



**NON-IONIZING RADIATION,  
PART 2: RADIOFREQUENCY  
ELECTROMAGNETIC FIELDS**

**VOLUME 102**

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opinions of an IARC Working Group on the  
Evaluation of Carcinogenic Risks to Humans,  
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**IARC MONOGRAPHS  
ON THE EVALUATION  
OF CARCINOGENIC RISKS  
TO HUMANS**

## 4. OTHER RELEVANT DATA

Data on specific absorption rate (SAR) and distribution of radiofrequency (RF) radiation inside tissues and organs and at the subcellular level are presented elsewhere in this Volume (Section 1.3, Dosimetry).

### 4.1 Genetic and related effects

#### 4.1.1 Humans

During the past decades, extensive research efforts have focused on determining the extent of DNA damage in eukaryotic and prokaryotic cells exposed to RF radiation. Several published reviews concluded that: (i) the existing data are not sufficiently strong to suggest that RF radiation is directly genotoxic; (ii) exposure to RF radiation probably does not enhance the damage induced by known genotoxic agents; and (iii) some of the reported “adverse effects” may be attributed to hyperthermia induced by RF radiation ([Brusick et al., 1998](#); [Verschaeve & Maes, 1998](#); [Moulder et al., 1999, 2005](#); [Heynick et al., 2003](#); [Meltz, 2003](#); [Vijayalaxmi & Obe, 2004](#); [Verschaeve, 2005](#); [Krewski et al., 2007](#); [Lai, 2007](#); [Vijayalaxmi & Prihoda, 2008](#); [Phillips et al., 2009](#); [Rüdiger, 2009a](#); [Verschaeve, 2009](#); [Verschaeve et al., 2010](#)). International organizations and expert scientific advisory groups in several countries, including Canada, France, the Netherlands, Sweden, the United Kingdom and the USA, have reached similar conclusions ([ICNIRP, 2009](#)).

This Section of the *Monograph* deals with studies on primary DNA damage in humans exposed occupationally or as mobile-phone users;

in these studies DNA damage was measured in peripheral blood lymphocytes and buccal cells by means of the alkaline or neutral single-cell gel electrophoresis assay (comet assay), which reveals alkali-labile lesions and single- and double-strand breaks in DNA, or by use of cytogenetic tests for chromosomal aberrations, micronucleus formation and sister-chromatid exchange (SCE). The studies reviewed below are summarized in [Table 4.1](#) and [Table 4.2](#) (with details of the exposure conditions).

#### (a) Peripheral blood lymphocytes

##### (i) Occupational exposure

[Garaj-Vrhovac et al. \(1990a\)](#) were the first to report an increased frequency of chromosomal aberrations in the form of chromatid and chromosome breaks, acentric fragments, dicentrics, rings and polycentric chromosomes, as well as micronuclei in 10 individuals employed in a radar service-station facility. The frequency of cells with chromosomal aberrations and micronuclei ranged from 1.6% to 31.5% and from 1.6% to 27.9%, respectively, in exposed subjects, while the corresponding values in controls were 1.8% and 1.5% [no range given].

In a study in Australia, [Garson et al. \(1991\)](#) collected lymphocytes from 38 radio linesmen,

**Table 4.1 Genetic and related effects of radiofrequency radiation in peripheral blood lymphocytes of occupationally exposed individuals**

End-point	No. of subjects	Occupation	Frequency	SAR or power density	Duration	Results	Reference
Aneuploidy	18	Air-traffic controllers; engineers	100 kHz to 300 GHz	-	10–27 yr	+	<a href="#">Othman <i>et al.</i> (2001)</a>
CA	10	Radar maintenance workers	0.2 MHz to 26 GHz	0.010–50 mW/cm <sup>2</sup>	8–25 yr	+	<a href="#">Garaj-Vrhovac <i>et al.</i> (1990a)</a>
CA	38	Radio linesmen	400 kHz to 20 GHz	614 V/m	5 yr	-	<a href="#">Garson <i>et al.</i> (1991)</a>
CA	6	Air-traffic radar-repairmen	1250–1350 MHz	0.01–20 mW/cm <sup>2</sup>	16 yr	+ [after a 30-wk follow-up, total aberrations had decreased]	<a href="#">Garaj-Vrhovac <i>et al.</i> (1993)</a>
CA	6	Transmission-antenna maintenance workers	450–900 MHz	NR	1 yr	-	<a href="#">Maes <i>et al.</i> (1995)</a>
CA	20	Workers in telecommunication and radio-relay stations	8 GHz	1 mW/cm <sup>2</sup>	6 yr (12 h/d)	+	<a href="#">Lalić <i>et al.</i> (2001)</a>
CA	50	Air-traffic controllers, engineers	100 kHz to 300 GHz	NR	8–27 yr	+	<a href="#">Aly <i>et al.</i> (2002)</a>
CA	49	Radio engineers	450–900 MHz	NR	2.3 yr (> 1 h/d)	-	<a href="#">Maes <i>et al.</i> (2006)</a>
CA	10	Radar maintenance workers	1250–1350 MHz	0.010–20 mW/cm <sup>2</sup>	7–29 yr	+	<a href="#">Garaj-Vrhovac &amp; Orescanin (2009)</a>
MN	10	Radar maintenance workers	0.2 MHz to 26 GHz	0.010–50 mW/cm <sup>2</sup>	8–25 yr	+	<a href="#">Garaj-Vrhovac <i>et al.</i> (1990a)</a>
MN	NR	Multiple occupations	1250–1350 MHz	0.01–20 mW/cm <sup>2</sup>	15 yr	+	<a href="#">Fucic <i>et al.</i> (1992)</a>
MN	12	Radar maintenance workers	1250–1350 MHz	0.01–20 mW/cm <sup>2</sup>	13 yr	+	<a href="#">Garaj-Vrhovac (1999)</a>
SB	40	Flight crew	NR	NR	5–18 yr	-	<a href="#">Cavallo <i>et al.</i> (2002)</a>
SB	49	Radio engineers	450–900 MHz	NR	2.3 yr (> 1 h/d)	-	<a href="#">Maes <i>et al.</i> (2006)</a>
SB	10	Radar maintenance workers	1250–1350 MHz	0.010–20 mW/cm <sup>2</sup>	7–29 yr	+	<a href="#">Garaj-Vrhovac &amp; Orescanin (2009)</a>
SCE	50	Air-traffic controllers	100 kHz to 300 GHz	NR	8–27 yr	-	<a href="#">Aly <i>et al.</i> (2002)</a>
SCE	49	Radio engineers	450–900 MHz	NR	2.3 yr (> 1 h/d)	-	<a href="#">Maes <i>et al.</i> (2006)</a>

+ increase; -, no effect; CA, chromosomal aberration; d, day; h, hour; MN, micronucleus formation; NR, not reported; SAR, specific absorption rate; SB, DNA single- and double-strand breaks; SCE, sister-chromatid exchange; wk, week; yr, year

**Table 4.2 Genetic and related effects of radiofrequency radiation in peripheral blood lymphocytes and buccal cells of mobile-phone users**

End-point	No. of subjects	Frequency	SAR	Duration	Results	Reference
<i>Peripheral blood lymphocytes</i>						
CA	24	890–960 MHz	NR	2 yr	+	<a href="#">Gadhia et al. (2003)</a>
CA	25	NR	0.1–1.9 W/kg	3–5 yr	+	<a href="#">Gandhi &amp; Singh (2005)</a>
MN	24	800–2000 MHz	0.6–1.6 W/kg	1–5 yr	+	<a href="#">Gandhi &amp; Anita (2005)</a>
SB	24	800–2000 MHz	0.6–1.6 W/kg	1–5 yr	+	<a href="#">Gandhi &amp; Anita (2005)</a>
SCE	24	890–960 MHz	NR	2 yr	+	<a href="#">Gadhia et al. (2003)</a>
<i>Buccal cells</i>						
MN	25	NR	0.1–1.9 W/kg	3–5 yr	+	<a href="#">Gandhi &amp; Singh (2005)</a>
MN	85	NR	0.3–1.0 W/kg	2.3 yr (1 h/d)	+	<a href="#">Yadav &amp; Sharma (2008)</a>
MN	112	NR	NR	5–10 yr (3 h/wk)	–	<a href="#">Hintzsche &amp; Stopper (2010)</a>

+, increase; –, no effect; CA, chromosomal aberration; d, day; h, hour; MN, micronucleus formation; NR, not reported; SAR, specific absorption rate; SB, DNA single- and double-strand breaks; SCE, sister-chromatid exchange; wk, week; yr, year

who erected and maintained broadcasting, telecommunication and satellite RF-transmission towers, and found no increase in the frequency of chromosomal aberrations compared with the frequency in 38 controls working as clerical staff. In this study, exposure to RF radiation was at or below occupational limits for Australia.

[Fucić et al. \(1992\)](#) measured the surface area of micronuclei in lymphocytes of workers in multiple occupations exposed to pulsed microwaves, X-rays (< 25 mSv during the previous 2 years) and vinyl-chloride monomer (VCM; average concentration, 50 ppm). The sample size in each category was not mentioned in the paper. There were increased numbers of smaller micronuclei in individuals exposed to X-rays and VCM, indicating a clastogenic effect. Increased numbers of smaller as well as larger micronuclei were found in individuals exposed to microwaves, suggesting a dual role of this type of radiation, as clastogen and aneugen.

In a regular 30-week follow-up investigation of six individuals who were acutely exposed to pulsed-wave RF radiation of high power density at an air-traffic radar-repair station,

[Garaj-Vrhovac et al. \(1993\)](#) observed a decline in the total number of chromosomal aberrations.

A preliminary study conducted by [Maes et al. \(1995\)](#) involved six workers in charge of maintaining transmission antennae linked to a mobile-phone network, and six matched controls. No increase in the frequency of chromosomal aberrations was observed in the maintenance workers. The authors mentioned the limited size of the study and the fact that exposure to RF radiation was intermittent. They then extended the study to 49 professionally employed radio engineers working in the field, and 11 administrative staff. Some of these had participated in the earlier study. No differences between exposed and controls were observed with the alkaline comet assay, the assay for chromosomal aberration, or the test for SCE ([Maes et al., 2006](#)).

[Garaj-Vrhovac \(1999\)](#) examined 12 subjects employed in repair services for radar equipment and antennae, and reported frequencies of 8–23 micronuclei per 500 cells in exposed workers compared with 2–7 per 500 cells in control subjects; this difference was statistically significant.

[Lalić et al. \(2001\)](#) investigated 20 workers in telecommunication and radio-relay stations who were exposed to non-ionizing electromagnetic fields, and 25 subjects employed as X-ray technicians, nurses and engineers in radiology, exposed to ionizing radiation. The analysis indicated an increased frequency of chromosomal aberration in both groups. The incidence of dicentric chromosomes was higher in the group exposed to non-ionizing radiation than in the group exposed to ionizing radiation.

[Othman et al. \(2001\)](#) studied professional air-traffic controllers and engineers exposed to RF radiation emitted by different pieces of equipment at the workplace. In a first study, blood lymphocytes were collected from 18 workers and 5 unexposed controls (all males), and cultured for 72 hours. Fluorescence *in situ* hybridization (FISH) with repetitive  $\alpha$ -satellite probes for chromosomes 7, 12, 17, and the heterochromatic region of the Y-chromosome, was used to determine the number of aneuploid cells. The results showed increased frequencies of monosomic cells containing a single copy of chromosome 7 (6.6%) or 17 (6.1%), and of cells lacking the Y-chromosome (8.4%); the corresponding values for the controls were 3.2%, 3.7% and 4.5%, respectively.

In a further study by the same group, [Aly et al. \(2002\)](#) examined lymphocytes from 26 air-traffic controllers, 24 engineers and 10 controls. Conventional cytogenetic techniques revealed an increase in the frequency of structural aberrations (2.7–5.3%) and numerical aberrations (8.9–9.3%) in exposed individuals relative to controls (0.8% and 3.2%, respectively). In subjects exposed to RF radiation, 90% of the cells were hypodiploid, i.e. showed loss of chromosomes. The frequency of SCE was also increased, but this increase did not reach statistical significance. [The Working Group noted that conventional cytogenetic techniques may be less reliable than the FISH technique for counting numerical aberrations.]

[Cavallo et al. \(2002\)](#) studied 40 airline pilots and flight technicians exposed to cosmic rays, electromagnetic fields from radar equipment, pollutants from jet-propulsion fluid etc. and 40 non-exposed individuals working on the ground. In the comet assay, visual examination of the results revealed a small increase in the frequency of DNA strand breaks in exposed individuals compared with ground staff, but this increase was not statistically significant.

[Garaj-Vrhovac & Orescanin \(2009\)](#) used the comet assay to measure DNA strand breaks and the test for sensitivity to bleomycin described by [Michalska et al. \(1998\)](#) to investigate genomic instability in 10 individuals working in radar-equipment and antenna-system services, and in 10 control subjects. In the latter method, the cells were treated with bleomycin (a drug used in clinical treatment of cancer) during the last 5 hours before harvesting after a culture period of 72 hours, to assess the incidence of chromosomal aberrations in the form of chromatid breaks. The results of the comet assay revealed increased DNA damage (tail length, 17.1  $\mu\text{m}$ , and tail moment, 14.4, in the exposed individuals compared with 14.2  $\mu\text{m}$  and 11.7, respectively, in the controls). The test for sensitivity to bleomycin showed a higher number of chromatid breaks (1.7 per cell in the exposed, compared with 0.5 per cell in the controls). All these differences were statistically significant.

[The Working Group noted the following limitations in the above-mentioned studies. Exposure assessment was poor or was not mentioned in many reports. The sample size in terms of number of individuals or number of cells analysed was not sufficient to allow robust statistical analysis. Except in one study, “blind” evaluation of microscope slides, and inclusion of positive controls (subjects or cells) while culturing the lymphocytes *in vitro*, was either not performed or not reported. Several investigations were conducted with blood samples collected from workers in one radar-service

facility in Croatia; it was unclear whether the same individuals had been included in more than one of these studies.]

[Although the reports from Australia ([Garson et al., 1991](#)) and Belgium ([Maes et al., 1995](#)) indicated no effect on the frequency of chromosomal aberrations from exposure to RF radiation, the Working Group noted that situations and exposure conditions in those countries may not have been comparable to those in other countries. Chromosomal changes are highly variable during carcinogenesis and are generally grouped into two categories: (i) reciprocal and balanced structural rearrangements resulting in translocations; and (ii) unbalanced and nonreciprocal structural or numerical changes in which genetic material may be lost or added: the latter can range from a single base pair to the entire chromosome. In the studies reviewed above, reciprocal and balanced structural rearrangements were either not observed or not reported in individuals exposed to RF radiation.]

(ii) *Personal exposure from mobile phones*

[Gadhia et al. \(2003\)](#) collected samples of peripheral blood from 24 users of digital mobile phones and 24 matched controls. Both groups comprised 12 nonsmokers/nondrinkers and 12 smokers/alcoholics [smokers consumed 10–15 cigarettes per day; data on alcohol consumption were not given]. Cytogenetic analysis of lymphocytes cultured for 72 hours indicated a significantly increased incidence ( $P < 0.05$ ) of chromatid gaps and dicentric chromosomes among mobile-phone users who smoked and drank alcohol, but not in nonsmokers/nondrinkers. A significantly increased frequency ( $P < 0.05$ ) of SCE was seen in mobile-phone users of both categories.

[Gandhi & Singh \(2005\)](#) studied G-banded chromosomes in lymphocytes (cultured for 72 hours) from 25 users of mobile phones and 25 non-users. There was a statistically significant increase in the frequency of aberrant metaphases,

including triploid chromosomes, acrocentric associations and centromere separation in lymphocytes of mobile-phone users (31.3%) compared with non-users (10.7%). In a subsequent study, [Gandhi & Anita \(2005\)](#) investigated DNA strand breaks by use of the comet assay in lymphocytes from 24 mobile-phone users and 10 controls. Unstimulated lymphocytes were also examined to record the frequency of micronuclei in 20 of those users and 8 non-users. In mobile-phone users, the frequency of damaged cells was 40%, with an average comet-tail length of 27  $\mu\text{m}$  (determined by visual examination with a micrometer), while these values were lower in non-users, at 10% and 8  $\mu\text{m}$ , respectively; both differences were highly significant. The total number of micronuclei was 100 in 40 000 cells in users, and 8 in 16 000 cells in non-users, *i.e.* 2.5% in the former and 0.5% in the latter ( $P < 0.05$ ). [The Working Group noted that the observations reported by [Gandhi & Singh \(2005\)](#) and [Gandhi & Anita \(2005\)](#) were questioned by others ([Vijayalaxmi et al., 2007](#)), pointing out several inconsistencies and weaknesses in laboratory methods, data collection, exposure assessment, etc. in both publications.]

(b) *Buccal cells: personal exposure from mobile phones*

The oral cavity is within the range of RF emissions from mobile phones used at the ear. Hence, examination of the cells in this region is relevant to evaluation of genotoxicity. The oral mucosa has a rich blood supply and is relatively permeable. It has an outer layer of stratified squamous epithelium that is approximately 40–50 cell-layers thick. These exfoliating cells can easily be obtained by non-invasive procedures (oral swabs) from adults, adolescents and children. The turnover of these cells is estimated at 1–3 weeks ([Harris & Robinson, 1992](#)).

The frequency of micronuclei in exfoliated buccal cells has been investigated in mobile-phone users. [Gandhi & Singh \(2005\)](#) collected

buccal cells from 25 mobile-phone users and 25 non-users. The average frequencies of micronuclei (in %) were  $0.82 \pm 0.09$  in users and  $0.06 \pm 0.003$  in non-users ( $P < 0.05$ ). [The Working Group noted the same limitations for this study as those mentioned above.]

[Yadav & Sharma \(2008\)](#) collected buccal cells from 85 mobile-phone users and 24 controls. In a total of 1000 cells from each donor, the frequency of micronuclei was determined, along with other indications of degeneration, i.e. karyolysis, karyorrhexis, “broken egg” effect, and binucleate cells. The mean frequency in users (10.7 per 1000 cells) was significantly higher than that in non-users (4.0 per 1000 cells). The changes in incidence of other end-points were not statistically significant. There was also a positive, albeit non-significant, correlation of the total number of micronuclei with increased duration of mobile-phone use.

[Hintzsche & Stopper \(2010\)](#) determined the frequency of micronuclei in buccal cells from 112 mobile-phone users and 13 non-users. Four patients receiving radiotherapy were included as positive controls, along with four healthy controls. The average frequency of micronucleus formation in users was not different from that in non-users. Also, there was no difference when the users were subdivided according to the number of hours of use per week and duration of use of up to 10 years. In contrast, the frequency of micronucleated cells in patients receiving radiotherapy was  $131 \pm 29.1$  per 1000 cells. The authors mentioned that the larger number of individuals studied, the use of DNA-specific staining, and the genotypic variation in the study populations may have contributed to the discrepancy between their results and those of [Yadav & Sharma \(2008\)](#).

[The Working Group noted that counting of 2000 differentiated cells and 200 basal cells is recommended for studies using buccal cells, ([Thomas & Fenech, 2011](#)); this was not accomplished in the studies discussed above. Known confounding factors such as tobacco smoking

and alcohol consumption were mentioned in some of the studies, but in view of the limited sample size the influence of such factors on the observed abnormalities is difficult to determine.]

[The Working Group further noted that studies of genotoxicity in humans exposed to RF radiation have been carried out by a limited number of research groups; that methodological weaknesses were found in many studies; and that confounding factors were generally not addressed. Overall, although there were studies with positive results for genotoxicity associated with occupational exposure to RF radiation or with the use of mobile phones, the Working Group concluded that the available evidence was not strong enough to draw firm conclusions.]

#### 4.1.2 Experimental systems: in vivo

The studies on experimental animals exposed to RF radiation were not uniformly clear in describing the rationale for choosing a specific dose.

##### (a) *Drosophila melanogaster*

Adult male fruit flies (*Drosophila melanogaster*) were exposed to RF radiation at either 146.34 MHz produced by a transmitter of 20 W, or 29.00 MHz produced by a transmitter of 300 W, for 12 hours ([Mittler, 1976](#)). Loss of the X or Y chromosomes, nondisjunction, and the induction of sex-linked recessive lethal mutations were investigated. There was no significant difference between exposed and non-exposed flies for any of these end-points.

In a subsequent study ([Mittler, 1977](#)), *D. melanogaster* were exposed to RF radiation at 98.5 MHz (field strength, 0.3 V/m) for 32 weeks. No significant difference in the incidence of sex-linked recessive lethal mutations was observed in the exposed group compared with the controls.

[Hamnerius et al. \(1979\)](#) examined the effect of exposure to RF radiation on somatic mutation of genes involved in eye pigmentation in

*D. melanogaster*. When embryos were exposed to continuous-wave RF radiation at 2450 MHz (average SAR, 100 W/kg) for 6 hours, no evidence of mutagenicity was found. The same investigators used the same test system to examine mutation frequency in *D. melanogaster* under different conditions of exposure for 6 hours: continuous-wave radiation at 2.45 GHz, pulsed-wave radiation at 3.1 GHz, and continuous-wave magnetic or electric fields at 27.12 MHz. Under none of these conditions was a change in mutation frequency observed ([Hamnerius et al., 1985](#)).

[Marec et al. \(1985\)](#) investigated the effect of repeated exposures to RF radiation on sex-linked recessive lethal mutations in *D. melanogaster* exposed to continuous-wave RF radiation at 2375 MHz (SAR values: 15 W/cm<sup>2</sup> for 60 minutes per day; or 20 W/cm<sup>2</sup> for 10 minutes per day; or 25 W/cm<sup>2</sup> for 5 minutes per day) for five consecutive days. The mutation frequency in the groups exposed to RF radiation was not significantly different from that in the control group.

In a series of studies from Greece, adverse effects were reported on the reproduction of *D. melanogaster* after exposure to RF radiation at non-thermal mobile-phone frequencies (900 or 1800 MHz). In these experiments commercially available mobile phones were used as exposure devices. The exposures were conducted with the mobile-phone antenna outside the glass vials containing the flies, either in contact with or at a certain distance from the glass wall. The daily duration of exposure varied from 1 to 20 minutes, depending on the experiment. Exposure always started on the day of eclosion and lasted for a total of 5 or 6 days. The temperature within the vials during exposure was monitored with a mercury thermometer with an accuracy of 0.05 °C. The authors explained the decreased reproductive ability as the result of RF radiation-induced DNA fragmentation in the gonads ([Panagopoulos, 2011](#); [Panagopoulos & Margaritis, 2008, 2010a, b](#); [Panagopoulos et al., 2004, 2007, 2010](#)).

[In reviewing these studies with *Drosophila*, the Working Group noted several shortcomings related to the methods of exposure assessment and temperature control, which could have influenced the results.]

(b) *Mouse*

See [Table 4.3](#)

(i) *900 MHz*

[Sykes et al. \(2001\)](#) studied somatic intrachromosomal recombination in the spleen of transgenic pKZ1 mice exposed to pulsed-wave RF radiation at 900 MHz (SAR, 4 W/kg) for 30 minutes per day, for 1, 5, or 25 days. There was a significant reduction in inversions below the spontaneous frequency in the group exposed for 25 days, whereas no effect was found in mice exposed for 1 or 5 days. The authors indicated that the number of mice in each treatment group in this study was small, and that repetition of this study with a larger number of mice was therefore required to confirm these observations.

[Aitken et al. \(2005\)](#) found a significant genotoxic effect on the epididymal spermatozoa of CD1 Swiss mice exposed to low-level RF radiation at 900 MHz (SAR, 0.09 W/kg) for 12 hours per day, for 7 days. No impact on male germ-cell development was observed. [The Working Group noted that insufficient information on dosimetry was provided in this study, which prevented a complete evaluation.]

Two cytogenetic studies were conducted with mice exposed to RF radiation from a mobile phone, with or without coexposure to X-rays or ultraviolet (UV) light. In the first study, female CBA/S mice were exposed for 78 weeks (1.5 hours per day, 5 days per week) either to continuous-wave RF radiation at 902.5 MHz (whole-body SAR, 1.5 W/kg) similar to that emitted by analogue NMT (Nordic Mobile Telephony) phones, or to a pulsed-wave signal at 902.4 MHz (SAR, 0.35 W/kg) similar to that emitted by digital GSM phones. All mice, except

the cage controls, were also exposed to X-rays ( $3 \times 1.33$  Gy; interval, 1 week) for the first 3 weeks of this experiment. In the second study, female transgenic mice (line K2) and their non-transgenic littermates were exposed to one of two digital mobile-phone signals at a frequency of 849 MHz GSM or 902 MHz DAMPS (Digital Advanced Mobile Phone System), with a SAR of 0.5 W/kg, for 1.5 hours per day, 5 days per week, for 52 weeks. All mice in the second study, except the cage controls, were also exposed to UV radiation mimicking the solar spectrum at 1.2 times the human minimal erythema dose (MED, 200 J/m<sup>2</sup>), three times per week. The results did not show any effects of RF fields on frequency of micronuclei in polychromatic erythrocytes or normochromatic erythrocytes, either alone or in combination with X-rays or UV radiation. The results were consistent in the two mouse strains (and in a transgenic variant of the second strain), after 52 or 78 weeks of exposure, at three SAR levels relevant to human exposure from mobile phones, and for three different mobile signals ([Juutilainen et al., 2007](#)).

(ii) 900 and 1800 MHz

In a study in B6C3F<sub>1</sub> mice exposed to RF radiation at 900 MHz or 1800 MHz (2 hours per day, for 1 week or 6 weeks) at different intensities (with SARs up to 33.2 W/kg in the 1-week experiment, and 24.9 W/kg in the 6-week experiment), the frequency of micronuclei was not increased in erythrocytes of peripheral blood or bone marrow, in keratinocytes or in spleen lymphocytes of the exposed animals compared with controls ([Görlitz et al., 2005](#)).

In a long-term study, micronucleus formation was measured in erythrocytes of B6C3F<sub>1</sub>/CrIBR mice exposed to RF radiation at 902 MHz GSM or 1747 MHz (DCS, Digital Cellular System), at SARs of 0.4, 1.3 or 4.0 W/kg, for 2 hours per day, 5 days per week, for 2 years. No differences were found in the frequencies of micronuclei in exposed, sham-exposed or cage-control mice ([Ziemann et al., 2009](#)).

(iii) 1500 MHz

Male Big Blue mice, which are transgenic for the *lacI* marker gene, were locally exposed (in the head region) to near-field RF radiation at 1500 MHz with SARs of 0.67 or 2.0 W/kg, for 90 minutes per day, 5 days per week, for 4 weeks. There was no significant difference between exposed and control mice in the frequency of mutation in the *lacI* transgene in the brain ([Takahashi et al., 2002](#)).

(iv) 450 MHz

[Sarkar et al. \(1994\)](#) found significant alterations in the length of a DNA microsatellite sequence in the brain and testes of Swiss albino mice exposed to RF radiation at 2450 MHz (power level, 1 mW/cm<sup>2</sup>; SAR, 1.18 W/kg) for 2 hours per day, for 120, 150 or 200 days. The authors hypothesized that a DNA fragment (7.7 kb) – generated by the restriction enzyme *HinfI* – that was found after exposure could represent a hypermutable locus and that exposure to these microwaves may have led to amplification of tandem sequences, generating more copies of 5'-GACA-3' sequences in this particular region. The authors also indicated that the radiation dose applied in the study was close to the prescribed safe limit for population exposure, according to Guidelines of the International Radiation Protection Association at the time ([IRPA, 1988](#)).

C3H/HeJ mice were exposed continuous-wave RF radiation at 2450 MHz in circularly polarized wave-guides (average whole-body SAR, 1.0 W/kg) for 20 hours per day, 7 days per week, for 18 months. Peripheral-blood and bone-marrow smears were examined for the presence of micronuclei in polychromatic erythrocytes. The initial publication reported no difference in micronucleus formation between exposed and sham-exposed mice, but a subsequent correction indicated that there was a slight but significant increase in the incidence of micronucleated cells in peripheral-blood and bone-marrow smears

of mice receiving long-term exposure to this RF radiation ([Vijayalaxmi et al., 1997a, 1998](#)).

Pregnant *lacZ*-transgenic mice (Muta<sup>TM</sup>Mouse) were exposed (16 hours per day) to intermittent (10 seconds on, 50 seconds off) RF radiation at 2450 MHz with an average whole-body SAR of 0.71 W/kg (4.3 W/kg during the exposure periods of 10 seconds), daily between day 0 and day 15 of gestation. Offspring were examined at age 10 weeks. Mutation frequencies at the *LacZ* gene in the spleen, liver, brain, and testis were similar to those observed in offspring of sham-exposed mice ([Ono et al., 2004](#)).

(v) 42 GHz (millimetre waves)

Adult male BALB/c mice were exposed (30 minutes per day) in the nasal region to RF radiation at 42 GHz (incident power density, 31.5 mW/cm<sup>2</sup>; peak SAR, 622 W/kg), on three consecutive days. The frequency of micronuclei in peripheral blood and in bone marrow was not increased in exposed mice compared with sham-exposed controls. One group of mice received a single injection of cyclophosphamide (15 mg/kg bw) immediately after the exposure to RF radiation on day 2. The micronucleus frequency in this group was not different from that in mice treated with cyclophosphamide only ([Vijayalaxmi et al., 2004](#)).

(vi) Ultra-wide band EMF

Male CF1 mice were exposed for 15 minutes to ultra-wide band (UWB) electromagnetic fields (600 pulses per second) at an estimated whole-body average SAR of 37 mW/kg. The mice were killed at 18 hours or 24 hours after exposure, and peripheral blood and bone marrow were collected and examined for the presence of micronuclei in polychromatic erythrocytes. Under the experimental conditions of this study, there was no evidence of cytogenetic effects in blood or bone marrow of the exposed mice ([Vijayalaxmi et al., 1999](#)).

(c) Rat

See [Table 4.3](#)

(i) 834 MHz

Micronucleus formation was investigated in the offspring of rats exposed to RF radiation. Wistar rats were placed in experimental cages on the first day of pregnancy and exposed (8.5 hours per day) to RF radiation at 834 MHz (26.8–40 V/m; vertical polarization; peak power, 600 mW; calculated SAR, 0.55–1.23 W/kg) from an analogue mobile telephone that was placed close to the plexiglass cage. Exposure was continued throughout gestation. Newborn pups (age, 2 days) showed a statistically significant increase ( $P < 0.003$ ) in micronucleus frequency in erythrocytes ( $1.23 \pm 0.17$  per 1000 cells) compared with controls ( $0.5 \pm 0.1$  per 1000 cells). Oxidative parameters measured in blood plasma or liver were not different between exposed and control rats ([Ferreira et al., 2006](#)).

(ii) 900–915 MHz

Wistar rats were exposed to RF radiation at 910 MHz (maximum SAR, 0.42 W/kg) for 2 hours per day on 30 consecutive days. Compared with non-exposed control rats, an almost threefold increase in the frequency of micronuclei was found in polychromatic erythrocytes of males and females ( $P < 0.001$  and  $P < 0.01$ , respectively); the induction was significantly lower in females than in males ( $P < 0.001$ ). An increase in micronucleus frequency was also observed in polymorphonuclear cells ([Demsia et al., 2004](#)).

Genotoxic effects of coexposure to RF radiation at 900 MHz with 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone (MX, a by-product of water chlorination; 19 µg/ml in drinking-water) were investigated in female Wistar rats ([Verschaeve et al., 2006](#)). The rats were exposed to RF radiation for 2 hours per day, 5 days per week, for 2 years, at an average SAR of 0.3 or 0.9 W/kg; exposure to MX was continuous. Blood samples were collected at 3, 6 and 24

**Table 4.3 Genetic and related effects of radiofrequency radiation, alone or in combination with chemical/physical mutagens: studies in experimental animals *in vivo***

End-point	Frequency	SAR	Duration	Chemical/physical mutagen	Results	Comments	References
<i>Mouse</i>							
MN formation in peripheral blood and bone-marrow cells in tumour-prone C3H/HeJ mice	2450 MHz, CW	1.0 W/kg	20 h/d, 7 d/wk, for 1.5 yr	None	+	Corrected statistical analysis in 1998 paper	Vijayalaxmi <i>et al.</i> (1997a, 1998)
MN formation in PCEs from peripheral blood and bone marrow of CF1 mice	Ultra-wide band radiation	0.037 W/kg	15 min	None	–		<a href="#">Vijayalaxmi <i>et al.</i> (1999)</a>
MN formation in peripheral-blood and bone-marrow cells of male BALB/c mice	42 200 MHz	622 ± 100 W/kg	30 min/d for 3 consecutive days	Coexposure with cyclophosphamide	–	No effect of RF radiation alone; no effect on MN induced by cyclophosphamide	<a href="#">Vijayalaxmi <i>et al.</i> (2004)</a>
MN formation in erythrocytes of blood or bone marrow, in keratinocytes and in spleen lymphocytes of B6C3F <sub>1</sub> mice	900 MHz (GSM) and 1800 MHz (DCS); AM	3.7, 11 and 33.2 W/kg (1-wk study); and 2.8, 8.3 and 24.9 W/kg (6-wk study)	2 h/d during 1 or 6 wk	None	–		<a href="#">Görlitz <i>et al.</i> (2005)</a>
MN formation in erythrocytes of female inbred CBA/S mice (taken from study by <a href="#">Heikkinen <i>et al.</i>, 2001</a> )	902.5 MHz (NMT), CW or 902.5 MHz (GSM), PW	1.5 W/kg or 0.35 W/kg	1.5 h/d, 5 d/wk, for 78 wk	Also exposed to X-rays (3 × 1.33 Gy, during first 3 wk)	–	No effect of RF radiation alone; no effect on MN induced by X-rays	<a href="#">Juutilainen <i>et al.</i> (2007)</a>
MN formation in erythrocytes of female K2 transgenic and non-transgenic mice (taken from <a href="#">Heikkinen <i>et al.</i>, 2003</a> )	Digital mobile-phone signals, GSM at 849 MHz and DAMPS at 902 MHz	0.5 W/kg	1.5 h/d, 5 d/wk, for 52 wk	Also exposed to UV radiation (1.2 MED), 3×/wk	–	No effect of RF radiation alone; no effect on MN induced by UV	<a href="#">Juutilainen <i>et al.</i> (2007)</a>
MN formation in erythrocytes of B6C3F <sub>1</sub> /Cr1BR male and female mice	GSM (902 MHz) or DCS (1747 MHz)	0.4, 1.3 or 4.0 W/kg	2 h/d, 5 d/wk, for 2 yr	None	–	No difference in MN frequency in exposed, sham-exposed or cage-control mice	<a href="#">Ziemann <i>et al.</i> (2009)</a>
Mutation assay ( <i>lacI</i> transgene) in brain tissue of Big Blue mice	1500 MHz	0, 0.67, or 2 W/kg	90 min/d, 5 d/wk, for 4 wk	None	–		<a href="#">Takahashi <i>et al.</i> (2002)</a>

**Table 4.3 (continued)**

End-point	Frequency	SAR	Duration	Chemical/physical mutagen	Results	Comments	References
Mutation frequency of the <i>lacZ</i> gene in cells from the spleen, liver, brain and testes of the offspring of <i>lacZ</i> - transgenic mice	2450 MHz (intermittent, 10 s on, 50 s off)	0.71 W/kg (average); 4.3 W/kg (for 10 s exposures)	Exposure <i>in utero</i> for 16 h/d on days 0–15 of gestation	None	–	Offspring was analysed at age 10 wk	<a href="#">Ono et al. (2004)</a>
DNA microsatellite analysis with synthetic oligonucleotide probes in cells of brain and testis of Swiss albino mice	2450 MHz, CW	1.2 W/kg	2 h/d, for 120, 150, 200 d	None	+	Change in length of a microsatellite sequence	<a href="#">Sarkar et al. (1994)</a>
DNA damage assessed by quantitative PCR (Q-PCR), alkaline- and pulsed-field electrophoresis in caudal epididymal spermatozoa of CD1 Swiss mice	900 MHz	0.09 W/kg	12 h/d, for 7 d	None	+	No effect on male germ-cell development; Q-PCR showed damage in mitochondrial genome and in nuclear $\beta$ -globin locus	<a href="#">Aitken et al. (2005)</a>
Somatic intrachromosomal recombination in spleen cells of pKZ1 transgenic mice	900 MHz, PW	4 W/kg	30 min/d for 1, 5, 25 d	None	–	Reduction in inversions below the spontaneous frequency in the group exposed for 25 d	<a href="#">Sykes et al. (2001)</a>
<i>Rat</i>							
MN formation in peripheral-blood and bone-marrow cells of male Sprague-Dawley rats	2450 MHz, CW	12 W/kg	24 h	None	–		<a href="#">Vijayalaxmi et al. (2001a)</a>
MN formation in peripheral blood cells of male Wistar rats	2450 MHz, CW	1 and 2 W/kg	2 h/d for up to 30 d	None	+	Only after 8 (not 2, 15, or 30) exposures of 2 h each	<a href="#">Trosic et al. (2002)</a>
MN formation in PCEs in bone marrow and peripheral blood of Wistar rats	2450 MHz	Whole-body SAR, 1.25 W/kg	2 h/d, 7 d/wk, 30 d	None	+	Increased MN frequency in PCEs in bone marrow on day 15, and in the peripheral blood on day 8	<a href="#">Trosic &amp; Busljeta (2006)</a>
MN formation in bone-marrow cells of male and female Wistar rats	910 MHz	Peak SAR, 0.42 W/kg	2 h/d for 30 consecutive days	None	+		<a href="#">Demsia et al. (2004)</a>

**Table 4.3 (continued)**

End-point	Frequency	SAR	Duration	Chemical/physical mutagen	Results	Comments	References
MN formation in bone-marrow cells of male Wistar rats	2450 MHz, CW	1.25 W/kg	2 h/d for up to 30 days (total exposure 4, 16, 30 or 60 h)	None	+	Increase in PCE in bone marrow on day 15 (exposure, 30 h). Transient effect on proliferation and maturation of erythropoietic cells	<a href="#">Trosic et al. (2004)</a> ; <a href="#">Busljeta et al. (2004)</a>
MN formation in blood from adult pregnant Wistar rats	834 MHz, mobile-phone antenna, 26.8–40 V/m	0.55–1.23 W/kg	From day 1 of gestation, for 8.5 h/d, until birth of offspring	None	+	Significant increase of MN frequency in erythrocytes of newborn pups exposed <i>in utero</i>	<a href="#">Ferreira et al. (2006)</a>
MN formation in blood of female Wistar rats	900 MHz, AM	0.3 and 0.9 W/kg	2 h/d, 5 d/wk, for 2 yr	Coexposure with MX in drinking-water	–	No increase in MN after coexposure to MX and RF radiation compared with MX [no group exposed to RF only]	<a href="#">Verschaeve et al. (2006)</a>
MN formation in blood cells of Wistar rats	10 000 MHz 50 000 MHz	0.04 W/kg 0.0008 W/kg	2 h/d for 45 d	None	+ +	Also significant increase of ROS in serum	<a href="#">Kumar et al. (2010)</a>
DNA breaks (SSB, DSB) measured with comet assay in brain cells of male Sprague-Dawley rats	2450 MHz, PW or CW	0.6 and 1.2 W/kg	2 h	None	+	Significant and SAR-dependent increase in SB immediately and at 4 h after exposure to CW; only at 4 h after exposure to PW	<a href="#">Lai &amp; Singh (1995)</a>
DNA breaks (SSB, DSB) measured with comet assay in brain cells of male Sprague-Dawley rats	2450 MHz, PW or CW	1.2 W/kg	2 h	None	+	Significant increase in SB at 4 h after exposure to either PW or CW	<a href="#">Lai &amp; Singh (1996)</a>
DNA breaks (SSB, DSB) measured with comet assay in brain cells of male Sprague-Dawley rats	2450 MHz, PW	1.2 W/kg	2 h	Melatonin or <i>N</i> -tert-butyl- $\alpha$ -phenylnitrone (free-radical scavengers)	+	Significant increase in SB at 4 h after exposure. Treatment with radical scavengers before and after exposure to RF prevented/reversed induction of SB	<a href="#">Lai &amp; Singh (1997)</a>

**Table 4.3 (continued)**

End-point	Frequency	SAR	Duration	Chemical/physical mutagen	Results	Comments	References
DNA breaks (SSB) measured with comet assay in brain cells of male Sprague-Dawley rats	2450 MHz, CW	1.2 W/kg	2 h	None	-		<a href="#">Malyapa et al. (1998)</a>
DNA breaks (SSB) measured with alkaline comet assay (with or without proteinase K) in brain cells of male Sprague-Dawley rats	2450 MHz, PW	1.2 W/kg	2 h	None	-		<a href="#">Lagroye et al. (2004a)</a>
DNA breaks (SSB, DSB) measured with comet assay in brain cells of male Sprague-Dawley rats	2450 MHz, CW, circular polarization	0.6 W/kg	2 h	None	+	Significant increase in SB at 4 h after exposure	<a href="#">Lai &amp; Singh (2005)</a>
DNA breaks (DSB) measured with pulsed-field electrophoresis. Changes in chromatin conformation detected with AVTD assay in brain cells from Wistar rats	915 MHz (GSM)	0.4 W/kg	2 h	None	-	Changes in gene expression were detected	<a href="#">Belyaev et al. (2006)</a>
DNA breaks (SSB) measured with alkaline comet assay in brain cells of male and female Wistar rats	2450 MHz or 16 500 MHz	1.0 W/kg or 2.01 W/kg	2 h/d, for 35 d	None	+	DNA breakage was observed at both frequencies	<a href="#">Paulraj &amp; Behari (2006)</a>
DNA breaks (SSB) measured with alkaline comet assay in blood, liver and brain of female Wistar rats	900 MHz, AM	0.3 or 0.9 W/kg	2 h/d, 5 d/wk for 2 yr	Co-exposure with MX in drinking-water	-	No increase in SB after co-exposure to MX and RF radiation compared with MX [no group exposed to RF only]	<a href="#">Verschaeve et al. (2006)</a>
DNA breaks (DSB) measured with neutral comet assay in brain of male Wistar rats	2450 MHz, from MW oven	0.11 W/kg (whole-body)	2 h/d, 35 d	None	+	Highly significant decrease in anti-oxidant enzymes and increase in catalase were also seen ( $P < 0.006$ )	<a href="#">Kesari et al. (2010)</a>

**Table 4.3 (continued)**

End-point	Frequency	SAR	Duration	Chemical/physical mutagen	Results	Comments	References
<i>Rabbit</i>							
Oxidative DNA damage (8-OHdG) in liver of pregnant and non-pregnant New Zealand White rabbits	1800 MHz (GSM-like)	NR	15 min/d for 1 wk (for pregnant rabbits: days 15–22 of gestation)	None	–	No difference in 8-OHdG/10 <sup>6</sup> dG between exposed and sham-exposed non-pregnant or pregnant rabbits, or between newborns exposed <i>in utero</i> and sham-exposed newborns	<a href="#">Tomruk et al. (2010)</a>
<i>Cow</i>							
MN formation in erythrocytes of Latvian Brown cows living in the Skrunda radio-station area	154–162 MHz, PW	NR	Cows had been living in the area for at least 2 yr	None	+	Significant increase in MN compared with cows in a control area. Frequencies of MN were low in all cases	<a href="#">Balode (1996)</a>

+, increase; –, no effect; AVTD, anomalous viscosity time-dependence; CW, continuous wave; d, day; DAMPS, Digital Advanced Mobile Phone System, DCS, Digital Cellular System; DSB, DNA double-strand breaks; GSM, Global System for Mobile Communications; h, hour; MED, minimal erythema dose; min, minute; MN, micronuclei; MW, microwave; MX, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone; NMT, Nordic Mobile Telephone; NR, not reported; PCE, polychromatic erythrocytes; PW, pulsed wave; s, second; SAR, specific absorption rate; SB, DNA strand breaks, SSB, DNA single-strand breaks; wk, week; yr, year

months and brain and liver samples were taken at the end of the study (24 months). The extent of DNA strand breaks in blood, liver and brain cells was determined by means of the alkaline comet assay; the frequency of micronuclei was measured in erythrocytes. Coexposure to MX and RF radiation did not significantly change the effects in blood, liver and brain cells compared with those seen with MX only [the Working Group noted that this study did not include a treatment group exposed to RF radiation only].

