000 binucleated cells with well-preserved cytoplasm were scored per donor per data point for MN.

In vitro cell cycling speed was determined in two ways. Firstly, the micronucleus assay slides were re-examined with 1000 cells per donor per point to determine the relative numbers of cells with 1–4 nuclei. This allows the calculation of a Nuclear Division Index (NDI) (Eastmond & Tucker 1989):

$$NDI = NI + 2NII + 3NIII + 4NIV/N \quad (1)$$

where N is the total number of cells and NI, NII, NIII and NIV are respectively the number of cells with one, two, three and four nuclei. Secondly, the differentially 'harlequin'-stained slides for the SCE assay were re-examined (200 metaphases per donor per point) to determine the relative numbers of metaphases that had completed 1–4 *in vitro* cell cycles by 72 h (Purrott et al. 1980). These data then permit an NDI to be calculated using the same form of equation as given above.

Decoded data were subjected to statistical analysis. For the CA, MN and SCE assays the distributions of damage among the scored cells from each donor were tested for conformity with the Poisson distribution. Over-dispersion for any data set was taken into account by an appropriate increase in the Poisson standard error (SE). The data from the individual donors in each laboratory were then pooled and tested for homogeneity using the chi-squared test for each of the exposure regimes. Inhomogeneity was taken into account by an appropriate increase to the SE on the mean of the pooled data point. For the comet assay, the two tailed paired Student's *t*-test was used to determine the statistical difference in the mean values, between treated and untreated samples and between co-exposed and X-ray only exposed samples. The calculation of uncertainties on the Nuclear Division Index were calculated by rewriting Equation 1 as:

$$NDI = (4N - 3NI - 2NII - NIII)/N \quad (2)$$

The standard error on NDI was then estimated by combining the binomial standard errors on NI, NII, and NIII assuming the variables are independent. The independence assumption is not true but a more rigorous method for estimating error does not appear to exist.

Results and discussion

In view of the several assays deployed, together with the duplication of some assays in two laboratories, the duplication of some assays at 2 SAR, the use of 14 blood donors and reversing the order that the specimens were exposed to x-rays and RFR, always accompanied with controls and shams, the study generated a large amount of data. These detailed data are preserved and could be made available on request. For presentation here in Tables I and II it has been necessary to combine data by pooling the results from replicate donors within each assay in each laboratory and calculating means with standard errors. These combined results are also plotted in summary Figures 1–6 where for ease of presentation the data from each laboratory have been normalized to an appropriate mean or a sham as described below.

Figure 1 presents the results for the alkaline comet assay performed at 1 W/Kg. The three endpoints from this assay; tail length, tail DNA fraction and tail moment have been normalized independently to the sham (S) result. Inspection of the data showed wide individual variations for all of the three assays. The data are therefore presented as the ratio of each exposure to the sham, averaging the ratio to the sham for each individual donor Uncertainties were then calculated as the standard error of the mean of the individual ratios, for the 10 donors used. The slight reductions in the values for RFR exposure only (F) compared with the sham (S) are not statistically significant. The comparison of F+X and S+X shows no difference while they both indicate a statistically significant increase of all damage parameters with respect to the sham controls, due to the effect of the ionizing radiation. The X+F and X+S comparison shows no significant difference either, but in this case their increase above the sham is less significant. This is because in this sequence of treatment, with x-rays first, the strand breaks that they induce are mostly repaired by the time (~24 h later) that the cells were subjected to the comet assay. Taking the comet assay results overall, RFR exposure alone is not genotoxic, nor is there any indication that in combination with x-rays it modifies their effect.

The comet assay is an indicator of primary DNA lesions whereas CA and MN represent the residue of unrepaired or misrepaired lesions. They are assayed after at least one full cell cycle has been completed by which time, repair processes are essentially finished. No significant induction of chromatid-type aberrations was noted for any of the exposure regimes. This was to be expected as the lymphocytes were in G_0 at this time. Chromosome-types; dicentrics, centric rings and excess acentric fragments, were however seen and, for convenience, the data for dicentrics

Table I. Mean yields per cell ± SE of pooled data from 4 HPA and 10 ENEA donors for various assays on blood exposed at an SAR of 1 W/kg with and without x-irradiation. S, sham; X, x-rays; F, radiofrequency field.

Exposure regime	HPA	ENEA	HPA	ENEA	HPA	ENEA		
	Dicentric yield ± SE	Dicentric yield ± SE	Micronucleus yield ± SE	Micronucleus yield ± SE	SCE yield ± SE	Mean tail length ± SE	Mean tail fraction ± SE	Mean tail moment ± SE
S	0.0005 ± 0.0005	0.002 ± 0.001	0.015 ± 0.00274	0.0051 ± 0.00071	5.03 ± 0.1585	7.41 ± 0.93	0.067 ± 0.008	1.99 ± 0.33
F	0	0.0005 ± 0.0005	0.0135 ± 0.0026	0.0058 ± 0.00076	5.33 ± 0.1632	6.25 ± 0.86	0.055 ± 0.007	1.63 ± 0.31
X+S	0.1715 ± 0.00923	0.0625 ± 0.00559	0.167 ± 0.01001	0.0551 ± 0.00235	5.16 ± 0.1606	9.74 ± 1.95	0.088 ± 0.017	2.98 ± 0.88
X+F	0.1585 ± 0.0089	0.059 ± 0.00543	0.1615 ± 0.00984	0.0461 ± 0.00215	5.27 ± 0.1623	8.23 ± 1.07	0.068 ± 0.01	2.08 ± 0.43
S+X	0.1755 ± 0.00937	0.0675 ± 0.00581	0.1605 ± 0.00981	0.0499 ± 0.00223	5.265 ± 0.1623	12.24 ± 1.56	0.107 ± 0.015	3.93 ± 0.83
F+X	0.1525 ± 0.00873	0.0645 ± 0.00568	0.1625 ± 0.00987	0.0495 ± 0.00223	4.97 ± 0.1576	11.59 ± 1.36	0.101 ± 0.013	3.55 ± 0.65

only scored in each laboratory at 1 W/Kg and at 2 W/Kg in one lab are shown in Figure 2. The results with the other unstable types of CA lead to identical conclusions. Figure 2 shows that dicentrics are only present for the exposure regimes that included x-rays. Exposure to only the RFR field (F) produced an aberration frequency consistent with the sham only (S) and these were in accordance with the expected background frequencies from the historical control databases in the two laboratories. The remaining data have been normalized separately for each of the two data sets at 1 W/Kg and one at 2 W/Kg. Thus the ratios 1.0 were calculated as the average in each data set of X + S and S + X, i.e., the dicentric yields in cells exposed to x-rays only. The uncertainties shown are standard errors calculated by using the Poisson distribution because there was no sign of over-dispersion or inhomogeneity. It is clearly apparent that the 4 sets of positive columns in Figure 2 are statistically indistinguishable; RFR exposure either before or after x-rays resulted in dicentric frequencies the same as from x-rays alone. The statistical strength of these data is such that differences of 15–20% would be detected and, because in some instances the yields in the field were actually slightly lower, albeit not significantly, than those in the sham, a genotoxic or epigenetic influence of RFR on the aberration frequencies of ~10% or higher can be excluded.

Figure 3 presents the results for the micronucleus assay using the same format and normalizing procedure as shown for dicentrics in Figure 2. The same conclusion can be drawn from the MN results; namely, that the RFR alone is not genotoxic and when combined with x-rays it does not moderate the genotoxicity of the ionizing radiation. With this assay many individual data points did exhibit significant over-dispersion. The average ratio of variance to mean was 1.2 and this is an expected feature for micronuclei (Prosser et al. 1988) and was reflected in the assignment of statistical uncertainties to the data. The data sets based on 4 donors showed homogeneity whilst that based on 10 donors was heterogeneous. Therefore for the latter, in comparing the different exposure regimes, a paired difference test was carried out. Only for one donor was there a difference between the field and sham exposures (X + F vs. X + S) and that represented a protective effect of the field. Overall, the pooled data from each lab showed no genotoxicity associated with RFR. The statistical strength of the data sets based on 4 donors could have detected a difference of about 20% and can reject a positive effect of the same magnitude. The pooled data from 10 donors in the other laboratory could also have detected a difference of about 20% but can reject the presence of a genotoxic effect of about 10%.

Table II. Mean yields per cell ± SE of pooled data from 4 HPA donors for various assays on blood exposed at an SAR of 2 W/kg with and without x-irradiation. S, sham; X, x-rays; F, radiofrequency field.

	HPA		
Exposure regime	Dicentric yield ± SE	Micronucleus yield ± SE	SCE yield ± SE
S	0.001 ± 0.00071	0.0095 ± 0.00218	5.405 ± 0.1644
F	0.0005 ± 0.0005	0.0095 ± 0.00218	5.175 ± 0.1609
X+S	0.082 ± 0.0064	0.151 ± 0.00869	5.22 ± 0.1616
X+F	0.0895 ± 0.00669	0.1495 ± 0.00865	5.36 ± 0.1637
S+X	0.085 ± 0.00652	0.1525 ± 0.00873	5.425 ± 0.1647
F+X	0.082 ± 0.0064	0.133 ± 0.00816	5.26 ± 0.1622

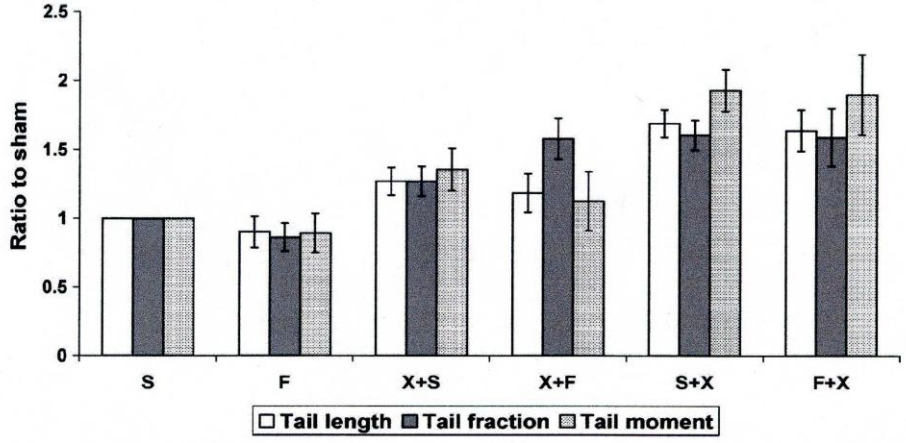

Figure 1. Effects of the different exposure regimes on comet parameters. Results for each assay are reported as ratios to its sham control value. Columns and error bars show the average of the ratios obtained from 10 donors and their standard errors of the mean. S, sham; X, x-rays; F, radiofrequency field.

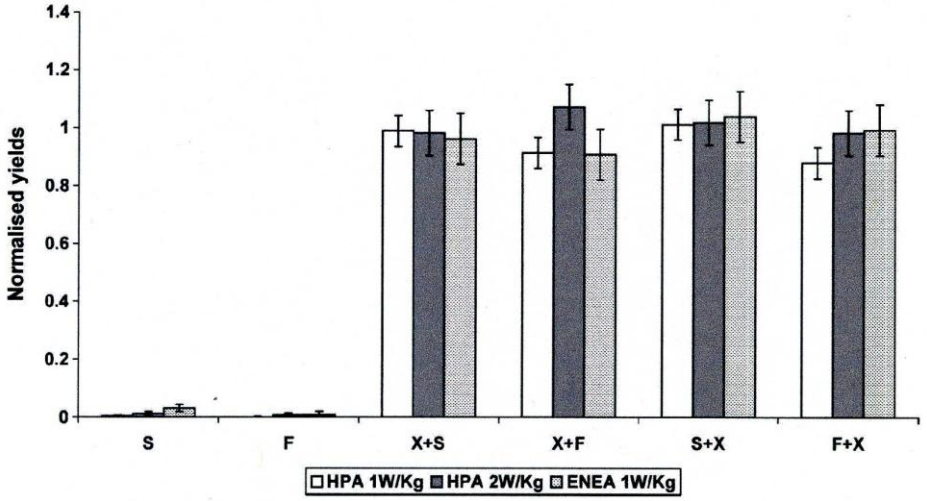

Figure 2. Pooled dicentric chromosomal aberration yields for the different exposure regimes normalised to the average for (X+S) and (S+X). Error bars are Poisson SE. S, sham; X, x-rays; F, radiofrequency field.

In Figure 4 the results for the SCE assay are shown and the data sets for each SAR have been normalized separately using the three sham values, i.e., the mean of S + (X + S) + (S + X). The grounds for doing this are that the SCE frequency was not dependent on x-ray dose, and indeed X-irradiation is acknowledged as being inefficient in inducing SCE. The rationale for including this assay was therefore primarily to explore for any genotoxicity of the combination of the two agents. At about 5 SCE/cell, the frequency was consistent throughout with the normal control level in that laboratory and thus no enhancement was observed with any of the exposure regimes.