Induction of DNA double-strand breaks was measured by means of pulsed-field gel electrophoresis, and changes in chromatin conformation were assessed by use of the anomalous viscosity time-dependence (AVTD) assay in brain tissue of Fisher rats exposed to RF radiation at 915 MHz (GSM; SAR, 0.4 W/kg) for 2 hours. No effects of exposure to RF radiation were found. Analysis of gene-expression profiles in the cerebellum of exposed rats revealed changes in genes associated with neurotransmitter regulation, melatonin production and regulation of the blood-brain barrier ([Belyaev et al., 2006](#)).

#### (iii) 1600 MHz

Timed-pregnant Fischer 344 rats were exposed from day 19 of gestation, and their nursing offspring until weaning at 3 weeks of age, to far-field RF radiation at 1600 MHz (iridium wireless-communication signal) for 2 hours per day, 7 days per week. The whole-body average SAR was 0.036–0.077 W/kg (0.10–0.22 W/kg in the brain). This first exposure was followed by long-term, head-only exposures of male and female offspring (starting at age 35 days) to a near-field 1600 MHz signal, with a SAR of 0.16 or 1.6 W/kg in the brain, for 2 hours per day, 5 days per week, for 2 years. The micronucleus frequency in polychromatic erythrocytes of the bone marrow was not significantly different between exposed, sham-exposed and cage-control rats ([Vijayalaxmi et al., 2003](#)).

#### (iv) 2450 MHz

In several publications from the same laboratory it was reported that brain cells of male Sprague-Dawley rats exposed for 2 hours to low-intensity pulsed-wave or continuous-wave RF radiation at 2450 MHz (SAR, 0.6 or 1.2 W/kg) showed an increased number of DNA single- and double-strand breaks – measured by the neutral and alkaline comet assays – at 4 hours after exposure. The authors suggested that this could be due either to a direct effect on DNA or to an effect on DNA repair ([Lai & Singh, 1995, 1996](#)). In subsequent experiments, treatment of the rats with free-radical scavengers appeared to block this effect of RF exposure, suggesting that free radicals may be involved in RF-radiation-induced DNA damage in the rat brain ([Lai & Singh, 1997](#)).

Male Sprague-Dawley rats were exposed to continuous-wave RF radiation at 2450 MHz (SAR of 1.2 W/kg) for 2 hours, which did not cause a rise in the core body-temperature of the rats. One group of rats was killed by carbon dioxide (CO<sub>2</sub>) asphyxia, another by decapitation. DNA breakage was assessed by means of the alkaline comet assay. No significant differences were observed in the comet length or the normalized comet moment of cells isolated from either the cerebral cortex or the hippocampus of irradiated rats and those from sham-exposed rats. This was independent of the method by which the rats were killed. However, there was more intrinsic DNA damage and more experiment-to-experiment variation in cells from the asphyxiated rats than from rats killed by decapitation. Therefore, the latter method appeared to be the most appropriate in this type of study ([Malyapa et al., 1998](#)). [The Working Group noted that this study was not a valid replication of the [Lai & Singh \(1995\)](#) study, contrary to the authors' intention, but it provided independent evidence contrary to those results. The Working Group also noted that the increased number of DNA strand breaks after

exposure to RF radiation *in vivo* was particularly protocol-dependent, specifically with respect to the method of killing the animals and the treatment of tissue samples between exposure of the animals and analysis of the tissues.]

[Vijayalaxmi et al. \(2001a\)](#) found no evidence for the induction of micronuclei in peripheral-blood and bone-marrow cells of Wistar rats exposed continuously to continuous-wave RF radiation at 2450 MHz, with an average whole-body SAR of 12 W/kg, for 24 hours.

[Lagroye et al. \(2004a\)](#) investigated the induction of DNA damage in brain cells of Sprague-Dawley rats exposed to pulsed-wave RF radiation at 2450 MHz, with a SAR of 1.2 W/kg, for 2 hours. The rats were decapitated 4 hours after exposure. No DNA damage was detected in separate samples of the same brain-cell preparation from exposed rats, assessed by two variants of the alkaline comet assay.

Wistar rats were exposed to non-thermal RF radiation at 2450 MHz for 2 hours per day on 7 days per week, for up to 30 days. The power-density range was 5–10 mW/cm<sup>2</sup>, which corresponded to an approximate SAR of 1–2 W/kg. Erythrocyte counts, haemoglobin concentrations and haematocrit values were significantly increased in peripheral blood on days 8 and 15, and anuclear cells and erythropoietic precursor cells in bone marrow were significantly decreased. The frequency of micronucleated cells in the bone marrow was significantly increased on day 15, not on days 2, 8, and 30 ([Busljeta et al., 2004](#)).

Adult male Wistar rats were exposed to continuous-wave RF radiation at 2450 MHz for 2 hours per day, 7 days per week, for up to 30 days. The power-density range was 5–10 mW/cm<sup>2</sup>, which corresponded to an approximate SAR of 1–2 W/kg. The frequency of micronuclei in polychromatic erythrocytes was significantly increased in the group that had received 8 irradiation treatments of 2 hours each, but not in the groups that received 2, 15 or 30 treatments, in comparison with the sham-exposed group.

These results would be in line with an adaptive or recovery mechanism that was triggered in this experimental model during treatment ([Trosic et al., 2002, 2004](#)). Similar results were presented in a later publication ([Trosic & Busljeta, 2006](#)).

[Paulraj & Behari \(2006\)](#) reported a significantly increased ( $P < 0.001$ ) level of DNA breakage – measured by means of the alkaline comet assay – in brain cells of rats exposed to RF radiation at 2450 MHz or 16.5 GHz (SAR, 1.0 or 2.01 W/kg) for 2 hours per day, for 35 days.

Wistar rats were exposed to RF radiation at 2450 MHz (power density, 0.34 mW/cm<sup>2</sup>) for 2 hours per day, for 35 days. The whole-body SAR was estimated to be 0.11 W/kg. After exposure, rats were killed and whole-brain tissue was dissected and used for analysis of DNA double-strand breaks by means of the neutral comet assay. A significant increase was observed in various comet parameters in exposed brain cells compared with controls. Statistically significant changes were also observed in the levels of different antioxidant enzymes, *i.e.* a decrease in glutathione peroxidase, superoxide dismutase and histone kinase, and an increase in catalase ([Kesari et al., 2010](#)).

#### (v) 10–50 GHz

Wistar rats were exposed continuously to RF radiation at 10 GHz or 50 GHz (SAR, 0.014 W/kg and 0.0008 W/kg, respectively) for 2 hours per day, for 45 days. In both cases, significant increases ( $P < 0.05$ ) in the frequency of micronuclei – deduced from a reduced polychromatic/normochromatic erythrocyte ratio – and in concentrations of reactive oxygen species (ROS) were found in blood cells and serum, respectively ([Kumar et al., 2010](#)).

#### (d) Rabbit

A study was performed with non-pregnant and pregnant New Zealand White rabbits. The rabbits were exposed (whole-body) to 1800 MHz RF radiation (GSM) for 15 minutes per day,

for 1 week. For the pregnant rats, this exposure period was between day 15 and day 22 of gestation. Control groups of non-pregnant and pregnant rabbits were sham-exposed. No difference was found in the level of 8-hydroxy-2'-deoxyguanosine (an indicator of oxidative DNA damage; expressed as 8-OHdG/10<sup>6</sup> dG) in DNA from liver tissue of exposed and sham-exposed rabbits (pregnant or non-pregnant). Changes in malondialdehyde concentration and ferrous oxidation in xylenol orange in the liver of exposed non-pregnant and pregnant rabbits indicated an effect on lipid peroxidation. In pups exposed *in utero*, a reduction in ferrous oxidation in xylenol orange was seen in the liver, but no change was observed in malondialdehyde concentration. These results supported the notion that 1800 MHz GSM-like RF radiation may induce oxidative stress in exposed tissues ([Tomruk et al., 2010](#)).

#### (e) Cow

Blood samples were obtained from 67 female Latvian Brown cows living on a farm in the vicinity of the Skrunda radio-location station (Latvia), and from 100 cows in a control area, which was selected on the basis of the similarity to the exposed area with regards to many factors except exposure. Frequencies of micronuclei were scored in the erythrocytes and found to be low but statistically significantly increased in the exposed cows compared with those in the controls (0.6/1000 cells compared with 0.1/1000 cells;  $P < 0.01$ ) ([Balode, 1996](#)).

### 4.1.3 Experimental systems: *in vitro*

#### (a) Humans: peripheral blood lymphocytes

The most widely used cell type for investigations *in vitro* is the peripheral blood lymphocyte. Some details on the exposure conditions to RF radiation and a short conclusion of the publications discussed below are presented in [Table 4.4](#).

#### (i) Studies with a single end-point

##### DNA-damage induction and repair

The effects of exposure to RF radiation at frequencies ranging from approximately 800 to 8000 MHz were examined by several investigators, who reported no significant effect on induction of DNA strand breaks ([Baohong et al., 2005, 2007](#); [Chemeris et al., 2006](#); [Sannino et al., 2006](#)).

[Vijayalaxmi et al. \(2000\)](#) assessed DNA strand breaks in human lymphocytes and also the capacity of these cells to repair such damage after exposure to RF radiation at 2450 MHz, and observed no effect on either parameter. [Zhijian et al. \(2009\)](#) also reported no effect of exposure to RF radiation at 1800 MHz, not only on induction of DNA strand breaks but also on the repair kinetics of X-irradiation-induced DNA strand breaks. [Tiwari et al. \(2008\)](#) exposed human lymphocytes to RF radiation at 835 MHz (SAR, 1.17 W/kg) and subsequently incubated the cells in the presence of aphidicolin (APC; an inhibitor of DNA repair) at a dose of 0.02 or 2 µg/ml. There was no effect on DNA strand breakage from exposure to RF radiation alone. APC (2 µg/ml) and combinations of RF radiation with APC (0.02 and 2 µg/ml) enhanced the number of DNA strand breaks; this damage is repairable (see Section 4.1.3c).

##### Chromosomal aberrations

[Maes et al. \(1995\)](#) found an increase in the frequency of chromosomal aberrations in the form of dicentrics and acentric fragments in human lymphocytes exposed to pulsed-wave RF radiation at 954 MHz, while using a cooled box to maintain the temperature at  $17 \pm 1$  °C. [Manti et al. \(2008\)](#) carried out FISH analysis with molecular probes specific for whole chromosomes 1 and 2 in lymphocytes from four donors. The cells were exposed to RF radiation at 1950 MHz (UMTS, Universal Mobile Telecommunications System) at SAR 0.5 or 2.0 W/kg, for 24 hours. There was no effect on the fraction of aberrant cells at a

**Table 4.4 Genetic and related effects in human peripheral blood lymphocytes exposed to radiofrequency radiation *in vitro***

End-point	Frequency	SAR or power density	Duration	Results	Comments	Reference
Aneuploidy	830 MHz, CW	2.0–8.2 W/kg	72 h	+ (chromosome 17)	Temperature kept at 33.5–37.5 °C. In control without RF, no aneuploidy was seen up to 38.5 °C	<a href="#">Mashevich et al. (2003)</a>
Aneuploidy	100 GHz, CW	0.31 mW/cm <sup>2</sup>	1–24 h	+ (chromosomes 11, 17) – (chromosomes 1, 10)	Direct effect questionable. High values in control cells.	<a href="#">Korenstein-Ilan et al. (2008)</a>
Aneuploidy	800 MHz, CW	2.9, 4.1 W/kg	24 h	+ (chromosomes 11, 17) at SAR of 2.9 W/kg + (chromosomes 1, 10) at SAR of 4 W/kg	High values in control cells. In control without RF, no aneuploidy was seen up to 40 °C	<a href="#">Mazor et al. (2008)</a>
Chromosomal aberration	7700 MHz, CW	0.5, 10, 30 mW/cm <sup>2</sup>	10, 30, 60 min	+	Abberations increased at 10 and 30 mW/cm <sup>2</sup> at all time-points	<a href="#">Garaj-Vrhovac et al. (1992)</a>
Chromosomal aberration	2450 MHz, PW	75 W/kg	30 min, 2 h	+	MW output was adjusted with a thermistor to keep cells at 36.1 °C	<a href="#">Maes et al. (1993)</a>
Chromosomal aberration	954 MHz, PW; GSM	1.5 W/kg	2 h	±	Questionable dosimetry (pylon from GSM base-station connected to indoor antenna); no statistics provided	<a href="#">Maes et al. (1995)</a>
Chromosomal aberration	440, 900, 1800 MHz, PW; GSM	1.5 W/kg	30–72 h	–		<a href="#">Eberle et al. (1996)</a>
Chromosomal aberration	935.2 MHz, PW; GSM	0.3–0.4 W/kg	2 h	–		<a href="#">Maes et al. (1997)</a>
Chromosomal aberration	2450 MHz, CW	12.5 W/kg	90 min or 3 × 30 min	–		<a href="#">Vijayalaxmi et al. (1997b)</a>
Chromosomal aberration	455.7 MHz, PW	6.5 W/kg	2 h	–	Cells were placed 5 cm from a car phone	<a href="#">Maes et al. (2000)</a>
Chromosomal aberration	900 MHz, PW; CDMA	0.4–10 W/kg	2 h	–		<a href="#">Maes et al. (2001)</a>
Chromosomal aberration	835.62 MHz, CW; FDMA	4.4, 5.0 W/kg	24 h	–		<a href="#">Vijayalaxmi et al. (2001a)</a>
Chromosomal aberration	847.74 MHz, CW; CDMA	4.9, 5.5 W/kg	24 h	–		<a href="#">Vijayalaxmi et al. (2001b)</a>
Chromosomal aberration	2500 MHz 10 500 MHz	627 W/kg 0.25 W/kg	40 s 5 min	– –	MW oven at 3 W	<a href="#">Figueiredo et al. (2004)</a>

**Table 4.4 (continued)**

End-point	Frequency	SAR or power density	Duration	Results	Comments	Reference
Chromosomal aberration	900 MHz, PW; GSM	0.3, 1 W/kg	2 h	-		<a href="#">Zeni et al. (2005)</a>
Chromosomal aberration	935 MHz, PW; GSM	1, 2 W/kg	24 h	-		<a href="#">Stronati et al. (2006)</a>
Chromosomal aberration	2450, 8200 MHz, PW	2.1, 21 W/kg	2 h	-		<a href="#">Vijayalaxmi et al. (2006)</a>
Chromosomal aberration	1950 MHz, PW; UMTS	0.5, 2 W/kg	24 h	- at SAR of 0.5 W/kg + at SAR of 2 W/kg	Frequency of aberrations/cell was increased at higher SAR; FISH technique was used	<a href="#">Manti et al. (2008)</a>
Chromosomal aberration	18 000 MHz, CW 16 500 MHz, PW	1 mW/cm <sup>2</sup> 10 mW/cm <sup>2</sup>	53 h	-		<a href="#">Hansteen et al. (2009a)</a>
Chromosomal aberration	2300 MHz, CW, PW	1 mW/cm <sup>2</sup>	53 h	-		<a href="#">Hansteen et al. (2009b)</a>
Micronucleus formation	7700 MHz, CW	0.5, 10, 30 mW/cm <sup>2</sup>	10, 30, 60 min	+	MN frequency increased at 30 mW/cm <sup>2</sup> , after 30 and 60 min of exposure	<a href="#">Garaj-Vrhovac et al. (1992)</a>
Micronucleus formation	2450 MHz, PW	75 W/kg	30 min, 2 h	+	MW output was adjusted with a thermistor to keep cells at 36.1 °C	<a href="#">Maes et al. (1993)</a>
Micronucleus formation	9000 MHz, CW, PW	90 W/kg	10 min	+ with PW - with CW	Temperature during exposure was 30–35 °C. Control cultures were kept at 37 °C	<a href="#">d'Ambrosio et al. (1995)</a>
Micronucleus formation	440, 900, 1800 MHz, PW; GSM	1.5 W/kg	30–72 h	-		<a href="#">Eberle et al. (1996)</a>
Micronucleus formation	2450 MHz, CW	12.5 W/kg	3 × 30 min	-		<a href="#">Vijayalaxmi et al. (1997b)</a>
Micronucleus formation	2450, 7700 MHz, CW	10, 20, 30 mW/cm <sup>2</sup>	15, 30, 60 min	+	Experiment carried out at 20–22 °C. Temperature-control measurements were made in water	<a href="#">Zotti-Martelli et al. (2000)</a>
Micronucleus formation	835.62 MHz, CW; FDMA	4.4, 5.0 W/kg	24 h	-		<a href="#">Vijayalaxmi et al. (2001a)</a>
Micronucleus formation	847.74 MHz, CW; CDMA	4.9, 5.5 W/kg	24 h	-		<a href="#">Vijayalaxmi et al. (2001b)</a>
Micronucleus formation	1748 MHz, CW, PW; GSM	5 W/kg	15 min	+ with PW - with CW	Temperature during exposure was 30–35 °C. Control cultures were kept at 37 °C	<a href="#">d'Ambrosio et al. (2002)</a>
Micronucleus formation	1900 MHz, CW, PW	0.1–10 W/kg	2 h	-		<a href="#">McNamee et al. (2002a)</a>

**Table 4.4 (continued)**

End-point	Frequency	SAR or power density	Duration	Results	Comments	Reference
Micronucleus formation	2450 MHz, PW	5 mW/cm <sup>2</sup>	2 h	-		<a href="#">Zhang et al. (2002)</a>
Micronucleus formation	837, 1909 MHz, CW, PW; CDMA, TDMA	1.0, 2.5, 5.0, 10.0 W/kg	3 h, 24 h	+ after 24 h, at SARs of 5 or 10 W/kg	Some exposures were from mobile telephones. Temperature variations were $\pm 0.3$ °C and $\pm 0.5$ °C at 3 h and 24 h, respectively. EMS was included as a positive control	<a href="#">Tice et al. (2002)</a>
Micronucleus formation	1900 MHz, CW, PW	0.1–10 W/kg	24 h	-		<a href="#">McNamee et al. (2003)</a>
Micronucleus formation	120, 130 GHz PW	1 and 0.6 mW average power	20 min	-		<a href="#">Scarfi et al. (2003)</a>
Micronucleus formation	900/925 MHz, CW, PW(i); GSM	1.6 W/kg 0.2 W/kg	14 $\times$ (6 min on, 3 h off) at 1.6 W/kg; 1 h/d for 3 d at 0.2 W/kg	-		<a href="#">Zeni et al. (2003)</a>
Micronucleus formation	1800 MHz, CW	5, 10, 20 mW/cm <sup>2</sup>	1, 2, 3 h	+	Large variation between individuals and repeat experiments	<a href="#">Zotti-Martelli et al. (2005)</a>
Micronucleus formation	900 MHz, PW; GSM	0.1–10 W/kg	24 h	-	Concordant results between two research groups in interlaboratory study	<a href="#">Scarfi et al. (2006)</a>
Micronucleus formation	935 MHz, PW; GSM	1, 2 W/kg	24 h	-		<a href="#">Stronati et al. (2006)</a>
Micronucleus formation	2450, 8200 MHz, PW	2.1, 21 W/kg	2 h	-		<a href="#">Vijayalaxmi et al. (2006)</a>
Micronucleus formation	1950 MHz, PW (c, i); UMTS	0.05–2 W/kg	4–48 h	-	Controversial data	<a href="#">Schwarz et al. (2008)</a>
Micronucleus formation	1950 MHz, PW (c, i); UMTS	2.2 W/kg	24–68 h	-		<a href="#">Zeni et al. (2008)</a>
Micronucleus formation	900 MHz, PW; GSM	1.25 W/kg	20 h	-	No effect of RF radiation alone. Reduction of MMC-induced micronucleus frequency. Data indicative of an adaptive response	<a href="#">Sannino et al. (2009a)</a>
Sister-chromatid exchange	2450 MHz, PW	75 W/kg	30 min, 2 h	-	MW output was adjusted with a thermistor to keep cells at 36.1 °C	<a href="#">Maes et al. (1993)</a>
Sister-chromatid exchange	954 MHz, PW; GSM	1.5 W/kg	2 h	-		<a href="#">Maes et al. (1996)</a>

**Table 4.4 (continued)**

End-point	Frequency	SAR or power density	Duration	Results	Comments	Reference
Sister-chromatid exchange	380, 900, 1800 MHz, PW; TETRA, DCS, GSM	0.08–1.7 W/kg	72 h	-		<a href="#">Antonopoulos et al. (1997)</a>
Sister-chromatid exchange	440, 900, 1800 MHz, PW; GSM	1.5 W/kg	30–72 h	-		<a href="#">Eberle et al. (1996)</a>
Sister-chromatid exchange	935.2 MHz, PW; GSM	0.3–0.4 W/kg	2 h	-		<a href="#">Maes et al. (1997)</a>
Sister-chromatid exchange	455.7 MHz, PW; car phone	6.5 W/kg	2 h	-		<a href="#">Maes et al. (2000)</a>
Sister-chromatid exchange	900 MHz, PW; GSM	0.4–10 W/kg	2 h	-		<a href="#">Maes et al. (2001)</a>
Sister-chromatid exchange	900 MHz, PW; GSM	0.3, 1 W/kg	2 h	-		<a href="#">Zeni et al. (2005)</a>
Sister-chromatid exchange	400–900 MHz, PW	-	-	-		<a href="#">Maes et al. (2006)</a>
Sister-chromatid exchange	935 MHz, PW; GSM	1, 2 W/kg	24 h	-		<a href="#">Stronati et al. (2006)</a>
DNA single- and double-strand breaks	935.2 MHz, PW; GSM	0.3–0.4 W/kg	2 h	-		<a href="#">Maes et al. (1997)</a>
DNA single- and double-strand breaks	2450 MHz, PW	2.1 W/kg	2 h	-	No effect, immediately or 4 h after exposure	<a href="#">Vijayalaxmi et al. (2000)</a>
DNA single- and double-strand breaks	1900 MHz, CW, PW	0.1–10 W/kg	2 h	-		<a href="#">McNamee et al. (2002a)</a>
DNA single- and double-strand breaks	2450 MHz, PW	5 mW/cm <sup>2</sup>	2 h	-		<a href="#">Zhang et al. (2002)</a>
DNA single- and double-strand breaks	837, 1909 MHz, CW, PW; CDM, TDM	1.0, 2.5, 5.0, 10.0 W/kg	3 h, 24 h	-	Some exposures were from mobile telephones. Temperature variations were $\pm 0.3$ °C and $\pm 0.5$ °C at 3 h and 24 h, respectively. EMS was included as a positive control.	<a href="#">Tice et al. (2002)</a>
DNA single- and double-strand breaks	1900 MHz, CW, PW	0.1–10 W/kg	24 h	-		<a href="#">McNamee et al. (2003)</a>

**Table 4.4 (continued)**

End-point	Frequency	SAR or power density	Duration	Results	Comments	Reference
DNA single- and double-strand breaks	1800 MHz, PW; GSM	3 W/kg	2 h	-		<a href="#">Baohong et al. (2005)</a>
DNA single- and double-strand breaks	900 MHz, PW; GSM	0.3, 1 W/kg	2 h	-		<a href="#">Zeni et al. (2005)</a>
DNA single- and double-strand breaks	8800 MHz, PW	1.6 kW/kg	40 min	-		<a href="#">Chemeris et al. (2006)</a>
DNA single- and double-strand breaks	1950 MHz, PW; UMTS	0.5, 2 W/kg	24 h	-		<a href="#">Sannino et al. (2006)</a>
DNA single- and double-strand breaks	935 MHz, PW; GSM	1, 2 W/kg	24 h	-		<a href="#">Stronati et al. (2006)</a>
DNA single- and double-strand breaks	1800 MHz, PW; GSM	3 W/kg	1.5, 4 h	-		<a href="#">Baohong et al. (2007)</a>
DNA single- and double-strand breaks	120 000, 130 000 MHz, PW; THz	0.2–2 W/kg	20 min	-		<a href="#">Zeni et al. (2007a)</a>
DNA single- and double-strand breaks	1950 MHz, PW(c, i); UMTS	0.05–2 W/kg	4–48 h	-	Controversial data	<a href="#">Schwarz et al. (2008)</a>
DNA single- and double-strand breaks	835 MHz, PW; CDMA	1.17 W/kg	1 h	-	RF radiation induced repairable DNA damage in the presence of aphidicolin	<a href="#">Tiwari et al. (2008)</a>
DNA single- and double-strand breaks	1950 MHz, PW(c, i); UMTS	2 W/kg	24–68 h	-		<a href="#">Zeni et al. (2008)</a>
DNA single- and double-strand breaks	1800 MHz, PW(i); GSM	2 W/kg	24 h	-	No effect of RF radiation on repair of X-ray-induced DNA damage	<a href="#">Zhijian et al. (2009)</a>
Mutation at <i>HPRT</i> locus	440, 900, 1800 MHz, PW; GSM	1.5 W/kg	30–72 h	-		<a href="#">Eberle et al. (1996)</a>

**Table 4.4 (continued)**

End-point	Frequency	SAR or power density	Duration	Results	Comments	Reference
Foci	915 MHz, PW; GSM	37 mW/kg	2 h	+	Decrease in 53BP1-foci (measured by immuno-staining); enhanced chromatin condensation (measured by AVTD)	<a href="#">Belyaev et al. (2005)</a>
Foci	905, 915 MHz, PW; GSM	37 mW/kg	1 h	+ at 915 MHz - at 905 MHz	Decrease in 53BP1- and $\gamma$ -H2AX-foci (measured by immunostaining) and enhanced chromatin condensation (measured by AVTD)	<a href="#">Markovà et al. (2005)</a>
Foci	905, 915, 1947 MHz, PW; GSM, UMTS	0.015–0.145 W/kg	1 h	+ at 915 MHz - at 905 MHz + t 1947 MHz	Decrease in 53BP1- and $\gamma$ -H2AX-foci (measured by immunostaining) and enhanced chromatin condensation (measured by AVTD). Strongest effect at 1947 MHz	<a href="#">Belyaev et al. (2009)</a>

+, increase;  $\pm$ , equivocal; -, no effect; APC, aphidicholin (inhibitor of DNA repair); AVTD, anomalous viscosity time-dependence; (c, i): continuous or intermittent exposure; CA, chromosomal aberration; CDMA, code-division multiple access; CW, continuous wave; d, day; DCS, Digital Communication System; EMS, ethylmethane sulfonate; FDMA, frequency-division multiple access; FISH, fluorescence *in situ* hybridization; GSM, Global System for Mobile Communication; h, hour; HPRT, hypoxanthine(guanine)phosphoribosyl transferase; min, minute; MMC, mitomycin C; MW, microwave; PW, pulsed wave; s, second; TDMA, time-division multiple access; TETRA, Trans European Trunked Radio; THz; teraHertz; UMTS, Universal Mobile Telecommunication System

SAR of 0.5 W/kg, while there was a small but statistically significant increase in the frequency of aberrations per cell at 2 W/kg. [Figueiredo et al. \(2004\)](#) and [Hansteen et al. \(2009a, b\)](#) carried out conventional analyses of chromosomal aberrations on Giemsa-stained slides prepared with lymphocytes exposed to RF radiation at 1800–10 500 MHz, and observed no effect.

#### *Micronucleus formation*

[Zotti-Martelli et al. \(2000\)](#) exposed whole blood from two volunteers to continuous-wave RF radiation at 2450 MHz or 7700 MHz, with power densities of 10, 20 and 30 mW/cm<sup>2</sup> for 15, 30, and 60 minutes, and reported an increased micronucleus frequency in exposed cells at 30 mW/cm<sup>2</sup>. In a subsequent study, [Zotti-Martelli et al. \(2005\)](#) observed an increase in the frequency of micronuclei in lymphocytes from nine different donors after exposure to RF radiation at 1800 MHz. This experiment was repeated after 3 months; there was significant variation between experiments. [The Working Group noted that temperature variation in the first study was not measured in blood samples during exposure, and the increased frequency of micronucleus formation may have been related to heating of the blood samples. Also, there were discrepancies between the data on micronuclei given in the text, figures, and tables]. [d'Ambrosio et al. \(1995, 2002\)](#) reported an increase in the formation of micronuclei in lymphocytes exposed to pulsed-wave RF radiation at 1748 MHz or 9000 MHz for 15 and 10 minutes, respectively, while no such increase was observed in cells exposed to continuous-wave RF at the same frequencies. [Zeni et al. \(2003\)](#) observed no significant effect on micronucleus formation in lymphocytes exposed to continuous or pulsed-wave RF radiation at 900 MHz (GSM). [Scarfi et al. \(2003\)](#) reported no micronucleus induction in lymphocytes exposed to continuous-wave RF radiation at 120–130 GHz. [Sannino et al. \(2009a\)](#) reported that a 20-hour pre-exposure of peripheral blood lymphocytes in the S-phase of the cell

cycle to pulsed-wave RF radiation at 900 MHz decreased the micronucleus frequency induced by mitomycin C (MMC), suggesting the existence of an adaptive response (see [Table 4.4](#) for details).

#### *Sister-chromatid exchange (SCE)*

[Maes et al. \(1996\)](#) did not find an effect on SCE in lymphocytes exposed to pulsed-wave RF radiation at 954 MHz, with a SAR of 1.5 W/kg, for 2 hours. Likewise, [Antonopoulos et al. \(1997\)](#) did not find an effect on SCE in lymphocytes exposed to RF radiation at 380–1800 MHz, with a SAR of 0.08–1.7 W/kg, for 72 hours.

#### *Phosphorylation of histone protein H2AX and TP53-binding protein 53BP1*

Over the past decade, several studies have demonstrated that two cellular check-point proteins, H2AX and TP53-binding protein 53BP1 are rapidly phosphorylated after induction of DNA damage in the form of double-strand breaks. These proteins then congregate to provide a scaffold structure to the repair sites ([Paull et al., 2000](#); [Schultz et al., 2000](#); [DiTullio et al., 2002](#); [Fernandez-Capetillo et al., 2002, 2004](#); [Sedelnikova et al., 2002](#); [Ismail et al., 2007](#)). By use of specific antibodies with fluorescent tags,  $\gamma$ -H2AX – the phosphorylated form of H2AX – and 53BP1 can be visualized as discrete foci, which can be counted directly with a fluorescence microscope.

The AVTD assay is used to detect stress-induced changes in chromatin conformation. [Shckorbatov et al. \(1998, 2009\)](#) and [Sarimov et al. \(2004\)](#) have reported changes in chromatin condensation in human lymphocytes exposed to RF radiation at 42.2 GHz, 35 GHz or 895–915 MHz, respectively, which prevented access of proteins involved in repair of DNA double-strand breaks. [Belyaev et al. \(2005\)](#) exposed human lymphocytes for 2 hours to pulsed-wave RF radiation at 915 MHz (GSM), with a SAR of 37 mW/kg, and reported significant

effects on chromatin condensation and a distinct reduction in the number of 53BP1-foci in samples from all individuals; these results were similar to those found after heat-shock treatment. The overall data suggested a reduced accessibility of 53BP1 to repair DNA double-strand breaks due to chromatin condensation. [Marková \*et al.\* \(2005\)](#) exposed human lymphocytes to pulsed-wave RF radiation at 905 MHz or 915 MHz (GSM), with a SAR of 37 mW/kg, for 1 hour. Chromatin condensation and decreased numbers of 53BP1- and  $\gamma$ -H2AX-foci were observed in cells after exposure at 915 MHz, but not at 905 MHz. The response was similar in healthy subjects and in subjects hypersensitive to RF radiation. [Belyaev \*et al.\* \(2009\)](#) exposed lymphocytes to pulsed-wave RF radiation at 905 MHz or 915 MHz (GSM), or 1947 MHz (UMTS), with a SAR of 15–145 mW/kg, for 1 hour. Chromatin condensation and reduction in numbers of 53BP1- and  $\gamma$ -H2AX-foci were much more pronounced in cells after exposure at 1947 MHz than at 915 MHz; there were no such effects after exposure at 905 MHz. The decrease in number of foci persisted for up to 72 hours after exposure, suggesting that not only the formation of double-strand breaks was affected, but also their repair. [Marková \*et al.\* \(2010\)](#) used VH10 primary fibroblasts established from human foreskin and mesenchymal stem cells isolated from adipose tissue of two healthy persons. These cells were exposed to pulsed-wave RF radiation at 905 MHz or 915 MHz (GSM; SAR, 37 mW/kg), or at 1947 MHz (UMTS; SAR, 39 mW/kg), as a single exposure for 1, 2 or 3 hours, or as repeated exposures for 1 hour per day, 5 days per week, for 2 weeks. The decrease in the number of 53BP1-foci was more pronounced in stem cells than in foreskin fibroblasts, and the stem cells did not adapt to long-term exposure to RF radiation.

### *Aneuploidy*

Peripheral blood lymphocytes from five individuals were stimulated with phytohaemagglutinin (PHA) and exposed for 72 hours

to continuous-wave RF radiation at 830 MHz (SAR, 1.6–8.8 W/kg), in an incubator set at temperatures between 33.5 °C (at the highest SAR value) and 37.5 °C. The incidence of aneuploidy of chromosome 17 was determined by use of a probe for  $\alpha$ -satellite DNA repeat-sequences present in its centromeric region. The data indicated a linear and SAR-dependent increase in aneuploidy in cells exposed to RF radiation at SAR 2.0–8.2 W/kg (6–9%) compared with control cells (4–5%). Control experiments without RF radiation were conducted at 34.5–41 °C, showing no change in aneuploidy at temperatures up to 38.5 °C. This indicates that the effect of RF radiation was produced via a non-thermal pathway ([Mashevich \*et al.\*, 2003](#)).

Peripheral blood lymphocytes from nine donors were stimulated with PHA for 1–6 hours, then exposed to continuous-wave RF radiation at 100 GHz (power density, 0.031 mW/cm<sup>2</sup>) for 1, 2 or 24 hours in an incubator in which CO<sub>2</sub> levels were not controlled. After exposure, the cells were incubated for a total culture period of 69–72 hours, with CO<sub>2</sub> levels at 5%. The cells were harvested and changes in chromosomes 1, 10, 11 and 17 were analysed by means of the FISH technique. For chromosomes 11 and 17, a 30% increase in aneuploidy was found after exposure for 2 or 24 hours, while chromosomes 1 and 10 were not affected. Asynchronous replication of centromeres 1, 11, and 17 was increased by 40% after 2 hours of exposure, while that of all four centromeres had increased by 50% after 24 hours of exposure. During the experiments, fibre-optic sensors were used to measure differences in temperature between exposed and sham-exposed samples; the difference never exceeded 0.3 °C ([Korenstein-Ilan \*et al.\*, 2008](#)).