The MN and SCE assays used cell culture times of 72 h during which the initially synchronized G_0 lymphocytes would have developed a degree of asynchrony. Thus some PHA responsive cells would have reached their first metaphase whilst the progeny of others would have progressed further into their second, third or even fourth cycles. The relative numbers of cells at these different stages gives

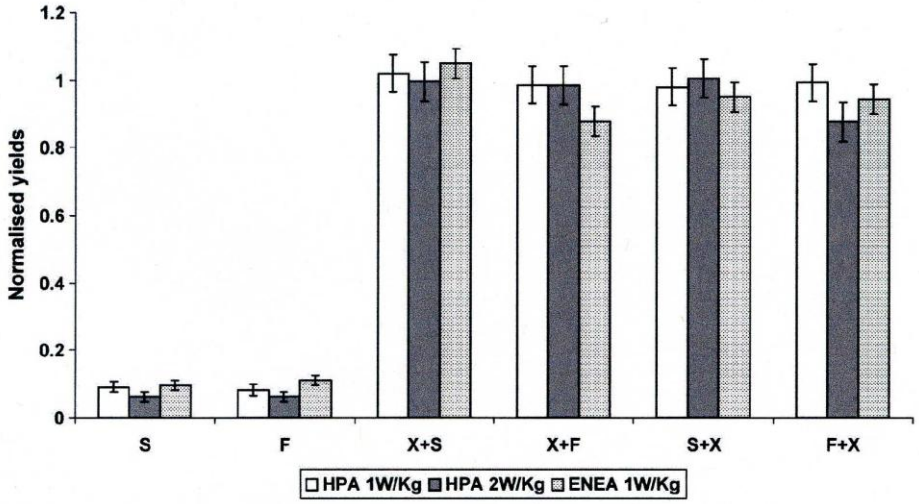

Figure 3. Pooled micronucleus yields for the different exposure regimes normalized to the average for X + S and S + X. Error bars are based on the Poisson distribution but enhanced to allow for over-dispersion. S, sham; X, x-rays; F, radiofrequency field.

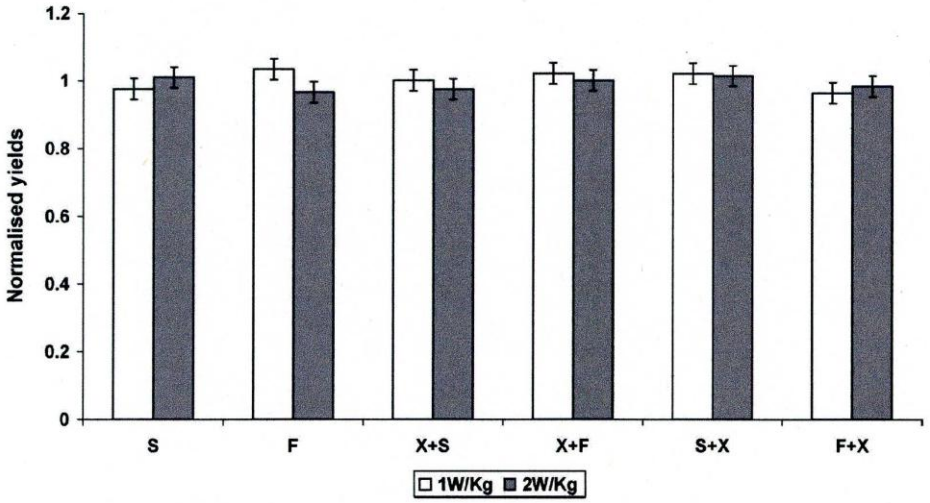

Figure 4. Pooled results for the sister chromatid exchange assay normalized for each data set to the average of S, X + S and S + X. Error bars are SE derived from the Poisson distribution. S, sham; X, x-rays; F, radiofrequency field.

a measure of the average speed of cell cycling – the proliferation or nuclear division index (NDI). Measuring the NDI gives the opportunity to determine whether exposure to mutagenic agents alters the rate of progression, perhaps by cells being held at cycle check points. Figures 5 and 6 respectively show the results of the NDI_{MN} and NDI_{SCE} measurements.

Because there are inherent differences in speed of cell cycling between individuals the sham value for each donor was subtracted from that person's other data points. The differences between the sham and the other exposure regimes were averaged over the donors and these values are shown in Figures 5 and 6. Standard errors on these mean differences were calculated by adding individual SEs in quadrature. Both Figures 5 and 6 show a tendency for values for irradiated samples to be negative indicating slower growth with respect to the shams. This is the expected mitotic delaying effect of the ionizing radiation (Purrott et al. 1980). In one of the data sets in Figure 5, the X + F sequence appears to have caused significant slowing. Inspection of the data showed that this was due to an odd value from one of the four donors. The effect was not seen with any other donor in either laboratory nor with that individual used in the 2 W/Kg data set. Overall therefore no effect of RFR could be found by these assays on the speed of *in vitro* cell cycling.

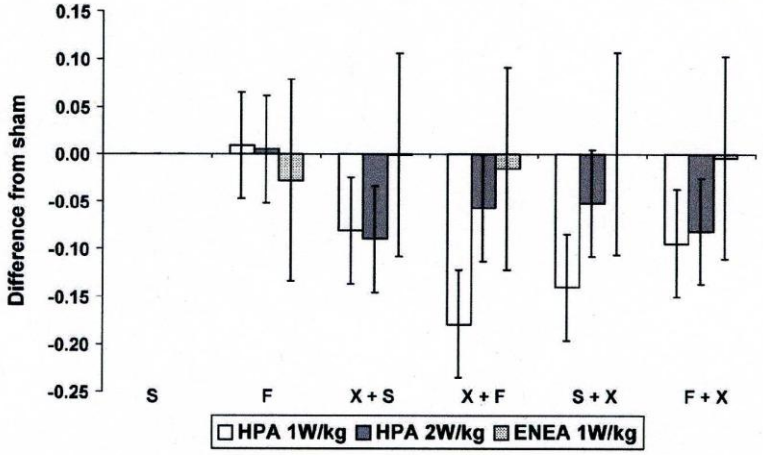

Figure 5. Changes in the nuclear division index derived from pooled donor data using the slides prepared for the micronucleus assay. Each data set is compared to its sham (S) value and binomial errors are shown. S, sham; X, x-rays; F, radiofrequency field.

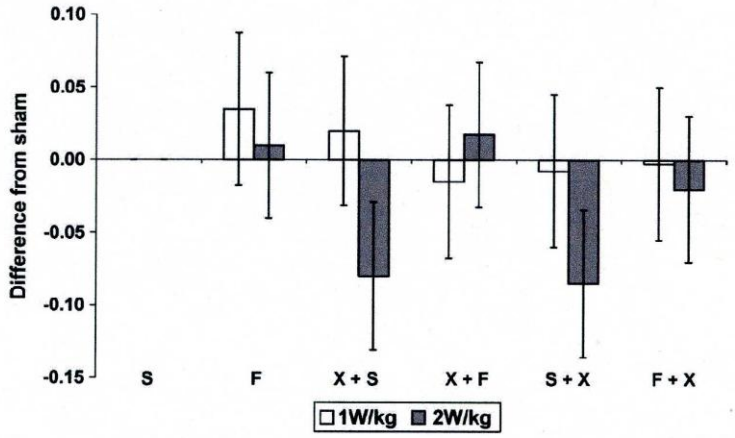

Figure 6. Changes in the nuclear division index derived from pooled donor data using slides prepared for the SCE assay. Each data set is compared to its sham (S) value and binomial errors are shown. S, sham; X, x-rays; F, radiofrequency field.

Conclusions

This study has used several standard *in vitro* tests for chromosomal and DNA damage in G_0 human lymphocytes exposed *in vitro* to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SARs of 1 or 2 W/Kg is genotoxic *per se* or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. In view of the large number of donors, assays and exposure regimes used, some data points were obtained indicating a reduction of genetic damage after RFR exposure. Generally these were statistically insignificant and are considered to reflect the normal spread of experimental data in such biological assays. Within the experimental parameters of the study in all instances no genotoxic or epigenetic effect from the RFR signal was observed.

Acknowledgements

We wish to thank the Information Technologies in Society Foundation, Zurich, for holding the blind coding of the experiments. The Mobile Manufacturers' Forum and the GSM Association are thanked for financial support for this study and the University of Helsinki for administering the sponsorship and for providing an academic 'firewall' between the sponsors and researchers.

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EXPOSURE TO LOW LEVEL GSM 935 MHz RADIOFREQUENCY FIELDS DOES NOT INDUCE APOPTOSIS IN PROLIFERATING OR DIFFERENTIATED MURINE NEUROBLASTOMA CELLS

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The aim of this study was to investigate whether radiofrequency (RF) fields characteristic of mobile phones at non-thermal levels can induce apoptosis in murine neuroblastoma (N2a) cells in both proliferating and differentiated states. Cells were exposed continuously for 24 h to one of the three 935-MHz RF signals: global system for mobile communication (GSM) basic, GSM talk and a continuous wave, unmodulated signal; all at a specific energy absorption rate of 2 W kg^{-1}. The measured increase in temperature of the cells due to the RF fields was around 0.06°C. At a number of time points between 0 and 48 h post-exposure, the cells were assessed for apoptosis under a fluorescence microscope using three independent assays: Annexin V, caspase activation and *in situ* end-labelling. No statistically significant differences in apoptosis levels were observed between the exposed and sham-exposed cells using the three assays at any time point post-exposure. These data suggest that RF exposures, characteristic of GSM mobile phones, do not significantly affect the apoptosis levels in proliferating and differentiated murine neuroblastoma cell line N2a.

INTRODUCTION

The rapid adoption of mobile phone use by the public has had very real benefits; however, concerns and fears relating to potential health risks associated with mobile phone use are widespread. For several years, the potential adverse health effects of exposure to radiofrequency (RF) fields have been considered by national and international bodies[1–6]. Apart from well characterised heating effects, no realistic biophysical mechanism for the interaction of RF fields with biological systems has been established[7]. Exposure guidelines are based upon these well established thermal effects, but the potential effects of low-level exposures remain an area of controversy and uncertainty. Two key areas of public concern relate to the possible effects of mobile phone use on brain function and on the development of brain cancer. Despite conflicting findings, these concerns have been raised by epidemiological studies, suggesting that long-term use of a mobile phone associates with the occurrence of acoustic neuroma[8–10]. However an over estimation of mobile phone use may have confounded the long-term findings of these studies. The evidence considered by, for example, the Independent Expert Group on Mobile Phones (IEGMP)[3] and the National Radiological Protection Board (NRPB)[4] led to the conclusion that there are no clear health risks associated with mobile phone use *per se*; except for the risk of driving while using a mobile phone. However, a somewhat precautionary approach was recommended and there remains a need for further biological research to provide a more robust evidence base.

Insufficient energy is delivered by RF fields to cause direct DNA damage. Thus, RF fields are unlikely to be initiators of tumourigenesis; however, less certainty is associated with potential promoting effects. Such promoting effects would likely be associated with proliferation of abnormal cells as a consequence of induced cell death. Brain injury, e.g. as a result of cerebral ischaemia, is associated with neuronal apoptosis[11] and, additionally, any loss of neurones due to apoptosis is likely to have an adverse effect on brain function. Apoptosis also plays a role in the process of carcinogenesis,[12] specifically brain carcinogenesis[13].

Apoptosis, or programmed cell death, follows a sequence of controlled steps[14]. An early event in the pathway is the translocation of phosphatidylserine from the inner to the outer face of the plasma membrane, while DNA fragmentation is a typical late-stage event and a central feature of the apoptotic process is a cascade of proteolytic enzymes called caspases. Assays to detect these distinct events in the apoptotic pathway have been used to study the induction of apoptosis by RF fields. Most published works consider the effects on haemopoietic cells, peripheral blood lymphocytes in particular[15–18] or lymphoblastoid cells[19] where no consistent association between the exposure to low levels of RF and apoptosis has been reported. More recently, additional cell types have been studied where it was noted that the induction of various effects was dependent on the type of cell studied[20,21]. It is also

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likely that the signalling and induction of apoptosis differs between proliferating undifferentiated and quiescent, differentiated cells. In this study, a cell type (neuroblastoma) relevant for brain effects has been used in both proliferating and differentiated states.

Given the involvement of apoptosis in brain disease and the sensitivity of the process to cellular insult, the aim of this study was to investigate whether RF fields characteristic of mobile phones can induce apoptosis in neuroblastoma cells in proliferating and differentiated states, by using three independent assays for apoptosis following a time course of 0–48 h post-exposure.

MATERIALS AND METHODS

Neuroblastoma cell culture

Murine neuroblastoma Neuro2a (N2a) cells[22] were obtained from J. Uney, Bristol University. Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen) supplemented with 10% heat-inactivated foetal bovine serum (FBS), (Invitrogen), 2 mM L-glutamine (Invitrogen), 5000 U ml^{-1} penicillin and 5000 μg ml^{-1} streptomycin (Invitrogen) in 75 cm^3 tissue culture flasks (Sarstedt) in a 5% CO_2 humidified atmosphere at 37°C. The flasks were split every 2–3 d when the cells had reached ~80% confluence. Cells were seeded into 35-mm dishes (Nunc) at a density of 10^5 cells in 3 ml of growth medium 24 h before any treatment.

N2a cells were induced to enter a post-mitotic state and differentiated into neurone-like cells by serum withdrawal[23]. Briefly, cells were maintained at confluence for at least 3 d before seeding them at a density of 2×10^5 cells into 35 mm dishes with 3 ml of growth medium containing only 1% FBS, 24 h before being placed into the RF exposure system.