[Mazor \*et al.\* \(2008\)](#) exposed PHA-stimulated lymphocytes from 10 individuals to continuous-wave RF radiation at 800 MHz (SAR, 2.9 or 4.1 W/kg) for 72 hours, with the incubator set at 33.5 °C to maintain the sample temperature at 36–37 °C, in particular at the high SAR value.

Aneuploidy was scored for chromosomes 1, 10, 11, and 17 by use of the FISH technique. An increased frequency of cells aneuploid for chromosomes 11 and 17 was observed at the lower SAR of 2.9 W/kg, and for chromosomes 1 and 10 at the higher SAR of 4.1 W/kg. Multisomy (chromosomal gain) was the primary contributor to the increase in aneuploidy. Control experiments – without exposure to RF radiation – were conducted in the temperature range 33.5–41 °C; there was no change in aneuploidy.

#### *Spindle disturbance (experiments with human-hamster hybrid cells)*

The well established human–hamster hybrid ( $A_L$ ) cell line, containing a single copy of human chromosome 11, was exposed to pulsed-wave RF radiation at 835 MHz, with increasing electric field strengths from 5 to 90 V/m, for 30 minutes ([Schmid & Schrader, 2007](#)). The results indicated a field strength-dependent increase in the frequency of spindle disturbances during anaphase/telophase of cell division. [The Working Group noted the absence of negative and positive controls.] [Schrader et al. \(2008\)](#) reported similar increases in spindle disturbances in  $A_L$  cells exposed for 30 minutes or 2 hours to RF radiation at 835 MHz (90 V/m) compared with non-exposed controls. [Schrader et al. \(2011\)](#) exposed  $A_L$  cells to RF radiation at 900 MHz (amplitude-modulated and unmodulated), at electric field strengths of 45 or 90 V/m, and with a SAR of 11.5 W/kg, for 30 minutes. The experiments were conducted with separate electric (E field) and magnetic (H field) components of RF radiation, at 20–22 °C. A significant increase in the frequency of spindle disturbances was observed in cells exposed to the E component, while no effect was seen in cells exposed to the H component (compared with non-exposed control cells). [Hintzsche et al. \(2011\)](#) also reported an increase in spindle disturbance during the anaphase/telophase of cell division in the same  $A_L$  cell line exposed to continuous-wave RF radiation at

106 GHz (power densities, 0.043–4.3 mW/cm<sup>2</sup>) for 30 minutes.

#### *(ii) Studies with two or more end-points*

[Tice et al. \(2002\)](#) reported a significant and reproducible increase in micronucleus formation in human lymphocytes exposed for 24 hours to RF radiation at 837 or 1909.8 MHz, with an average SAR of 5.0 or 10.0 W/kg. There was no increase in the number of DNA strand breaks in leukocytes, as measured with the alkaline comet assay. [McNamee et al. \(2002a, 2003\)](#) reported no effects on DNA strand-break induction or micronucleus formation in cells exposed to continuous- or pulsed-wave RF radiation at 1900 MHz, with SARs of up to 10 W/kg, for 2 or 24 hours. [Zhang et al. \(2002\)](#) observed no induction of DNA strand breaks or formation of micronuclei in human lymphocytes exposed to pulsed-wave RF radiation at 2450 MHz compared with controls. [Zeni et al. \(2008\)](#) reported no increase in DNA strand breaks or micronucleus formation in human lymphocytes exposed to intermittent (6 minutes on, 2 hours off) RF radiation at 1900 MHz (SAR, 2.2 W/kg) for 24–68 hours. Likewise, [Schwarz et al. \(2008\)](#), reported no increase in DNA strand-break induction or micronucleus formation in PHA-stimulated or non-stimulated human lymphocytes exposed for 16 hours to intermittent (5 minutes on, 10 minutes off) RF radiation at 1950 MHz (SAR, 0.1 W/kg).

[Garaj-Vrhovac et al. \(1992\)](#) reported significantly increased frequencies of chromosomal aberrations and micronuclei in human peripheral blood lymphocytes exposed for up to 60 minutes to continuous-wave RF radiation at 7700 MHz, with power densities up to 30 mW/cm<sup>2</sup>.

In a series of studies from one laboratory, no increase in the frequency of chromosomal aberrations or micronuclei was reported in human lymphocytes exposed to RF radiation at 2450 MHz for 90 minutes, to continuous-wave RF radiation at 835 or 847 MHz for 24 hours, or

to RF radiation at 2450 or 8200 MHz for 2 hours ([Vijayalaxmi et al., 1997b](#), [2001b](#), [c](#), [2006](#)).

[Maes et al. \(1993\)](#) found a time-dependent increase in the frequencies of chromosomal aberrations and micronuclei in peripheral blood lymphocytes exposed to pulsed-wave RF radiation at 2450 MHz (SAR, 75 W/kg) for 30 or 120 minutes. Both effects were statistically significant for the exposure of 120 minutes. No induction of SCE was found. In this study, the microwave output was adjusted by use of a thermistor thermometer to maintain the temperature of the cells at 36.1 °C. In subsequent experiments, [Maes et al. \(2000, 2001\)](#) examined human lymphocytes exposed to pulsed-wave RF radiation at 455.7 MHz (SAR, 6.5 W/kg) or 900 MHz (SAR, 0.4–10 W/kg) for 2 hours; no increase in chromosomal aberrations or SCE was observed.

[Stronati et al. \(2006\)](#) did not report significant changes in DNA strand-break induction, chromosomal aberrations, micronucleus formation or SCE in blood cells exposed to pulsed-wave RF radiation at 935 MHz (SAR, 1 or 2 W/kg). [Eberle et al. \(1996\)](#) measured chromosomal aberrations, micronucleus formation, SCE, and mutations at the *HPRT* locus in human lymphocytes exposed to RF radiation at 440, 900, or 1800 MHz (SAR, 1.5 W/kg). Exposure times varied (39, 50, 70 hours), depending on the experiment. No significant effects were observed for any of these end-points in RF-exposed cells compared with controls.

(b) *Humans: other primary and continuously growing cultured cells*

Some details on the exposure conditions to RF radiation and a short conclusion for each publication are presented in [Table 4.5](#).

(i) *Amniotic cells*

Human amniotic cells were exposed to RF radiation at 900 MHz (GSM; SAR, 0.25 W/kg) for 24 hours. Chromosomes were stained by use of the R-banding method and examined to determine

the incidence of structural and numerical aberrations. Exposure to RF radiation had no effect ([Bourthoumieu et al., 2010](#)). [The Working Group noted that R-banding is not recommended for analysis of chromosomal aberrations.] In a subsequent study by the same authors, amniotic cells were collected during amniocentesis from three separate donors. The cells were cultured for 15 days before being exposed to RF radiation at 900 MHz (GSM, pulsed-wave; pulse duration, 0.577 ms; pulse-repetition rate, 217 Hz; SAR, 0.25, 1, 2 or 4 W/kg) for 24 hours in a wire-patch cell at exposure temperatures of  $36.3 \pm 0.4$  °C,  $37.0 \pm 0.2$  °C,  $37.5 \pm 0.4$  °C and  $39.7 \pm 0.8$  °C, respectively, for the four SAR levels. The cells were processed for analysis by two-colour FISH with centromeric  $\alpha$ -satellite repetitive probes for chromosomes 11 and 17 in interphase cells. No significant differences were observed between exposed and sham-exposed cells in the percentages of monosomic, trisomic cells or the total number of cells aneuploid for chromosomes 11 or 17 ([Bourthoumieu et al., 2011](#)).

(ii) *Glioblastoma and neuroblastoma cells*

No effects on DNA strand-break induction were observed in human U87MG glioblastoma cells exposed for up to 24 hours to continuous-wave or pulsed-wave RF radiation at 835, 847, or 2450 MHz (SAR, 0.6 W/kg at 835/847 MHz, and 0.7 or 1.9 W/kg at 2450 MHz) ([Malyapa et al. \(1997a, b\)](#)).

[Miyakoshi et al. \(2002\)](#) did not find an effect on DNA strand-break induction in human MO54 glial cells – derived from a patient with a brain tumour – exposed to RF radiation at 2450 MHz (average SAR, 50 or 100 W/kg) for 2 hours. Likewise, [Sakuma et al. \(2006\)](#) reported no effect on DNA strand-break induction in human A172 glioblastoma cells exposed to pulsed-wave RF radiation at 2142.5 MHz (SAR, up to 800 mW/kg) for 2 or 24 hours, and [Luukkonen et al. \(2009, 2010\)](#) found no effects on DNA strand-break induction in cultured human SH-SY5Y

**Table 4.5 Genetic and related effects in human cells (other than lymphocytes) exposed to radiofrequency radiation *in vitro***

End-point	Cells	Frequency	SAR or power density	Duration	Results	Comments	Reference
Aneuploidy	HAC	900 MHz, PW; GSM	0.25, 1, 2, 4 W/kg	24 h	-	Chromosomes 11 and 17 were included in this study	<a href="#">Bourthoumieu <i>et al.</i> (2011)</a>
Chromosomal aberration	HAC	900 MHz, PW; GSM	0.25 W/kg	24 h	-		<a href="#">Bourthoumieu <i>et al.</i> (2010)</a>
Micronucleus formation	BUC	PW; mobile phone	NR	1 h/d for 2.3 yr	+		<a href="#">Yadav &amp; Sharma (2008)</a>
Micronucleus formation	BUC	PW; mobile phone	NR	3 h/wk for 5–10 yr	-		<a href="#">Hintzsche &amp; Stopper (2010)</a>
Micronucleus formation	HSF	1800 MHz, CW, PW(i); GSM	2 W/kg	1, 4, 24 h	-	Replication study. Previous results not confirmed.	<a href="#">Speit <i>et al.</i> (2007)</a>
Micronucleus formation	SHF	1950 MHz, PW(c-i); UMTS	0.05–2 W/kg	4–48 h	+ after 12 h exposure	Controversial data	<a href="#">Schwarz <i>et al.</i> (2008)</a>
Micronucleus formation	SHF	900 MHz, PW; GSM	1 W/kg	24 h	-		<a href="#">Sannino <i>et al.</i> (2009b)</a>
DNA single- and double-strand breaks	GLB	2450 MHz, CW	0.7 W/kg	2–24 h	-		<a href="#">Malyapa <i>et al.</i> (1997a)</a>
DNA single- and double-strand breaks	GLB	835, 847 MHz, CW, PW; FMCW, CDMA	0.6 W/kg	2–24 h	-		<a href="#">Malyapa <i>et al.</i> (1997b)</a>
DNA single- and double-strand breaks	GLB	2450 MHz	13–100 W/kg	2 h	-		<a href="#">Miyakoshi <i>et al.</i> (2002)</a>
DNA single- and double-strand breaks	GLB	2000 MHz, PW; CW, IMT	0.08, 0.25, 0.80 W/kg	2 h, 24 h	-		<a href="#">Sakuma <i>et al.</i> (2006)</a>
DNA single- and double-strand breaks	HSF	1800 MHz, CW, PW(i); GSM	2 W/kg	1, 4, 24 h	-	Replication study. Previous results not confirmed.	<a href="#">Speit <i>et al.</i> (2007)</a>
DNA single- and double-strand breaks	HTR	1817 MHz, PW; GSM	2 W/kg	1 h	-		<a href="#">Valbonesi <i>et al.</i> (2008)</a>

**Table 4.5 (continued)**

End-point	Cells	Frequency	SAR or power density	Duration	Results	Comments	Reference
DNA single- and double-strand breaks	HTR	1800 MHz, CW, PW(i); GSM	2 W/kg	4–24 h	– with CW – with PW at 4 h + with PW at 16 h and 24 h	Differential response between CW and PW and exposure duration	<a href="#">Franzellitti et al. (2010)</a>
DNA single- and double-strand breaks	LEP	1800 MHz, PW; GSM	1, 2, 3 W/kg	2 h	– at 1 and 2 W/kg + at 3 W/kg		<a href="#">Lixia et al. (2006)</a>
DNA single- and double-strand breaks	LEP	1800 MHz, PW(i); GSM	1, 2, 3, 4 W/kg	2 h	– at 1 and 2 W/kg + at 3 and 4 W/kg		<a href="#">Yao et al. (2008)</a>
DNA single- and double-strand breaks	LUF	2000 MHz, PW, CW; IMT	0.08 W/kg	2, 24 h	–		<a href="#">Sakuma et al. (2006)</a>
DNA single- and double-strand breaks	LYB	813, 836 MHz, PW; iDEN, TDMA	2.4–26 mW/kg	2–21 h	±	Inconsistent results	<a href="#">Phillips et al. (1998)</a>
DNA single- and double-strand breaks	LYB	813, 836, 835, 847 MHz, CW, PW; iDEN, TDMA, FDMA, CDMA	0.0024–0.026 W/kg, 3.2 W/kg	2–21 h	–		<a href="#">Hook et al. (2004a)</a>
DNA single- and double-strand breaks	LYB	1800 MHz, PW; GSM	2 W/kg	6–24 h	–		<a href="#">Zhijian et al. (2010)</a>
DNA single- and double-strand breaks	NUB	872 MHz, CW, PW; GSM	5 W/kg	1 h	–	Temperature-controlled conditions	<a href="#">Luukkonen et al. (2009)</a>
DNA single- and double-strand breaks	NUB	872 MHz, CW, PW; GSM	5 W/kg	3 h	–	Temperature-controlled conditions	<a href="#">Luukkonen et al. (2010)</a>
DNA single- and double-strand breaks	SHF	1800 MHz, PW (c, i)	2 W/kg	4–24 h	+	Controversial data	<a href="#">Diem et al. (2005)</a>
DNA single- and double-strand breaks	SHF	1950 MHz, PW(c-i); UMTS	0.05–2 W/kg	4–48 h	+	Controversial data	<a href="#">Schwarz et al. (2008)</a>

**Table 4.5 (continued)**

End-point	Cells	Frequency	SAR or power density	Duration	Results	Comments	Reference
DNA single- and double-strand breaks	SHF	900 MHz, PW; GSM	1 W/kg	24 h	–		<a href="#">Sannino <i>et al.</i> (2009b)</a>
Foci	HFB, MST	905, 915, 1947 MHz, PW; GSM, UMTS	0.037, 0.039 W/kg	1–3 h	+ at 915MHz – at 905 MHz + at 1947 MHz	Decrease in 53BP1-foci, measured by immunostaining	<a href="#">Marková <i>et al.</i> (2010)</a>
Spindle disturbance	HHH	835 MHz, PW; GSM	5–90 V/m	30 min	+	Mitotic cell fraction was scored on slides stained with 2% acetic orcein.	<a href="#">Schmid &amp; Schrader (2007)</a>
Spindle disturbance	HHH	835 MHz, PW	62.5 mW/kg	10 min to 2 h	+	Mitotic cell fraction was scored on slides stained with 2% acetic orcein.	<a href="#">Schrader <i>et al.</i> (2008)</a>
Spindle disturbance	HHH	1060 MHz, CW	0.043–4.3 mW/cm <sup>2</sup>	30 min	+	Mitotic cell fraction was scored on slides stained with 2% acetic orcein.	<a href="#">Hintzsche <i>et al.</i> (2011)</a>
Spindle disturbance	HHH	900 MHz, CW, PW	0.0115 W/kg	30 min	+	Mitotic cell fraction was scored on slides stained with 2% acetic orcein.	<a href="#">Schrader <i>et al.</i> (2011)</a>
8-OHdG, oxidative damage in DNA	SPR	1800 MHz	0.4–27.5 W/kg	16 h	+	Temperature controlled at 21 °C; maximum increase 0.4 °C during exposure.	<a href="#">De Iuliis <i>et al.</i> (2009)</a>

+, increase; ±, equivocal; –, no effect; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; BUC, human buccal cells; (c, i), continuous and intermittent exposure; d, day; FDMA, frequency-division multiple access; FMCW, frequency-modulated continuous wave; GLB, glioblastoma cells; h, hour; HAC, human amniotic cells; HFB, human foreskin fibroblasts; HHH, hamster–human hybrid cells; HTR, trophoblast cells; iDEN, Integrated Digital Enhanced Network; IMT, International Mobile Telecommunication; LEP, lens epithelial cells; LUF, human fibroblasts from fetal lung; LYB, lymphoblastoid cells; min, minute; MST, mesenchymal stem cells; NUB, neuroblastoma cells; NR, not reported; SHF, skin human fibroblasts; SPR, sperm cells; TDAM, time-division multiple access; yr, year

neuroblastoma cells exposed to continuous- or pulsed-wave RF radiation at 872 MHz, with a SAR of 5 W/kg. In the studies mentioned above the alkaline comet assay was used to measure strand breakage in DNA.

#### (iii) *Lens epithelial cells*

Immortalized SRA01/04 human lens epithelial cells were exposed to pulsed-wave RF radiation at 1800 MHz (SAR, 1, 2 or 3 W/kg) for 2 hours to investigate induction of DNA breakage, which was measured by means of the alkaline comet assay. DNA-damage repair was evaluated by further incubation of the exposed cells for 30, 60, 120 or 240 minutes. There was a significant increase ( $P < 0.05$ ) in DNA strand-breaks at a SAR of 3 W/kg immediately after exposure, which had decreased at 30 minutes, and had diminished to control levels at later time-points. At SARs of 1 and 2 W/kg, there were no significant differences between exposed cells and sham-exposed controls ([Lixia et al., 2006](#)).

In a similar study, DNA strand breaks were measured in SRA01/04 human lens epithelial cells exposed to intermittent (5 minutes on, 10 minutes off) pulsed-wave RF radiation at 1800 MHz (SAR, 1, 2, 3, or 4 W/kg) for 2 hours. There was no effect on DNA single-strand breaks – measured with the alkaline comet assay – at SARs of 1 or 2 W/kg, but a significant increase at SARs of 3 or 4 W/kg ( $P < 0.001$ ). At these two higher SAR values, there was no difference in the induction of DNA double-strand breaks, measured with the  $\gamma$ H2AX-focus formation assay ([Yao et al., 2008](#)).

#### (iv) *Lung fibroblasts*

[Sakuma et al. \(2006\)](#) exposed human IMR-90 fetal lung fibroblasts to pulsed-wave RF radiation at 2000 MHz (SAR, 80 mW/kg) for 24 hours, and observed no effect on induction of DNA strand breaks.

#### (v) *Lymphoblastoid cells*

[Phillips et al. \(1998\)](#) studied DNA strand-break induction in human Molt-4 lymphoblastoid cells exposed for 2, 3 or 21 hours to RF radiation at 813 or 835 MHz as iDEN (Integrated Digital Enhanced Network) and TDMA (time-division multiple access) signals, with very low SARs of 2.4, 24, 2.6 and 26 mW/kg. There was a general decrease in the number of strand breaks at lower SARs at 2 and 21 hours (but not at 3 hours), and inconsistent results at higher SARs, depending on the type of RF signal, power intensity and duration of exposure. [Hook et al. \(2004a\)](#) examined DNA strand-break induction in Molt-4 cells exposed to the same and additional signals (813.56–847.74 MHz) at the same and higher SARs (2.4 mW/kg–3.4 W/kg) than in the study by [Phillips et al. \(1998\)](#). No effect on DNA strand-break induction was noted. [Zhijian et al. \(2010\)](#) did not find any effect on DNA strand-break induction when human HMy2.CIR lymphoblastoid B-cells were exposed to pulsed-wave RF radiation at 1800 MHz (SAR, 2 W/kg), for 6–24 hours.

#### (vi) *Skin fibroblasts*

[Diem et al. \(2005\)](#) exposed human ES-1 skin fibroblasts to continuous or intermittent (5 minutes on, 10 minutes off) RF radiation at 1800 MHz (SAR, 1.2 or 2 W/kg) for 4–24 hours. The cells were examined visually and subjectively to evaluate DNA single- and double-strand breaks by use of the alkaline and neutral comet assays. A “tail factor” was devised to express the results. The authors concluded that: there was a significant increase in tail factor after a 16-hour exposure, with no further increase after 24 hours; and that intermittent exposure produced a stronger effect than continuous exposure.

In a study from the same group, [Schwarz et al. \(2008\)](#) used ES-1 cells exposed for 4–48 hours to continuous and intermittent RF radiation at 1950 MHz (UMTS), with a range of SAR values (0.05–2 W/kg). The results from the analyses of

DNA strand breaks by means of the comet assay indicated a significant increase in tail factor ( $P < 0.02$ ) at SAR 0.05 W/kg. In addition, there was a significant increase ( $P < 0.02$ , at SAR 0.05 W/kg) in the frequency of micronuclei, which turned out be centromere-negative (suggesting a clastogenic effect). In a similar experiment in peripheral blood lymphocytes, there was no effect on the comet tail factor or micronucleus formation (see above). Several discussions about the mode of data acquisition in these two studies subsequently appeared in scientific journals ([Rüdiger et al., 2006](#); [Vijayalaxmi et al., 2006](#); [Tuffs, 2008](#); [Vogel, 2008](#); [Wolf, 2008](#); [Balzano, 2008](#); [Kuster, 2008](#); [Drexler & Schaller, 2009](#); [Rüdiger, 2009b, c](#); [Lerchl & Wilhelm, 2010](#); [Baan, 2009](#)).

[Speit et al. \(2007\)](#) performed independent experiments to replicate and confirm the results of the two studies mentioned above, by use of the same ES-1 skin fibroblasts, the same exposure system supplied by the same company, and the same laboratory protocols. The comet tail factor as well as computerized image-analysis were used to quantify the DNA strand breaks. The experiments were also performed in Chinese hamster V79 cells. The results showed no effect on DNA breakage either by the alkaline comet assay or by the micronucleus test, in either fibroblasts or V79 cells.

Skin fibroblasts established from healthy individuals or from subjects with Turner syndrome were exposed to 900 MHz pulsed-wave RF radiation (SAR, 1 W/kg). It was suggested that cells from patients with Turner syndrome were sensitive to the effects of weakly genotoxic agents ([Scarfi et al., 1997a, b](#)). No effects on DNA strand-break induction or micronucleus formation were observed in either cell line ([Sannino et al., 2009b](#)).

[Markovà et al. \(2010\)](#) observed no effect on 53BP1 foci in skin fibroblasts exposed to RF radiation at 905 MHz (SAR, 37 mW/kg). In contrast, a decrease was seen when the same cells were exposed at 915 or 1947 MHz at similar SAR

levels [the Working Group noted that this technique assesses repair foci of DNA double-strand breaks; it is different from the comet assay used for analysis of DNA strand breaks in the other studies with skin cells discussed above].

#### (vii) Mesenchymal stem cells

[Markovà et al. \(2010\)](#) observed a decrease in the number of 53BP1 foci in mesenchymal stem cells exposed to RF radiation at 915 or 1947 MHz (GSM; SAR 37 and 39 mW/kg, respectively) for 1, 2, or 3 hours; no effect was noted after exposure at 905 MHz (SAR, 37 mW/kg).

#### (viii) Sperm cells

[De Iuliis et al. \(2009\)](#) studied purified human spermatozoa exposed to RF radiation at 1800 MHz (SAR, 0.4–27.5 W/kg) for 16 hours at 21 °C. With increasing SAR values, motility and vitality of the sperm cells were significantly reduced, while mitochondrial production of reactive oxygen species was significantly increased ( $P < 0.001$ ). There was also a significant increase ( $P < 0.05$ ) in formation of 8-OHdG adducts (measured immunochemically) and DNA fragmentation (measured with the TUNEL – terminal deoxynucleotidyl transferase dUTP nick end labelling – assay) at SARs of 2.8 W/kg and higher. The temperature during these experiments was kept at 21 °C; the highest observed exposure-induced temperature increase was +0.4 °C, at a SAR of 27.5 W/kg.

#### (ix) Trophoblast cells

[Valbonesi et al. \(2008\)](#) observed no effects on DNA strand-break induction in human HTR-8/SVneo trophoblast cells exposed to pulse-modulated RF radiation at 1817 MHz (SAR, 2 W/kg) for 1 hour.

[Franzellitti et al. \(2010\)](#) observed no effects on DNA strand-break induction in HTR-8/SVneo trophoblast cells exposed to continuous-wave RF radiation at 1800 MHz (SAR, 2 W/kg) for 4, 16 or 24 hours. Exposure to this radiation as pulsed-wave amplitude-modulated signals (GSM-217 Hz

and GSM-Talk), caused a significant increase in DNA strand breakage after all three treatment periods when the results of the comet assay were expressed as “% DNA in tail.” The number of DNA strand breaks decreased rapidly during the 2 hours after exposure.

(c) *Humans: interaction of RF radiation with known genotoxic agents*

Some details on the exposure conditions to RF radiation and a short conclusion for each publication are presented in [Table 4.6](#). Unless otherwise mentioned, the results discussed below refer to those observed in human peripheral blood lymphocytes exposed to RF radiation before, during or after exposure to a genotoxic agent.

(i) *Chemotherapeutic drugs*

[Gadhia et al. \(2003\)](#) reported a synergistic increase in chromosomal aberrations (rings, dicentrics) and SCE in lymphocytes collected from mobile-phone users and treated with mitomycin C (MMC) *in vitro*, compared with cells from controls (non-phone users) treated with MMC. This effect was stronger in mobile-phone users who smoked and consumed alcohol.

[Maes et al. \(2006\)](#) found no effect of treatment with MMC on induction of DNA strand breaks, chromosomal aberrations or SCE in lymphocytes obtained from workers at a mobile-phone company. In a series of experiments *in vitro*, the same authors reported a highly reproducible synergistic effect ([Maes et al., 1996](#)), a weak synergistic effect ([Maes et al., 1997](#)), an inconsistent synergistic effect ([Maes et al., 2000](#)), or no synergistic effect ([Maes et al., 2001](#)) of exposure to RF radiation on MMC-induced SCE. [The Working Group noted that the authors made several suggestions regarding possible mechanistic explanations for their findings, which were not pursued in detail. The authors also mentioned the possibility of a thermal effect, and indicated

the incomplete characterization of the exposure conditions in their studies.]

[Zhang et al. \(2002\)](#) investigated a possible synergistic effect in human lymphocytes exposed to RF radiation at 2450 MHz (5 mW/cm<sup>2</sup>; 2 hours) followed by treatment with MMC (0.0125–0.1 µg/ml; 24 hours). While RF radiation had no effect by itself, it significantly increased the effect of the higher doses of MMC on DNA strand-break induction and micronucleus formation. Since the temperature increase during the 2-hour exposure was less than 0.5 °C, the synergy was not likely to be due to thermal effects.

[Baohong et al. \(2005\)](#) exposed human lymphocytes to pulsed-wave RF radiation at 1800 MHz (SAR, 3 W/kg) for 2 hours, before, together with, or after incubation for 3 hours with four different chemicals. After these treatments, the cells were washed and processed for measurement of DNA strand-break induction at once or after further incubation for 21 hours. Exposure to RF radiation alone had no effect. All combinations of MMC or 4-nitroquinoline-1-oxide (4NQO) with RF radiation showed a significant increase in DNA breakage, compared with the results after incubation with the chemical alone. No such effect was observed when exposure to RF radiation was combined with treatment with bleomycin or methylmethane sulfonate (MMS), suggesting that interaction between RF radiation and different chemical mutagens could vary.

[Hansteen et al. \(2009a\)](#) found no effect on MMC-induced chromosomal aberrations after exposure of human lymphocytes to pulsed-wave RF radiation at 16.5 GHz (power density, 10 W/m<sup>2</sup>) or 18 GHz continuous-wave RF radiation (power density 1 W/m<sup>2</sup>) for 53 hours, with MMC added after 30 hours. Similar results were reported by the same authors for exposures to continuous-wave or pulsed-wave RF radiation at 2.3 GHz (power density, 10 W/m<sup>2</sup>) in combination with MMC ([Hansteen et al., 2009b](#)).

[Sannino et al. \(2009a\)](#) reported that pre-exposure of human lymphocytes to pulsed-wave RF

**Table 4.6 Interaction between radiofrequency radiation and known genotoxic agents in human cells *in vitro***

End-point	Cells	Genotoxic agent	Frequency (MHz)	SAR or power density	Duration	Results	Comments	Reference
Chromosomal aberration	PBL	MMC or X-rays	900 MHz, PW; GSM	0.4–10 W/kg	RF radiation for 2 h, followed by X-rays for 1 min (1 Gy) or MMC for 72 h	–	No effect of RF radiation; no synergistic effects of RF radiation and MMC or X-rays	<a href="#">Maes et al. (2001)</a>
Chromosomal aberration	PBL from phone users	MMC	890–960 MHz, PW; GSM	NR	Phone use for 1–3 h/d for 2 yr, MMC for 48 h	+	Increased gaps/dicentrics after RF radiation; synergistic effect of RF radiation with MMC	<a href="#">Gadhia et al. (2003)</a>
Chromosomal aberration	PBL	Gamma-rays	2500 MHz 10 500 MHz, PW	627 W/kg 0.25 W/kg	40 s 5 min	–	MW oven used as 2.5 GHz source. No effect of RF radiation; no synergistic effect with gamma-rays	<a href="#">Figueiredo et al. (2004)</a>
Chromosomal aberration	PBL	MMC	400–900 MHz, PW	NR	2.3 yr (> 1h/d) MMC for 72 h	–	Lymphocytes from exposed workers. No synergistic effect with MMC	<a href="#">Maes et al. (2006)</a>
Chromosomal aberration	PBL	X-rays	935 MHz, PW; GSM	1 or 2 W/kg	1 min (1 Gy) X-rays, 24 h RF radiation	–	No effect of RF radiation; no synergistic effect with X-rays	<a href="#">Stronati et al. (2006)</a>
Chromosomal aberration	PBL	X-rays	1950 MHz, PW, UMTS	0.5, 2 W/kg	X-rays 5 min, RF radiation for 24 h	+ at 2 W/kg – at 0.5 W/kg	No effect of RF radiation; synergistic effect of RF radiation with X-rays (4 Gy) at the higher SAR	<a href="#">Manti et al. (2008)</a>
Chromosomal aberration	PBL	MMC	1800, 1650 MHz, CW, PW	0.1, 1 mW/cm <sup>2</sup>	53 h; MMC added at 30 h	–	No effect of RF radiation; no synergistic effect with MMC	<a href="#">Hansteen et al. (2009a)</a>
Chromosomal aberration	PBL	MMC	2300 MHz, CW, PW	1 mW/cm <sup>2</sup>	53 h; MMC added at 30 h	–	No effect of RF radiation; no synergistic effect with MMC	<a href="#">Hansteen et al. (2009b)</a>
Micronucleus formation	PBL	MMC	2450 MHz, PW	5 mW/cm <sup>2</sup>	2 h, then MMC for 24 h	+	No effect of RF radiation; synergistic effect with MMC	<a href="#">Zhang et al. (2002)</a>
Micronucleus formation	PBL	X-rays	935 MHz, PW; GSM	1 or 2 W/kg	1 min (1 Gy) X-rays, 24 h RF	–	No effect of RF radiation; no synergistic effect with X-rays	<a href="#">Stronati et al. (2006)</a>
Micronucleus formation	PBL	MMC	900 MHz, PW; GSM	1.25 W/kg	20 h; MMC for 24 h	+	Reduction of MMC-induced MN frequency (adaptive response?) in lymphocytes from 4 out of 5 donors	<a href="#">Sannino et al. (2009a)</a>

**Table 4.6 (continued)**

End-point	Cells	Genotoxic agent	Frequency (MHz)	SAR or power density	Duration	Results	Comments	Reference
DNA single- and double-strand breaks	PBL	MMC	2450 MHz, PW	5 mW/cm <sup>2</sup>	2 h, then MMC for 24 h	+	No effect of RF radiation; synergistic effect with MMC	<a href="#">Zhang et al. (2002)</a>
DNA single- and double-strand breaks	PBL	MMC, 4NQO	1800 MHz, PW; GSM	3 W/kg	2 h RF irradiation, 3 h with the chemical	+	No effect of RF radiation. Exposure to chemicals before, during or after RF irradiation showed a synergistic effect with MMC and 4NQO	<a href="#">Baohong et al. (2005)</a>
DNA single- and double-strand breaks	PBL	BLM, MMS	1800 MHz, PW, GSM	3 W/kg	2 h RF radiation 3 h with the chemical	-	No effect of RF radiation. Exposure to chemicals before, during or after RF irradiation showed no synergistic effect with BLM and MMS	<a href="#">Baohong et al. (2005)</a>
DNA single- and double-strand breaks	PBL	MMC	400–900 MHz, PW	NR	2.3 yr (> 1 h/d) MMC for 72 h	-	Lymphocytes from exposed workers. No synergistic effect with MMC	<a href="#">Maes et al. (2006)</a>
DNA single- and double-strand breaks	PBL	X-rays	935 MHz, PW; GSM	1 or 2 W/kg	1 min (1 Gy) X-rays, 24 h RF	-	No effect of RF radiation; no synergistic effect with X-rays	<a href="#">Stronati et al. (2006)</a>
DNA single- and double-strand breaks	PBL	UV	1800 MHz, PW; GSM	3 W/kg	1.5 or 4 h; just after UVC at 0.25–2.0 J/m <sup>2</sup>	+ at 4 h + at 1.5 h	Effect with UV depended on exposure duration: decrease at 1.5 h, increase at 4 h	<a href="#">Baohong et al. (2007)</a>
DNA single- and double-strand breaks	PBL	APC	835 MHz, PW; CDMA	1.2 W/kg	1 h RF irradiation and APC at 0.2 or 2 µg/ml	+	No effect of RF radiation; synergistic RF effect on aphidicolin-induced repairable DNA damage.	<a href="#">Tiwari et al. (2008)</a>
DNA single- and double-strand breaks	NUB	Menadione	872 MHz, CW, PW; GSM	5 W/kg	1 h RF and 50 µM menadione	+ with CW - with PW	Differential effect of CW and PW with menadione	<a href="#">Luukkonen et al. (2009)</a>
DNA single- and double-strand breaks	HSF	MX	900 MHz, PW; GSM	1 W/kg	24 h RF, 1 h MX at 25 µM	-	No synergistic effect on MX-induced SB	<a href="#">Sannino et al. (2009b)</a>
DNA single- and double-strand breaks	PBL	X-rays	1800 MHz, PW(i); GSM	2 W/kg	24 h (on/off for 5/10 min) then 0.25–2 Gy of X-rays	-	No effect of RF radiation; no synergistic effect with X-rays on SB induction or repair	<a href="#">Zhijian et al. (2009)</a>

**Table 4.6 (continued)**

End-point	Cells	Genotoxic agent	Frequency (MHz)	SAR or power density	Duration	Results	Comments	Reference
DNA single- and double-strand breaks	NUB	FeCl <sub>2</sub> + DEM	872 MHz, CW, PW; GSM	5 W/kg	1 h or 3 h RF; 1 h FeCl <sub>2</sub> ± DEM	–	No effect of RF radiation; no synergistic effect with free radical-inducing chemicals	<a href="#">Luukkonen et al. (2010)</a>
DNA single- and double-strand breaks	LYB	DOX	1800 MHz, PW; GSM	2 W/kg	6–24 h RF; 2 h DOX	–	No effect of RF radiation; no synergistic effect with doxorubicin on induction of single- or double-strand breaks; effect on repair (?)	<a href="#">Zhijian et al. (2010)</a>
Sister-chromatid exchange	PBL	MMC	954 MHz, PW; GSM	1.5 W/kg	2 h RF radiation 72 h MMC	+	No effect of RF radiation; highly reproducible synergistic effect with MMC	<a href="#">Maes et al. (1996)</a>
Sister-chromatid exchange	PBL	MMC	935.2 MHz, PW; GSM	0.3–0.4 W/kg	2 h RF radiation 72 h MMC	+	No effect of RF radiation: weak synergistic effect with MMC	<a href="#">Maes et al. (1997)</a>
Sister-chromatid exchange	PBL	MMC	455.7 MHz, PW; car phone	6.5 W/kg	2 h (72 h MMC)	±	No effect of RF radiation; inconsistent synergistic effect with MMC	<a href="#">Maes et al. (2000)</a>
Sister-chromatid exchange	PBL	MMC, X-rays	900 MHz, PW; GSM	0.4–10 W/kg	2 h (72 h MMC)	–	No effect of RF radiation; no synergistic effect with MMC or with X-rays	<a href="#">Maes et al. (2001)</a>
Sister-chromatid exchange	PBL from phone users	MMC	890–960 MHz, PW; GSM	NR	1–3 h/d for 2 yr 48 h MMC	+	Increased SCE after RF radiation; synergistic effect with MMC	<a href="#">Gadhia et al. (2003)</a>
Sister-chromatid exchange	PBL	MMC	400–900 MHz, PW	NR	72 h	–	No effect of RF radiation; no synergistic effect with with MMC	<a href="#">Maes et al. (2006)</a>
Sister-chromatid exchange	PBL	X-rays	935 MHz, PW; GSM	1, 2 W/kg	24 h	–	No effect of RF radiation; no synergistic effect with X-rays	<a href="#">Stronati et al. (2006)</a>

+, increase; ±, equivocal; –, no effect; 4NQO, 4-nitroquinoline-1-oxide; APC, aphidicolin; BLM, bleomycin; CW, continuous wave; d, day; DEM, diethyl maleate; FeCl<sub>2</sub>, ferrous chloride; h, hour; HSF, human skin fibroblasts; (i), intermittent exposure; LYB, lymphoblastoid cells; min, minute; MMC, mitomycin C; MMS, methylmethane sulfonate; MX, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone; NUB, neuroblastoma cells; NR, not reported; PBL, peripheral blood lymphocytes; PW, pulsed wave

radiation at 900 MHz (peak SAR, 10 W/kg) for 20 hours reduced the incidence of MMC-induced micronucleus formation, suggesting that non-ionizing radiation is capable of inducing an “adaptive response” similar to that observed in several studies of ionizing radiation.

[Zhijian et al. \(2010\)](#) treated cultured human lymphoblastoid cells with doxorubicin (DOX) for 2 hours before, during and after exposure to pulsed-wave RF radiation at 1800 MHz (SAR, 2 W/kg). No significant effects on DOX-induced DNA strand-break formation were found.

#### (ii) Genotoxic chemicals

[Tiwari et al. \(2008\)](#) exposed human peripheral blood lymphocytes to RF radiation at 835 MHz (SAR, 1.17 W/kg) for 1 hour, with and without treatment with aphidicolin (APC; 0.2 or 2 µg/ml), an inhibitor of DNA repair. There was no effect on DNA strand-break induction of RF radiation by itself, or of the low dose of APC alone. There was a significant increase in DNA breakage after combined exposure of the cells to RF radiation with the low ( $P = 0.025$ ) and the high dose ( $P = 0.002$ ) of APC. [Sannino et al. \(2009b\)](#) found no effect on the number of DNA strand breaks induced by 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in skin fibroblasts from healthy individuals or from subjects with Turner syndrome exposed to pulsed-wave RF radiation at 900 MHz (SAR, 1 W/kg) for 24 hours, followed by treatment with MX for 1 hour.

[Luukkonen et al. \(2009\)](#) found a significant increase ( $P < 0.01$ ) in the number of menadione-induced DNA strand breaks in cultured human SH-SY5Y neuroblastoma cells exposed to continuous-wave RF radiation at 872 MHz (SAR, 5 W/kg) and menadione (25 µM) for 1 hour, but not in cells exposed to pulsed-wave RF radiation (GSM) and menadione. In a subsequent study with the same cell type, the same authors did not observe an increase in the number of DNA strand breaks after exposure to continuous- or pulsed-wave RF radiation at the same frequency

and SAR (872 MHz; 5 W/kg), with or without ferrous chloride and diethyl maleate (the latter compound was added to enhance the free-radical production induced by the former) ([Luukkonen et al., 2010](#)).