RF exposure system

Exposures to RF fields were carried out in a sXc900 exposure setup based on two identical rectangular waveguide cavities (Information Technologies in Society Foundation (IT'IS), Zurich), installed in a tissue culture incubator maintained at 37°C with a humidified atmosphere of 5% CO_2 in air. A specially designed holder allowed eight 35-mm Petri dishes to be placed accurately inside each chamber. Good exposure and environmental control was achieved using field sensors, temperature sensors for the air environment and an optimised airflow system. A computer-controlled signal unit allowed the application of various global system for mobile communication (GSM) modulated and non-modulated signals. Field strengths, temperature and fan currents as well as all commands were continuously logged to encrypted files. Temperature was measured using an integrated TP100 temperature probe, which was extensively tested within the experimental setup. The exposure apparatus has been demonstrated to lead to temperature changes of <0.1°C within 3.1 ml of culture medium in a 35-mm Petri dish. The computer-controlled random assignment of waveguide was made as either sham or active, and the coding for this was held at IT'IS and was only broken when the assay data were complete. Schuderer *et al.*[24] described the complete detail of the apparatus, the signal profile, the exposure geometry and dosimetry.

RF exposure protocol

One hour before the RF field exposure, dishes containing the proliferating or differentiated N2a cells were placed inside the waveguides to allow the temperature to stabilise to 37°C. Cells were exposed or sham exposed for 24 h to a 935-MHz signal at a mean specific energy absorption rate (SAR) of 2 W kg^{-1}. Three signal types were used to investigate the effects of different modulation characteristics on the cells. First, GSM basic, which combines the carrier frequency with an amplitude modulation that has a repetitive frequency of 217 Hz and is the signal emitted when a person is talking into a mobile phone. Secondly, GSM talk, which switches between GSM basic and GSM DTX; the latter being the signal emitted while the person is listening. The computer randomly changes the duration of each mode in order to simulate a person having a conversation. Both GSM talk and basic reflect the 'typical' fields to which a mobile phone user would be exposed. Thirdly, a continuous wave (CW) field, which is an unmodulated signal comprising just the carrier frequency. The measured increase in temperature of the cells due to the RF field was around 0.06°C (range, 0.03–0.1°C), which is considered sufficiently low and any effect observed could be attributed to non-thermal processes.

Cells irradiated with 4.0 Gy of 250 kVp X-rays at a dose rate of 1.0 Gy min^{-1} were used as a positive control together with a zero dose control. The irradiations were carried out at Medical Research Council, Harwell, at room temperature, and the samples were maintained at room temperature while being conveyed (~5 min) between the X-ray facility and laboratory. Following all treatments, the dishes were held in an incubator and assessed for the presence of cells undergoing apoptosis at several time points.

Assessment of apoptosis

Three independent assays for apoptosis were employed using commercially available kits. These were the Annexin V-FITC apoptosis detection kit (BD Pharmingen), CaspaTag pan-caspase *in situ*

assay kit (Chemicon International) and the *in situ* end labelling Apo-Direct kit (BD Pharmingen).

Annexin V-binding assay

The kit was used according to the manufacturer's protocol, but modified for use with adherent cells as described by van Engeland *et al.*[25]. Briefly, cells were washed twice in cold phosphate-buffered saline (PBS) with the medium and PBS being transferred to a centrifuge tube to recover any non-adherent cells. Both the adherent and the recovered non-adherent cells were directly labelled with fluorescein isothiocyanate (FITC)-labelled Annexin V in its specific binding buffer along with propridium iodide (PI) at room temperature in the dark for 20 min. Following the incubation, cold binding buffer was added to the cells, which were then carefully scraped off the dish using the bulb of a plastic pipette and transferred to a centrifuge tube. The dish was washed again in cold binding buffer to remove all the cells. After centrifugation the cell pellet was pipetted onto a cold slide and viewed under the fluorescence microscope. The number of apoptotic cells, which appeared Annexin V–FITC positive and PI negative, was recorded in a total of 1000 cells. Cells were assayed at 0, 2, 4, 8, 24 and 48 h post-exposure along with a corresponding control. Each experiment was repeated two more times with fresh exposures of cells.

Measurement of caspase activation

Following exposure/sham-exposure and post-exposure incubation, the cells were washed twice in DMEM medium. Both the culture and the wash medium were transferred to a centrifuge tube to collect any cells that had detached from the growth surface. The recovered and the adherent cells were then directly incubated with the fluorescein-labelled FAM-VAN-FMK inhibitor for 70 min at 37°C in an atmosphere of 5% CO_2 in air. Cells were incubated for a further 5 min after the addition of Hoechst stain. The cells were then washed with cold buffer, and the bulb of a plastic pipette was used to carefully scrape the adherent cells off the surface of the dish and transferred to a centrifuge tube.

After rinsing the dish again, the cells were centrifuged and the pellet mounted on a cold microscope slide with a drop of wash buffer containing PI. Caspase positive cells (i.e. those having entered apoptosis) were stained with the fluorescent inhibitor and the number of such cells, in a total of 1000, was recorded. Cells were assayed at 0, 4, 8, 24 and 48 h post-exposure along with a corresponding control. Each experiment was repeated two more times with fresh exposures of cells.

In situ end-labelling assay

Following exposure and post-incubation, cells that had detached from the growth surface were collected in the culture medium and combined with the adherent cells harvested by trypsinisation. They were then fixed with 1% paraformaldehyde for 1 h on ice. After washing in PBS, the cells were incubated in PBS with 0.2% Tween at room temperature for 5 min. Following another wash in PBS, the cells were suspended in 70% ice-cold ethanol and kept at −20°C for at least 48 h. Cells from three replicate dishes were pooled for staining and analysis. After centrifugation and removal of the 70% ethanol, cells were washed twice with buffer. The fixed cells were then incubated with terminal deoxynucleotidyl transferase (TdT) in the presence of FITC-labelled deoxyuridine triphosphates (dUTP) for 75 min at 37°C to detect DNA breaks. At the end of the incubation, cells were washed twice with rinse buffer and the cell pellet added to a drop of Vectashield antifade solution (Vector) containing 100 ng ml^{-1} Hoechst stain on a microscope slide. Viewed under a fluorescent microscope, the apoptotic cells appeared green (FITC +) and the rest appeared blue. A total of 3000 cells were scored at time points of 0, 24 and 48 h post-exposure. Each experiment was repeated two more times with fresh exposures of cells.

Data analysis

All the data presented for the Annexin V, the caspase activation and the *in situ* end-labelling assays are the combined values ± SE from three experiments. Chi-squared tests for homogeneity and repeated measures analysis of variance tests were carried out to ensure that the data from the three experimental repeats could be pooled. The Student's *t*-test was then used to determine the significance of any difference between the control and exposed cells. All statistical parameters were calculated using Microsoft Excel® and Minitab® 15. A *P*-value of <0.05 was considered significant.

RESULTS

Annexin V assay

With proliferating cells, the positive controls showed an X-ray-induced increase in apoptotic cells that peaked at 2 h post-exposure. The level of apoptosis remained significantly higher than that in the unirradiated controls between 4 and 8 h post-exposure (Table 1). At 24 h post-exposure, the level of apoptosis was not significantly different from the unirradiated control, but at 48 h the level of apoptosis significantly increased again. The results with X-ray irradiated differentiated cells were essentially the same where the level of apoptosis increased

Table 1. Measurement of apoptosis levels using the Annexin V binding assay in proliferating (P) and differentiated (D) N2a cells given a 24 h exposure to GSM basic, GSM talk or GSM CW signals (2 W/kg^{-1}) / sham exposures, together with positive controls exposed to 4 Gy X-rays and unirradiated controls.