#### (iii) Ionizing radiation

[Figueiredo et al. \(2004\)](#) reported no effects of RF radiation on the induction of chromosomal aberration by gamma radiation in human lymphocytes exposed to pulsed-wave RF radiation at 2.5 or 10.5 GHz (SAR, 627 and 0.25 W/kg, respectively) for 40 seconds or 5 minutes, respectively, followed 2 hours later by exposure to 1.5 Gy gamma radiation from a cobalt-60 source. No effect was observed after exposure to RF radiation alone. [Stronati et al. \(2006\)](#) found no effect of RF radiation on X-ray-induced DNA strand breaks, chromosomal aberrations, micronucleus formation or SCE in human peripheral blood lymphocytes exposed to pulsed-wave RF radiation at 935 MHz (SAR 1 or 2 W/kg) for 24 hours, combined with 1.0 Gy of 250 kVp X-rays, given for 1 minute immediately before or after exposure to RF radiation. In the FISH assay used by [Manti et al. \(2008\)](#), there was no effect of RF radiation on X-ray-induced chromosomal aberrations in human lymphocytes exposed to 4 Gy of X-rays immediately before exposure to pulsed-wave RF radiation at 1950 MHz (SAR, 0.5 W/kg), while a small but statistically significant increase ( $P = 0.036$ ) was observed at a SAR of 2 W/kg. [Zhijian et al. \(2009\)](#) did not find an effect of RF radiation on DNA strand breaks induced by X-rays, or their repair, in lymphocytes exposed to intermittent (5 minutes on, 10 minutes off) pulsed-wave RF radiation at 1800 MHz (SAR, 2 W/kg) for 24 hours, followed by exposure to X-rays (0.25–2.0 Gy).

#### (iv) Ultraviolet radiation

[Baohong et al. \(2007\)](#) exposed peripheral blood lymphocytes to 254 nm ultraviolet radiation (UVC) at 0.25–2.0 J/m<sup>2</sup>, followed by RF

radiation at 1800 MHz (SAR, 3 W/kg), for 1.5 or 4 hours. The number of UV-induced DNA strand breaks decreased after exposure to RF radiation for 1.5 hours, and increased after exposure for 4 hours.

(d) *Mammalian cells (non-human)*

See [Table 4.7](#)

(i) *800–1800 MHz*

Mouse C3H 10T½ fibroblast cells (both exponentially growing and in plateau phase) were exposed to RF radiation at 835.62 MHz as a frequency-modulated continuous-wave (FMCW) signal, or to RF radiation as a code-division multiple access (CDMA) signal at 847.74 MHz (SAR, 0.6 W/kg), for up to 24 hours. The alkaline comet assay was used to measure induction of DNA strand breaks. No significant differences were observed between the results obtained with FMCW or CDMA radiation and the sham-exposed negative controls ([Malyapa et al., 1997b](#)). The same authors did not find any effects in a similar experiment with continuous-wave RF radiation at 2450 MHz (SAR, 0.7 or 1.9 W/kg) ([Malyapa et al., 1997a](#)).

C3H 10T½ fibroblast cultures (exponentially growing or in the plateau phase) were exposed to RF radiation at 847.74 MHz as a CDMA signal, or to RF radiation at 835.62 MHz as a FDMA signal (SAR, 3.2–5.1 W/kg) for 2, 4, or 24 hours. The alkaline comet assay was used to measure induction of DNA strand breaks. No statistically significant change was found in tail moment or tail length for cells that had been exposed to RF radiation (CDMA or FDMA), compared with sham-exposed controls. Furthermore, in cells exposed for 2 hours to RF radiation, a post-incubation of 4 hours did not result in significant changes in tail moment or tail length ([Li et al., 2001](#)).

Exponentially growing or plateau-phase C3H 10T½ cells – derived from mouse-embryo fibroblasts – were exposed to RF radiation at

835.62 MHz, as CDMA (SAR, 3.2 or 4.8 W/kg) signal, or at 847.74 MHz as frequency-division multiple access (FDMA) signal (SAR, 3.2 or 5.1 W/kg), for 3, 8, 16 or 24 hours. No significant exposure-related differences in micronucleus formation were found for either plateau-phase cells or exponentially growing cells ([Bisht et al., 2002](#)).

[Diem et al. \(2005\)](#) reported the results of an alkaline comet assay with SV40-transformed rat granulosa cells exposed to continuous or intermittent (5 minutes on, 10 minutes off) RF radiation at 1800 MHz (SAR, 1.2 or 2 W/kg) for 4–24 hours. Both continuous and intermittent exposures induced DNA single- and double-strand breaks, with the greatest effect found with intermittent exposure. [Speit et al. \(2007\)](#) independently repeated some of the experiments with V79 Chinese hamster cells, using the same equipment and exposure conditions (1800 MHz; 2 W/kg SAR; continuous wave with intermittent exposure). No effects of exposure to RF radiation were found in assays for DNA strand-break induction and micronucleus formation.

Chinese hamster lung cells exposed to intermittent (5 minutes on, 10 minutes off) RF radiation at 1800 MHz (SAR, 3 W/kg) for 24 hours contained an increased number of  $\gamma$ -H2AX foci – a measure of DNA double-strand breaks – compared with sham-exposed cells. There was no effect after a 1-hour exposure to RF radiation ([Zhang et al., 2006](#)).

Because auditory cells could be exposed to RF radiation at frequencies at which mobile phones operate, [Huang et al. \(2008\)](#) used HEI-OC1 immortalized mouse auditory hair cells to characterize their response to exposure to RF radiation at 1763 MHz (SAR, 20 W/kg), in a CDMA exposure chamber for 24 or 48 hours. No changes were found in the phase-distribution of the cell cycle, DNA strand-break induction, stress response, or gene-expression profiles in the exposed cells, compared with sham-exposed controls.

**Table 4.7 Genetic and related effects of exposure to radiofrequency radiation in experimental systems *in vitro***

Test system, end-point	Exposure conditions	Genotoxic agent	Results and comments	Reference
pBluescript SK(+) plasmid, DNA strand breaks (DNA degradation <i>in vitro</i> )	835 MHz, CW; SAR, 4 W/kg; 48 h	-	Exposure to RF radiation did not change the rate of degradation of plasmid pBluescript SK(+) exposed to H <sub>2</sub> O <sub>2</sub> (Fenton-type reaction) as an indicator.	<a href="#">Chang et al. (2005)</a>
<i>Escherichia coli</i> WP2 <i>uvrA</i> , reverse mutation	835 MHz, CW; SAR, 4 W/kg; 48 h	4-NQO, for 48 h during exposure to RF radiation	RF radiation increased 4-NQO-induced mutation rate in <i>Escherichia coli</i> WP2.	<a href="#">Chang et al. (2005)</a>
<i>Escherichia coli</i> WP2 <i>uvrA</i> , reverse mutation	2450 MHz; SAR, 5–200 W/kg; 30 min	-	No effect on mutagenicity	<a href="#">Koyama et al. (2007)</a>
<i>Salmonella typhimurium</i> TA98, TA100, TA102 and TA1535, reverse mutation	835 MHz, CW; SAR, 4 W/kg; 48 h	4-NQO, cumene hydroperoxide (CHP) sodium azide (SA) for 48 h during exposure to RF radiation	RF radiation increased CHP-induced mutation rate in TA102, had no effect on SA-induced revertants in TA100, and reduced SA-induced mutation rate in TA1535	<a href="#">Chang et al. (2005)</a>
<i>Salmonella typhimurium</i> TA98, TA100, TA1535 and TA1537; reverse mutation	2450 MHz; SAR, 5–200 W/kg; 30 min	-	No effect on mutagenicity	<a href="#">Koyama et al. (2007)</a>
<i>Saccharomyces cerevisiae</i> , gene-specific forward mutation at <i>CAN1</i>	900 MHz, GSM pulsed-wave; SAR, 0.13; 1.3 W/kg	MMS	No significant effect on mutation rates at <i>CAN1</i> ± MMS	<a href="#">Gos et al. (2000)</a>
<i>Saccharomyces cerevisiae</i> , induction of respiration-deficient ( <i>petite</i> ) clones (loss of mitochondrial function)	900 MHz, GSM pulsed-wave; SAR, 0.13; 1.3 W/kg	MMS	No significant effect on the frequency of <i>petite</i> colony formation ± MMS	<a href="#">Gos et al. (2000)</a>
<i>Saccharomyces cerevisiae</i> , intrachromosomal deletion-formation assay	900 MHz, GSM pulsed-wave; SAR, 0.13; 1.3 W/kg	MMS	No significant effect on formation of intrachromosomal deletions ± MMS	<a href="#">Gos et al. (2000)</a>
<i>Saccharomyces cerevisiae</i> , intra-genic recombination assay in the <i>ADE2</i> gene	900 MHz, GSM pulsed-wave; SAR, 0.13; 1.3 W/kg	MMS	No significant effect on rates of intragenic recombination ± MMS	<a href="#">Gos et al. (2000)</a>
<i>Xenopus laevis</i> ; DNA SSB measured in erythrocytes sampled immediately after exposure	8800 MHz pulsed-wave; peak power, 65 kW; SAR, 1.6 W/kg; 40 min	-	No indication of non-thermal effects. Observed DNA damage probably due to temperature rise	<a href="#">Chemeris et al. (2004)</a>
C3H10T½ mouse; DNA SSB measured in fibroblasts, sampled immediately and up to 4 h after exposure	2450 MHz CW; SAR, 0.7 and 1.9 W/kg; 2, 4 and 24 h	-	No effect	<a href="#">Malyapa et al. (1997a)</a>

**Table 4.7 (continued)**

Test system, end-point	Exposure conditions	Genotoxic agent	Results and comments	Reference
C3H10T½ mouse; DNA SSB measured in fibroblasts, sampled immediately exposure	835.62 MHz FMCW and 847.7 MHz, CDMA CW; SAR, 0.6 W/kg; 2, 4, 24 h	-	No effect	<a href="#">Malyapa et al. (1997b)</a>
C3H10T½ mouse; DNA SSB measured in fibroblasts, sampled immediately and 4 h after exposure	835.6 MHz FDMA and 847.7 MHz FDMA; SAR, 3.2 and 5.1 W/kg; 2, 4 and 24 h exposure	-	No effect	<a href="#">Li et al. (2001)</a>
C3H10T½ mouse; DNA SB, DNA–DNA and DNA–protein cross-links measured in fibroblasts	2450 MHz CW; SAR, 1.9 W/kg; 2 h exposure followed by 4 Gy gamma-rays	Gamma radiation	No effect of RF radiation on SB. No reduction by RF radiation of DNA migration induced by gamma-rays. No induction of DNA–protein crosslinks or changes in amount of DNA-associated protein by RF radiation	<a href="#">Lagroye et al. (2004b)</a>
HEI-OC1 (immortalized mouse auditory hair) cells; DNA damage, stress response and gene expression	1763 MHz; SAR, 20 W/kg; CDMA; continuous exposure for 24 or 48 h	-	No effect on cellular responses, including cell-cycle distribution, DNA damage, stress response or gene expression	<a href="#">Huang et al. (2008)</a>
L5178Y <i>Tk</i> <sup>-/-</sup> mouse lymphoma cells (DNA damage) and Chinese hamster lung fibroblasts (chromosomal aberrations, CA)	835 MHz; SAR, 4 W/kg; exposure for 48 h, alone or combined with chemicals	CPA, 4-NQO	No effect on DNA damage or CA. No effect on EMS-induced CA. Significant increase in CPA- and 4NQO-induced DNA damage.	<a href="#">Kim et al. (2008a)</a>
Chinese hamster V79 cells; DNA damage (SSB, DSB)	1800 MHz; CW or pulsed-wave; continuous or intermittent (5 min on, 10 min off); SAR, 2 W/kg; exposure 1–24 h	-	No induction of DNA damage found in independent repeat experiments.	<a href="#">Speit et al. (2007)</a>
Rat granulosa cells; DNA damage (SSB, DSB), sampled immediately after exposure	1800 MHz; CW or pulsed-wave; continuous and intermittent (5 min on, 10 min off); SAR, 2 W/kg; exposure 1–24 h	-	Induction of DNA SSB and DSB after 16 h intermittent exposure, at different mobile-phone modulations. Objections were raised to the analysis of the data	<a href="#">Diem et al. (2005)</a>
Chinese hamster V79 cells; DNA synthesis, incorporation of [ <sup>3</sup> H] thymidine	7700 MHz; 300 mW/cm <sup>2</sup> ; 15, 30, 60 min	-	Cells are blocked in entering S-phase	<a href="#">Garaj-Vrhovac et al. (1990b)</a>

**Table 4.7 (continued)**

Test system, end-point	Exposure conditions	Genotoxic agent	Results and comments	Reference
Chinese hamster lung cells; DNA damage, gamma-H2AX focus formation	1800 MHz; intermittent (5 min on, 10 min off); SAR, 3.0 W/kg; for 1 or 24 h	-	RF radiation (24 h exposure, not 1 h) caused gamma-H2AX focus formation. A cell was classified positive when it contained more than five foci.	<a href="#">Zhang et al. (2006)</a>
L5178Y <i>Tk</i> <sup>+/-</sup> mouse lymphoma cells; gene mutation	2450 MHz, pulsed-wave; power density, 488 W/m <sup>2</sup> ; SAR, 30 or 40 W/kg; 4 h, together with MMC (at lower SAR) of proflavin (at higher SAR).	-	No effect of RF radiation alone. No influence by RF radiation on cell-growth inhibition or on MMC- or proflavin-induced mutagenesis	<a href="#">Meltz et al. (1989, 1990)</a>
Chinese hamster ovary CHO K1 cells; gene mutation <i>Hprt</i> locus	2450 MHz, SAR, 5–200 W/kg; 2 h	Bleomycin, for 1 h before irradiation	RF radiation (200 W/kg) increased <i>Hprt</i> mutation frequency by itself, and increased bleomycin-induced <i>Hprt</i> mutations (100 and 200 W/kg). Effects may be due to hyperthermia	<a href="#">Koyama et al. (2007)</a>
Chinese hamster ovary cells; SCE	2450 MHz, pulsed-wave; 490 W/m <sup>2</sup> ; SAR, 33.8 W/kg; 2 h	Simultaneous exposure to adriamycin	No effect of RF radiation alone. No effect on adriamycin-induced SCE	<a href="#">Ciaravino et al. (1991)</a>
C3H10T $\frac{1}{2}$ mouse fibroblasts; micronucleus formation	835.6 MHz CW, FDMA and 847.7 MHz CW, CDMA; SAR, 3.2 and 5.1 W/kg	-	No increase in frequency of micronucleus formation	<a href="#">Bisht et al. (2002)</a>
Chinese hamster V79 cells; micronucleus formation	7700 MHz; 30 mW/cm <sup>2</sup> ; 15, 30, 60 min	-	Increased micronucleus formation	<a href="#">Garaj-Vrhovac et al. (1991)</a>
Chinese hamster ovary CHO-K1 cells; micronucleus formation	2450 MHz; SAR, 13, 39, 50, 78, 100 W/kg; 18 h	Bleomycin	Increased micronucleus frequency after RF radiation, and potentiation by RF radiation of bleomycin-induced micronucleus formation, both at SARs $\geq$ 78 W/kg	<a href="#">Koyama et al. (2003)</a>
Chinese hamster ovary CHO-K1 cells; micronucleus formation	2450 MHz; SAR, 5, 10, 20, 50, 100, 200 W/kg; 2 h	Bleomycin	Increased micronucleus formation at SARs of 100 and 200 W/kg. No combined effect of RF and bleomycin	<a href="#">Koyama et al. (2004)</a>
Chinese hamster V79 cells; micronucleus formation	1800 MHz; CW or pulse-wave; continuous and intermittent (5 min on, 10 min off), 1–24 h; SAR, 2 W/kg	-	No effect found. This study was aimed at replicating earlier findings.	<a href="#">Speit et al. (2007)</a>
Bovine lymphocytes; micronucleus formation	9000 MHz, 70 W/kg CW; 10 min	MMC	Increased micronucleus frequency after RF radiation; significant increase by RF radiation of MMC-induced micronuclei.	<a href="#">Scarfi et al. (1996)</a>

**Table 4.7 (continued)**

Test system, end-point	Exposure conditions	Genotoxic agent	Results and comments	Reference
Mouse m5S cells; chromosomal aberrations	2450 MHz CW or PW; SAR, 5, 10, 20, 50, 100 W/ kg; 2 h	-	No effect	<a href="#">Komatsubara <i>et al.</i> (2005)</a>
Chinese hamster V79 cells; chromosomal aberrations	7700 MHz, 300 mW/cm <sup>2</sup> ; 15, 30, 60 min	-	Induction of chromosomal aberrations	<a href="#">Garaj-Vrhovac <i>et al.</i> (1990b)</a>
Chinese hamster ovary cells; chromosomal aberrations	2450 MHz pulsed-wave; 490 mW/cm <sup>2</sup> , SAR, 33.8 W/kg, 2 h	Simultaneous exposure to adriamycin or MMC	No effect of RF radiation alone No effect by RF radiation on aberrations induced by adriamycin or MMC	<a href="#">Kerbacher <i>et al.</i> (1990)</a>
Chinese hamster V79 cells; chromosomal aberrations (structural)	7700 MHz, 30 mW/cm <sup>2</sup> , 15, 30, 60 min	-	Increased frequency of chromosomal aberrations, including dicentric and ring chromosomes	<a href="#">Garaj-Vrhovac <i>et al.</i> (1991)</a>
Chinese hamster ovary cells; cell-cycle progression	2450 MHz pulsed-wave; 490 mW/cm <sup>2</sup> , SAR, 33.8 W/kg, 2 h	Simultaneous exposure to adriamycin	No effect of RF radiation alone No influence on cell-cycle progression caused by adriamycin	<a href="#">Ciaravino <i>et al.</i> (1991)</a>
Chinese hamster V79 cells; cell growth (cell count, microtubule structure)	935 MHz; SAR: 0.12 W/kg; 1, 2, 3 h	-	Alteration of microtubule structure after a 3-h exposure; significantly decreased growth was noted in cells exposed for 3 h, at 3 d after exposure	<a href="#">Pavicic &amp; Trosic, (2008)</a>
Chinese hamster V79 cells; cell-proliferation kinetics, analysis of microtubule structure, mitotic index	935 MHz CW; SAR, 0.12 W/kg; 1, 2, 3 h	-	Alteration of microtubule structure; no effect on mitotic index. Cell proliferation was reduced at 72 h after exposure in cells exposed for 3 h. Slower cell-division kinetics	<a href="#">Trosić &amp; Pavčić (2009)</a>
Chinese hamster V79 cells; survival	7700 MHz; 0.5, 10, 30 mW/cm <sup>2</sup> ; 10, 20, 30, 60 min	-	After 8 days of post-incubation: reduced cell survival related to power density and exposure time	<a href="#">Garaj-Vrhovac <i>et al.</i> (1991)</a>

4-NQO, 4-nitroquinoline 1-oxide; CA, chromosomal aberrations; CDMA, code-division multiple access; CHP, cumene hydroperoxide; CPA, cyclophosphamide; CW, continuous wave; DSB, DNA double-strand breaks; FDMA, frequency-division multiple access; h, hour; *Hprt*, hypoxanthine-guanine phosphoribosyl transferase gene; min, minute; MMC, mitomycin C; MMS, methylmethane sulfonate; MN, micronuclei; SA, sodium azide; SCE, sister-chromatid exchange; SSB, DNA single-strand breaks

The alkaline comet assay and a test for chromosome aberrations *in vitro* were used to investigate the effects of 835 MHz RF radiation (4 W/kg), alone and in combination with the clastogens cyclophosphamide (CP), 4NQO and ethylmethane sulfonate (EMS), in L5178Y *Tk*<sup>+</sup> mouse-lymphoma cells (to assess DNA breakage) and in Chinese hamster lung fibroblasts (to measure chromosome aberrations). In the latter cells, no effect was observed from RF radiation, alone or in combination with CP or EMS, but in the mouse-lymphoma cells a potentiating effect was noted on DNA strand-break induction after exposure to RF radiation following treatment with CP or 4NQO (Kim *et al.*, 2008a).

V79 Chinese hamster cells were exposed for 1, 2, or 3 hours to RF radiation at 935 MHz, generating an electric field-strength of  $8.2 \pm 0.3$  V/cm and an average SAR of 0.12 W/kg. The microtubule structure in these cells was analysed by use of an immunocytochemical method. After 3 hours of exposure, microtubules in exposed cells were found to be altered compared with those in unexposed control cells. Three days after exposure, cell proliferation was significantly decreased in samples that had been exposed for 3 hours. Exposure to RF radiation at 935 MHz affects the structure of microtubule proteins, which consequently may obstruct cell growth (Pavicic & Trosic, 2008; Trosic & Pavicic, 2009).

#### (ii) 2450 MHz

The assay for forward mutation at the thymidine kinase locus in L5178Y mouse lymphoma cells was used to investigate the effects of a 4-hour exposure to RF radiation at 2450 MHz (power density, 48.8 mW/cm<sup>2</sup>; SAR, 30 W/kg), alone and in the presence of the chemical mutagen MMC (0.1, 0.2, 0.3 µg/ml). Exposure to RF radiation alone was not mutagenic, and it did not alter the effects of MMC with regards to cell proliferation or mutation induction (Meltz *et al.*, 1989). A similar experiment involving exposure to RF radiation combined with proflavin – a

DNA-intercalating drug – gave similar results (Meltz *et al.*, 1990).

In a cytogenetic study, CHO cells were exposed to pulsed-wave RF radiation at 2450 MHz (SAR, 33.8 W/kg), for 2 hours in the absence or presence of MMC (0.075 or 0.1 µg/ml) or adriamycin (0.175 µg/ml). The experimental conditions resulted in a maximum temperature increase of 3.2 °C. With respect to the induction of chromosomal aberrations, no effect was found that could be ascribed to the exposure to RF radiation (Kerbacher *et al.*, 1990).

CHO cells were exposed simultaneously to adriamycin (10<sup>-6</sup> M) and pulsed-wave RF radiation at 2450 MHz (SAR, 33.8 W/kg) for 2 hours, or to adriamycin only. There was no effect of exposure to RF radiation on adriamycin-induced changes in cell progression or SCE frequency (Ciaravino *et al.*, 1991).

Micronucleus formation in Chinese hamster ovary (CHO) K1 cells was measured after exposure of the cells to RF radiation at 2450 MHz in four different scenarios: (1) exposure for 18 hours at average SARs of 13, 39 or 50 W/kg (input power, 7.8 W), which had no effect on micronucleus formation; (2) exposures corresponding to SARs of 78 or 100 W/kg (input power, 13 W), which produced a significant increase ( $P < 0.01$ ) in micronucleus frequency; (3) treatment with the clastogenic compound bleomycin alone, or with bleomycin followed by irradiation for 18 hours at SARs of 25, 78 or 100 W/kg, which resulted in enhancement by RF radiation (at SAR values of 78 and 100 W/kg) of the effect of bleomycin alone; and (4) incubation at 39 °C for 18 hours as a high-temperature control; this last experiment also showed an increase in micronucleus frequency, albeit less strong than that after exposure to RF radiation. In a subsequent study, the authors reported a significant increase in micronucleus formation in cells exposed to RF radiation at 2450 MHz at SARs of 100 or 200 W/kg for 2 hours, but no effect of the combined exposure to RF radiation and bleomycin. Sham-exposures

at higher temperatures (38–42 °C) also increased the frequency of micronuclei, which indicates that the effects at the high SAR levels may have been thermal in nature ([Koyama et al., 2003, 2004](#)).

C3H 10T½ mouse fibroblasts were exposed to continuous-wave RF radiation at 2450 MHz (SAR, 1.9 W/kg) for 2 hours and processed for measurement of alkali-labile DNA damage and/or DNA–protein or DNA–DNA crosslinks. No effect was noted for any of these end-points ([Lagroye et al., 2004b](#)).

The induction of chromosomal aberrations was investigated in murine m5S cells exposed to continuous- or pulsed-wave RF radiation at 2450 MHz (average SARs of 5, 10, 20, 50 or 100 W/kg) for 2 hours. No significant differences were observed following exposure at any SAR compared with sham-exposed controls. There was also no difference between exposure to continuous-wave and pulsed-wave RF radiation ([Komatsubara et al., 2005](#)).

CHO-K1 cells were exposed to RF radiation at 2450 MHz (SAR, 5–200 W/kg) for 2 hours, after which *Hprt* gene mutations were scored. There was no mutation induction by exposure to RF radiation alone. An increase in the mutation frequency was found in cells exposed to RF radiation (SAR, 100 or 200 W/kg) in combination with bleomycin, but this may have been a thermal effect ([Koyama et al., 2007](#)).

### (iii) 7000–9000 MHz

Cultured V79 Chinese hamster cells were exposed to continuous-wave RF radiation at 7700 MHz (power density, 30 mW/cm<sup>2</sup>) for 15, 30, or 60 minutes. In comparison with the controls, there was a higher frequency of specific chromosome lesions and a reduction in the incorporation of [<sup>3</sup>H]thymidine, showing inhibition of entry into S-phase ([Garaj-Vrhovac et al., 1990b](#)).

In a further study, the same authors reported a decrease in the number of V79 cell colonies, which was related to the power density and

the duration of exposure. Significantly higher frequencies of specific chromosomal aberrations – dicentrics, ring chromosomes – and micronuclei were observed in the exposed cells ([Garaj-Vrhovac et al., 1991](#)).

Cultures of bovine (*Bos taurus* L.) peripheral blood lymphocytes were exposed to RF radiation at 9000 MHz (SAR, 70 W/kg) for 10 minutes. To evaluate possible cooperative effects with a chemical mutagen, some exposed cultures were also treated with MMC. Exposure to RF radiation induced a statistically significant increase in micronucleus formation, both in the presence ( $P < 0.01$ ) and absence ( $P < 0.001$ ) of MMC ([Scarfi et al., 1996](#)).

### (e) Non-mammalian cells

See [Table 4.7](#)

Mutagenic or recombinogenic effects of RF radiation at 900 MHz (GSM; SAR, 0.13 and 1.3 W/kg) were investigated in the yeast *Saccharomyces cerevisiae*. Mutation rates were monitored with a widely used gene-specific assay for forward mutation in the *CAN1* gene, which encodes arginine permease (gene-inactivating mutations lead to canavanine resistance) and with an assay measuring induction of respiration-deficient “*petite*” clones (small colonies) that have lost mitochondrial function. The recombinogenic effect of RF radiation was investigated with an assay for intrachromosomal deletion and an assay for intragenic recombination at the *ADE2* gene, which encodes an enzyme involved in purine (adenine) biosynthesis. Exposure of *S. cerevisiae* to RF radiation under these conditions did not result in recombinogenic or mutagenic effects ([Gos et al., 2000](#)).

The effects of a 40-minute exposure to pulsed-wave RF radiation at 8800 MHz (SAR, 1.6 W/kg; pulse width, 180 ns; peak power, 65 kW; repetition rate, 50 Hz) were investigated in erythrocytes of the frog *Xenopus laevis* by means of the alkaline comet assay. The temperature rise in the blood samples at steady-state was  $3.5 \pm 0.1$  °C.

The results showed that the increase in DNA damage after exposure was associated with the increase in temperature; in this experiment, no non-thermal effects on frog erythrocytes *in vitro* were noted ([Chemeris et al., 2004](#)).

The effects of exposure to RF radiation at 835 MHz (SAR, 4 W/kg) for 48 hours were examined in assays for mutagenicity in bacteria. RF radiation was not directly mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537, or in *Escherichia coli* strain WP2 *uvrA*. It significantly enhanced the mutagenicity of 4NQO in *E. coli* strain WP2 *uvrA* and of cumene hydroperoxide in *S. typhimurium* strain TA102. In a test for DNA degradation, no change in the rate of degradation (formation of DNA strand breaks) was observed with plasmid pBluescript SK(+) exposed to H<sub>2</sub>O<sub>2</sub> (Fenton-type reaction) as an indicator ([Chang et al., 2005](#)).

Mutagenicity tests were conducted in different bacterial strains (*S. typhimurium* TA98, TA100, TA1535 and TA1537, and *E. coli* WP2 *uvrA*) exposed to RF radiation at 2450 MHz (SAR, 5–200 W/kg) for 30 minutes. No effects were found in any of the strains tested ([Koyama et al., 2007](#)).

[The Working Group noted that while several studies showed positive responses at high SAR values, some of these were due to thermal effects. The Working Group concluded that there was weak evidence that exposure to RF radiation is genotoxic in experimental systems in mammalian and non-mammalian cells *in vitro*.]

## 4.2 Effects of low-level exposure to RF radiation on the immune system

In this section, some studies that assess the effects of RF radiation on the immune system are discussed (see review by [Jauchem, 2008](#)).

### 4.2.1 Immunotropic effects of exposure to RF radiation in humans

[In general, occupational studies in this Section included small numbers of subjects and generally failed to control for possible confounders.]

[Dmoch & Moszczyński \(1998\)](#) measured immunoglobulin concentrations and proportions of different subsets of T lymphocytes in blood samples from 52 workers at television-retransmission and satellite-communication centres, exposed to RF radiation at 6–12 GHz. Concentrations of IgG and IgA immunoglobulins, and cell counts of total lymphocytes and T8 lymphocytes were increased, whereas the number of natural killer (NK) cells and the ratio of T-helper/T-suppressor cells were decreased, compared with the values in 30 non-exposed controls. There was no change in IgM concentrations. In an extension of this study, [Moszczyński et al. \(1999\)](#) performed a similar analysis with blood samples from radar operators. In this case, IgM concentrations were elevated and T8 lymphocyte cell-counts were decreased. The different results obtained in these two professional groups with respect to immunological parameters and blood-cell counts suggested that the effect of RF radiation on the immune system depends on the character of the exposure.

[Tuschl et al. \(1999\)](#) investigated the effects of long-term handling of various types of diathermy equipment – operating at frequencies of 27, 434, or 2450 MHz – on the immune system of medical personnel, by analysis of blood samples collected from physiotherapists operating these devices. Eighteen exposed subjects and 13 controls matched for sex and age were examined. Total leukocyte/lymphocyte counts and the proportion of leukocyte subpopulations were determined by use of flow cytometry and monoclonal antibodies to cell-surface antigens. In addition, lymphocyte activity was measured to quantify subpopulations of immunocompetent cells.

Lymphocytes were stimulated by the mitogen PHA and proliferation was measured by flow cytometry. No statistically significant differences between the exposed personnel and the controls were found. In both groups, all immune parameters were within normal ranges.

[Radon et al. \(2001\)](#) investigated the effects of RF radiation at 900 MHz (pulse frequency, 217 Hz; power density, 1 W/m<sup>2</sup>) used in modern digital wireless telecommunication (GSM standard), in eight healthy male volunteers exposed in a specifically designed, shielded experimental chamber. The circularly polarized electromagnetic field applied was transmitted by an antenna positioned 10 cm behind the head of the volunteer, who was sitting upright. In double-blind trials, each volunteer underwent a total of 20 randomly allotted 4-hour periods of exposure and sham exposure, equally distributed during day and night. The salivary concentrations of IgA – as well as those of melatonin, cortisol and neopterin – did not differ significantly between the exposed and the sham-exposed subjects.

[Yuan et al. \(2004\)](#) investigated the effect of low-intensity, 170 MHz RF radiation on immune parameters in occupationally exposed workers. Blood-sample analysis showed no marked change in IgA concentrations, whereas those of IgM and IgG were significantly increased ( $P < 0.01$ ) in the exposed group compared with those in non-exposed controls.

[Kimata \(2005\)](#) exposed 15 patients with atopic eczema dermatitis syndrome (AEDS) to RF radiation from a mobile phone (SAR, 1.62 W/kg) for 30 minutes. A second group of 15 patients was sham-exposed. In a repeat experiment 2 weeks later, the groups were switched with respect to exposure/sham-exposure. Before and after each study, mononuclear cells were stimulated with latex, the allergen to which the patients were sensitive. The production of latex-specific immunoglobulin E (IgE) was significantly increased ( $P < 0.01$ ) after exposure to RF radiation.

[The Working Group noted that studies of humans exposed to RF radiation provided weak evidence for effects on the humoral immune system.]

#### 4.2.2 Immunotropic effects of exposure to RF radiation in experimental animals: studies in vivo

See [Table 4.8](#)

##### (a) Mouse

[Smiałowicz et al. \(1983\)](#) exposed male CBA/J mice to 2450 MHz continuous-wave RF radiation (power density, 5, 15, 30 mW/cm<sup>2</sup>; SAR, 3.5, 10.5, 21 W/kg, respectively) for 90 minutes per day for 2 or 9 days, and studied the effects on the activity of NK cells and the mitogen-induced response of lymphocytes. There was no consistent difference in the mitogen response of spleen cells from irradiated mice and sham-irradiated mice, while a significant suppression of NK activity was seen at the highest exposure intensity. NK activity returned to normal within 24 hours after exposure.

[Veyret et al. \(1991\)](#) exposed BALB/c mice to pulsed-wave RF radiation at 9400 MHz (1  $\mu$ s pulses at 1000/second), both with and without amplitude modulation (AM) by a sinusoid signal at discrete frequencies between 14 and 41 MHz. Mice were immunized with sheep erythrocytes and exposed to RF radiation (30  $\mu$ W/cm<sup>2</sup>; whole-body SAR, 0.015 W/kg) for 10 hours per day, for 5 days. The antibody response to sheep erythrocytes was measured by the plaque-forming assay. In the absence of AM, there was not much change in immune responsiveness. Exposure to RF radiation with AM at 21 or 32 MHz led to significant enhancement of the response, while there was a decrease in the number of plaque-forming cells with AM at 14, 36, or 41 MHz.

[Elekes et al. \(1996\)](#) studied the effects of continuous-wave (CW) or amplitude-modulated (AM) RF radiation at 2450 MHz in male

**Table 4.8 Immunotropic effects of exposure to radiofrequency radiation in experimental animals *in vivo***

Experimental system	Exposure conditions	Results	Reference
CBA/J mice	2450 MHz PW; SAR, 3.5, 10.5 and 21 W/kg; 1.5 h/d for 2, 3, 9 d	No increase in mitogenic response of splenic lymphocytes	<a href="#">Smiałowicz <i>et al.</i> (1983)</a>
BALB/c mice	9400 MHz PW, AM; 30 $\mu$ W/cm <sup>2</sup> ; whole-body SAR, ~0.015 W/kg; 10 h/d for 5 d	Significant increase in numbers of PFC at AM frequencies 21 and 32 MHz; significant decrease at 14, 36, 41 MHz.	<a href="#">Veyret <i>et al.</i> (1991)</a>
BALB/c mice	2450 MHz CW or AM (50 Hz square wave); SAR, 0.14 W/kg; 3 h/d for 6 d	Increase in the number of antibody-producing cells in the spleen of male mice; no effect in female mice.	<a href="#">Elekes <i>et al.</i> (1996)</a>
C57BL/6 mice	900 MHz (GSM); SAR, 1 or 2 W/kg; 2 h/d for 1, 2, 4 wk	No substantial effect on T- and B-cell compartments. Transient increase of interferon- $\gamma$ after 1 week of exposure, not at 2 or 4 wk	<a href="#">Gatta <i>et al.</i> (2003)</a>
Mice [strain not given]	42 GHz; 105 $\mu$ W/cm <sup>2</sup> ; 20 min/d for 1–14 d	Strong effect on indices of non-specific immunity. Phagocytic activity of neutrophils was suppressed by 45–50% within 2–3 h after a single exposure, remained suppressed for 1 d, and was restored to normal during 3 d. Blood leukocytes were increased after exposure for 5 d.	<a href="#">Kolomytseva <i>et al.</i> (2002)</a>
NMR1 mice, exposed in the far-field zone of horn antenna	42 GHz; 150 $\mu$ W/cm <sup>2</sup> ; 20 min (single exposure), 20 min/d for 5 or 20 successive days, before or after immunization	No effect of single exposure or five repeat exposures. Daily exposure for 20 d before immunization with SRBC resulted in significant reductions in thymic and renal cellularity	<a href="#">Lushnikov <i>et al.</i> (2001)</a>
C57BL/6 mice	900 MHz (GSM); whole-body average SAR, 2 W/kg; 2 h/d for 4 wk	No changes in frequencies of various B cell types or in IgM/IgG serum levels. Production of IgM/IgG by B cells from exposed mice, challenged <i>in vitro</i> with lipopolysaccharides, was comparable to that in controls	<a href="#">Nasta <i>et al.</i> (2006)</a>
NMRI mice	1.8–81.5 GHz; 1 $\mu$ W/cm <sup>2</sup> ; 5 h	Increased production of TNF in peritoneal macrophages and splenic T lymphocytes. Increased mitogenic response in T lymphocytes.	<a href="#">Novoselova &amp; Fesenko (1998)</a> , <a href="#">Novoselova <i>et al.</i> (1999)</a>
NMRI mice	8.15–18 GHz; 1 $\mu$ W/cm <sup>2</sup> ; 5 h –7 d	Increased NK cell activity, which persisted up to 24 h after exposure. Increased TNF production in peritoneal macrophages and splenic T lymphocytes after exposures of 5 h – 3 d, and reduced TNF production in peritoneal macrophages after an exposure of 7 d.	<a href="#">Fesenko <i>et al.</i> (1999b)</a>
Rats [strain not given]	2450 MHz PW; SAR, 0.15–0.4 W/kg; 25 mo	Transient increase in the number of B and T lymphocytes and their response to the mitogen PHA after exposure for 13 mo	<a href="#">Guy <i>et al.</i> (1985)</a>
Sprague-Dawley rats	900 MHz (GSM); SAR, 0.075 and 0.27 W/kg; 2h/d for 10 d	No alterations in the surface phenotype of splenic lymphocytes or in their concavalin A-stimulated mitogenic activity	<a href="#">Chagnaud &amp; Veyret (1999)</a>
Belgian White rabbits	2.1 GHz; 5 mW/cm <sup>2</sup> ; 3 h/d, 6 d/wk for 3 mo	Suppression of T-lymphocyte numbers at 2 mo; stronger response of T-cell-mediated immunity (delayed-type hypersensitivity response)	<a href="#">Nageswari <i>et al.</i> (1991)</a>

AM, amplitude modulation; CW, continuous wave; d, day; GSM, Global System for Mobile Communications; h, hour; LPS, lipopolysaccharides; min, minute; mo, month; MW, microwave; NK, natural killer; PHA, phytohaemagglutinin; PFC, plaque-forming cells; PW, pulsed-wave; TNF, tumour necrosis factor; wk, week.

and female BALB/c mice. The time-averaged power density was  $100 \mu\text{W}/\text{cm}^2$ , with a SAR of  $0.14 \pm 0.02 \text{ W}/\text{kg}$ . Exposure to RF radiation as CW or AM (3 hours per day for 6 days) induced a non-significant increase in the number of antibody-producing cells in the spleen of male mice. No effects were seen in female mice.

[Novoselova & Fesenko \(1998\)](#) and [Novoselova et al. \(1999\)](#) exposed male NMRI mice to RF radiation at 8150–18 000 MHz (power density,  $1 \mu\text{W}/\text{cm}^2$ ) for 5 hours, and observed a significantly enhanced ( $P < 0.05$ ) production of TNF in peritoneal macrophages and in T-cells in the spleen, and an increased mitogenic response in T lymphocytes.