Time post exposure (h)	X-ray			RF fields								
	0 Gy number of apoptotic cells ± SE	4 Gy number of apoptotic cells ± SE	*P*-value	Sham number of apoptotic cells ± SE	Basic number of apoptotic cells ± SE	*P*-value	Sham number of apoptotic cells ± SE	Talk number of apoptotic cells ± SE	*P*-value	Sham number of apoptotic cells ± SE	CW number of apoptotic cells ± SE	*P*-value
P 0	45 ± 6.66	59 ± 7.61	0.16	54 ± 7.28	50 ± 7.01	0.3	50 ± 7.01	55 ± 7.35	0.4	42 ± 6.44	41 ± 6.36	0.48
P 2	53 ± 7.22	128 ± 11.07	0.003	65 ± 7.97	54 ± 7.28	0.37	46 ± 6.73	42 ± 6.44	0.37	51 ± 7.08	59 ± 7.61	0.23
P 4	45 ± 6.66	86 ± 9.14	0.04	53 ± 7.22	47 ± 6.80	0.27	52 ± 7.15	48 ± 6.87	0.43	37 ± 6.05	46 ± 6.73	0.15
P 8	52 ± 7.15	86 ± 9.14	0.02	57 ± 7.48	51 ± 7.08	0.38	43 ± 6.51	50 ± 7.01	0.38	40 ± 6.28	37 ± 6.05	0.31
P 24	49 ± 6.94	73 ± 8.44	0.1	50 ± 7.01	57 ± 7.48	0.32	49 ± 6.94	42 ± 6.44	0.28	39 ± 6.20	37 ± 6.05	0.45
P 48	34 ± 5.80	62 ± 7.79	0.04	46 ± 6.73	45 ± 6.66	0.46	33 ± 5.71	38 ± 6.13	0.26	41 ± 6.36	40 ± 6.28	0.44
D 0	137 ± 11.43	158 ± 12.23	0.18	109 ± 10.25	123 ± 10.86	0.28	104 ± 10.02	109 ± 10.25	0.39	134 ± 11.31	126 ± 10.99	0.39
D 2	137 ± 11.43	210 ± 13.97	0.07	109 ± 10.25	114 ± 10.47	0.42	123 ± 10.86	118 ± 10.65	0.43	133 ± 11.27	126 ± 10.99	0.4
D 4	129 ± 11.11	234 ± 14.69	0.04	123 ± 10.86	148 ± 11.86	0.08	127 ± 11.03	120 ± 10.73	0.19	126 ± 10.99	137 ± 11.43	0.29
D 8	134 ± 11.31	199 ± 13.63	0.07	101 ± 9.88	118 ± 10.65	0.26	142 ± 11.63	111 ± 10.34	0.2	122 ± 10.82	105 ± 10.07	0.35
D 24	116 ± 10.56	130 ± 11.15	0.3	106 ± 10.11	102 ± 9.93	0.4	123 ± 10.86	110 ± 10.29	0.4	107 ± 10.16	110 ± 10.29	0.44
D 48	108 ± 10.20	175 ± 12.84	0.06	123 ± 10.86	129 ± 11.11	0.4	138 ± 11.47	135 ± 11.35	0.45	119 ± 10.69	115 ± 10.52	0.44

Each data point shows the combined total ± SE of apoptotic cells from assaying 1000 cells in each of three experiments. *P*-values, obtained by the t-test, for each pair of exposed/sham data points are shown.

between 2 and 8 h post-exposure compared with the unirradiated controls although this was only significant at 4 h. The level of apoptosis then returned to control levels at 24 h and although it rose again at 48 h post-exposure the rise was not significant.

Both proliferating and differentiated cells exposed to RF fields in all three modes showed no obvious peak in apoptosis levels and at no time point was there a significant difference ($P > 0.05$) between an exposed and an individually matched sham-exposed control.

Caspase activation assay

In the X-irradiated positive controls, using proliferating cells, there was a steady and significant rise in apoptosis levels from 4 h post-exposure, up to 24 h and, although it was lower at 48 h, the level was still significantly above that seen in the unirradiated samples (Table 2). With differentiated cells a significant difference in the level of apoptosis occurred at 8 and 24 h post-exposure compared with the unirradiated control samples. The level remained elevated 48 h post-exposure, but was not significantly different from the unirradiated control.

With one exception, for each RF signal and at all time points, both proliferating and differentiated cells showed no significant difference ($P > 0.05$) in apoptosis levels from its matched sham-exposed control. The Student's *t*-test revealed one statistically significant difference ($P = 0.03$) between the sham- and RF GSM talk-exposed proliferating cells at 8 h (indicated with a * in Table 2). Here, the exposed cells showed a decrease in the level of apoptosis compared with the sham, which was consistent in all three replicates. As this result seemed to be due to high sham values compared with other time points, rather than a low exposed value, the exposure regime was repeated a further three times. No significant difference was found in the level of apoptosis between the sham and exposed cells (combined number of apoptotic cells from the three repeat samples were 313 ± 16.74 and 341 ± 16.68 for the sham and GSM talk exposures, respectively; $P = 0.65$). This, together with the lack of significant changes in apoptosis for this exposure signal/time point in the other assays, tends to suggest that it was a chance finding.

In situ end-labelling (Apo Direct) assay

The X-irradiated positive controls using both proliferating and differentiated cells showed a significant difference in the level of apoptosis at 24 and 48 h post-exposure compared with their unirradiated controls (Table 3). However, no significant differences were seen in proliferating or differentiated cells exposed to any RF signal at any time point post-exposure.

DISCUSSION

In this study, exposure of mouse neuroblastoma cells, either proliferating or in the differentiated state, to a GSM basic, talk or CW signal of 935 MHz for 24 h at an SAR of 2 W kg^{-1} did not induce significant differences in the levels of apoptosis compared with sham-exposed/control samples; as measured by any of the three assays between 0 and 48 h post-exposure. The Student's *t*-test revealed one statistically significant difference ($P = 0.03$) between the sham- and RF GSM talk-exposed proliferating cells at 8 h (marked with a * in Table 2). However, this finding was not reproduced in three further repeats. Also, the total number of experiments carried out for RF exposure was 84 (basic, talk, CW exposures; Annexin V, caspase and Apo Direct assays). Using 5% as the level of significance, statistically approximately four results (5%) could be expected to be falsely revealed as positive in 84 experiments. The fact that only one positive was revealed by these analyses indicates that the weight of evidence is in favour of their being no effect of RF exposure. Furthermore, as expected from the results of the *t*-tests, full general linear model analysis of variance, for exposure time, X-ray and RF status, revealed that X-ray was the only important factor ($P << 0.001$).