Male NMRI mice received whole-body exposure to RF radiation at 10 GHz (average power density,  $1 \mu\text{W}/\text{cm}^2$ ) for different time periods (1 hour to 7 days). A significant enhancement of the production of tumour necrosis factor (TNF) in peritoneal macrophages and in splenic T lymphocytes was seen after exposures of 5–72 hours. Prolonged irradiation after 72 hours resulted in a decrease in production of TNF. In mice exposed to RF radiation at 8.15–18 GHz (average power density,  $1 \mu\text{W}/\text{cm}^2$ ) for 24 hours, TNF production in T-cells and macrophages was significantly increased ( $P < 0.05$ ); in the latter cell type, this increase persisted for 3 days after termination of exposure ([Fesenko et al., 1999b](#)).

[Lushnikov et al. \(2001\)](#) exposed male NMRI mice to RF radiation at 42.0 GHz (energy-flux density,  $150 \mu\text{W}/\text{cm}^2$ ) for 20 minutes per day, on five or twenty successive days before immunization with sheep erythrocytes, or for 20 minutes per day during five successive days after immunization. The response was estimated on day 5 after immunization by the number of antibody-forming splenic cells and by antibody titres. Humoral immunity and cellularity of the lymphoid organs did not change significantly after the single exposure, or after the series of five exposures before and after immunization. However, after daily exposure for 20 days before

immunization, statistically significant reductions ( $P < 0.05$ ) of thymic and splenic cellularity were observed.

[Kolomytseva et al. \(2002\)](#) exposed mice to RF radiation at 4200 MHz (power density,  $150 \mu\text{W}/\text{cm}^2$ ) for 20 minutes. The phagocytic activity of neutrophils was suppressed by about 50% in the 2–3 hours after a single exposure. The effect persisted for 1 day, and phagocytic activity then returned to normal within 3 days. A significant modification of the leukocyte profile in mice exposed for 5 days was observed after cessation of exposure: the number of leukocytes increased, mostly due to an increase in lymphocyte content.

[Gatta et al. \(2003\)](#) exposed C57BL/6 mice to GSM-modulated RF radiation at 900 MHz (SAR, 1 or 2 W/kg) for 2 hours per day for 1, 2 or 4 weeks. The number of spleen cells, the percentage of B and T-cells, and the distribution of T-cell subpopulations (CD4 and CD8) were not affected by the exposure. There was no difference in stimulation of T or B lymphocytes with specific monoclonal antibodies or lipopolysaccharides (LPS) between sham-exposed and exposed mice. After 1 week of exposure at a SAR of 1 or 2 W/kg, there was an increase in the production of interferon-gamma (IFN- $\gamma$ ), which was no longer observed when exposure was prolonged to 2 or 4 weeks.

[Nasta et al. \(2006\)](#) examined the effects of GSM-modulated RF radiation at 900 MHz (average SAR, 2 W/kg) on peripheral differentiation of B-cells and antibody production in female C57BL/6 mice exposed *in vivo*. Whole-body exposure for 2 hours per day, for 4 weeks, did not affect the frequencies of T1 and T2 B-cells, or of mature follicular B-cells and marginal zone B-cells in the spleen. Serum concentrations of IgM and IgG were not significantly affected. B-cells from mice exposed to RF radiation, which were then challenged *in vitro* with lipopolysaccharide (LPS) produced comparable amounts of IgM and IgG. Exposure to RF radiation did not alter the

ongoing antigen-specific immune response in immunized mice.

(b) *Rat*

In a study with rats receiving lifelong exposure to pulsed-wave RF radiation at 2450 MHz (SAR, 0.15–0.4 W/kg), [Guy et al. \(1985\)](#) found a significant increase in the number of splenic B and T lymphocytes at 13 months, but this effect had disappeared by the end of the study at 25 months. The exposed rats also showed a significant increase in their response to LPS and pokeweed mitogen after 13 months of exposure (no data available at 25 months).

[Chagnaud & Veyret \(1999\)](#) examined the effects of exposure to GSM-modulated RF radiation at 900 MHz (55 and 200  $\mu\text{W}/\text{cm}^2$ ; SAR, 0.075 and 0.279 W/kg; repetition rate, 217 Hz) for 2 hours per day for 10 days, on lymphocyte subpopulations in female Sprague-Dawley rats. The mitogenic response of the exposed rats was analysed by flow cytometry and a colorimetric method. No alterations were found in cell-surface markers (CD4, CD8 and IaAg) of splenic lymphocytes of exposed rats, or in their mitogenic activity when stimulated with concanavalin A.

(c) *Rabbit*

[Nageswari et al. \(1991\)](#) exposed male Belgian White rabbits to RF radiation at 2100 MHz (power density, 5  $\text{mW}/\text{cm}^2$ ; calculated average SAR, 0.83 W/kg) for 3 hours per day, 6 days per week, for 3 months, in specially designed miniature anechoic chambers. One group of rabbits was tested for T-lymphocyte-mediated cellular immune-response, being initially sensitized with bacille Calmette–Guérin (BCG) vaccine and challenged with tuberculin after termination of exposure. A second group was assessed for B-lymphocyte-mediated humoral immune-response. Samples of peripheral blood were collected each month during exposure or sham exposure and during follow-up at 5 and 14 days

after termination of exposure (second group only). Significant suppression of numbers of T lymphocytes was noted in the exposed rabbits at 2 months and during the follow-up period. Rabbits in the group initially sensitized with BCG showed an increase in foot-pad thickness, which is indicative of a good T-lymphocyte-mediated immune response (a delayed-type hypersensitivity response).

[The Working Group noted that the available evidence from the numerous experimental studies *in vivo* that have assessed the effects of short-term and prolonged low-level exposure to RF radiation on the function and status of the immune system, clearly indicates that various shifts in the number and/or activity of immunocompetent cells can be detected. However, results have been inconsistent between experiments, despite comparable exposure conditions at similar intensities and radiation parameters. Short-term exposure to weak RF fields may temporarily stimulate certain humoral or cellular immune functions, while prolonged irradiation inhibits the same functions. The relevance of these observations to carcinogenicity was unclear.]

4.2.3 *Immunotropic effects of exposure to RF radiation in experimental systems: studies in human cells in vitro*

See [Table 4.9](#)

[Cleary et al. \(1990\)](#) studied human peripheral blood cells that were sham-exposed or exposed *in vitro* to RF radiation at 27 MHz (SAR, 0–196 W/kg) or 2450 MHz (SAR, 0–50 W/kg) for 2 hours under isothermal conditions ( $37 \pm 0.2$  °C). Immediately after exposure, peripheral blood mononuclear cells were isolated by Ficoll density-gradient centrifugation and cultured for 3 days at 37 °C with or without mitogenic stimulation by PHA. Lymphocyte proliferation was assayed at the end of the culture period by a 6-hour pulse-labelling with [ $^3\text{H}$ ]thymidine. Exposure to radiation at

**Table 4.9 Immunotropic effects of exposure to radiofrequency radiation in experimental systems *in vitro***

Experimental system	Exposure conditions	Results	Reference
Mouse PBMC; assessment of IL-2-dependent cytolytic T-lymphocyte proliferation (CTLL-2)	2450 MHz, CW (SAR, 5–50 W/kg) or PW (SAR, 5 W/kg), for 2 h	Statistically significant reduction in CTLL-2 proliferation after CW-RF radiation at low IL-2 levels and at SAR $\geq 25$ W/kg; increase after PW-RF radiation	<a href="#">Cleary <i>et al.</i> (1996)</a>
Rat basophilic leukaemia RBL-2H3 cells (a mast cell line)	835 MHz; 81 W/m <sup>2</sup> ; 3 × 20 min/d for 7 d	From day 4 onwards, the rates of DNA synthesis and cell replication continued to increase in exposed cells, but decreased in controls; cell morphology was also altered	<a href="#">Donnellan <i>et al.</i> (1997)</a>
Human PBMC, microculture with mitogen (PHA) stimulation	27 MHz (SAR, 0–196 W/kg) or 2450 MHz (SAR, 0–50 W/kg); isothermal conditions (37 ± 0.2 °C); 2 h	Dose-dependent, statistically significant increase in [ <sup>3</sup> H] thymidine uptake in PHA-activated or unstimulated lymphocytes at SAR < 50 W/kg; uptake was suppressed at SAR $\geq 50$ W/kg	<a href="#">Cleary <i>et al.</i> (1990)</a>
Human lymphocytes; transformation of PBMC exposed to RF radiation or heated conventionally	2450 MHz CW or PW, at non-heating (37 °C) and various heating levels (temperature increases of 0.5, 1.0, 1.5, and 2 °C); SARs up to 12.3 W/kg	Both conventional and CW heating enhanced cell transformation to the same extent, which was correlated with the increase in incubation temperature. Exposure to PW RF radiation enhanced transformation at non-heating conditions.	<a href="#">Czerska <i>et al.</i> (1992)</a>
Human mast cell line, HMC-1	864.3 MHz; average SAR, 7 W/kg; 3x20 min/d for 7 d	Effect on localization of protein kinase C (migration towards the cell membrane), upregulation of <i>c-kit</i> , down-regulation of <i>NDPK</i> -beta, and the apoptosis-associated gene <i>DAD-1</i> .	<a href="#">Harvey &amp; French (1999)</a>
Human PBMC, microculture with mitogens, assessment of interleukin release, T-cell suppression (SAT)	1300 MHz PW; SAR, 0.18 W/kg; 1 h	Decreased spontaneous incorporation of [ <sup>3</sup> H]thymidine; no change in response to PHA or concanavalin A; no change in SAT index and saturation of IL-2 receptors; production of IL-10 by lymphocytes increased. Pulse-modulated MWs have immunotropic effects.	<a href="#">Dąbrowski <i>et al.</i> (2003)</a>
Human lymphocytes; analysis of CD25, CD95, CD28 antigens in unstimulated and stimulated CD4+ or CD8+ T-cells from PBMC	1800 MHz (10 min on, 20 min off); SAR, 2 W/kg; 44 h. Microculture with or without antibody anti-CD3 mitogenic stimulation	No significant difference in proportion of cell subsets between exposed and sham-exposed lymphocytes from young or elderly donors. Slight but significant downregulation of CD95 expression in stimulated CD4+ T lymphocytes from elderly (average age, 88 yr) but not from younger (average age, 26 yr) donors.	<a href="#">Capri <i>et al.</i> (2006)</a>

**Table 4.9 (continued)**

Experimental system	Exposure conditions	Results	Reference
Human PBMC, microculture with mitogens, assessment of interleukin (IL) release, T-cell suppression (SAT)	900 MHz (GSM); SAR, 0.024 W/kg; 15 min	Significantly increased response to mitogens and enhanced immunogenic activity of monocytes (LM index). The results suggest that immune activity of responding lymphocytes and monocytes can be enhanced by 900 MHz MW.	<a href="#">Stankiewicz <i>et al.</i> (2006)</a>
Human PBMC, microculture with mitogens, assessment of several immune functions	1950 MHz (GSM; 5 min on, 10 min off); SAR, 1 W/kg; 8 h	No effects of RF radiation on immune functions: (i) the intracellular production of IL-2 and INF- $\gamma$ in lymphocytes, and IL-1 and TNF- $\alpha$ in monocytes; (ii) the activity of immune-relevant genes (IL 1- $\alpha$ and $\beta$ , IL-2, IL-2-receptor, IL-4, MCSF-receptor, TNF- $\alpha$ , TNF- $\alpha$ -receptor); or (iii) the cytotoxicity of lymphokine-activated killer cells (LAK cells) against a tumour cell line.	<a href="#">Tuschl <i>et al.</i> (2006)</a>

d, day; h, hour; IL-2, interleukin 2; IL-10, interleukin 10; INF- $\gamma$ , interferon  $\gamma$ ; LM, lymphocytes-monocytes; MCSF, macrophage colony-stimulating factor; MW, microwave; min, minute; mo, month; NDPK, nucleoside diphosphate kinase; PBMC, peripheral blood mononuclear cells; PHA, phytohaemagglutinin; PW, pulsed-wave; SAR, specific absorption rate; SAT index, a measure of the suppressive activity of T cells; TNF, tumour necrosis factor; yr, year

either frequency at SARs < 50 W/kg resulted in a dose-dependent, statistically significant increase in [<sup>3</sup>H]thymidine uptake in PHA-activated or non-stimulated lymphocytes. Exposure at SARs of ≥ 50 W/kg suppressed [<sup>3</sup>H]thymidine uptake. There were no detectable effects of RF radiation on lymphocyte morphology or viability.

[Czerska et al. \(1992\)](#) determined the effects of continuous- and pulsed-wave RF radiation at 2450 MHz (average SARs up to 12.3 W/kg) on spontaneous lymphoblastoid transformation of human lymphocytes *in vitro*. Peripheral blood mononuclear cells from healthy donors were exposed for 5 days to conventional heating, or to continuous- or pulsed-wave RF radiation at 2450 MHz under non-heating (37 °C) or various heating conditions (temperature increases of 0.5, 1.0, 1.5, or 2 °C). The pulsed exposures involved pulse-repetition frequencies from 100 to 1000 pulses per second at the same average SARs as the continuous exposures. At the end of the incubation period, spontaneous lymphoblastoid-cell transformation was detected by use of an image-analysis system. At non-heating levels, continuous-wave exposure did not affect transformation compared with sham-exposed cultures. Under heating conditions, both conventional heating and exposure to continuous-wave RF radiation enhanced transformation to the same extent, and correlated with the increases in incubation temperature. Exposure to pulsed-wave RF radiation enhanced transformation under non-heating conditions. At heating levels, it enhanced transformation to a greater extent than did conventional heating or continuous-wave exposure. The results indicate that pulsed-wave RF radiation at 2450 MHz had a different action on the process of lymphoblastoid cell transformation *in vitro* than continuous-wave radiation at 2450 MHz and at the same average SARs.

Human HMC-1 mast cells were exposed to RF radiation at 846.3 MHz (average SAR, 7.3 W/kg) for 20 minutes, three times per day

(at 4-hour intervals) for 7 days. During the 20 minutes of exposure, the cells were outside the incubator and the temperature in the cell-culture medium dropped to 26.5 °C. Effects were seen on the localization of protein kinase C (migration to the cell membrane), and on expression of three genes: the proto-oncogene *c-kit* (upregulated 36%), the gene encoding transcription factor nucleoside diphosphate kinase B (downregulated 38%), and the apoptosis-associated gene *DAD-1* (downregulated 47%) ([Harvey & French, 1999](#)).

[Dąbrowski et al. \(2003\)](#) exposed peripheral blood mononuclear cells from healthy donors ( $n = 16$ ) to pulse-modulated RF radiation at 1300 MHz (power density, 1 mW/cm<sup>2</sup>; SAR, 0.18 W/kg) for 1 hour. This exposure decreased the spontaneous incorporation of [<sup>3</sup>H]thymidine, but the proliferative response of lymphocytes to PHA and concavalin A, the T-cell suppressive activity (SAT index), and the saturation of IL-2 receptors did not change. The IL-10 production by the lymphocytes increased ( $P < 0.001$ ), and the concentration of interferon-gamma (IFN $\gamma$ ) remained unchanged or slightly decreased in the culture supernatants. Exposure to RF radiation modulated monokine production by monocytes. The production of IL-1 $\beta$  increased significantly, the concentration of its antagonist (IL-1ra) dropped by half and the concentration of tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) remained unchanged. These changes in monokine proportion (IL-1 $\beta$  versus IL-1ra) resulted in a significant increase in the immunogenic activity of the monocytes, *i.e.* the influence of monokines on the lymphocyte mitogenic response, which reflects the activation of monocyte immunogenic function. The results indicated that pulse-modulated microwaves have the potential to influence immune function, stimulating preferentially the immunogenic and pro-inflammatory activity of monocytes at relatively low levels of exposure.

[Capri et al. \(2006\)](#) analysed CD25, CD95, CD28 molecules in non-stimulated and stimulated CD4+ or CD8+ T-cells *in vitro*. Peripheral

blood mononuclear cells from 10 young (age,  $26 \pm 5$  years) and 8 elderly (age,  $88 \pm 2$  years) donors were sham-exposed or exposed to intermittent (10 minutes on, 20 minutes off) RF radiation at 1800 MHz (SAR, 2 W/kg) for 44 hours, with or without mitogenic stimulation. No significant changes in the percentage of these subsets of cells were found between exposed and sham-exposed non-stimulated lymphocytes in young or elderly donors. A small, but statistically significant downregulation of CD95 expression was noted in stimulated CD4+ T lymphocytes from elderly, but not from younger donors, after exposure to RF radiation.

[Stankiewicz et al. \(2006\)](#) investigated whether cultured human immune cells induced into the active phases of the cell cycle (G1, S) were sensitive to exposure to RF radiation at 900 MHz (GSM; 27 V/m; SAR, 0.024 W/kg) for 15 minutes. The exposed microcultures of peripheral blood mononuclear cells showed a significantly higher proliferative response to PHA or concanavalin A, a stronger response to mitogens, and a higher immunogenic activity of monocytes than sham-exposed control cultures.

[Tuschl et al. \(2006\)](#) exposed peripheral blood mononuclear cells to RF radiation at 1950 MHz, with a SAR of 1 W/kg, in an intermittent mode (5 minutes on, 10 minutes off) for 8 hours. Numerous immune parameters were evaluated, including: intracellular production of IL-2 and INF $\gamma$  in lymphocytes, and IL-1 and TNF- $\alpha$  in monocytes; activity of immune-relevant cytokines (IL-1- $\alpha$  and  $\beta$ , IL-2, IL-2-receptor, IL-4, macrophage colony-stimulating factor (MCSF)-receptor, TNF- $\alpha$ , TNF- $\alpha$ -receptor); and cytotoxicity of lymphokine-activated killer cells (LAK cells) against a tumour cell line. For each parameter, blood samples from at least 15 donors were evaluated. No statistically significant effects of exposure were found.

[The Working Group concluded that exposure *in vitro* to non-thermal intensities of RF

radiation provided weak evidence for effects on immunocompetent cells.]

### 4.3 Effects of exposure to RF radiation on gene and protein expression

#### 4.3.1 Gene expression

##### (a) Humans

There were no studies examining gene or protein expression after exposure to RF radiation in humans.

##### (b) Experimental animals

See [Table 4.10](#)

##### (i) *Caenorhabditis elegans*

No effect was found on the transgene expression of *hsp16* (encoding heat-shock protein hsp16, the equivalent of human hsp27) in the nematode *C. elegans* – transgenic for *hsp16* – exposed to continuous-wave or pulsed-wave RF radiation at 1.8 GHz (SAR, 1.8 W/kg) for 2.5 hours at 25 °C ([Dawe et al., 2008](#)). In a second study, *C. elegans* was exposed to continuous-wave RF radiation at 1 GHz (SAR, 0.9–3 mW/kg; power input, 0.5 W) for 2.5 hours at 26 °C. In this exposure set-up, with very low SAR, the difference in temperature between exposed and sham-exposed samples did not exceed 0.1 °C. In a gene-expression array, no statistically significant effects on the gene-expression pattern were found ([Dawe et al., 2009](#)). [The Working Group noted that experiments at these low SAR levels may favour a no-effect outcome.]

##### (ii) *Drosophila melanogaster*

Using a semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR), [Lee et al. \(2008\)](#) showed that exposure of fruit flies (*D. melanogaster*) to RF radiation at 835 MHz (SAR, 1.6 or 4.0 W/kg) for up to 36 hours (resulting in 90% or 10% survival, respectively, at low and high SAR) affected the

**Table 4.10 Effects on gene expression in animal models after exposure to radiofrequency radiation *in vivo***

Biological model	Exposure conditions	Assessment of gene expression	Results	Comments	Reference
<i>Caenorhabditis elegans</i> (strain PC72)	1800 MHz (GSM); CW or DTX; SAR, 1.8 W/kg; 2.5 h at 25 °C	Stress-inducible reporter gene $\beta$ -galactosidase under control of <i>hsp16</i> heat-shock promoter, measured as $\beta$ -galactosidase activity	No effect on expression of <i>hsp16</i>		<a href="#">Dawe et al. (2008)</a>
<i>Caenorhabditis elegans</i> wild-type (N2)	1000 MHz (CW); SAR, 0.9–3 mW/kg; 2.5 h	Affymetrix <i>C. elegans</i> Genome GeneChip array (> 22 000 probes)	21 upregulated and 6 downregulated genes; less than expected by chance		<a href="#">Dawe et al. (2009)</a>
<i>Drosophila melanogaster</i> F, age 3 d	835 MHz; SAR, 1.6 and 4.0 W/kg; 12, 18, 24, 30, 36 h	Semi-quantitative RT-PCR; analysis of stress genes <i>rolled</i> ( <i>Erk</i> ), <i>Jra</i> ( <i>Jun</i> ), <i>Dfos</i> ( <i>Fos</i> ) and apoptosis-related genes: <i>Bcl2</i> , <i>Dmp53</i> ( <i>TP53</i> ), <i>reaper</i> , <i>hid</i>	Increased expression of <i>rolled</i> (1.6 W/kg) and <i>Jra</i> and <i>Dfos</i> (4.0 W/kg); protein-expression changes confirmed gene-expression changes; increased expression of <i>Bcl2</i> (1.6 W/kg) and <i>Dmp53</i> , <i>reaper</i> , <i>hid</i> (4.0 W/kg)		<a href="#">Lee et al. (2008)</a>
Mouse brain (BALB/c) age, 5–6 wk	800 MHz (GSM); SAR, 1.1 W/kg (whole-body); SAR, 0.2 W/kg (brain); 1 h	Affymetrix Mouse Expression Array 430A (22 600 probe sets)	Filtering microarray results for fold-changes > 1.5 and > 2.0 provided; respectively 301 and 30 differentially expressed probe sets	No consistent evidence of modulation of gene expression in whole brain	<a href="#">Paparini et al. (2008)</a>
Rat brain (Wistar, M)	900 MHz (GSM); SAR, 0.3 or 1.5 W/kg; 900 MHz (CW), SAR, 7.5 W/kg; 4 h	Gene expression assessed immediately after exposure. Hybridization <i>in situ</i> ; <i>hsp70</i> , <i>c-fos</i> , <i>c-jun</i> , <i>GFAP</i> ; optical-density analysis	<i>hsp70</i> mRNA: increase at 7.5 W/kg (CW); <i>c-fos</i> mRNA: increase at all exposures; <i>c-jun</i> mRNA: decline at 1.5 W/kg (GSM) and 7.5 W/kg (CW). <i>GFAP</i> mRNA: no effect	Exposure by use of a mobile phone	<a href="#">Fritze et al. (1997a)</a>
Rat brain (F344)	1600 MHz; SAR, 0.16, 1.6, 5 W/kg; 2 h	Northern blot for ornithine decarboxylase, <i>Fos</i> and <i>Jun</i> in total brain RNA; normalization to $\alpha$ -actin probe	No effect on mRNA expression		<a href="#">Stagg et al. (2001)</a>
Rat brain (F344)	915 MHz GSM (DTX); average whole-body SAR, 0.4 W/kg; 2 h	Affymetrix U34A GeneChip (8800 genes)	11 upregulated genes; 1 downregulated gene		<a href="#">Belyaev et al. (2006)</a>

**Table 4.10 (continued)**

Biological model	Exposure conditions	Assessment of gene expression	Results	Comments	Reference
Rat brain (F344)	1800 MHz (GSM); whole-body SAR, 0.013 W/kg (brain SAR, 0.03 W/kg); 6 h	Affymetrix rat 2302 chip (31 099 genes); categories: upregulated > 1.05-fold; downregulated < 0.95-fold; unaffected, 0.95–1.05-fold	Numerous upregulated and downregulated genes in nearly all 4956 gene ontologies analysed, especially regulatory genes of membrane integrity and cell signalling.	Less reliable due to small “fold-change” criteria; information on affected genes not given	<a href="#">Nittby et al. (2008)</a>
Rat brain, facial nerves (Sprague-Dawley)	1.9 GHz (GSM); SAR, 0.9, 1.18, 1.8 W/kg; 6 h/d, for 126 d	RT-PCR analysis of mRNA for calcium ATPase, N-CAM, NGF-B, VEGF in brain and in facial nerves	Statistically significant upregulation of all mRNAs	Radiation source was a mobile phone; less reliable dosimetry	<a href="#">Yan et al. (2008, 2009)</a>
Rat (newborn) kidney (pregnant Sprague-Dawley rats)	9.4 GHz (GSM); SAR, 0.5 mW/kg; continuously on days 1–3 or 4–7 after mating	RT-PCR analysis of mRNA expression of bone morphogenetic proteins (Bmp) and their receptors (Bmpr)	Increased mRNA expression of Bmp4 and Bmpr1a, and decreased expression of Bmpr2 in kidneys of newborns from rats exposed on days 1–3 or 4–7 of gestation. No effect on expression of Bmp7.	These changes may reflect a delay in renal development	<a href="#">Pyrpasopoulou et al. (2004)</a>

CW, continuous wave; d, day; DTX, discontinuous transmission mode; GSM, Global System for Mobile communication; h, hour; N-CAM, neural cell-adhesion molecule; NGF, neural growth factor; RT-PCR, reverse-transcriptase polymerase chain reaction; SAR, specific absorption rate; VEGF, vascular endothelial growth factor; wk, week

expression of genes encoding stress-response kinases and proteins involved in the regulation of apoptosis. Interestingly, some of these genes – involved in cell-survival signalling pathways – responded to the lower SAR, while others – involved in apoptotic pathways – were activated by the higher SAR. The changes in gene expression were followed by similar changes in expression of the corresponding proteins ([Table 4.11](#)), which strengthens the validity of the findings.

#### (iii) Mouse

[Paparini et al. \(2008\)](#) exposed BALB/c mice to RF radiation at 1800 MHz (whole-body SAR, 1.1 W/kg; brain-averaged SAR, 0.2 W/kg) for 1 hour, and analysed gene expression in total brain homogenate. The array analysis did not show any significant modulation of gene expression in the exposed mice compared with sham-exposed controls. Under less stringent conditions, 42 genes were found to be upregulated, while 33 were downregulated. However, these results could not be confirmed with RT-PCR. [The Working Group noted that analysing mRNA from a whole-brain homogenate might obscure the detection of any effect in specific brain regions.]

#### (iv) Rat

Groups of 30 male Wistar rats were exposed to RF radiation at 900 MHz (GSM; brain-averaged SAR, 0.3 or 1.5 W/kg) or to continuous-wave RF radiation at 900 MHz (brain-averaged SAR, 7.5 W/kg), for 4 hours. To mimic actual life exposure as closely as possible, the signal was generated with a commercial mobile GSM phone, and a telephone conversation was simulated by repeatedly playing the first half of H. von Kleist's comedy *Der zerbrochene Krug* ([Von Kleist, 1811](#)). Subgroups of 10 rats were processed immediately after exposure, or 24 hours or 7 days later. Enhanced expression of Hsp70 mRNA was observed in the brain at the higher SAR of 7.5 W/kg, and a small but significant increase

was seen in c-Fos expression in the brain at the two lower SAR values ([Fritze et al., 1997a](#)). [The Secretariat was pleased to learn that the spoken text to which the rats were exposed in this study mimicked actual life exposure of the authors, but was uncertain about confounding effects on the rat brain.]

Fischer 344 rats were exposed to RF radiation at 1600 MHz (brain-averaged SAR, 0.16, 1.6, and 5.0 W/kg) for 2 hours. No changes were seen in core body temperature and corticosterone or adrenocorticotrophic hormone levels in the brain that could be attributed to exposure to RF radiation. Also the levels of *Odc*, *Fos* and *Jun* mRNA in brain tissue showed no differences with sham-exposed controls that could be ascribed to RF radiation ([Stagg et al., 2001](#)).

Three groups of pregnant Wistar rats were sham-exposed, or exposed to pulsed-wave RF radiation at 9.4 GHz (SAR, 0.5 mW/kg) continuously during days 1–3 after mating, or during days 4–7 after mating, respectively. In 20–26 newborns collected from each of these groups, significantly altered expression and localization of proteins involved in bone morphogenesis were observed in the kidney. These changes may reflect a delay in renal development ([Pyrpasopoulou et al., 2004](#)).

Whole-body exposure of Fischer 344 rats to RF radiation at 915 MHz (GSM; SAR, 0.4 W/kg) for 2 hours led to significantly ( $P < 0.0025$ ) increased expression (1.34–2.74-fold) of eleven genes and reduced expression (0.48-fold) of one gene in the cerebellum of the exposed rats. Only these genes showed significantly increased/decreased expression in all nine comparisons between three exposed and three sham-exposed rats ([Belyaev et al., 2006](#)).

[Nittby et al. \(2008\)](#) reported a strong response and changes in the expression of numerous genes after whole-body exposure of Fischer 344 rats to RF radiation at 1800 MHz (GSM; SAR, 13 mW/kg) for 6 hours. In this study, changes in gene expression were considered when expression

**Table 4.11 Effects on protein expression in human and animal models after exposure to radiofrequency radiation *in vivo***

Biological model	Exposure conditions	Assessment of protein expression	Results	Comments	Reference
Human skin, female volunteers	900 MHz (GSM); SAR, 1.3 W/kg; local exposure, 1 h; punch-biopsies collected immediately after exposure	Protein expression by 2DE-based proteomics	Expression was significantly increased for 7 proteins, reduced for 1; 2 proteins – one up, one down – were affected in all 10 volunteers	Proteins not identified	<a href="#">Karinen et al. (2008)</a>
<i>Drosophila melanogaster</i>	1900 MHz (GSM); SAR, 1.4 W/kg; 2 × 1 h per day for 10 d	Immunocytochemistry; serum response element (SRE)-binding, ELK1 phosphorylation, hsp70	Increase in expression of all measured proteins	Unreliable dosimetry: exposure by placing vials next to mobile-phone antenna; unreliable data analysis, single experiments; no statistical analysis	<a href="#">Weisbrot et al. (2003)</a>
<i>Drosophila melanogaster</i>	835 MHz; SAR, 1.6 and 4.0 W/kg; 12, 18, 24, 30, 36 h	Immunocytochemistry; phospho-JNK, phospho-ERK, phospho-p38MAPK	Activation of ERK (at SAR 1.6 W/kg), activation of JNK (at SAR 4.0 W/kg); no effect on p38MAPK		<a href="#">Lee et al. (2008)</a>
<i>Drosophila melanogaster</i>	900 MHz; SAR 0.64 W/kg; continuous/intermittent exposure	Immunofluorescence; phalloidin detection of actin stress fibres	Increase in disorganization of actin network	Unreliable dosimetry: exposure by placing vials next to mobile-phone antenna	<a href="#">Chavdoula et al. (2010)</a>
Mouse brain (C57BL/6NTac) age, 8 wk	900 MHz (GSM); SAR, 4 W/kg. Mice were restrained for 1 h during exposure; brains perfusion-fixed immediately after exposure	Immunocytochemistry; c-fos	Non-significant decline (~50%) in c-fos expression in exposed cingulate cortex; no effects in other parts of the brain	Significant difference of exposed/sham-exposed with cage-controls; effects may be due to immobilization	<a href="#">Finnie (2005)</a>
Fetal mouse brain (BALB/c)	900 MHz (GSM); SAR, 4 W/kg; 1 h daily, on days 1–19 of gestation. Mice were restrained during exposure	Immunocytochemistry; c-fos	Average expression of c-fos was non-significantly increased in basal ganglion and reduced in pyriform cortex		<a href="#">Finnie et al. (2006a)</a>
Mouse brain (C57BL/6NTac)	900 MHz (GSM); SAR, 4 W/kg; 1 h/d, 5 d/wk, 104 wk. Mice were restrained during exposure	Immunocytochemistry; c-fos	No effects on c-fos expression, but no numerical analysis shown	No statistical details given. Significant difference of exposed/sham-exposed with cage-controls; effects may be due to immobilization	<a href="#">Finnie et al. (2007)</a>

**Table 4.11 (continued)**

Biological model	Exposure conditions	Assessment of protein expression	Results	Comments	Reference
Fetal mouse brain (BALB/c)	900 MHz (GSM); SAR, 4 W/kg; 1 h daily on days 1–19 of gestation. Mice were restrained during exposure	Immunocytochemistry; Hsp25, Hsp32, Hsp70	No effect on expression of Hsp; no numerical analysis shown	No statistical details given; shown only examples of stained brain slices	<a href="#">Finnie et al. (2009a)</a>
Mouse brain [strain NS]	900 MHz (GSM); SAR, 4.0 W/kg; 1 h single exposure or 1 h/d, 5 d/wk, 104 wk	Immunocytochemistry; aquaporin (AQP4, marker of blood–brain barrier function)	No effect on aquaporin expression; no numerical analysis shown	No statistical details given; shown only examples of stained brain slices	<a href="#">Finnie et al. (2009b)</a>
Mouse brain [strain NS]	900 MHz (GSM); SAR, 4.0 W/kg; 1 h single exposure or 1 h/d, 5 d/wk, 104 wk	Immunocytochemistry; ionized calcium-binding adaptor molecule Iba1 (microglia activation marker)	No effect on Iba1 expression		<a href="#">Finnie et al. (2010)</a>
Transgenic mouse (hsp70.1-deficient)	849 MHz or 1763 MHz; whole-body average SAR, 0.04 W/kg; 2 × 45 min/d with 15-min interval, 5 d/wk, for up to 10 wk; mice killed after 4, 8, 10 wk of exposure	Immunocytochemistry: PCNA Western blot: actin, HSP90, HSP70, HSP25, ERK and phospho-ERK, JNK and phospho JNK, p38MAPK and phospho-p38MAPK	No effect on HSP90, HSP70, HSP25 expression No effect on phosphorylation of ERK, JNK and p38MAPK		<a href="#">Lee et al. (2005)</a>
Mouse brain (C57BL/6N)	849 MHz or 1763 MHz; brain average SAR, 7.8 W/kg; 1 h/d, 5 d/wk, for 6 or 12 mo	Immunocytochemistry: PCNA, GFAP, NeuN	No effect on expression of PCNA, GFAP, NeuN	No numerical data, no statistical details given Visual evaluation only	<a href="#">Kim et al. (2008b)</a>
Mouse brain (ICR, M)	835 MHz; SAR, 1.6 and 4.0 W/kg; whole-body exposure; 5 h (single), 1 h/d for 5 d; daily [no time given] for 1 mo (1.6 W/kg only)	Immunocytochemistry: calbindin, calretinin	Changes in expression of calbindin and calretinin after 1 mo exposure, particularly in the inner molecular layer of the dentate gyrus of the brain	Alterations in calcium-binding proteins affect cellular Ca <sup>2+</sup> levels and hippocampal functions associated with neuronal connectivity and integration	<a href="#">Maskey et al. (2010)</a>
Rat brain (Wistar, M)	900 MHz (GSM, SAR 0.3 and 1.5 W/kg; CW, SAR 7.5 W/kg CW); 4 h; protein expression examined 24 h after exposure	Immunocytochemistry; c-fos, fos B, c-jun, jun B, jun D, krox-20, krox-24, Hsp70, Gfap, MHCclass II;	No effect on expression of any of the proteins examined	Exposure by use of a mobile telephone; only visual inspection and evaluation of samples; no statistical details	<a href="#">Fritze et al. (1997a)</a>

**Table 4.11 (continued)**

Biological model	Exposure conditions	Assessment of protein expression	Results	Comments	Reference
Rat brain (F344, M)	915 MHz (GSM, DTX); average whole-body SAR, 0.4 W/kg; 2 h	Western blot: Hsp70	No effect on expression of hsp70 protein		<a href="#">Belyaev et al. (2006)</a>
Rat brain (Wistar albino)	900 MHz (GSM); SAR, 2.0 W/kg; 2 h/d, 7 d/wk, for 10 mo	Immunocytochemistry: caspase-3, Tp53	No effect on Tp53; caspase-3 re-localized to nucleus	Protein expression scored by visual inspection and evaluation	<a href="#">Dasdag et al. (2009)</a>
Rat brain (Sprague-Dawley)	900 MHz (GSM); SAR, 6 and 1.5 W/kg; exposure 15 min/d for 7 d at high SAR, and 45 min/d for 7 d at low SAR	Cytochrome- <i>c</i> oxidase activity in brain slices by staining with di-amino-benzidine and horse-heart cytochrome- <i>c</i> as substrate	Decreased cytochrome- <i>c</i> oxidase activity in prefrontal, frontal and posterior cortex, septum, and hippocampus, at SAR 6 W/kg	Exposure may affect brain metabolism and neuronal activity	<a href="#">Ammari et al. (2008)</a>
Rat brain (Sprague-Dawley)	900 MHz (GSM); SAR, 6 and 1.5 W/kg; exposure 15 min/d for 8 wk at high SAR, and 45 min/d for 8 wk at low SAR; samples taken 3 and 10 d after exposure	Immunocytochemistry: glial fibrillary acidic protein (Gfap)	Increase in Gfap expression	Gfap increase may be a sign of gliosis	<a href="#">Ammari et al. (2010)</a>
Rat skin (hairless rat, F)	900 MHz or 1800 MHz (GSM); local skin SAR, 5 W/kg; 2 h; sampling immediately after exposure	Immunocytochemistry: Ki67, filaggrin, collagen, elastin	No effect on number of cells expressing Ki-67; no effect on density of filaggrin, collagen and elastin		<a href="#">Masuda et al. (2006)</a>
Rat skin (hairless rat)	900 MHz or 1800 MHz (GSM); local skin SAR, 2.5 and 5 W/kg; 2 h/d, 5 d/wk, for 12 wk; samples taken 72 h after the last exposure	Immunocytochemistry: Ki67, filaggrin, collagen, elastin	No effect on number of cells expressing Ki-67, no effect on density of filaggrin, collagen and elastin		<a href="#">Sanchez et al. (2006a)</a>
Rat skin (hairless rat)	900 MHz or 1800 MHz (GSM); local skin SAR, 5 W/kg; 2 h; sampling immediately after exposure. Multiple exposures to 900 MHz or 1800 MHz (GSM); local skin SAR, 2.5 or 5 W/kg; 2 h/d, 5 d/wk, for 12 wk; samples taken 72 h after the last exposure	Immunocytochemistry: Hsc70, Hsp70 and Hsp25	No effect on expression of stress proteins	Analysis of three areas on three photographs per stained skin slice, quantified by image-analysis software	<a href="#">Sanchez et al. (2008)</a>

**Table 4.11 (continued)**

Biological model	Exposure conditions	Assessment of protein expression	Results	Comments	Reference
Rat kidney (Wistar; newborn)	9.4 GHz; 5 $\mu$ W/cm <sup>2</sup> ; 0.5 mW/kg; continuously exposed on days 1–3 or 4–7 after mating	Kidneys from newborns of exposed rats were investigated by means of immunocytochemistry (Bmp4 and Bmp7) and <i>in situ</i> hybridization (receptors Bmpr2 and Bmpr1a)	Significant increase in expression and change in localization of Bmp4. Increase in Bmpr1a, decrease in Bmpr2 expression. Effects were stronger after exposure <i>in utero</i> on days 1–3 of gestation (embryogenesis) than on days 4–7 (organogenesis)	Effects dependent on timing of exposure <i>in utero</i>	<a href="#">Pyrpasopoulou et al. (2004)</a>
Rat thyroid (Wistar)	900 MHz (GSM); SAR, 1.35 W/kg; 20 min/d, 3 wk	Immunocytochemistry, transmission electron microscopy; Casp3 and Casp9 (markers of apoptosis)	Significant increase in expression of Casp3 and Casp9; thyroid hypertrophy; reduced thyroid-hormone secretion; formation of apoptotic bodies	Histomorphometry of thyroid tissue	<a href="#">Eşmekaya et al. (2010)</a>
Rat testis (Sprague-Dawley)	848.5 MHz (CDMA signal); SAR, 2.0 W/kg; 2 $\times$ 45 min/d with a 15-min interval; 12 wk	Western blot; p21, Tp53, Bcl2, Casp3, PARP	No effect for Tp53, Bcl2, Casp3; no result given for PARP or p21		<a href="#">Lee et al. (2010)</a>

2DE, two-dimensional gel electrophoresis; CDMA, code division multiple access; d, day; DTX, discontinuous transmission mode; F, female; GSM, Global System for Mobile Communications; h, hour; M, male; min, minute; mo, month; NS, not specified; SAR, specific absorption rate; wk, week

had risen or declined by 5%, compared with controls. [The genes investigated in this study were not identified, and the changes in gene expression were not validated by RT-PCR.]