The present work showed that a 4-Gy X-ray exposure of murine N2a cells, in both proliferating and differentiated states, produced a peak in apoptosis levels, which occurred at different times post-exposure depending on the assay used to assess apoptosis. The Annexin V assay showed a peak at 2 and 4 h for proliferating and differentiated cells, respectively. In contrast, the peaks in apoptosis levels for both proliferating and differentiated cells arose at 24 and 48 h post-exposure for the caspase activation and the *in situ* end-labelling assays, respectively. The relative timings of these peaks are entirely as expected because the Annexin V-binding assay detects an early membrane change event and the *in situ* end-labelling assay detects DNA fragmentation, which is typical of late-stage apoptotic cells[13]. The caspase activation assay detects the cascade of proteolytic caspases, an intermediate feature of the apoptotic process. Also, the X-ray results show that the three apoptosis assays are sensitive enough to pick up an effect in both proliferating and differentiated N2a cells. It was possible that small temperature gradients and/or hot spots could have been induced in the cell culture and these could have affected the results, as changes in temperature would be an indicator of unequal energy deposition within the dish. However, previous analyses[24] of the experimental setup found no hot spots in the medium, and at all times the temperature gradients

Table 2. Measurement of apoptosis levels using the caspase activation assay in proliferating (P) and differentiated (D) N2a cells given a 24 h exposure to GSM basic, GSM talk or GSM CW signals (2 W/kg^{-1}) / sham exposures, together with positive controls exposed to 4 Gy X-rays and unirradiated controls.

Time post exposure (h)	X-ray			RF fields								
	0 Gy number of apoptotic cells ± SE	4 Gy number of apoptotic cells ± SE	*P*-value	Sham number of apoptotic cells ± SE	Basic number of apoptotic cells ± SE	*P*-value	Sham number of apoptotic cells ± SE	Talk number of apoptotic cells ± SE	*P*-value	Sham number of apoptotic cells ± SE	CW number of apoptotic cells ± SE	*P*-value
P 0	335 ± 17.25	446 ± 19.49	0.07	350 ± 17.58	353 ± 17.65	0.47	345 ± 17.47	362 ± 17.84	0.41	379 ± 18.20	384 ± 18.30	0.45
P 4	355 ± 17.69	562 ± 21.37	0.02	336 ± 17.27	333 ± 17.21	0.47	364 ± 17.88	394 ± 18.50	0.34	422 ± 19.04	377 ± 18.16	0.16
P 8	341 ± 17.39	738 ± 23.59	0.02	392 ± 18.46	361 ± 17.82	0.35	427 ± 19.14	365 ± 17.91	0.03*	384 ± 18.30	420 ± 19.01	0.21
P 24	312 ± 16.72	821 ± 24.42	0.01	425 ± 19.10	420 ± 19.01	0.42	394 ± 18.50	409 ± 18.79	0.38	410 ± 18.81	380 ± 18.22	0.35
P 48	255 ± 15.27	577 ± 21.59	0.01	289 ± 16.16	267 ± 15.60	0.13	321 ± 16.93	353 ± 17.65	0.29	386 ± 18.34	392 ± 18.46	0.43
D 0	111 ± 10.34	128 ± 11.07	0.2	93 ± 9.49	90 ± 9.34	0.44	91 ± 9.39	102 ± 9.93	0.37	99 ± 9.78	102 ± 9.93	0.44
D 4	90 ± 9.34	116 ± 10.56	0.12	119 ± 10.69	117 ± 10.60	0.48	105 ± 10.07	115 ± 10.52	0.35	90 ± 9.34	78 ± 8.72	0.24
D 8	103 ± 9.97	181 ± 13.04	0.03	132 ± 11.23	122 ± 10.82	0.43	109 ± 10.25	114 ± 10.47	0.37	100 ± 9.83	78 ± 8.72	0.21
D 24	89 ± 9.29	178 ± 12.94	0.05	85 ± 9.09	94 ± 9.54	0.28	113 ± 10.43	106 ± 10.11	0.3	95 ± 9.59	82 ± 8.93	0.27
D 48	96 ± 9.64	202 ± 13.73	0.09	105 ± 10.07	115 ± 10.52	0.33	97 ± 9.69	102 ± 9.93	0.36	77 ± 8.66	82 ± 8.93	0.37

Each data point shows the combined total ± SE of apoptotic cells from assaying 1000 cells in each of three repeated experiments. *P*-values, obtained by the *t*-test, for each pair of exposed/sham data points are shown.

Table 3. Measurement of apoptosis levels using the *in situ* end labelling assay in proliferating (P) and differentiated (D) N2a cells given a 24 h exposure to GSM basic, GSM talk or GSM CW signals (2 W/kg^{-1}) / sham exposures, together with positive controls exposed to 4 Gy X-rays and unirradiated controls.

Time post exposure (h)	X-ray			RF fields								
	0 Gy number of apoptotic cells ± SE	4 Gy number of apoptotic cells ± SE	*P*-value	Sham number of apoptotic cells ± SE	Basic number of apoptotic cells ± SE	*P*-value	Sham number of apoptotic cells ± SE	Talk number of apoptotic cells ± SE	*P*-value	Sham number of apoptotic cells ± SE	CW number of apoptotic cells ± SE	*P*-value
P 0	99 ± 9.89	88 ± 9.33	0.39	114 ± 10.61	123 ± 11.01	0.49	131 ± 11.36	144 ± 11.90	0.4	104 ± 10.14	106 ± 10.23	0.85
P 24	120 ± 10.88	288 ± 16.70	0.04	126 ± 11.15	164 ± 12.69	0.16	145 ± 11.94	172 ± 12.99	0.23	135 ± 11.53	125 ± 11.10	0.47
P 48	182 ± 13.35	628 ± 24.17	0.02	145 ± 11.94	188 ± 13.57	0.15	225 ± 14.81	230 ± 14.97	0.75	205 ± 14.15	177 ± 13.17	0.24
D 0	341 ± 18.11	288 ± 16.70	0.17	388 ± 19.27	379 ± 19.05	0.67	411 ± 19.80	362 ± 18.64	0.2	336 ± 17.98	353 ± 18.42	0.46
D 24	407 ± 19.71	728 ± 25.87	0.04	484 ± 21.40	477 ± 21.25	0.76	429 ± 20.21	466 ± 21.02	0.27	468 ± 21.06	461 ± 20.91	0.75
D 48	459 ± 20.87	899 ± 28.45	0.03	528 ± 22.29	595 ± 23.57	0.17	522 ± 22.17	529 ± 22.31	0.77	515 ± 22.03	484 ± 21.40	0.33

Each data point shows the combined total ± SE of apoptotic cells from assaying 3000 cells in each of three repeated experiments. *P*-values, obtained by the *t*-test, for each pair of exposed/sham data points are shown.

were found to be small, much lower than those required to induce convection.

The result of the present study is in agreement with several recent studies that have also used neuroblastoma cells and failed to demonstrate apoptosis after exposure to RF fields. Gurisik *et al.*[26] exposed the human neuroblastoma cell line SK-N-SH for 2 h to a 900-MHz signal at an SAR of 0.2 W kg^{-1}. Apoptosis was measured 24 h post-exposure by using flow cytometry to measure the uptake of the DNA staining dye YO-PRO-1. No significant differences between the sham- and RF-exposed cells were observed. Merola *et al.*[27] exposed the human neuroblastoma cell line Lan-5 to a 900-MHz GSM signal in a wire-patch cell, at an SAR of 1 W kg^{-1}. Following a 24 or 48 h exposure to RF fields, again no significant differences in apoptosis between the exposed and the sham-exposed cells were found using the caspase assay. Joubert *et al.*[28] also exposed another human neuroblastoma cell line, SH-SY5Y, to a 900-MHz GSM and a CW signal in a wire-patch cell, at SARs of 0.25 and 2 W kg^{-1}, respectively, for 24 h. As in the present study three methods were used to detect apoptosis, DAPI staining, caspase 3 activity and terminal deoxynucleotidytransferase dUTP nick end-labelling (TUNEL) at 0 and 24 h post-exposure. No significant differences in the levels of apoptosis were observed between the exposed and control cells with any signal, time point or assay used.

The above results are also in agreement with studies using human lymphocytes exposed *in vitro* to RF fields. All of the following studies have exposed non-cycling lymphocytes in the G_0 stage of the cell cycle. Belyaev *et al.*[18] exposed lymphocytes from healthy donors and persons who claimed to be hypersensitive to electromagnetic fields to a 915-MHz RF field with an SAR of 37 mW kg^{-1} for 2 h. No significant differences in apoptosis levels were detected by comparison with unexposed controls. An extensive study by Capri *et al.*[16] also used lymphocytes from young and old donors and exposed them to several 1800 MHz GSM-modulated signals; GSM basic, talk and DTX. The authors found in all cases that the susceptibility to apoptosis was unaffected by exposure to RF fields. In a second study by Capri *et al.*[17] in which human lymphocytes were exposed to a 900-MHz GSM or CW signal (SAR 70–76 W kg^{-1}) for 1 h d^{-1} for 3 d, apoptosis levels were again found to be unaffected by RF fields. Using human Molt-4 cells, Hook *et al.*[19] showed that the frequency of apoptotic cells, as measured by the Annexin V assay, was not affected by RF fields exposure. Two further studies have also used the Annexin V assay immediately after RF field exposure. Lantow *et al.*[29] exposed human Mono Mac 6 cells to a 1800-MHz GSM DTX signal (SAR 2 W kg^{-1}) for 12 h and Lagroye *et al.*[15] exposed human U937 lymphoma cells to a 900-MHz GSM signal (SAR 0.7 W kg^{-1}) for 48 h. Again, in both studies, no significant induction of apoptosis was detected.

In contrast to these negative results, other researchers using other types of cells and exposure conditions have reported RF-induced apoptosis. Caraglia *et al.*[20] exposed human oropharyngeal epidermal carcinoma KB cells to a 1.95-GHz RF signal, at an SAR of 3.6 mW kg^{-1} for 1, 2 and 3 h. By using the Annexin V assay immediately after exposure, a time-dependent increase in the levels of apoptosis was found, together with a time-dependent decrease in the heat shock protein, HSP90. The authors considered the increase in apoptosis rate was attributable to the inactivation of the Ras → Erk survival signalling with enhanced deregulation of Ras and Raf1. However, Lee *et al.*[30] found no change in HSP90 expression, as well as HSP70 and HSP27, in human T-lymphocyte Jurkat cells or rat primary astrocytes following a 1-h exposure to a 1763-MHz RF field at SARs of 2 and 20 W kg^{-1} for 6, 12 or 24 h post-exposure. Previously, Marinelli *et al.*[31] had exposed human T-lymphoblastoid leukaemia cells CCRF-CEM to a 900-MHz CW signal for 2 h at an SAR of 3.5 mW kg^{-1} and shown an increase in the percentage of cells undergoing apoptosis. Here, however, the increase was thought to be the result of early activation of the p53-dependent, as well as the p53-independent, apoptotic pathways. However, at longer exposure times (24 and 48 h), the effect decreased, although the differences between the exposed and unexposed cells were still statistically significant.

Although only a few studies have used neuroblastoma cell lines to examine the RF-induced apoptosis, several more have used other brain-derived cell lines. As with the present study, most have found that RF field failed to induce apoptosis. Hirose *et al.*[32] used proliferating human glioblastoma A172 cells (and also human fibroblasts IMR-90) to assess apoptosis levels using the Annexin V assay immediately after exposure to 2.1425 GHz W-CDMA or CW RF fields associated with mobile phone base stations. The authors concluded that low levels of RF fields, SARs of up to 800 mW kg^{-1}, did not induce p53-dependent apoptosis. In two recent studies, Joubert *et al.*[33,21] exposed primary rat cortical neurones to RF fields for 24 h and used several methods to assess apoptosis at 0 and 24 h post-exposure. Joubert *et al.*[33] used a GSM 900 MHz signal at an SAR of 0.25 mW kg^{-1} and assessed apoptosis using 4′,6-diamidino-2-phenylindole (DAPI) staining, TUNEL and caspase 3 activity. They were unable to demonstrate any difference in apoptosis between the exposed and unexposed cells. This was in agreement with Lagroye *et al.*[15] who found that apoptosis levels, as measured immediately by the Annexin V assay, were

not increased in primary neuronal cells or astrocytes after a 1-h exposure to a GSM 900 MHz field with an SAR of 2 W kg^{-1}. However, in a second study, Joubert *et al.*[21], using a 900-MHz CW signal with an SAR of 2 W kg^{-1}, did report a significant difference in the apoptosis frequency between the exposed and the sham-exposed cells assayed by DAPI staining and TUNEL. During these RF exposures, a temperature rise of 2°C was noted and therefore control experiments with neurones exposed to 37 and 39°C were also performed. Overall, the results suggested that the induction of apoptosis is independent of changes in temperature. As the apoptosis rate in the RF-exposed cells was significantly different from these controls, they concluded that they may have seen an effect of RF fields. However, the authors concede that localised thermal effects of the RF field could not be ruled out.

CONCLUSION

Exposure of N2a mouse neuroblastoma cells, either in a proliferating or a differentiated state, to a GSM basic, talk or CW signal of 935 MHz for 24 h at an SAR of 2 W kg^{-1} did not induce differences in the levels of apoptosis compared with sham-exposed/control samples; as measured by the Annexin V, the caspase activation and the Apo direct assays between 0 and 48 h post-exposure. The data presented here serve to strengthen the case that RF fields associated with mobile phones do not cause apoptosis in brain cells.

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