Sprague-Dawley rats were exposed to RF radiation at 1.9 GHz (with SARs of 0.9, 1.18, or 1.8 W/kg at a distance of 2.2 cm) from a mobile phone operating in three different modes, for  $2 \times 3$  hours per day, for 18 weeks. A statistically significant upregulation of the mRNAs for calcium ATPase, neural cell-adhesion molecule, neural growth factor, and vascular endothelial growth factor was measured in the brain of these rats. In addition, these mRNAs were upregulated in the mandibular and buccal branches of the facial nerve. These results suggest that neurological damage may be associated with long-term mobile-phone use ([Yan et al., 2008, 2009](#)).

#### 4.3.2 Protein expression

See [Table 4.11](#)

##### (a) Humans

In a pilot study, a small skin area of one forearm of 10 volunteers was exposed to RF radiation at 900 MHz (GSM; SAR, 1.3 W/kg) for 1 hour. Immediately after exposure, punch biopsies were taken from the exposed area and from the other non-exposed forearm of the same person. Proteins were extracted and analysed by means of 2D-gel electrophoresis. Changes in the expression of eight proteins were found; two of these proteins were observed in all 10 volunteers. Identity and function of these proteins were not given ([Karinen et al., 2008](#)).

##### (b) Experimental animals

###### (i) *Drosophila melanogaster*

Exposure of fruit flies (*D. melanogaster*) to RF radiation at 1900 MHz from a mobile phone (GSM; SAR, 1.4 W/kg) for  $2 \times 1$  hour per day, for 10 days, resulted in an increase of 3.6–3.9-fold in the expression of heat-shock protein hsp70,

the phosphorylation of ELK1 kinase, and the DNA-binding activity of the serum-response element (SRE) ([Weisbrot et al., 2003](#)).

As indicated above, exposure of *D. melanogaster* to RF radiation at 835 MHz (GSM; SAR, 1.6 or 4.0 W/kg) for up to 36 hours affected the expression of genes encoding stress-response kinases and proteins involved in the regulation of apoptosis. The expression of the corresponding proteins was confirmed by Western blotting with protein-specific antibodies ([Lee et al., 2008](#)).

[Chavdoula et al. \(2010\)](#) exposed *D. melanogaster* to continuous or intermittent RF radiation at 900 MHz (GSM) from a digital mobile phone (SAR, 0.64 W/kg) for 6 minutes per day, for 6 days. The phone was fully charged and its antenna was in contact with the glass vials containing the flies, and parallel to the vial axis. Exposure to RF radiation caused an increased disorganization of the actin network of the egg chambers. This effect was due to DNA fragmentation, as measured with the TUNEL assay.

###### (ii) Mouse

Nine studies were performed in mice on changes in protein expression after exposure to RF radiation. The mice were of different age (fetus, or adults aged 6–8 weeks) and different strains (C57BL/6N, C57BL/6NTac, hsp70.1-deficient, BALB/c, ICR); mouse strain and age were not specified in two studies ([Finnie et al., 2009b, 2010](#)). Changes in protein expression were assessed by use of immunocytochemistry with monoclonal and polyclonal antibodies.

ICR mice were exposed to RF radiation at 835 MHz (SAR, 1.6 W/kg and 4.0 W/kg) for 5 hours, 1 hour per day for 5 days, or for 1 month. Changes in the expression of the calcium-binding proteins calbindin D28-k (CB) and calretinin (CR) were measured in the hippocampus by use of immunohistochemistry. Exposure for 1 month produced almost complete loss of pyramidal cells in the CA1 area of the brain. These alterations in calcium-binding proteins may cause

changes in cellular  $\text{Ca}^{2+}$  levels, which could affect hippocampal functions associated with neuronal connectivity and integration ([Maskey et al., 2010](#)).

Six of the published studies came from a single research group. Most of these studies were based on the same biological material that was separately stained to detect different proteins. Studies from this group have reported no effects on the expression of the following proteins after exposure to RF radiation: c-Fos in adult and fetal mouse brain, stress proteins Hsp25, Hsp32, and Hsp70 in fetal brain, aquaporin 4 in adult brain, and ionized calcium-binding adaptor molecule Iba1 in brain [age not given]. Others have reported similar findings (see [Table 4.11](#)). [The Working Group noted that these studies generally provided very few numerical and technical details.]

### (iii) Rat

Eleven studies were performed with rats of different ages (newborn to adult) and different strains (Wistar, Fisher 344, hairless rat, Sprague-Dawley). In addition, different tissues were examined (brain, skin, kidney, testis, thyroid). Detection of changes in protein expression was mostly by immunocytochemistry with protein-specific monoclonal and polyclonal antibodies, and in some studies by Western blotting.

Five studies assessed the effects of exposure to RF radiation in rat brain ([Fritze et al., 1997a](#); [Belyaev et al., 2006](#); [Dasdag et al., 2009](#); [Ammari et al., 2008, 2010](#)). These studies considered a limited number of proteins, generally gave negative results for changes in expression, and provided limited statistical detail. Samples were often analysed visually and without calculating statistical significance. For this reason the results were considered less reliable (see comments in [Table 4.11](#)).

In three studies, the effects of mobile-phone radiation on the skin of hairless rats were investigated ([Masuda et al., 2006](#); [Sanchez et al., 2006a](#),

[2008](#)). No effects were observed on any of the proteins analysed.

[Pyrpasopoulou et al. \(2004\)](#) used immunocytochemistry and hybridization in situ to examine the effects of exposure to RF radiation on kidneys of newborn rats and found that exposure affected the expression of bone morphogenic protein (Bmp4) and bone morphogenic protein receptors (Bmpr2, Bmpr1a). Similar changes were observed in the expression of the corresponding genes, as noted above (Section 4.3.1).

[Eşmekaya et al. \(2010\)](#) observed increased expression and activity of the apoptosis-regulating proteins caspase 3 (Casp3) and caspase 9 (Casp9) by use of light microscopy, electron microscopy, and immunohistochemical methods in the thyroid of Wistar rats exposed to RF radiation at 900 MHz (SAR, 1.35 W/kg) for 20 minutes per day, for 3 weeks.

[Lee et al. \(2010\)](#) examined the effects on rat testis of exposure to RF radiation at 848.5 MHz (SAR, 2.0 W/kg) twice per day for 45 minutes, 5 days per week, for 12 weeks. No significant effects were found on any of the apoptosis-associated proteins tested (p21, Tp53, Bcl2, Casp3, PARP).

[The Working Group noted that only few studies in experimental animals have examined the effects of RF radiation on gene and protein expression. These studies used a variety of biological models, and had mixed and inconsistent results. Many proteins that are known to be important for the initiation and development of cancer in humans were not evaluated. The Working Group concluded that the available studies on gene and protein expression in humans and animals exposed to RF radiation did not provide evidence to support mechanisms of carcinogenesis in humans.]

### (c) *In-vitro studies in human cells*

#### (i) *Heat-shock proteins*

See [Table 4.12](#)

Heat-shock proteins (HSPs) are a highly conserved family of chaperone proteins that are found in all cell types; they are expressed abundantly and have diverse functions. HSPs are expressed in response to cold, heat and other environmental stress factors, although some are expressed constitutively. HSPs increase heat tolerance and perform functions essential to cell survival under these conditions. Some HSPs serve to stabilize proteins in specific configurations, while others play a role in the folding and unfolding of proteins, acting as molecular chaperones. Stress-induced transcription of HSPs requires activation of heat-shock factors that bind to the heat-shock promoter element, thereby activating its transcription activity. Overexpression of HSPs has been linked to oncogenic development and poor prognostic outcome for multiple cancers, possibly through the roles of HSPs as mediators of signal transduction and inhibitors of oncogene-mediated senescence ([Evans et al., 2010](#)). Since markedly increased expression of HSPs is co-incident with exposure of cells to a variety of stress factors, expression of HSP genes and proteins in response to exposure to RF radiation has been extensively investigated in a variety of cell models.

Since the effects of RF radiation on HSP expression have been reviewed previously ([Cotgreave, 2005](#)), only recent publications on this issue are reviewed in detail in this Volume. Several studies have reported changes in HSP expression in human cell lines exposed to RF radiation.

[Tian et al. \(2002\)](#) exposed human glioma (MO54) cells to RF radiation at 2.45 MHz (SAR, 5–100 W/kg) for up to 16 hours. An increase in HSP70 protein levels at SARs of 25 and 78 W/kg was observed, but no effect was seen at SARs below 20 W/kg. [The Working Group noted that thermal confounding cannot be ruled out in this study due to the high relative SARs tested, the highly non-uniform SAR distribution within the exposure system, and the considerable reduction

in cell viability (~70%) in some samples during exposure.]

[Leszczynski et al. \(2002\)](#) exposed a human endothelial cell line (EA.hy926) to RF radiation at 900 MHz (GSM; SAR, 2 W/kg) for 1 hour. The phosphorylation status of several proteins was altered. Specifically, HSP27 was found to undergo a transient increase in expression and phosphorylation immediately after exposure, but this effect had disappeared at 1 or 4 hours after exposure.

[Lim et al. \(2005\)](#) exposed human peripheral blood cells to RF radiation at 900 MHz (average SAR, 0.4, 2.0 or 3.6 W/kg) for 20 minutes, 1 hour, or 4 hours. No statistically significant differences were detected in the number of lymphocytes or monocytes expressing stress proteins HSP27 or HSP70 after exposure, compared with the numbers in sham-exposed samples.

[Miyakoshi et al. \(2005\)](#) exposed human malignant glioma MO54 cells to RF radiation at 1950 MHz (SAR, 1, 2, or 10 W/kg) for up to 2 hours. Exposed cells did not show increased expression of HSP27 or HSP70 protein, but levels of phosphorylated HSP27 had decreased significantly in cells exposed at a SAR of 10 W/kg for 1 or 2 hours.

The transcription of HSPs is regulated by the DNA-binding activity of heat-shock transcription factors (HSFs). These factors bind to specific regulatory elements in the promoter region of HSP genes. In a study by [Laszlo et al. \(2005\)](#), no DNA-binding activity of HSF protein was detected in hamster (HA-1), mouse (C3H 10T $\frac{1}{2}$ ) and human cells (HeLa S3) exposed to 835.62 MHz (SAR, ~0.6 W/kg) or 847.74 MHz (SAR, ~5 W/kg) RF radiation, for up to 24 hours.

[Lee et al. \(2006\)](#) observed no detectable alterations in the expression of HSP27, HSP70 or HSP90 transcripts after exposure of human T-lymphocyte Jurkat cells to RF radiation at 1763 MHz (SAR, 2 or 20 W/kg) for 30 minutes or 1 hour.

**Table 4.12 Effects on heat-shock proteins in human cell lines exposed to radiofrequency radiation *in vitro***

Tissue/cell line	Exposure conditions	End-point and target	Results	Comments	Reference
MO54 glioma cells	2450 MHz, CW; SARs, 5, 20, 50, 100 W/kg; 2, 4, 8, 16 h	HSP70 protein expression	Increased expression of HSP70 only at SARs > 20 W/kg	SAR values very high; thermal confounding possible	<a href="#">Tian et al. (2002)</a>
EA.hy926 endothelial cells	900 MHz (GSM); SAR, ~2 W/kg; 1 h	p-HSP27 protein level	Transient change in p-HSP27 and phosphorylation of other unidentified proteins; transient change in HSP27 protein level	Effect had disappeared at 1 or 4 hours after exposure	<a href="#">Leszczynski et al. (2002)</a>
EA.hy926 endothelial cells	1800 MHz (GSM); SAR, 2.0 W/kg; 1 h	Protein HSP27 expression	No effect		<a href="#">Nylund et al. (2009)</a>
Human lens epithelial cells (hLEC)	1800 MHz (GSM); SAR, 1, 2, 3 W/kg; 2 h	HSP70 mRNA and protein expression	Increased expression of HSP70 protein at SAR 2 and 3 W/kg; no change in mRNA levels		<a href="#">Lixia et al. (2006)</a>
HeLa, S3 and EA.hy296 cell lines	837 MHz (TDMA); SAR, 5 W/kg; 1, 2, 24 h; or 900 MHz (GSM); SAR, 3.7 W/kg; for 1, 2, 5 h	p-HSP27 protein levels	No effect		<a href="#">Vanderwaal et al. (2006)</a>
A172 cells and IMR-90 fibroblasts	2142.5 MHz (CW or W-CDMA); SAR, 0.08 and 0.8 W/kg; 2–48 h	HSP27, HSP40, HSP70, HSP105 mRNA and protein expression, p-HSP27 protein levels	No effect		<a href="#">Hirose et al. (2007)</a>
Human blood	900 MHz (CW or GSM); SAR, 0.4, 2 or 3.6 W/kg; 20 min, 1 h, or 4 h	HSP27, HSP70 protein expression	No effect		<a href="#">Lim et al. (2005)</a>
A172 cells	2450 MHz (CW); SAR, 5–200 W/kg; 1–3 h	HSP27, HSP70 protein expression; p-HSP27 protein levels	No effect	SAR values very high (thermal confounding possible)	<a href="#">Wang et al. (2006)</a>
MO54 cells	1950 MHz (CW); SARs, 1, 2, 10 W/kg; 1 or 2 h	HSP27, HSP70 protein expression; p-HSP27 protein levels	Decrease in p-HSP27 at highest SAR		<a href="#">Miyakoshi et al. (2005)</a>
Mono Mac 6 cells	1800 MHz (CW and GSM); SAR, 2 W/kg; 1 h	HSP70 protein expression	No effect		<a href="#">Simkó et al. (2006)</a>
Mono Mac 6 and K562 cells	1800 MHz (CW and GSM); SARs, 0.5, 1, 1.5, or 2 W/kg; 45 min	HSP70 protein expression	No effect		<a href="#">Lantow et al. (2006a)</a>
U-251MG cells	6000 MHz (CW); power density 5.4 µW/cm <sup>2</sup> or 0.54 mW/cm <sup>2</sup> ; 1–33 h	HSP70 mRNA and protein	No effect		<a href="#">Zhadobov et al. (2007)</a>

**Table 4.12 (continued)**

Tissue/cell line	Exposure conditions	End-point and target	Results	Comments	Reference
HTR-8/ neo; human trophoblast cell line	1817 MHz (GSM); SAR, 2.0 W/kg; 1 h	HSP70, HSC70 mRNA expression	No effect		<a href="#">Valbonesi et al. (2008)</a>
Human keratinocytes, fibroblasts and reconstructed epidermis	900 MHz (GSM); SAR, 2 W/kg; 48 h	HSP27, HSC70, and HSP70 protein expression	Keratinocytes: no effect Epidermis: slight but significant increase in HSP70 Fibroblasts: significant decrease in HSC70		<a href="#">Sanchez et al. (2006b)</a>
Human primary keratinocytes and fibroblasts	1800 MHz (GSM); SAR, 2 W/kg; 48 h	HSP27, HSP70 and HSC70 protein expression	No effect		<a href="#">Sanchez et al. (2007)</a>
HeLa S3, HA-1, C3H 10T½	835 MHz (FDMA) and 847 MHz (CDMA); SAR, 0.6 W/kg (low dose) and 5 W/kg (high dose); 5–60 min, 24 h	HSF protein DNA- binding activity	No effect		<a href="#">Laszlo et al. (2005)</a>
Jurkat cells	1763 MHz (CDMA), SAR, 2 or 20 W/kg; 30 min or 1 h	HSP27, HSP70, HSP90 protein expression	No effect		<a href="#">Lee et al. (2006)</a>
MO54, A172 and T98 cell lines	1950 MHz (CW); SAR, 1 or 10 W/kg; 1 h	HSP27 mRNA and protein expression, p-HSP27 protein levels	No effect on HSP27 expression Slight decrease in p-HSP27 levels in MO54 cells		<a href="#">Ding et al. (2009)</a>
TK6 cells	1900 MHz (pulsed-wave; 5 min on, 10 min off); SAR, 1 and 10 W/kg; 6 h	HSP27, HSP70 mRNA expression	No effect		<a href="#">Chauhan et al. (2006a)</a>
HL60 and Mono Mac 6 cells	1900 MHz (pulse-wave; 5 min on, 10 min off); SAR, 1 and 10 W/kg; 6 h	HSP27, HSP70 mRNA expression	No effect		<a href="#">Chauhan et al. (2006b)</a>
Mono Mac 6 and U87MG cells	1900 MHz (pulsed-wave; 5 min on, 10 min off); SAR, 0.1, 1 and 10 W/kg; 6–24 h	HSP27, HSP40, HSP70, HSP90, HSP105 mRNA expression	No effect		<a href="#">Chauhan et al. (2007a)</a>
U87MG cells	1900 MHz; SAR, 0.1, 1, 10 W/kg; 4 h	HSP27, HSP40, HSP70, HSP86, HSP105 mRNA expression	No effect		<a href="#">Qutob et al. (2006)</a>

CDMA, code-division multiple access; CW, continuous wave; FDMA, frequency-domain multiple access; GSM, Global System for Mobile Communications; h, hour; HSC, heat-shock cognate; HSF, heat-shock factor; HSP, heat-shock protein; p-HSP27, phosphorylated-HSP27; min, minute; RF, radiofrequency; SAR, specific absorption rate; SRE, serum-response element; TDMA, time-domain multiple access; WCDMA, wideband code-division multiple access

[Lixia et al. \(2006\)](#) exposed human lens epithelial cells to RF radiation at 1800 MHz (GSM; SAR, 1, 2, or 3 W/kg) for 2 hours. The authors noted increased expression of HSP70 protein at the higher SARs, but no corresponding change was observed in mRNA expression.

[Simkó et al. \(2006\)](#) exposed a human monocyte-derived cell line (Mono-Mac-6) to RF radiation at 1800 MHz (SAR, 2 W/kg) for 1 hour, either alone or with ultra-fine particles. The authors observed no effect on the expression of HSP70 protein. In a follow-up study, [Lantow et al. \(2006a\)](#) investigated whether exposure to RF radiation at 1800 MHz (SAR, 0.5–2.0 W/kg) for 45 minutes had an effect on expression of HSP70 in Mono-Mac-6 and K562 cells. No significant effects of exposure to RF radiation were detected in the expression of HSP70 protein in either cell line under any of the conditions tested.

[Vanderwaal et al. \(2006\)](#) found no evidence of altered HSP27 phosphorylation in three human cell lines (HeLa, S3 and EA.hy296) after exposure to RF radiation at 837 MHz (SAR, 5.0 W/kg) for 1, 2, or 24 hours, or at 900 MHz (SAR, 3.7 W/kg) for 1, 2 or 5 hours.

[Wang et al. \(2006\)](#) did not detect any alterations in HSP27, HSP70 or expression of phosphorylated-HSP27 protein in human A172 cells – derived from a malignant glioblastoma – exposed to RF radiation at 2450 MHz (SARs of up to 50 W/kg) for 0–3 hours.

[Sanchez et al. \(2006b\)](#) evaluated possible stress-related effects in isolated human skin cells and in reconstructed human epidermis exposed to RF radiation at 900 MHz (SAR, 2 W/kg) for 48 hours. Immunohistochemical analysis did not reveal any detectable changes in expression of HSP27 or inducible HSP70 in exposed keratinocytes. However, levels of HSC70 (heat shock cognate) protein were significantly decreased in dermal fibroblasts isolated from human skin after exposure to RF radiation. Such results were not seen in reconstructed human epidermis. Human skin cells may thus react to exposure by

modulating the expression of some HSPs, but this response may depend on the cell model. In a follow-up study, the same investigators found that primary human skin cells (keratinocytes and fibroblasts) did not display any alterations in inducible HSP27, HSP70 or HSC70 protein levels after exposure at 1800 MHz (SAR, 2 W/kg) for 48 hours ([Sanchez et al., 2007](#)). [The authors did not discuss the different responses observed in these two studies.]

[Hirose et al. \(2007\)](#) examined HSP27 phosphorylation, gene and protein expression in human glioblastoma A172 cells and human IMR-90 fetal lung fibroblasts exposed to RF radiation at 2142.5 MHz (SARs up to 0.8 W/kg) for 2–48 hours. No evidence of altered HSP27 phosphorylation or increased mRNA expression of a variety of HSPs was found in either cell line.

[Zhadobov et al. \(2007\)](#) investigated the expression of stress-sensitive genes and proteins in a human glial cell line (U-251MG) exposed to RF radiation at 60 GHz (power density, 5.4  $\mu\text{W}/\text{cm}^2$  or 0.54  $\text{mW}/\text{cm}^2$ ) for 1–33 hours. No evidence was found for altered expression of stress-response genes, as determined by reporter assays and RT-PCR. Western-blot analysis indicated no effects of RF radiation on levels of clusterin or HSP70 protein.

[Valbonesi et al. \(2008\)](#) observed no change in expression of HSP70 in the human HTR-8/SVneo trophoblast cell-line exposed to RF radiation at 1800 MHz (SAR, 2 W/kg) for 1 hour.

Exposure of the human endothelial cell line EA.hy926 to 1.8 GHz RF radiation (SAR, 2.0 W/kg) for 1 hour did not result in altered HSP protein expression; phosphorylation status was not assessed in this study ([Nylund et al., 2009](#)).

[Ding et al. \(2009\)](#) studied three human glioma cell-lines (MO54, A172, T98) and found no evidence of altered HSP expression or phosphorylation after exposure to RF radiation at 1950 MHz (SAR, 1 or 10 W/kg) for 1 hour. These findings were supported by results of a series of earlier studies by [Chauhan et al. \(2006a, b, 2007a\)](#)

and [Qutob et al. \(2006\)](#), in which exposure at 1900 MHz (SAR, 0.1–10 W/kg) for 4–24 hours did not alter the transcript expression of HSP27, HSP40, HSP70, HSP90 or HSP105, in several human cell lines (MM6, U87MG, HL60, TK6).

[The Working Group noted that a small number of studies reported altered expression of HSPs in certain cell lines ([Leszczynski et al., 2002](#); [Tian et al., 2002](#); [Miyakoshi et al., 2005](#); [Lixia et al., 2006](#); [Sanchez et al., 2006b](#)). However, it was not clear whether these responses were specific to the cell line, the frequency, the modulation or model used, or were false-positives, e.g. artefacts caused by irregularities in the exposure system. The majority of studies conducted in cultured human cells to date have found no evidence that exposure to RF radiation under non-thermal conditions elicits alterations in the expression of HSP genes or proteins.]

(ii) *Proto-oncogenes and signal-transduction pathways*

See [Table 4.13](#)

Several studies have investigated the ability of RF radiation to mediate the expression of proto-oncogenes and proteins involved in the regulation of signal-transduction pathways. Proto-oncogenes are genes with the capacity to induce cellular proliferation and/or transformation. While these genes are constitutively expressed at low levels, they are rapidly and transiently induced in response to external stress stimuli. Similarly, transcriptional activity in response to stress factors can be mediated by mitogen-activated protein kinase (MAPK) pathways, which include the extracellular signal-regulated kinase (ERK), p38 and the c-Jun N-terminal kinase (JNK) cascades. These pathways are complex and regulate a variety of cellular processes, including proliferation, differentiation, metabolism and the stress response. Upon phosphorylation of these kinases, a large number of regulatory proteins and transcription factors can become activated, thereby altering

cellular processes and allowing further gene transcription.

[Li et al. \(1999\)](#) exposed human fibroblasts to continuous-wave RF radiation at 837 MHz (SAR, 0.9–9.0 W/kg) for 2 hours. No evidence of altered expression of TP53 protein was found.

[Leszczynski et al. \(2002\)](#) exposed a human endothelial cell line (EA.hy926) to RF radiation at 900 MHz (SAR, 2 W/kg) for 1 hour. A transient increase was noted in p38-MAPK and in phosphorylation of HSP27. This effect could be inhibited by SB203580 (a specific inhibitor of p38-MAPK). Since accurate measurements indicated no alterations in cell-culture temperature during the exposure period, activation of the p38-MAPK stress-response pathway might be a potential mode of non-thermal molecular interaction of RF radiation with biological tissue.

[Caraglia et al. \(2005\)](#) exposed human epidermoid-cancer KB cells to RF radiation at 1950 MHz (SAR, 3.6 W/kg) for 1–3 hours. Decreased expression was noted for the proteins Ras, Raf-1 and Akt. The activity of Ras and ERK1/2 was determined by their phosphorylation status, and found to be reduced. This exposure to RF radiation increased JNK1/2 activity and expression of HSP27 and HSP70, but caused a reduction in p38-MAPK activity and HSP90 expression. [The Working Group noted that details on the exposure system were incompletely described, and that these observations may have been due to thermal effects.]

[Miyakoshi et al. \(2005\)](#) exposed human glioma cells (MO54) to RF radiation at 1950 MHz (SAR, 10 W/kg) for 2 hours. A decrease was noted in the phosphorylation of HSP27 at serine-78, indicating repression of the p38-MAPK cascade or activation of an HSP27 phosphatase.

[Lee et al. \(2006\)](#) exposed Jurkat cells to RF radiation at 1763 MHz (SAR, 2 or 20 W/kg) for 30 minutes to 1 hour in the presence or absence of the phorbol-ester, 12-O-tetradecanoylphorbol-13-acetate (TPA). There was no evidence of an altered phosphorylation status of ERK1/2,

**Table 4.13 Studies on the effect of radiofrequency radiation on the expression of proto-oncogenes in human cells *in vitro***

Tissue/cell line	Exposure	End-point and target	Results	Comments	Reference
Human endothelial EA.hy926 cells	900 MHz (GSM); ~2 W/kg; 1 h	p38MAPK protein expression	Transient change		<a href="#">Leszczynski et al. (2002)</a>
Rat1, HeLa cells	800/875/950 MHz; power density 0.07–0.31 mW/cm <sup>2</sup> ; 5–30 min	ERK1/2, JNK1/2, p38MAPK, EGFR, Hb-EGF protein expression, phosphorylation status	Transient increase of ERK1/2 phosphorylation at 0.10 mW/cm <sup>2</sup> . Phosphorylation of p38MAPK and JNK1/2 (stress-activated cascades) is not changed. Phosphorylation is ROS-dependent	Stress-activated cascades are not affected, which may indicate that effects are non-thermal. Temperature remained constant within 0.05 °C.	<a href="#">Friedman et al. (2007)</a>
Human epidermoid KB cell line	1950 MHz; SAR, 3.6 W/kg; 1, 2, 3 h	Ras, Raf-1, Akt, ERK1/2, JNK1/2, HSP27, HSP70, HSP90 protein expression, phosphorylation status	Expression of ras, Raf-1, Akt, and HSP90 was reduced; expression of HSP27 and HSP70 was increased. Phosphorylation of ERK1/2, ras, p38MAPK was reduced, while that of JNK1/2 was increased	Incomplete details on RF exposure; no temperature control; possible thermal confounding	<a href="#">Caraglia et al. (2005)</a>
Human neuroblastoma (SH-SY5Y) cells	900 MHz (GSM); SAR, 1 W/kg; 5, 15, 30 min, 6 h, 24 h	EGR1, ERK1/2, SAPK/JNK, p38MAPK, ELK1, BCL2, survivin mRNA and protein expression, phosphorylation status	Transient increase in EGR1 and ELK1 transcript levels; transient increase in ERK1/2, SAPK/JNK phosphorylation. Evidence of apoptosis after 24 h exposure	Confounding due to environmental factors unclear	<a href="#">Buttiglione et al. (2007)</a>
Jurkat cells	1763 MHz (CDMA); SAR, 2 or 20 W/kg; 30 min or 1 h	p38MAPK, ERK1/2, JNK1/2 protein expression, phosphorylation status	No effect on protein expression for HSP90, HSP70, HSP27; no effect on phosphorylation with/without TPA	Exposure conditions and temperature properly controlled	<a href="#">Lee et al. (2006)</a>
Human glioma MO54 cells	1950 MHz (CW); SAR, 10 W/kg; 1h and 2h	Phosphorylated HSP27 protein levels	Decrease in phosphorylation of HSP27		<a href="#">Miyakoshi et al. (2005)</a>
TK6, MM6, HL-60 cells	1900 MHz (PW; 5 min on, 5 min off); SAR, 1 or 10 W/kg; 6 h	c-fos, c-myc, c-jun mRNA expression	No effect		<a href="#">Chauhan et al. (2006a, b)</a>
WS1neo human foreskin fibroblasts	837 MHz (CW); SAR, 0.9 or 9 W/kg; 2 h	TP53 protein expression	No effect		<a href="#">Li et al. (1999)</a>
Human glioblastoma A172, human lung IMR-90 fibroblasts	2142.5 MHz (CW, W-CDMA); SAR, 80, 250, 800 mW/kg; 24, 28, 48 h	APAF1, TP53, TP53BP2 and CASP9 protein levels, phosphorylation status	No effect	Temperature control unclear	<a href="#">Hirose et al. (2006)</a>

CDMA, code-division multiple access; CW, continuous-wave; FDMA, frequency-division multiple access; FMCW, frequency-modulated continuous wave; GSM, Global System for Mobile communications; h, hour; min, minute; RF, radiofrequency; SAR, specific absorption rate; TDMA, time-division multiple access; TPA, 12-O-tetradecanoylphorbol-13-acetate; W-CDMA, wideband code-division multiple access

JNK1/2 or p38-MAPK after exposure to RF radiation, with or without TPA.

[Chauhan \*et al.\* \(2006a, b\)](#) exposed three human-derived cell lines (TK6, MM6, HL-60) to intermittent (5 minutes on/10 minutes off) RF radiation at 1900 MHz (SAR, 1 or 10 W/kg) for 6–24 hours. No significant differences were observed in relative expression levels of the proto-oncogenes *c-JUN*, *c-FOS* and *c-MYC* in any of the cell lines examined.

[Hirose \*et al.\* \(2006\)](#) examined gene-transcript levels in human A172 and IMR-90 cells following exposure to RF radiation. A series of genes known to be involved in TP53-mediated apoptosis (including *APAF1*, *TP53*, *TP53BP2* and *CASP9*) were assessed after the cells had been exposed at 2142.5 MHz (SAR, 0.08–0.8 mW/kg) for up to 48 hours. No significant differences were observed in the expression of these TP53-related apoptosis genes, relative to the sham-exposed control groups, under any of the conditions tested.

[Buttiglione \*et al.\* \(2007\)](#) assessed the expression levels of several transcription factors (EGR1, BCL2, ELK1) downstream of the MAPK pathways. EGR1 transcript expression and phosphorylation of ERK1/2 and JNK in human SH-SY5Y neuroblastoma cells were evaluated after exposure to 900 MHz RF radiation (SAR, 1 W/kg) for 5 minutes up to 24 hours. There was a transient increase in EGR1 levels at 5–30 minutes after exposure; this effect was no longer evident at 6–24 hours after exposure. Phosphorylation of ERK1/2, JNK1/2 and ELK1 was also transiently increased after various exposure times (5 minutes to 6 hours), while a significant decrease in the transcript levels of BCL2 and survivin was observed after 24 hours of exposure. However, a significant decrease in cell viability (as determined by the MTT assay) was noted, as well as the appearance of subG<sub>1</sub> nuclei and a G<sub>2</sub>-M block (as determined by flow cytometry) after 24 hours of exposure. [The Working Group noted that the appearance of subG<sub>1</sub> nuclei is indicative of possible induction of apoptosis in the cell

culture. It was unclear whether this effect was thermal or non-thermal in nature.]

[Friedman \*et al.\* \(2007\)](#) reported that low-level exposure of serum-starved HeLa cells to RF radiation at 875–950 MHz (power densities, 0.07–0.31 mW/cm<sup>2</sup>) for 5–30 minutes, significantly activated the ERK1/2 signal-transduction pathway via generation of ROS through NADPH-oxidase activation. Neither the p38-MAPK nor the JNK1/2 stress-response pathways were activated by RF radiation. [The Working Group noted that the description of the exposure conditions in this study was poor.]

[The Working Group noted that there was weak evidence from studies with human cell lines that non-thermal RF exposure could result in alterations in the expression or phosphorylation of proto-oncogenes or proteins involved in signal-transduction pathways. Most studies that report altered expression of genes or proteins, or phosphorylation of proteins involved in cell homeostasis, proliferation and signal-transduction pathways, appeared to have been conducted under unique exposure conditions, with results that show no clear dose- and time-response.]

#### (d) *High-throughput studies of gene and protein expression*

See [Table 4.14](#)

In recent years, many studies have employed high-throughput techniques to analyse differential gene/protein expression in human cells in response to exposure to RF (reviewed by [Vanderstraeten & Verschaeve, 2008](#); [McNamee & Chauhan, 2009](#)). While such technology offers ample opportunity for understanding potential biological interactions of RF radiation in a hypothesis-free testing approach, it is also subject to generating a large number of “false-positive” results. For this reason, it is fundamentally important that such high-throughput studies employ rigorous statistical-inference analysis, include an appropriate number of biological replicates, and validate the differential expression of gene

**Table 4.14 High-throughput studies on the effects of radiofrequency radiation on gene and protein expression**

Tissue/cell line	Exposure	Platform	Results	Comments	Reference
C3H 10T½ mouse cells	847.7 MHz (CDMA) or 835.6 MHz (FDMA); SAR, 5 W/kg; 24 h	Affymetrix GeneChip U74Av2	Differential expression of ~200 genes	Not confirmed by RT-PCR	<a href="#">Whitehead et al. (2006)</a>
Mouse embryo primary cultured neurons/astrocytes	1900 MHz (GSM); SAR not reported; 2 h	GEArray Q Series Mouse Apoptosis gene array, RT-PCR	Neurons: upregulation of <i>Casp2</i> , <i>Casp6</i> , <i>Pycard</i> ; <i>Casp9</i> and <i>Bax</i> mRNA levels unchanged Astrocytes: upregulation of <i>Casp2</i> , <i>Casp6</i> , <i>Pycard</i> , <i>Bax</i>	Uncontrolled experimental conditions (exposure from mobile phone). Confirmed by RT-PCR	<a href="#">Zhao et al. (2007a)</a>
Rat neurons	1800 MHz (GSM PW; 5 min on, 10 min off); SAR, 2 W/kg; 24 h	Affymetrix GeneChip Rat Neurobiology U34 Array	Of 1200 screened genes, 24 were upregulated and 10 were downregulated	Confirmed by RT-PCR; fair agreement with microarray data	<a href="#">Zhao et al. (2007b)</a>
EA.hy926 human endothelial cells	900 MHz (GSM); SAR, 2.4 W/kg; 1 h	2DE protein analysis (silver staining), MALDI-MS	Found 38 altered spots; 4 spots identified by MALDI-MS: 2 spots (increased expression) were identified as vimentin isoforms (confirmed by Western blot) 2 spots (downregulated expression) were identified as IDH3A and HNRNPH1		<a href="#">Nylund &amp; Leszczynski (2004)</a>
EA.hy926, EA.hy926v1,	900 MHz (GSM); SAR, 2.8 W/kg; 1 h	Atlas Human v1.2 cDNA arrays (1167 genes screened); 2DE protein analysis (silver staining)	EA.hy926 cells: 1 gene downregulated, 38 altered protein spots in EA.hy926 EA.hy926v1 cells: 13 genes upregulated, 45 altered protein spots	No confirmation of gene-expression results with RT-PCR, or of proteome results with Western blotting; minimum number of biological replicates	<a href="#">Nylund &amp; Leszczynski (2006)</a>
MCF7 cells	849 MHz (CDMA); SAR, 2 or 10 W/kg; 1 h/d for 3 d	2DE protein analysis (silver staining), electrospray ionization MS-MS, Western blotting, RT-PCR	No reproducible changes in protein expression; GRP78 protein/RNA not differentially expressed	Exposure conditions and temperature properly controlled. Minimum number of biological replicates	<a href="#">Kim et al. (2010)</a>
Human lens epithelial cells (hLEC)	1800 MHz (GSM); SAR, 1, 2, 3.5 W/kg; 2 h	2-DE protein analysis (silver staining), electrospray ionization MS-MS	More than 1600 protein spots were differentially expressed in each condition vs sham-exposed control. Of four upregulated proteins (at SAR 2 and 3.5 W/kg), two were identified by MS (hnRNP K, HSP70)	Number of independent experiments unclear; no confirmation by Western blotting	<a href="#">Li et al. (2007)</a>

**Table 4.14 (continued)**

Tissue/cell line	Exposure	Platform	Results	Comments	Reference
Jurkat cells, fibroblasts, leukocytes	1800 MHz (GSM PW, 5 min on, 10 min off); SAR, 2 W/kg; 8 h	2DE protein analysis (fluorescence), ion-trap MS-MS	No differentially expressed protein spots by fluorescence 2DE. Increased rate (> 2-fold) of <i>de novo</i> protein synthesis in exposed cells	Not corrected for multiple comparisons; no confirmation by Western blotting; minimum number of biological replicates	<a href="#">Gerner et al. (2010)</a>
NB69, U937 EA.hy926, CHME5, HL60, lymphocytes, used pooled RNA	900 or 1800 MHz (GSM); SAR, 0.77 or 1.8–2.5 W/kg; 1, 24, and 44 h	Human Unigene RZPD-2 cDNA array (~75 000 probes screened)	Differential gene expression in three cell lines (EA.hy926, U937, HL60)	No confirmation of results with RT-PCR; insufficient number of biological replicates. Exposure conditions and temperature properly controlled	<a href="#">Remondini et al. (2006)</a>
Jurkat cells	1763 MHz (CDMA); SAR, 10 W/kg; 1 h/d for 3 d	Applied Biosystems 1700 full genome array (30000 probes)	No gene-expression changes > 2-fold; 10 genes changed > 1.3-fold ( $P < 0.1$ )	No confirmation of results with RT-PCR	<a href="#">Huang et al. (2008a)</a>
A172, H4 and IMR90 cell lines	2142.5 MHz (CW and W-CDMA); SAR, 0.08, 0.25, 0.80 W/kg; 96 h	Affymetrix Human Genome HG-U133A and B arrays	Differential expression (>2-fold) of 8 genes (H4 cells), 5 genes (A172 cells) and 1 gene (IMR90 cells)	Genes not all identified; insufficient number of independent experiments; no confirmation by RT-PCR	<a href="#">Sekijima et al. (2010)</a>
MCF7 cells	1800 MHz (GSM PW; 5 min on, 10 min off); SAR, 2 or 3.5 W/kg; 24 h	Affymetrix GeneChip Test3 arrays (~22 000 probes screened)	No effect at 2 W/kg; five genes upregulated at 3.5 W/kg	RT-PCR analysis did not confirm differential expression of the five candidate genes identified by microarray analysis. Insufficient number of biological replicates	<a href="#">Zeng et al. (2006)</a>
A172 and IMR90 cells	2142.5 MHz (CW and W-CDMA); SAR, 0.08, 0.25, 0.8 W/kg; 24, 28, 48 h	Affymetrix Human Genome U133 Plus 2.0 GeneChip (38 000 probes screened)	No consistent changes in gene expression in two experiments. Lack of response for TP53-related gene expression ( <i>TP53</i> , <i>TP53BP2</i> , <i>APAF1</i> and <i>CASP9</i> ) confirmed by microarray hybridization and RT-PCR	Insufficient number of biological experiments	<a href="#">Hirose et al. (2006)</a>
A172 cells and IMR90 fibroblasts	2142.5 MHz (CW and W-CDMA); SAR, 0.08 or 0.8 W/kg; 2–48 h	Affymetrix Human Genome U133 Plus 2.0 GeneChip (38 000 probes screened)	No effect	No parallel experiments with RT-PCR; insufficient number of biological replicates	<a href="#">Hirose et al. (2007)</a>

**Table 4.14 (continued)**

Tissue/cell line	Exposure	Platform	Results	Comments	Reference
U87MG glioblastoma cells	1900 MHz (PW); SAR 0.1, 1, 10 W/kg; 4 h	Agilent Human 1A arrays (~22 000 probes screened)	No effect	Lack of effect on several HSPs confirmed by RT-PCR; multiple doses of RF radiation tested; concurrent positive, negative and sham controls; exposure conditions and temperature properly controlled	<a href="#">Outob <i>et al.</i> (2006)</a>
TK6, HL60, Mono Mac 6 cells	1900 MHz (pulsed-wave; 5 min on, 10 min off); SAR, 0.1, 1, 10 W/kg; 6 or 24 h	Agilent Human 1Av2 arrays (~22 000 probes screened)	No effect	No parallel experiments with RT-PCR; multiple doses of RF radiation tested; concurrent positive, negative and sham controls; exposure conditions and temperature properly controlled	<a href="#">Chauhan <i>et al.</i> (2007a)</a>
Glial cell line (SVGp12)	2450 MHz (CW); SAR, 1, 5, 10 W/kg; 1, 2, 24 h	AceGene Premium Human DNA Array, RT-PCR	Microarray analysis identified 23 differentially expressed genes and showed 5 unassigned gene spots: 17 genes were upregulated, 11 were downregulated	RT-PCR analysis with 22 of the 23 genes did not confirm microarray data. Minimum number of biological replicates	<a href="#">Sakurai <i>et al.</i> (2011)</a>
EA.hy926 cells	1800 MHz (GSM); SAR, 2 W/kg; 1 h	2DE protein analysis, MALDI-TOF MS analysis, Western blotting	Eight differentially expressed protein spots; three identified as SRM, GRP78 and PSA1	Western blot found no response in GRP78, or changes in HSP27 and vimentin expression	<a href="#">Nylund <i>et al.</i> (2009)</a>
HUVEC, HBMEC cells	1800 MHz (GSM); SAR, 2 W/kg; 1 h	2DE-DIGE	No differentially expressed spots in either cell line when corrected for multiple comparisons (correction for false-discovery rate)	Exposure conditions and temperature properly controlled	<a href="#">Nylund <i>et al.</i> (2010)</a>

2DE, two-dimensional gel electrophoresis; CDMA, code-domain multiple access; CW, continuous wave; d, day; DIGE, difference gel electrophoresis; FDMA, frequency domain multiple access; GSM, Global System for Mobile Communications; h, hour; HSC, heat-shock cognate; HSF, heat-shock factor; HSP, heat-shock protein; min, minute; MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; MALDI-TOF MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; MS-MS, tandem mass spectrometry; p-HSP27, phosphorylated-HSP27; PW, pulsed wave; RT-PCR, reverse-transcriptase polymerase chain reaction; SAR, specific absorption rate; SRE, serum response element; W-CDMA, wideband-code division multiple access

and proteins by use of alternative techniques (e.g. RT-PCR or Western blotting).

(i) *Proteomics studies in human cells*

[Nylund & Leszczynski \(2004\)](#) reported altered expression of 38 protein spots – observed in a two-dimensional (2D) electrophoresis gel – and identified 4 proteins by matrix-assisted laser desorption/ionization–mass spectrometry (MALDI-MS) in the human endothelial cell line EA.hy926, exposed to RF radiation at 900 MHz (SAR, 2.4 W/kg) for 1 hour. Of particular interest was that two of the spots identified were isoforms of the cytoskeletal protein, vimentin. In a subsequent genomics/proteomics study, [Nylund & Leszczynski \(2006\)](#) observed that 1 gene was downregulated in the EA.hy926 cell line and 13 genes were upregulated in a related EA.hy926v1 cell line exposed to RF radiation at 900 MHz (SAR, 2.8 W/kg) for 1 hour. Proteome analysis indicated 38 differentially expressed proteins in the EA.hy926 cell line and 45 altered proteins in the EA.hy926v1 cell line. The identity of the differentially expressed proteins was not determined. More recent studies by these authors, with exposure of the cells at 1800 MHz (SAR, 2.0 W/kg) did not show the altered expression of, e.g. vimentin ([Nylund et al., 2009, 2010](#)). [The Working Group noted that the observations reported in these studies were either not confirmed by Western blotting, or were identified as artefacts upon further investigation. The discrepancy in the results with RF radiation at 900 and 1800 MHz may be attributable to the different exposure frequencies; the different distribution of SAR within the cell cultures, *i.e.* less uniform SAR distribution at 900 MHz; and the occurrence of false positives when using the silver-stain-based 2D gel-electrophoresis technique.]

[Li et al. \(2007\)](#) exposed human lens epithelial cells to RF radiation at 1800 MHz (SAR, 1, 2, and 3.5 W/Kg) for 2 hours. In the 2D-electrophoresis pattern, enhanced expression was noted of two stress-related proteins, namely HSP70 and

ribonucleoprotein K. [The Working Group noted that failure to confirm the identity of the spots by Western blotting made the results of this study difficult to interpret.]

[Kim et al. \(2010\)](#) employed 2D gel-electrophoresis to examine the proteome of human MCF7 breast-cancer cells exposed to RF radiation at 849 MHz (SAR, 2 or 10 W/kg) for 1 hour per day, on three consecutive days. At 24 hours after exposure, no significant differences in protein expression were identified between exposed and sham-exposed cells.

[Gerner et al. \(2010\)](#) assessed relative protein expression in Jurkat cells, human fibroblasts and primary mononuclear cells (leukocytes) exposed to intermittent (5 minutes on, 10 minutes off) RF radiation at 1800 MHz (SAR, 2 W/kg during the “on” phase) for 8 hours, in growth medium containing [<sup>35</sup>S]methionine/cysteine. No significant differences were observed between sham-exposed and RF-exposed samples in the expression of any particular proteins by use of 2D gel-electrophoresis with fluorescence detection. However, cells exposed to RF radiation for 8 hours displayed a significant increase in protein synthesis, measured as enhanced incorporation of <sup>35</sup>S in autoradiographs of the 2D gel: in Jurkat cells, 14 proteins showed a doubling of the spot intensity in the autoradiograph. All these proteins were identified by ion-trap mass spectrometry. Of these 14 proteins, 13 were also enhanced in 2D autoradiographs prepared with samples from exposed fibroblasts. Several stress-responsive proteins were particularly affected, including Hsp70 and Hsp90. The enhancement of the signals in the leukocytes (stimulated/non-stimulated) were much weaker, with only heat-shock protein Hsp60 showing a more than twofold increase. These results suggest increased synthesis *de novo* of these proteins in cells exposed to RF radiation. None of these observations were validated with other techniques.

[The Working Group noted that the studies assessing proteomic changes in human cells

were limited in number, and shortcomings were evident in some.]

(ii) *Transcriptomics studies in human cells*

[Remondini et al. \(2006\)](#) isolated RNA from six human-derived cell lines (NB69, EA.hy926, T lymphocytes, U937, CHME5, and HL-60) after exposure to RF radiation at 900 MHz or 1800 MHz (SAR, 1.0, 1.3, 1.4, 1.8–2.5, and 2.0) for 1, 2, or 44 hours. In some cases, the exposure at 1800 MHz was intermittent with 5/5, 5/10, or 10/20 minutes on/off. Total RNA was isolated and processed for transcriptome analysis, i.e. to detect changes in gene expression. There was no evidence of differential gene expression in three of the cell lines tested (NB69, T lymphocytes, CHME5), but alterations in gene expression (12–34 differentially expressed genes) were observed in EA.hy926, U937, and HL-60 cells under various exposure conditions. [The Working Group noted that the conclusions that could be drawn from this study were limited since the data analysis was carried out using a single RNA pool for each condition, making it impossible to estimate the true biological variance for statistical inference testing. Furthermore, no validation of results by RT-PCR was performed.]

[Zeng et al. \(2006\)](#) exposed human MCF7 breast-cancer cells to intermittent (5 minutes on, 10 minutes off) RF radiation at 1800 MHz (SAR, 2.0 or 3.5 W/kg) for 24 hours. No statistically significant differences were observed at the lower SAR, but five differentially expressed genes were detected in cells exposed at the SAR of 3.5 W/kg. [These findings were not validated with RT-PCR.]

[Hirose et al. \(2006\)](#) observed no noticeable changes in *TP53*-related gene expression in human A172 or IMR-90 cells exposed to RF radiation at 2142.5 MHz (SAR, 0.08–0.8 W/kg) for 24–48 hours. In this study the authors confirmed the absence of a response in the microarray analysis for four genes (*APAF1*, *TP53*, *TP53BP2* and *CASP9*) involved in *TP53*-mediated apoptosis

by use of RT-PCR. In a similar study, [Hirose et al. \(2007\)](#) exposed the same two cell lines to RF radiation at 2142.5 MHz (SAR, 0.08–0.8 W/kg) for 2–28 hours. Despite assessing a variety of exposure conditions, including exposure duration, signal modulation and SAR levels, the authors reported no differential expression in *hsp*-related genes under any of the conditions tested in either cell line.

[Qutob et al. \(2006\)](#) exposed human glioblastoma-derived (U87MG) cells to pulsed-wave RF radiation at 1900 MHz (SAR, 0.1, 1 or 10 W/kg) for 4 hours. There was no evidence for differential gene expression in any of the exposed samples relative to the sham-exposed cells. As a positive control, exposure to heat-shock (43 °C, 1 hour) did induce several stress-responsive genes. In an extension of this study, the same research group exposed U87MG cells to RF radiation at 1900 MHz (SAR, 0.1, 1 or 10 W/kg) for 24 hours, and harvested RNA at 6 hours after exposure. In addition, the human-derived monocyte cell line (Mono-Mac-6) was exposed under similar conditions for 6 hours, and RNA was harvested either immediately or 18 hours after exposure. No evidence for differential gene expression was observed in either cell line, at any SAR or time-point tested ([Chauhan et al., 2007a](#)).

[Huang et al. \(2008a\)](#) exposed human-derived Jurkat cells to RF radiation at 1763 MHz (SAR, 10 W/kg) for 1 hour per day, for 3 days. Genome-wide analysis did not identify any genes that were differentially expressed at a significant level ( $P < 0.05$ ) with a greater than twofold change, but 10 genes were identified with a 1.3-fold change, with  $P < 0.1$ .

[Sekijima et al. \(2010\)](#) exposed three human cell lines (A172, glioblastoma; H4, neuroglioma; IMR-90 fibroblasts) to continuous-wave or W-CDMA-modulated RF radiation at 2142.5 MHz (SAR, 0.08, 0.25 or 0.8 W/kg) for up to 96 hours. Differential expression of a small number of genes was observed in each cell line. Ribosomal protein S2, growth arrest-specific

transcript 5, and integrin beta 5 were differentially expressed in H4 cells at the two higher SARs tested. [These findings were not validated with RT-PCR.]

[Sakurai et al. \(2011\)](#) assessed differential gene expression in a normal human astroglia cell-line (SVGp12) exposed to continuous-wave RF radiation 2450 MHz at (SAR, 1, 5 or 10 W/kg) for 1, 4, or 24 hours. With the high-throughput microarray, this study identified 17 genes that were upregulated and 11 that were downregulated in response to exposure to RF radiation. However, RT-PCR analysis found that the expression of these genes was not statistically different from that in the sham-exposed control group. [The Working Group noted that these results highlight the importance of proper validation of results generated by means of high-throughput screening.]

### (iii) Transcriptomics studies in cultured mammalian cells

[Whitehead et al. \(2006\)](#) exposed C3H 10T $\frac{1}{2}$  mouse cells to RF radiation at 847.74 MHz (CDMA) or at 835.2 MHz (FDMA) (SAR, 5 W/kg) for 24 hours. Three independent experiments were conducted for each of the signal modulations, and matching samples were exposed to X-radiation (0.68 Gy) as positive controls. By intercomparison of the six sham-exposed samples an empirical estimate was made of the false-discovery rate. From the results of this analysis, the authors concluded that all of the gene-expression changes found after exposure to RF radiation were false positives, and that exposure to RF radiation had no effect on gene expression. No validation with RT-PCR was conducted. [The Working Group noted that genes responding to RF radiation were disregarded on the basis of the calculated false-discovery rate, rather than validated by means of RT-PCR. This was not scientifically justified as genes that were not false-positives may have been accidentally

disregarded. Therefore, this study provided little useful information.]

[Zhao et al. \(2007a\)](#) investigated the expression of genes related to apoptosis in primary cultured neurons and astrocytes isolated from ICR mouse embryos aged 15 days. The cells were exposed to GSM-modulated RF radiation at 1900 MHz (SAR not given) from a mobile phone placed over the culture dish for 2 hours. Upregulation of several genes involved in the apoptotic pathway was observed, including *Casp2*, *Casp6* and *Pycard*. For the astrocytes, these effects were exposure-dependent, and not observed after sham-exposure (with the mobile phone on “stand-by”). These results were confirmed by RT-PCR analysis. [The Working Group noted that this study had some methodological deficiencies. The cells were exposed to RF radiation from a mobile phone under poorly defined experimental conditions with regards to control for electromagnetic-field components, such as SAR levels within the cell cultures during exposure.]

In a second study, [Zhao et al. \(2007b\)](#) observed significant changes in gene expression in primary rat neurons exposed to intermittent (5 minutes on, 10 minutes off) GSM-modulated RF radiation at 1800 MHz (SAR, 2 W/kg) for 24 hours. Ten downregulated and 24 upregulated genes were identified among the 1200 genes that were screened, with “fold-change” as the analysis criterion. These findings were confirmed by RT-PCR analysis of 17 of the upregulated and 8 of the downregulated genes, showing fair agreement with the microassay data.

[Nylund et al. \(2009\)](#) examined the proteome of human endothelial cells (EA.hy926) exposed to GSM-modulated RF radiation at 1800 MHz (SAR, 2 W/kg) for 1 hour. In 2D gel-electrophoresis, eight proteins were found to be differentially expressed in exposed cells, three of which were identified as SRM, GRP78, and PSA1. Western blotting did not confirm the response of GRP78 [SRM and PSA1 not tested due to lack of specific antibodies]. No effect was seen on the

expression of vimentin or HSP27 protein, which were found to respond to radiation at 900 MHz in earlier studies (see above). In a subsequent study, [Nylund et al. \(2010\)](#) exposed umbilical vein endothelial cells (HUVEC) and human brain microvascular endothelial cells (HBMEC) to the same type of RF radiation. No effects on protein expression were reported.

[Of the numerous studies that investigated the potential for RF radiation to modify gene-transcription and protein-expression levels in a variety of animal models *in vivo* and human models *in vitro*, some reported effects under conditions where the possibility of thermal confounding could not be excluded. Other studies reported alterations in gene/protein expression under non-thermal exposure conditions, but typically in single, usually unreplicated experiments, or under experimental conditions with methodological shortcomings. There were no studies in human populations. Overall, there was weak evidence that exposure to RF radiation affects gene and protein expression.]

## 4.4 Other relevant effects

### 4.4.1 Humans

#### (a) Neuroendocrine system

The majority of studies on the effects of exposure to RF radiation on the endocrine system in volunteers have focused on hormones released into the blood stream by the pineal and pituitary neuroendocrine glands. Both are situated in the brain and are intimately connected with and controlled by the nervous system. Some studies have investigated urinary excretion of the major melatonin metabolite: 6-sulfatoxymelatonin (aMT6s). Fewer studies have been carried out on circulating concentrations of pituitary hormones or hormones released from other endocrine glands, such as the adrenal cortex. The pituitary hormones exert a profound influence on body metabolism and physiology, particularly during

development and reproduction, partly via their influence on the release of hormones from other endocrine glands situated elsewhere in the body. The main pituitary hormones investigated in studies on electromagnetic fields are thyroid-stimulating hormone (TSH), adrenocorticotrophic hormone (ACTH), which regulates the function of the adrenal cortex and particularly the release of cortisol, and growth hormone (GH). Pituitary hormones with important sexual and reproductive functions have also been studied, particularly follicle-stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL). ACTH, cortisol and prolactin are also involved in the response to stress, and were often used as a marker for the effects of exposure to RF radiation.

No cumulative effects on serum melatonin or pituitary hormones were observed after repeated exposure to RF radiation for 1 month. Most studies did not report an effect after a single exposure, but the statistical power of these studies was often insufficient because of the small number of volunteers involved ([Mann et al., 1998](#); [de Seze et al., 1999](#); [Radon et al., 2001](#); [Bortkiewicz et al., 2002](#); [Braune et al., 2002](#); [Jarupat et al., 2003](#); [Wood et al., 2006](#)).

#### (b) Neurobehavioural effects

##### (i) Electrical activity of the brain

The electroencephalogram (EEG) reflects synchronous activity in relatively large populations of cortical neurons. The “spontaneous” EEG of subjects who are awake is generally divided into several frequency bands, in which the relative amount of activity depends on the psychological state of the subject and the nature of the cognitive function in which she or he is engaged. The designation of the frequency bands is not always strictly applied, which results in specific frequencies sometimes being assigned to different bands in different studies. Generally, the following division is used: delta ( $\delta$ ) < 4 Hz; theta ( $\theta$ ) 4–8 Hz; alpha ( $\alpha$ ) 8–12 Hz; beta ( $\beta$ ) 12–30 Hz;

and gamma ( $\gamma$ ) > 30 Hz. Slightly different band designations are used by some authors, which are also cited in this Volume. The functional significance of these different components of the normal “waking” EEG is poorly understood. Thus, while a demonstration that mobile-phone signals influence these components would be indicative of a biological effect of such signals, interpretation of the effect would be uncertain. In addition, intra-individual variability is very high. In contrast, EEG patterns associated with sleep are well characterized and routinely used as indices of the different sleep stages that a typical healthy individual will experience during the night. Only studies on EEG during sleep are discussed here.

A review of studies on EEG during sleep and RF radiation was compiled by [Hamblin & Wood \(2002\)](#) and more recently, with a broader scope, by [Kwon & Hämäläinen \(2011\)](#). They cited studies by [Mann & Röschke \(1996\)](#), [Mann et al. \(1998\)](#), [Wagner et al. \(1998, 2000\)](#), [Borbély et al. \(1999\)](#), [Huber et al. \(2000, 2002, 2003\)](#), [Loughran et al. \(2005\)](#), [Fritzer et al. \(2007\)](#), [Hung et al. \(2007\)](#), [Regel et al. \(2007b\)](#), and [Lowden et al. \(2011\)](#). Some but not all studies on exposure to RF radiation during sleep have indicated increased EEG power in  $\alpha$  or  $\beta$  bands. A reported shortening of sleep latency could not be reproduced. Other studies that looked at exposure to RF radiation for 30 minutes before going to sleep also showed variable results, sometimes reporting increases in  $\alpha$  and  $\beta$  band power. In one study this was observed only after exposure to a modulated but not a continuous RF radiation signal, while in another study a dose-dependent increase in  $\alpha$  and  $\beta$  power was seen. Two studies reported an increase in time taken to fall asleep. A recent study by [Lowden et al. \(2011\)](#) indicated that self-reported differences in sensitivity to emissions from mobile-phone use were not reflected in sleep parameters.

[The Working Group concluded that exposure to a GSM-type signal may result in minor effects on brain activity during sleep.]

### (ii) Auditory and vestibular systems

As mobile phones are held close to the ear, various studies have checked for possible effects of exposure to mobile-phone type (GSM) RF radiation on the vestibular (balance) and cochlear (auditory) organs that comprise the inner ear. The hair-cell receptors present in each organ respond to head movement or to audible sound. This topic was recently reviewed by [Kwon & Hämäläinen \(2011\)](#), who concluded that neurophysiological studies showed no significant effects on cochlear and brainstem auditory processing, or on the vestibular system. [The Working Group noted that the results on spontaneous and evoked electrical activity in the brain were inconsistent.]

### (iii) Cognitive performance

Studies on cognitive performance in relation to exposure to RF radiation have been carried out in healthy adult volunteers, in adults who self-reported a variety of symptoms such as headaches in the vicinity of RF sources, and in children and adolescents, following the recommendations of [IEGMP \(2000\)](#).

Dynamic changes in brain anatomy occur throughout childhood and adolescence. The amount of white matter, which corresponds to myelination of nerve axons and is related to the speed of neuronal processing, increases linearly throughout adolescence. Changes in the amount of grey matter are thought to reflect changes in size and complexity in neurons, such as the number of synaptic connections, rather than changes in number of neurons themselves. These changes are considered to be related to maturation of behaviour; they are more complex and continue into the early 20s ([Giedd, 2004](#)).

Reviews of studies on neurobehavioural effects of exposure to RF radiation have been compiled by [Barth et al. \(2008\)](#) and more recently

by [Kwon & Härmäläinen \(2011\)](#). The latter authors indicated that improvement of cognitive performance after exposure to RF radiation, as reported in earlier studies, had not been confirmed in more recent behavioural studies with improved analyses.

#### (iv) *Subjective symptoms*

Some people self-report having a variety of subjective complaints, including headaches and migraines, fatigue, skin itches, and sensations of heat, after exposure to RF radiation ([Frey, 1998](#); [Hocking, 1998](#); [Chia et al., 2000](#); [Hocking & Westerman, 2000](#); [Sandström et al., 2001](#); [Santini et al., 2002a, b](#)). These symptoms are attributed to exposures at home or at work to RF radiation emitted by mobile phones, nearby base stations, digital enhanced cordless telecommunications (DECT) cordless phones and, more recently, wireless local area network (LAN) systems. Less commonly reported symptoms include dizziness, blurred vision, memory loss, confusion and vagueness, toothaches, and nausea. An increasing number of these people consider themselves to be electrosensitive. Provocation studies provide the most direct way of studying a possible effect of exposure to RF radiation on the occurrence of such symptoms. A weakness of these studies is that they focus on direct, short-term interactions, while symptoms may only occur after a longer exposure. In their review, [Kwon & Härmäläinen \(2011\)](#) conclude that provocation studies provided no evidence that the subjective symptoms could be attributed to mobile-phone use, which suggests that there are other explanations for the induction of such symptoms in hypersensitive people.

#### (c) *Thermal effects and thermoregulation*

There is an established literature on cardiovascular responses to heating associated with exposure to RF radiation, such as those involved in thermoregulation. Several studies addressed these end-points in connection with

thermoregulation and heat-stress disorders, to place the possible health consequences of such heating into a broader occupational and environmental context ([ICNIRP, 2009](#)).

RF energy is absorbed by the body, resulting in the production of heat due to an increase in molecular rotational and translational kinetic energy. The absorbed heat energy is distributed throughout the body in the circulation and is partially lost to the external environment. Significant whole-body heating has a major impact on cardiovascular physiology. In addition, the ability to carry out cognitive tasks is compromised before physiological limits of tolerance are reached ([Hancock & Vasmatazidis, 2003](#)). [ICNIRP \(2009\)](#) has indicated that adequately hydrated, inactive, healthy volunteers exposed to RF radiation under laboratory conditions will accommodate whole-body heat loads of approximately 1 W/kg for 45 minutes at environmental temperatures of up to 31 °C, to 6 W/kg for at least 15 minutes at ambient temperatures, with increased skin blood-flow and profuse local sweating, but with minimal changes in core temperature. With regard to local heating of the skin, skin blood-flow and local sweating increase with increasing skin temperature by up to 4 °C in response to a local peak SAR of about 15 W/kg at the irradiated site, but it is not known how less superficial and less vascular tissues may respond.

A full assessment of whole-body heat stress can only be properly derived from a consideration of all sources of heat and from the ease with which heat can be lost from the body, as given by the heat-balance equation. Heat gain through solar radiation or other sources of radiant heat may also have to be taken into account. The main adverse health effects expected to result from excessive heat loads are heat-related disorders such as heat exhaustion and, in elderly people, an increase in the risk of heat-related mortality ([Lakatta, 2002](#)). These effects are well documented in people exposed to hot environments and in elderly people during prolonged periods

of hot weather, but have not been associated with exposure to RF radiation. In addition, adverse effects on cognitive function may be expected to result from increased body temperature, with the potential to increase accident rates, but this has proven to be difficult to quantify in studies with volunteers. Several studies of acute exposure have been carried out to assess the adverse effects of increased tissue temperature in experimental animals, often in the context of providing guidance on the use of ultrasound or hyperthermia treatments in clinical practice ([Ryan et al., 1997](#)). Lesions, including those that result from cell death, generally occur when temperatures exceed 42 °C for more than about 1 hour. The central nervous system and testes appear to be particularly susceptible to heat-induced damage and show significant changes in cell numbers after exposures to 40–41 °C and higher.

Studies on mobile-phone use by volunteers have investigated the effects of RF radiation from mobile phones at levels generally assumed to be too low to induce significant heating. In principle, such “athermal” effects on the cardiovascular centres of the brainstem, which regulate the heart and circulation via outflow in the sympathetic and parasympathetic systems, are possible ([Benham et al., 2003](#); [Patapoutian et al., 2003](#); [Moran et al., 2004](#); [Glaser, 2005](#); [Bandell et al., 2007](#); [Foster & Glaser, 2007](#)). Several studies focused on possible effects on heart rate, heart-rate variability, blood pressure and cerebral blood flow. There is no clear evidence of an effect of such exposure on resting heart rate or blood pressure. However, small but inconsistent variations in heart-rate variability have been reported.

#### (d) *Cerebral blood flow and neural biochemical activity*

Changes in regional cerebral blood flow could reflect (or cause) local changes in neural activity. There are some indications of changes in regional cerebral blood flow during and after exposure to RF radiation. In their review, [Kwon](#)

[& Hämäläinen \(2011\)](#) concluded that approaches such as measurement of the haemodynamic response in the brain were promising, but the findings were few and not conclusive. The studies reviewed were [Braune et al. \(1998, 2002\)](#), [Reid & Gettinby \(1998\)](#), [Borbély et al. \(1999\)](#), [Huber et al. \(2000, 2002, 2003, 2005\)](#), [Haarala et al. \(2003a\)](#), [Sandström et al. \(2003\)](#), [Tahvanainen et al. \(2004\)](#), [Aalto et al. \(2006\)](#), [Nam et al. \(2006\)](#), [Barker et al. \(2007\)](#), and [Parazzini et al. \(2007\)](#). Also linked to cerebral blood flow, a more recent study by [Volkow et al. \(2011\)](#) using glucose-uptake positron-emission tomography (PET) showed an increase in local cerebral metabolism after exposure to a mobile phone in reception mode.

[The small changes seen in electrical activity in the brain and possibly in regional cerebral blood flow may not have functional significance. No consistent effects on cognitive performance have been found, although the use of a large variety of techniques to assess cognitive performance makes it difficult to directly compare the results of different studies. No research data were available that would link these findings to cancer.]

#### 4.4.2 *Experimental systems: in vivo*

##### (a) *Oxidative stress*

Numerous experiments have been conducted to explore the possibility that exposure to RF radiation may trigger oxidative stress in tissues of exposed animals (most frequently rats). Markers of oxidative stress include increased levels of malondialdehyde (indicative of lipid peroxidation), nitric oxide (NO), and reduced glutathione (GSH), and the activities of antioxidant enzymes such as SOD, catalase, or GSH-Px, or of pro-oxidant enzymes such as xanthine oxidase (XO).

(i) *Brain*

[Many of the studies in this section used a mobile phone as the source of exposure to RF radiation, which limits the value of these studies in hazard identification.]

[Irmak et al. \(2002\)](#) exposed male rabbits to radiation from a commercially available GSM mobile phone (900 MHz; peak power, 2 W; average power density, 0.02 mW/cm<sup>2</sup>) for 30 minutes per day, for 7 days. The telephones were positioned “in close contact with the rabbits.” The concentrations of malondialdehyde and NO, and activities of several relevant enzymes were measured in brain and serum of exposed and sham-exposed rabbits. No significant changes were noted in any parameter in the brain; a significant increase in SOD activity ( $P = 0.042$ ) and a significant decrease in concentrations of NO ( $P = 0.004$ ) were observed in the serum of exposed rabbits.

[Ilhan et al. \(2004\)](#) exposed female rats to a GSM signal from a mobile phone (900 MHz; continuous wave; analogue phone), 1 hour per day, for 7 days, at SARs of 2 W/kg (brain) or 0.25 W/kg (whole body), with or without administration of a *Ginkgo biloba* extract. Treatment with this extract by daily oral gavage started 2 days before and was continued throughout the 7 days of exposure to RF radiation. Immediately after exposure, histopathological changes and biochemical markers of oxidative stress were evaluated in the brain. “Dark” neurons (degenerative neurons that can be visualized by staining with cresyl violet) were detected in all locations, particularly in the cortex, hippocampus and basal ganglia. The concentrations of NO and malondialdehyde, and the activities of the enzymes XO and adenosine deaminase were increased in brain tissues, while the activities of SOD and glutathione peroxidase were decreased. Co-exposure with the *Ginkgo biloba* extract prevented these effects. [The Working Group noted that the experimental protocol in this study

was imprecise. The SAR was given without any information on how it was derived; the mention of analogue with GSM was contradictory.]

[Elhag et al. \(2007\)](#) exposed rats of unspecified strain and sex to RF radiation from a GSM mobile phone (900 MHz) for either 1 hour, or for 15 minutes per day, for 4 days, at a SAR of 0.25 W/kg, and reported a reduction in concentrations of vitamins C and A in serum, a decreased level of vitamin E in erythrocytes, and a reduction in the activities of catalase and SOD and concentrations of reduced glutathione in erythrocytes. [The Working Group noted the imprecise experimental protocol of this study, and did not take the results into further consideration.]

[Meral et al. \(2007\)](#) exposed guinea-pigs to RF radiation at 890–915 MHz (SAR, 0.95 W/kg) from a mobile phone for 12 hours per day (11 hours 45 minutes “stand-by” and 15 minutes “on”) for 30 days. At the end of the exposure period, lipid peroxidation, enzymatic activities and vitamins in blood and brain tissue were measured biochemically, and compared between exposed and non-treated controls. Increased concentrations of malondialdehyde, and reduced glutathione concentrations and catalase enzyme activity were observed in brain tissue, but there was no change in levels of vitamins A, E and D<sub>3</sub> in the brain. In the blood of the exposed animals, increased concentrations of malondialdehyde, vitamins A, D<sub>3</sub> and E, and catalase enzyme activity were seen, as well as decreased levels of glutathione. [The Working Group noted the lack of sham-exposed controls.]

[Ammari et al. \(2008\)](#) studied the activity of cytochrome oxidase in the brain of rats exposed to RF radiation at 900 MHz (GSM) from an RF generator, for 15 minutes per day for 7 days at a SAR (brain) of 6 W/kg, or for 45 minutes per day for 7 days at a SAR of 1.5 W/kg. While exposure at the lower SAR had no effect, exposure at a SAR of 6 W/kg induced a decrease in the activity of cytochrome oxidase in some areas of the rat brain (frontal cortex, posterior

cortex, hippocampus and septum). [This result showed that GSM signals at high SAR may affect the activity of cytochrome oxidase in the brain, which is a metabolic marker of neuronal activity.]

[Sokolovic et al. \(2008\)](#) exposed male rats to continuous-wave RF radiation at 900 MHz (GSM) from a mobile phone placed in the cage, for 4 hours per day during the light period (06:00–18:00) for 20, 40 or 60 days, at an estimated whole-body SAR of 0.043–0.135 W/kg, with or without daily intraperitoneal injections of melatonin (2 mg/kg bw) or saline. A false phone was placed in the cages of the control groups and the groups receiving melatonin only. A significant 20–50% increase in brain concentrations of malondialdehyde and carbonyl groups was observed during exposure. Catalase activity was decreased (–20%) during exposure, while the activity of XO was increased (15–25%) after 40 and 60 days of exposure. Treatment with melatonin prevented increases in malondialdehyde content and XO activity in brain tissue after 40 and 60 days of exposure.

[Dasdag et al. \(2009\)](#) exposed male Wistar rats to RF radiation at 900 MHz (GSM) delivered to the head for 2 hours per day, 7 days per week, for 10 months. No difference was found in oxidative-stress indexes between the groups, while total oxidant capacities and catalase in the brain were significantly higher ( $P < 0.05$ ) in the exposed group than in the sham-exposed group.

[Imge et al. \(2010\)](#) exposed female rats to RF radiation at 900 MHz (GSM) from a mobile phone (SAR, 0.95 W/kg) placed 10 cm above the cages, for 4 × 10 minutes per day, for 4 weeks, with or without daily oral administration of vitamin C (250 mg/kg bw). The activities in brain tissue of 5'-nucleotidase and catalase were significantly reduced compared with those of the non-treated control group, and there was a non-significant reduction in the activity of glutathione peroxidase and in concentrations of malondialdehyde in the brain. Vitamin C had a protective effect

in some of these analyses. [The Working Group noted the lack of sham-exposed controls.]

### (ii) *Kidney*

The justification for studying oxidative stress in the kidney following exposure to electromagnetic fields stems from the fact that the kidney would be the organ with the greatest exposure when a mobile phone is worn at the belt.

[Oktem et al. \(2005\)](#) exposed groups of eight Wistar albino rats to RF radiation at 900 MHz (GSM; average power density, 1.04 mW/cm<sup>2</sup>) for 30 minutes per day for 10 days, with or without treatment with melatonin (100 µg/kg bw; subcutaneous injection) before the daily exposure to RF radiation. SAR values were not reported. Increases in tissue concentrations of malondialdehyde and urinary *N*-acetyl-β-D-glucosaminidase (NAG), a marker of renal tubular damage, were observed. The activities of SOD, catalase, and GSH-Px were reduced. Administration of melatonin reversed or prevented these effects.

The same group ([Ozguner et al., 2005b](#)) compared the protective effects of melatonin (100 µg/kg bw; subcutaneous injection) and of caffeic acid phenethyl ester (CAPE; dose unclear), a component of honey-bee propolis used in traditional medicine, in Sprague-Dawley rats exposed to RF radiation. The experimental protocol was similar to that of [Oktem et al. \(2005\)](#), with antioxidants being injected daily for 10 days before exposure to RF radiation at 900 MHz (GSM; average power density, 1.04 mW/cm<sup>2</sup>). Urinary NAG and renal MDA were increased, while renal SOD and GSH-Px were decreased. Melatonin and CAPE reversed or prevented many of these effects, with melatonin being the more potent antioxidant. The results were similar to those reported previously, with the exception of catalase, the activity of which was not modified.

*(iii) Myocardium*

[Ozguner et al. \(2005a\)](#) assessed the protective effects of CAPE in myocardium of Sprague-Dawley rats exposed to RF radiation at 900 MHz, using an experimental protocol similar to that used for studies in the kidney (see above) and found comparable results.

*(iv) Eye*

[Ozguner et al. \(2006\)](#) compared the protective effects of melatonin and CAPE (a component of honey-bee propolis used in traditional medicine) on oxidative stress induced in rat retina by exposure to RF radiation at 900 MHz (whole-body SAR, 0.016 W/kg; local SAR at the head, 4 W/kg). The experimental protocol was similar to that in [Ozguner et al. \(2005b\)](#): antioxidants were injected daily for 60 days (rather than 10 days) before exposure to RF radiation for 30 minutes per day for 60 days (rather than 10 days). Significantly increased ( $P < 0.0001$ ) retinal concentrations of NO and MDA were found in exposed rats, which remained at control values after pre-treatment with melatonin and CAPE. Likewise, the activities of SOD, GSH-Px and CAT were significantly reduced in the retina of exposed rats. Again, prior treatment with melatonin and CAPE prevented this reduction in the activities of these antioxidant enzymes. These data indicated that antioxidants reduce oxidative stress in the rat retina caused by long-term exposure to RF radiation. [The Working Group was uncertain about the dosimetry in this study, and noted the lack of a cage-control group to assess the effect on the rats of being restrained in a tube during the exposures.]

[Balci et al. \(2007\)](#) exposed female rats to RF radiation at 900 MHz from a mobile phone (GSM; SAR, 1.2 W/kg), placed 10 cm above the cages, for 4 × 10 minutes per day, for 4 weeks, with or without daily oral administration of vitamin C (250 mg/kg bw). In the cornea, a significant increase was found in the concentration of malondialdehyde and in the activity of

catalase compared with the control group and with the exposed group receiving vitamin C, while the activity of SOD was decreased. In the lens tissues, the malondialdehyde concentration was significantly increased, but no significant differences in the activities of SOD, GSH-Px or catalase were observed. The presence of vitamin C generally diminished the effects of exposure to RF radiation. [The Working Group noted several design flaws in this study (e.g. the exposure system, the absence of dosimetry, absence of sham-exposed controls) and did not further consider these results.]

*(v) Liver*

[Ozguner et al. \(2010\)](#) investigated oxidative damage and antioxidant-enzyme status in the liver of guinea-pigs exposed to RF radiation at 1800 MHz (GSM; SAR, 0.38 W/kg) for 10 or 20 minutes per day, for 7 days. In this study the potential protective effects of *N*-acetylcysteine (NAC) and epigallocatechin-gallate (EGCG) were also investigated. A significant increase in the concentrations of malondialdehyde and nitrogen oxides (NO<sub>x</sub>) and a reduction in the activities of SOD, myeloperoxidase and GSH-Px were observed in the liver of exposed guinea-pigs. Some of these changes appeared to be proportional to the duration of exposure). In addition, treatment with NAC induced an increase in hepatic GSH-Px activities, whereas treatment with EGCG attenuated concentrations of malondialdehyde.

[Tomruk et al. \(2010\)](#) evaluated the effects of whole-body exposure to RF radiation at 1800 MHz (GSM) for 15 minutes per day, for 1 week, on oxidative DNA damage and lipid peroxidation in the liver of nonpregnant or pregnant New Zealand White rabbits, and in their newborns. Concentrations of malondialdehyde increased significantly in exposed nonpregnant and pregnant animals compared with nonpregnant controls, but there was no difference between exposed and sham-exposed pregnant rabbits. The same results were observed with lipid

peroxidation, measured by means of the ferrous oxidation-xylenol orange [FOX] assay. Exposure to RF radiation had no effect on the amount of oxidative DNA damage (8-OHdG adducts) in the liver of RF-exposed and sham-exposed nonpregnant and pregnant rabbits. No differences in concentrations of malondialdehyde and 8-OHdG were found in the liver of newborns exposed to RF radiation *in utero* compared with newborns of sham-exposed mothers. However, a significant reduction in lipid peroxidation, *i.e.* reduced FOX levels, in the liver of RF-exposed newborns was observed. [The Working Group noted that SAR values were not stated.]

(vi) *Miscellaneous*

[Mailankot et al. \(2009\)](#) exposed adult male Wistar albino rats to RF radiation at 900/1800 MHz (SAR not given) from a GSM mobile phone “in active mode” for 1 hour per day for 28 days, while control rats were exposed to a mobile phone “without battery.” There was no difference in sperm counts in the epididymis between exposed and control rats, but a 40% reduction in the proportion of motile sperm was observed after exposure. In addition, the concentration of malondialdehyde was significantly increased and intracellular GSH was significantly reduced in the testis and epididymis of exposed rats, compared with sham-exposed controls, together with a significant decrease in intracellular GSH in both testis and the epididymis of RF-exposed rats.

[Kumar et al. \(2010\)](#) exposed male Wistar rats to continuous RF radiation at 10 or 50 GHz (SAR, 0.014 and 0.0008 W/kg, respectively) for 2 hours per day, for 45 days. Total levels of ROS and catalase activity were higher and the proliferative index, and the activities of SOD and reduced GSH-Px in the serum were lower in exposed rats than in sham-exposed controls.

(b) *Differentiation and apoptosis*

[Dasdag et al. \(2003\)](#) exposed male Sprague-Dawley rats to RF radiation at 900 MHz from commercially available mobile phones (average calculated whole-body SAR, 0.52 W/kg; peak SAR, 3.13 W/kg) for 20 minutes per day, 7 days per week, for 1 month. The mobile phones were placed 0.5 cm under the cages. There were no differences between exposed and sham-exposed groups in terms of structure of testes, sperm counts, phospholipid composition or Tp53 immunoreactivity. [The Working Group noted the ill-defined exposure set-up and the approximate SAR calculations.]

In a study mentioned before, [Dasdag et al. \(2009\)](#) exposed male Wistar rats to RF radiation at 900 MHz (GSM; SAR, 0.19–0.58 W/kg) delivered to the head for 2 hours per day, 7 days per week, for 10 months. The apoptosis score – based on immunostaining of active caspase-3 – in the brain of the exposed rats was significantly lower than in sham-exposed or cage-control rats.

Apoptosis induced in the endometrium was studied by [Oral et al. \(2006\)](#) by exposing female Wistar albino rats in a plastic tube to RF radiation at 900 MHz (GSM) (SAR, 0.016–4 W/kg) for 30 minutes per day, for 30 days. Different group of rats received vitamin E (50 mg/kg bw) or vitamin C (20 mg/kg bw) by intramuscular or intraperitoneal injection, respectively, just before the daily exposure to RF radiation. Increased concentrations of malondialdehyde (indicative of lipid peroxidation) and enhanced apoptosis were observed in endometrial tissue (stromal cells) of exposed rats. These effects were partly reverted by vitamin treatment. Using the same experimental protocol, [Guney et al. \(2007\)](#) observed an increase in oxidation products (NO, malondialdehyde), a decrease in activities of antioxidant enzymes (SOD, catalase, GSH-Px), and diffuse and severe apoptosis in the endometrial surface epithelial and glandular cells and in

stromal cells. [Both studies lacked details on SAR measurement.]

[Odaci et al. \(2008\)](#) examined paraffin-embedded sections of the brain of rats aged 4 weeks born from females exposed to RF radiation at 900 MHz (GSM; calculated whole-body SAR, 2 W/kg), for 60 minutes per day during the entire gestation period. A slight but statistically significant reduction in the number of granule cells in the dentate gyrus of pups of exposed dams was observed; this reduction may affect postnatal behavioural and cognitive functions. [The Working Group noted the apparent lack of a sham-exposed control group.]

More recently, [Sonmez et al. \(2010\)](#) examined paraffin-embedded sections of the cerebellum of female rats aged 16 weeks exposed to RF radiation at 900 MHz (calculated average SAR, 0.016 and 2 W/kg, respectively, for whole-body or head-only) for 1 hour per day, for 28 days. A significant reduction in the number of Purkinje cells was observed in the cerebellum of exposed rats compared with sham-exposed controls and cage controls.

[The Working Group concluded that there was weak evidence that exposure to RF radiation at 900 MHz induces differentiation or apoptosis in the brain or endometrium of exposed rats.]

### (c) Blood–brain barrier

The blood–brain barrier regulates exchange between blood and the brain. An increase in the normally low permeability of this barrier for hydrophilic and charged molecules after exposure to RF radiation could potentially be detrimental by enabling the extravasation of substances that could potentially act as brain carcinogens.

*In vivo*, several methods have been used to evaluate the integrity of the blood–brain barrier. These methods are based either on assessment of the permeability of the barrier to endogenous molecules such as albumin, which can be visualized by immunohistochemistry on brain sections, or on the injection of dyes (Evans

blue) or labelled molecules that do not cross the blood–brain barrier under normal physiological conditions and hence may serve as permeability markers. Models of brain injury (e.g. cold injury or chemical injury) are informative positive controls in these experiments. Another method comprises the evaluation of alterations in nervous tissue by detecting degenerating neurons (“dark neurons”) through staining with cresyl violet, or with the fluorescent molecule Fluoro-Jade B, which is more specific for neurons.

Dozens of experiments in rodents have assessed the functioning of the blood–brain barrier in animals exposed to various intensities of RF radiation at frequencies  $\geq$  900 MHz (for reviews, see [Stam, 2010](#) and [Nittby et al., 2011](#)). Here are described only experimental studies of exposure at frequencies  $\geq$  900 MHz and at exposure levels that did not – or were unlikely to – produce a thermal effect: in the rat brain, hyperthermia of  $> 1$  °C induces alterations in the blood–brain barrier. It should be noted also that anaesthesia itself may modify the permeability of the blood–brain barrier.

One research group has reported effects on the permeability of the blood–brain barrier and alterations in nervous tissue (dark neurons) after exposure of Fisher 344 rats (males and females) to continuous or GSM-modulated RF radiation at 900 and 915 MHz, with SARs of 2–5 W/kg. Among recently published studies from this group, three ([Eberhardt et al., 2008](#); [Nittby et al., 2009, 2011](#)) reported an increase in permeability to albumin at 1 or 2 weeks after 2 hours of exposure to a 900 MHz GSM signal (SAR, 0.0001–0.13 W/kg). Another study from this group ([Grafström et al., 2008](#)) assessed permeability of the blood–brain barrier 5–7 weeks after exposure to a GSM signal (SAR, 0.0006–0.6 W/kg) for 2 hours per week for 55 weeks, and found no increase in permeability using several markers, and no appearance of dark neurons.

[Masuda et al. \(2009\)](#) did not observe albumin extravasation or appearance of dark neurons

in experiments in two-compartment transverse electromagnetic (TEM) transmission line cells. Male Fischer F344 rats were exposed to a 915 MHz GSM signal (whole-body SAR, 0.02, 0.2 or 2 W/kg) for 2 hours. Positive controls (cold and chemical injury) were included. Analyses were performed 14 and 50 days after exposure.

[McQuade et al. \(2009\)](#) did not observe any leakage of albumin across the blood–brain barrier in male Fischer 344 rats sham-exposed or exposed to 915 MHz RF radiation (SAR, 0.0018–20 W/kg) for 30 minutes in TEM cells. Both continuous-wave and pulsed modes of 16 and 217 Hz were used, with pulse parameters based on those in studies from the research group mentioned above ([Persson et al., 1997](#)). Positive controls (hyperthermia at 43 °C, and urea 10 M) were included. Albumin extravasation was investigated by immunohistochemical staining of brain sections. A subset of the microscopic slides was sent to Sweden and analysed by scientists associated with the original studies. No alterations in the blood–brain barrier were observed at any exposure level.

[De Gannes et al. \(2009\)](#) found no changes in the integrity of the blood–brain barrier or neuronal degeneration in Fischer 344 rats exposed head-only to a 900 MHz GSM signal (brain-averaged SAR, 0.14 or 2 W/kg) for 2 hours. Complete numerical and experimental dosimetry was included in this study. Albumin leakage, dark neurons, or changes in neuronal apoptosis were not observed. [It is worthy of note that in these three studies, homogeneous samples of male rats of the same age and weight were used. The SAR values tested were higher or of a wider power range than in experiments of the Swedish group.]

[The Working Group concluded that despite consistent results from one laboratory, the experimental evidence did not support the notion that non-thermal RF radiation affects the permeability of the blood–brain barrier.]

#### 4.4.3 Experimental systems: in vitro

##### (a) Human cells

##### (i) Free radicals and ROS

Free radicals are highly reactive molecules that carry unpaired electrons in the outer orbit. Free radicals that are derived from oxygen metabolism are known as reactive oxygen species (ROS). These radicals are continuously neutralized by antioxidants present in body tissues. When production of these species exceeds the scavenging capacity of antioxidants, oxidative stress results. Production of radicals is a known pathway involved in the development of cancer.

[Lantow et al. \(2006a, c\)](#) measured production of ROS and expression of HSPs (described in section 4.3.2.c (i)) in human Mono Mac 6 and K562 cells exposed to RF radiation at 1800 MHz (SAR, 0.5, 1.0, 1.5 or 2.0 W/kg) as three different GSM modulation signals, for 45 minutes. Heat and phorbol 12-myristate-13-acetate (PMA) induced a significant increase in superoxide radical anions and in the production of ROS. In general, no effects were observed from exposure to RF radiation alone or in combination with PMA or lipopolysaccharide. [Lantow et al. \(2006b\)](#) used human umbilical cord blood-derived monocytes and lymphocytes to examine release of ROS after continuous or intermittent (5 minutes on, 5 minutes off) exposure at 1800 MHz (SAR, 2 W/kg) for 30 or 45 minutes. Exposure to RF radiation did not enhance the effects of PMA. In another study from the same group, [Simkó et al. \(2006\)](#) exposed human Mono Mac 6 cells to RF radiation under similar conditions, but combined exposures were carried out with ultrafine particles. Exposure to RF radiation alone had no effect on radical production. In addition, RF radiation did not enhance the production of superoxide anion radicals induced by ultrafine particles.

[Luukkonen et al. \(2009\)](#) investigated intracellular production of ROS and DNA-damage induction in human SH SY5Y neuroblastoma

cells exposed to continuous-wave or pulsed-wave RF radiation at 872 MHz (SAR, 5 W/kg) for 1 hour. The experiments also involved combined exposure to RF radiation and menadione. The production of ROS was measured by use of the fluorescent probe dichlorofluorescein. No effects were seen from exposure to RF radiation alone. Consistent with the increase in DNA damage (described in Section 4.1.3.b.ii), the level of ROS measured after treatment with menadione was higher in cells exposed to a continuous-wave RF field. However, no effects of the pulsed-wave RF radiation were seen at identical SARs. In a second study using identical exposure conditions and the same cell line, [Luukkonen et al. \(2010\)](#) found no effects on ROS production induced by ferrous choride from continuous-wave or pulsed-wave RF radiation. This finding was consistent with lack of effect on DNA-damage induction in the same study, as described earlier.

[Höytö et al. \(2008a\)](#) exposed human SH-SY5Y neuroblastoma cells and mouse L929 fibroblasts to continuous-wave or GSM-modulated RF radiation at 872 MHz (SAR, 5 W/kg) for 1 hour or 24 hours, under isothermal conditions. To investigate possible combined effects with other agents, menadione was used to induce ROS, and *tert*-butylhydroperoxide (*t*-BOOH) was used to induce lipid peroxidation. After the 1-hour exposure, there was a statistically significant enhancement by RF radiation of *t*-BOOH-induced lipid peroxidation in SH-SY5Y cells exposed to the GSM-modulated signal. After the 24-hour exposure, there was a statistically significant increase by RF radiation of menadione-induced caspase-3-like protease activity in mouse L929 fibroblasts exposed to the GSM-modulated signal. No effects were seen in any of the other experimental conditions, or from exposure to RF radiation alone.

Purified human spermatozoa were exposed to RF radiation at 1800 MHz (SAR, 0.4 W/kg to 27.5 W/kg) ([De Iuliis et al., 2009](#)). With increasing SAR, motility and vitality of the sperm cells were significantly reduced after exposure,

while the mitochondrial generation of ROS and DNA fragmentation were significantly elevated. Furthermore, highly statistically significant relationships between SAR, the oxidative DNA damage biomarker 8-OHdG, and DNA fragmentation were observed in exposed cells. The temperature during these experiments was kept at 21 °C; the highest observed exposure-induced temperature increase was +0.4 °C, at SAR 27.5 W/kg; control experiments in which spermatozoa were incubated at 21 °C–50 °C – without RF radiation – indicated that the end-points measured were only significant above 40 °C.

Human sperm was exposed *in vitro* for 1 hour to RF radiation at 850 MHz (SAR, 1.46 W/kg) from a mobile phone in talk mode, and markers of oxidative stress were evaluated ([Agarwal et al., 2009](#)). The results showed a significant increase in production of ROS in exposed samples and a decrease in sperm motility, viability, and in the ROS-total antioxidative capacity (ROS-TAC) score in exposed samples.

[The Working Group concluded that there was weak evidence that RF radiation activates a stress response or production of ROS in human cells under non-thermal conditions.]

#### (ii) Cell proliferation

[Kwee & Raskmark \(1998\)](#) exposed human AMA epithelial amnion cells to RF radiation at 960 MHz (GSM; SAR, 0.021, 0.21 or 2.1 mW/kg) for 20, 30, and 40 minutes at 37 °C. Cellular proliferation was assessed by means of the formazan test, and found to decrease linearly with exposure time at the lowest and highest SAR level. In a follow-up study, [Velizarov et al. \(1999\)](#) exposed human AMA cells to RF radiation at 960 MHz (GSM; SAR, 2.1 mW/kg) for 30 minutes at two different temperatures (39 °C and 35 °C), to evaluate whether the earlier results (see above) were temperature-dependent. There was a marginally significant reduction in cellular proliferation rate – measured with the formazan test – after the 30-minute exposure at both

temperatures ( $P = 0.086$  and  $0.072$ , respectively, based on 11 independent experiments); the change in proliferation rate of the sham-exposed cells was not different at the two temperatures tested. The authors considered it unlikely that the effect of exposure to RF radiation on cell proliferation was a thermal effect.

[Pacini et al. \(2002\)](#) exposed human Detroit 550 skin fibroblasts to RF radiation at 960 MHz (GSM; estimated SAR, 0.6 W/kg) for 60 minutes. The radiation source was a mobile phone placed underneath the culture dish. No changes in the rate of cell replication were seen, as tested by [ $^3\text{H}$ ] thymidine incorporation. [The use of a mobile phone as a radiation source made this study difficult to interpret; with only three replicates, the sample size was small.]

[Capri et al. \(2004a\)](#) exposed peripheral blood mononucleated cells from healthy volunteers to RF radiation at 900 MHz (GSM or continuous-wave; average SAR, 70–76 mW/kg) for 1 hour per day, for 2 or 3 days. Cells were treated with the mitogens PHA or alphaCD3 to stimulate replication. A statistically significant ( $P = 0.04$ ) decrease in cell replication – as judged by [ $^3\text{H}$ ] thymidine incorporation – was seen only for the cells exposed to the GSM RF-radiation and stimulated with the lowest dose of PHA; all other differences were non-significant. There was no effect at all after exposure to the continuous-wave RF radiation.

[Marinelli et al. \(2004\)](#) exposed human CCRF-CEM T-lymphoblastic leukaemia cells to continuous-wave RF radiation at 900 MHz (SAR, 3.5 mW/kg) for 2, 4, 12, 24, or 48 hours. There was a significant decrease in total viable cell number after 24 and 48 hours of exposure, and a significant increase in the percentage of apoptotic cells – measured by fluorescence-activated cell sorting (FACS) analysis – after 2 hours, which gradually diminished but remained significant after 24 and 48 hours of exposure. In addition, after 48 hours the number of cells that had started S-phase had increased, while the percentage of

cells in growth-arrest diminished. These data support the notion that RF radiation may lead cancer cells to acquire an advantage to survive and proliferate. [The Working Group had some difficulty in understanding the description of the exposure conditions in this study.]

[Sanchez et al. \(2006b\)](#) exposed reconstructed human primary keratinocytes to RF radiation at 900 MHz (GSM; SAR, 2 W/kg) for 48 hours. No apoptosis was induced in these cells, and there was no alteration of cell proliferation. A small increase in expression of heat-shock protein (Hsp) 70 was noted after 3 and 5 weeks of culture. [Merola et al. \(2006\)](#) exposed human LAN-5 neuroblastoma cells to RF radiation at 900 MHz (GSM; SAR, 1 W/kg) for 24, 48 or 72 hours, and found no effects on cellular replication. [Gurisik et al. \(2006\)](#) exposed human SK-N-SH neuroblastoma cells and monocytic U937 cells to 900 MHz (GSM-modulated) RF radiation (SAR of 0.2 W/kg) for 2 hours. There were no effects on cell-cycle distribution, apoptosis, or HSP levels. [Lantow et al. \(2006c\)](#) exposed human macrophagic Mono Mac 6 cells to pulse-modulated RF radiation at 1800 MHz (GSM-DTX; SAR, 2 W/kg) for 12 hours. No changes in cell-cycle distribution or cell proliferation were reported. [Takashima et al. \(2006\)](#) exposed human MO54 glioma cells to 2450 MHz continuous-wave RF radiation (SAR, 0.05, 0.5, 5, 50, 100, 200 W/kg) for 2 hours, or to intermittent RF radiation at 2450 MHz (mean SAR, 50 or 100 W/kg) for 2 hours. Exposure to continuous-wave RF radiation at 200 W/kg caused a decrease in cell replication and cell survival. Other exposures had no effect. [It should be noted that the temperature of the medium increased to 44.1 °C at exposures with SAR of 200 W/kg.] [Sun et al. \(2006\)](#) exposed human lens epithelial cells to GSM-modulated RF radiation at 1800 MHz (SAR, 1, 2, 3, 4 W/kg) for 2 hours. No effects of RF exposure were observed on cell proliferation (incorporation of bromodeoxyuridine) up to 4 days after exposure. [Chauhan et al. \(2007b\)](#) exposed human lymphoblastoid TK6,

lymphoblastic HL60 and myeloid Mono Mac 6 cells to intermittent (5 minutes on, 10 minutes off) pulse-modulated RF radiation at 1900 MHz (SAR, 1 and 10 W/kg) for 6 hours. There were no effects on cell-cycle progression.

[The Working Group concluded that there was weak evidence that exposure to RF radiation affects cell proliferation.]

### (iii) Apoptosis

Defects in apoptosis-signalling pathways are common in cancer cells; apoptosis is an important mechanism by which damaged cells are removed, thus preventing the proliferation of potential cancer cells.

[Marinelli et al. \(2004\)](#) reported increased apoptosis, determined by flow cytometry and DNA-ladder analysis, in human CCRF-CEM T-lymphoblastoid leukaemia cells exposed to continuous-wave RF radiation at 900 MHz (SAR, 0.0035 W/kg) for 2–48 hours. Measurement of gene expression indicated activation of both TP53-dependent and -independent apoptotic pathways after shorter exposures (2–12 hours), while decreased pro-apoptotic signals were seen at longer exposure times (24–48 hours). As indicated above, these data support the notion that RF radiation may lead cancer cells to acquire an advantage to survive and proliferate. [The Working Group noted that the statistical comparisons with respect to FACS analysis were with unexposed, not sham-exposed cells.]

[Port et al. \(2003\)](#) exposed human myeloid leukaemia cells (HL-60) to pulsed-wave RF radiation at 400 MHz (SAR not given) for 6 minutes. The electric-field strength was 50 kV/m. No effects on the number of apoptotic cells or micronuclei were found. [The Working Group noted that interpretation of these findings was difficult due to the lack of SAR values and very short exposure times.]

[Capri et al. \(2004a\)](#) exposed human peripheral blood mononuclear cells to continuous-wave or GSM-modulated RF radiation at 900 MHz

(average SAR, 70–76 mW/kg) for 1 hour per day, for 2 or 3 days. In general, no differences were detected in apoptosis – measured by means of annexin V-FITC staining – between exposed and sham-exposed cells, irrespective of whether or not the cells were treated with 2-deoxy-D-ribose, an inducer of apoptosis. In a similar study ([Capri et al., 2004b](#)), the cells were exposed intermittently (10 minutes on, 20 minutes off) to RF radiation at 1800 MHz with three different GSM-modulation schemes (SAR, 1.4 or 2 W/kg) for 44 hours. No effects on apoptosis were observed from RF radiation alone or from RF radiation combined with the apoptosis-inducing agent, 2-deoxy-D-ribose.

[Hook et al. \(2004a\)](#) reported no effects on apoptosis, detected by use of the annexin V affinity assay, in human Molt-4 lymphoblastoid leukaemia cells exposed to RF radiation at 847.74 MHz as CDMA, 835.62 MHz as FDMA, 813.56 MHz as iDEN, or 836.55 MHz as TDMA signals, for up to 24 hours. The SARs were 3.2 W/kg for CDMA and FDMA, 0.0024 or 0.024 W/kg for iDEN, and 0.0026 or 0.026 W/kg for TDMA.

[Gurisik et al. \(2006\)](#) exposed human neuroblastoma SK-N-SH cells to RF radiation at 900 MHz (GSM; SAR, 0.2 W/kg) for 2 hours. Apoptosis was measured by means of propidium iodide/YO-PRO-1 staining. No differences were detected between sham-exposed and exposed samples.

[Hirose et al. \(2006\)](#) reported no effects on apoptosis, measured by the annexin V-FITC affinity assay, or on apoptosis-related gene expression, in human glioblastoma A172 or human IMR-90 fibroblasts exposed to RF radiation at 2142.5 MHz (SAR, 0.08–0.8 W/kg), with or without W-CDMA modulation, for 24–48 hours.

[Joubert et al. \(2006\)](#) studied apoptosis in human neuroblastoma SH-SY5Y cells exposed to GSM-modulated RF radiation at 900 MHz (SAR, 0.25 W/kg) or continuous-wave RF radiation at 900 MHz (SAR of 2 W/kg) for 24 hours.

No effects on apoptosis were detected, either immediately or 24 hours after exposure, with three different techniques, *viz.* 4',6-diamino-2-phenylindole (DAPI) staining of nuclei, flow cytometry with double staining (TUNEL and propidium iodide), or measurement of caspase-3 activity by fluorometry.

[Lantow et al. \(2006c\)](#) reported no effects on apoptosis – measured with the annexin V-FITC assay – in human Mono Mac 6 cells exposed to 1800 MHz GSM-modulated RF radiation (SAR, 2.0 W/kg) for 12 hours, either alone or in combination with the apoptosis-inducing agents PMA or gliotoxin.

[Merola et al. \(2006\)](#) exposed human neuroblastoma LAN-5 cells to RF radiation at 900 MHz (GSM; SAR of 1 W/kg) for 24 or 48 hours. This exposure did not affect apoptosis, measured by an assay for caspase activation. In addition, RF-radiation did not enhance camptothecin-induced apoptosis.

[Sanchez et al. \(2006b\)](#) exposed human epidermal keratinocytes and fibroblasts to RF radiation at 900 MHz (GSM; SAR, 2 W/kg) for 48 hours. No alteration in apoptosis was detected in the annexin V/FITC affinity assay, while a very clear response was seen for UVB radiation, which was used as a positive control. In a subsequent study, [Sanchez et al. \(2007\)](#) exposed the same types of cell to RF radiation at 1800 MHz (GSM; SAR, 2 W/kg) for 2 hours. No effects on apoptosis were observed in the annexin V-FITC affinity assay.

[Chauhan et al. \(2007b\)](#) reported that apoptosis assessed by the neutral comet assay to detect DNA double-strand breaks was not affected in human TK6, HL-60, or Mono Mac 6 cells exposed to intermittent (5 minutes on, 10 minutes off) pulsed-wave RF radiation at 1900 MHz (SAR, 1, 10 W/kg) for 6 hours.

[Höytö et al. \(2008a\)](#) exposed human SH-SY5Y neuroblastoma cells to continuous-wave or GSM-modulated RF radiation at 872 MHz (SAR, 5 W/kg) for 1 or 24 hours under isothermal

conditions, with or without the apoptosis-inducing agent menadione. No direct effects of RF radiation on apoptosis, or on menadione-induced apoptosis were observed in assays for caspase-3 activity and DNA fragmentation.

Human KB oropharyngeal epidermoid carcinoma cells were exposed to RF radiation at 1.95 GHz (SAR, 3.6 mW/kg) for 1, 2, or 3 hours. The exposure caused a time-dependent increase in apoptosis (45% after 3 hours), along with a 2.5-times decrease in the expression of the genes *RAS* and *RAF1* and in the activity of the proteins *RAS* and *ERK-1/2*. The overall results showed that RF radiation can induce apoptosis via inactivation of the ras–Erk survival-signalling pathway ([Caraglia et al., 2005](#)) [the Working Group noted the lack of specific control of the temperature of the cells during the exposure periods in this study].

[The Working Group concluded that there was weak evidence that RF radiation induces apoptosis in human cells *in vitro*.]

#### (b) Other mammalian cells

See [Table 4.15](#)

##### (i) Stress response and ROS formation

Exposure of J774.16 mouse macrophages stimulated with  $\gamma$ -interferon and bacterial lipopolysaccharide to RF radiation at 835.62 MHz as FMCW, or to at 847.74 MHz as a CDMA signal (SAR, 0.8 W/kg) for 20–22 hours at  $37 \pm 0.3$  °C did not alter the concentrations of intracellular oxidants (NO, glutathione disulfide), or activities of the enzymes CuZnSOD, MnSOD, catalase, or GSH-Px ([Hook et al., 2004b](#)).

[Zmysłony et al. \(2004\)](#) reported an increase in cellular ROS production in rat lymphocytes coexposed to RF radiation and iron ions. The cells were exposed to continuous-wave RF radiation at 930 MHz (SAR, 1.5 W/kg) for 5 or 15 minutes in the presence of  $\text{FeCl}_2$  (10  $\mu\text{g/ml}$ ). Intracellular ROS production, measured with the fluorescent probe 2',7'-dichlorofluoresceindiacetate

**Table 4.15 Effects of exposure to radiofrequency radiation in cultured mammalian cells *in vitro***

Cell type	Exposure conditions	End-points	Results	Comments	Reference
<i>Stress response and formation of reactive oxygen species</i>					
Mouse, J774.16 macrophages	835.62 MHz (FMCW) or 847.74 MHz (CDMA); SAR, 0.8 W/kg; 20–22 h; 37.0 ± 0.3°C; stimulation with IFN and bacterial LPS	Oxidative stress evaluated by oxidant and antioxidant levels, oxidative damage and NO production. Oxidation of thiols measured by accumulation of GSSG. Cellular antioxidant defenses evaluated by SOD activity (CuZnSOD and MnSOD), CAT and GSH-Px activities.	No effect on parameters indicative of oxidative stress, levels of intracellular oxidants, accumulation of GSSG, or induction of antioxidant defences in IFN/LPS-stimulated cells. No toxicity was observed.		<a href="#">Hook et al. (2004b)</a>
Hamster ovary HA-1 fibroblasts, Mouse C3H10T½, Human HeLa S3 cells	835.62 MHz (FMCW), 847.74 MHz (CDMA); SAR, 0.6 or 5 W/kg; 50–60 min, or 24 h; at 37.0 ± 0.28 °C, 36.9 ± 0.18 °C and 37.1 ± 0.28 °C for FDMA-, sham-, and CDMA-exposure, respectively.	DNA-binding activity of HSF – a necessary condition for induction of a heat-shock response – monitored with a gel-shift assay.	No increase in the DNA-binding ability of HSF after any exposure tested in any of the cell lines	A 10% increase was detectable after a 1 °C temperature increase	<a href="#">Laszlo et al. (2005)</a>
Mouse L929 fibroblasts Human SH-SY5Y neuroblastoma cells	872 MHz (CW or GSM); SAR, 5 W/kg; 1 h or 24 h.	Co-exposure (1 hour) with menadione (to induce ROS) or <i>t</i> -BOOH (to induce lipid peroxidation). Assessment of apoptosis (caspase3-like protease activity and DNA-fragmentation analysis) after 24 h exposure to RF	No effects of RF radiation alone. Menadione induced caspase-3 activity in L929 (not in SH-SY5Y) cells; lipid peroxidation was induced by <i>t</i> -BOOH in SH-SY5Y (not in L929) cells. Effects significant only for GSM-RF. Other end points not affected.	The positive findings may reflect effects that occur in cells sensitized by chemical stress.	<a href="#">Höytö et al. (2008a)</a>
Mouse L929 fibrosarcoma cells	900 MHz (CW or GSM); SAR, 0.3 or 1 W/kg; 10 or 30 min; with/without co-exposure to 500 µM of MX.	ROS formation measured by a fluorimetric method just after the exposure and at different times until 1 h after exposure	No effect of RF radiation (with or without MX) on formation of ROS		<a href="#">Zeni et al. (2007b)</a>
Wistar rat; primary cortical astrocytes	900 MHz (CW or amplitude-modulated); electric field 10V/m; 5, 10, 20 min. Electric field at the sample position: 10 V/m.	Evaluation of intracellular ROS production, and of DNA damage (comet assay).	Increased ROS levels and DNA fragmentation after a 20-min exposure to AM-RF radiation. No effects of CW	Few details of experimental procedures; no temperature control	<a href="#">Campisi et al. (2010)</a>
Rat lymphocytes	930 MHz (CW); SAR 1.5 W/kg; 5 or 15 min	Intracellular ROS measured with the fluorescent probe dichlorofluorescein diacetate (DCF-DA).	No effect on ROS formation		<a href="#">Zmysłony et al. (2004)</a>

**Table 4.15 (continued)**

Cell type	Exposure conditions	End-points	Results	Comments	Reference
Rat, age 1–2 d, primary cortical astrocytes	1763 MHz (CDMA); average SAR, 2 or 20 W/kg; 30 min or 1 h.	Assessment of expression of HSPs and activation of MAPKs	No detectable effect on expression of HSP90, HSP70, HSP27; no change in MAPK phosphorylation, ERK1/2, JNK1/2, p38; no effect on TPA-induced MAPK phosphorylation	Temperature-control at 37 ± 0.2°C	<a href="#">Lee et al. (2006)</a>
Newborn rat, primary cortical neurons	1800 MHz; average SAR 2 W/kg; (5 min on, 10 min off); 24 h	Melatonin was given 4 h before exposure to RF radiation. Immunostaining and HPLC analysis of 8-OHdG in mitochondria; number of copies of mtDNA; levels of mtDNA transcripts.	RF radiation induced a significant 2-fold increase in 8-OHdG in the mitochondria of neurons, and a reduction in the copy number of mtDNA and the amount of mtRNA transcripts. The effects could be partly reversed by pre-treatment with melatonin.		<a href="#">Xu et al. (2010)</a>
<i>Cell proliferation and cell cycle</i>					
Mouse C3H 10T½ cells	835.62 MHz (CW or CDMA); average SAR, 0.6 W/kg; 13 h (short exposure), up to 100 h (long-term exposure)	Cell-cycle parameters (transit of cells through G1, G2, S phase; probability of cell division) evaluated immediately after cells were exposed for 3 h, or after 100 h exposure	No changes in cell-cycle parameters after exposure to either CW or CDMA	Controlled exposure and temperature	<a href="#">Higashikubo et al. (2001)</a>
Mouse pre-neoplastic CLS1 mammary epithelial cells	Nanopulse electric-field strength of 18 kV/m; repetition rate 1–1000 kHz; up to 6 h. Cells cultured in the presence EGF (10 ng/ml) and insulin (10 µg/ml) as comitogens. After exposure, cells in all treatment groups were returned to the incubator for 72 h	After exposure, cells in all treatment groups were returned to the incubator for 72 h; cell growth and viability were assessed	No effect on CLS1 cell growth or viability during the subsequent culture period of 72 h after 0.25–3 h exposure to nanopulse radiation. Prolonged exposure (4–6 h) caused a significant increase in cell proliferation.	Radar-type signal. Increase in cell proliferation associated with MAPK activation in EGF-supplemented medium.	<a href="#">Sylvester et al. (2005)</a>
Mouse embryonic stem cells	1720 MHz; SAR, 1.5 W/kg (5 min on, 30 min off); 6 or 48 h	Transcript levels of cell-cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation, apoptosis, and cytogenetic effects (quantitative RT-PCR and comet assay).	No difference in rates of cell proliferation between exposed and sham-exposed cells		<a href="#">Nikolova et al. (2005)</a>

**Table 4.15 (continued)**

Cell type	Exposure conditions	End-points	Results	Comments	Reference
Mouse HEI-OC1 immortalized auditory hair cells	1763 MHz (CDMA); SAR, 20 W/kg; 24 or 48 h	Cell cycle (flow cytometry), DNA damage (comet assay: tail length, tail moment) were evaluated. Stress response (HSP) and gene activation were analysed with Western blotting and DNA microarray (Affymetrix full mouse genome chips, 32 000 genes)	No cell-cycle change or DNA damage. No change in expression of HSP or in phosphorylation of MAPK; minimal changes in gene expression: only 29 genes down- or upregulated; no consistent group of functional categories.		<a href="#">Huang et al. (2008b)</a>
Mouse CTLL-2 cytolytic T lymphocytes	2450 MHz (CW, PW); SAR, 25 or 50 W/kg (CW) and 5 W/kg (PW); 2 h	Effects of exposure on IL-2-dependent proliferation.	Consistent and statistically significant reduction in cell proliferation at low concentrations of IL-2.	Large sample size: 24 replicates per exposure group	<a href="#">Cleary et al. (1996)</a>
Chinese hamster ovary (CHO) cells	27 MHz; SAR, 5 or 25 W/kg; 2 h	Cell-cycle alterations determined by flow-cytofluorometric DNA determinations	Significant SAR-dependent changes in cell-cycle progression, with maximum change occurring 3 d after the initial exposure		<a href="#">Cao et al. (1995)</a>
Chinese hamster lung fibroblasts (V79 cells)	935 MHz (CW); SAR, 0.12 W/kg; 1, 2, 3 h	Microtubule protein morphology determined by immunocytochemistry immediately after exposure; cell proliferation examined by cell counts up to 5 d after exposure	No changes after 1 or 2 h exposure. After 3 h exposure, microtubules appeared morphologically grainy, comparable to those in colchicine-treated positive controls; no consistent change in proliferation.	Only one proliferation value decreased 3 d after exposure (but not at 4 and 5 d) in cells exposed for 3 h	<a href="#">Pavicic &amp; Trosic (2008)</a>
Chinese hamster ovary (CHO) cells	2450 MHz PW; SAR, 33.8 W/kg; 2 h; simultaneous exposure to adriamycin	Evaluation of percentage of first- and second-division mitotic cells after treatment with BrdU.	Exposure did not affect changes in cell progression caused by adriamycin.		<a href="#">Ciaravino et al. (1991)</a>

**Table 4.15 (continued)**

Cell type	Exposure conditions	End-points	Results	Comments	Reference
Chinese hamster ovary CHO-K1 cell line	2450 MHz, continuous or intermittent; SAR, 0.05–200 W/kg; 2 h	Cell survival, growth and cell cycle (flow cytometry at 0–24 h) were determined	Exposure to CW RF radiation (SAR $\leq$ 100 W/kg) did not affect cell growth, survival, or cell-cycle distribution. At 200 W/kg, cell growth was suppressed and cell survival decreased. Exposure to intermittent RF radiation caused no significant effects. Exposures $\leq$ 200 W/kg (continuous) or $\leq$ 900 W/kg (intermittent) did not affect cell-cycle distribution.	Effects on proliferation due to temperature rise at SAR 50–200 W/kg	<a href="#">Takashima et al. (2006)</a>
Chinese hamster V79 cells	7700 MHz, CW; power density, 30 mW/cm <sup>2</sup> ; 15, 30, 60 min	Incorporation of [ <sup>3</sup> H]thymidine and autoradiography	Decreased [ <sup>3</sup> H]thymidine incorporation immediately after exposure. Between 4 and 24 h after exposure, incorporation returns to control values; labelling index decreased after exposure, returned to normal after 24 h.	Normal incorporation rate is recovered within one cell generation; no information on temperature control	<a href="#">Garaj-Vrhovac et al. (1990)</a>
Chinese hamster V79 cells	7700 MHz, CW; power density, 0.5, 10, 30 mW/cm <sup>2</sup> ; 10, 20, 30, 60 min	Cell survival assessed by colony-forming ability	Surviving fraction reduced in a time- and energy-dependent manner.	Exposure system kept under controlled temperature conditions at 22°C.	<a href="#">Garaj-Vrhovac et al. (1991)</a>
Rat RBL-2H3 mast cells	835 MHz; estimated power density, 81 W/m <sup>2</sup> ; for 3 $\times$ 20 min/day for 7 days	Effects on cell proliferation, morphology and secretion	Increased [ <sup>3</sup> H]thymidine uptake and increased cell counts at days 6 and 7. Increased release of calcium	Exposure was variable across the chamber, based on temperature variation	<a href="#">Donnellan et al. (1997)</a>
Transformed C6 rat glioma and normal primary glial cells (from d 17 rat embryos)	836.55 MHz (TDMA); power density 0.09, 0.9, 9 mW/cm <sup>2</sup> ; SAR, 0.15–59 mW/kg; 24 h	Monitoring of cell growth, DNA synthesis assay ([ <sup>3</sup> H]thymidine).	No difference in growth curves and doubling times between sham-exposed and exposed cells		<a href="#">Stagg et al. (1997)</a>

**Table 4.15 (continued)**

Cell type	Exposure conditions	End-points	Results	Comments	Reference
Rabbit lens epithelial cells	2450 MHz (CW); intensity, 0.5–20 W/m <sup>2</sup> ; 2–8 h	Cell cycle (flow cytometry), cell viability (MTT assay); cell-cycle regulatory RNA and proteins (RT-PCR and Western blot).	Decreased number of cells in S-phase (decreased cellular replication) at exposures > 0.5 W/m <sup>2</sup> after 8 h	Inadequate description of the exposure conditions	<a href="#">Yao et al. (2004)</a>
<i>Apoptosis</i>					
Mouse-embryo primary neurons and astrocytes	1900 MHz (GSM) from a mobile phone; SAR not given; 2 h, mode “on” (exposure) or “stand-by” (sham).	Expression of apoptosis-related genes studied by array analysis. Genes showing ≥ 35% decrease or increase further studied by real time RT-PCR.	Up-regulation of <i>Pycard</i> , <i>Casp2</i> , and <i>Casp6</i> genes, both in “on” and “stand-by” modes, in neurons. <i>Pycard</i> , <i>Casp2</i> , <i>Casp6</i> , and <i>Bax</i> were upregulated in astrocytes, when cell phone in the “on” mode, but not in the “stand by” mode.	Cell phone placed over the culture dish; no dosimetry; no temperature control	<a href="#">Zhao et al. (2007a)</a>
Mouse neuroblastoma N2a cells	935 MHz (GSM basic, GAM “talk”, CW); SAR, 2 W/kg; 24 h. Cells were in proliferative and differentiated states.	Apoptosis assessed – up to 48 h after exposure – by fluorescence microscopy: annexin V, caspase activation, and <i>in situ</i> end-labelling.	No differences in apoptosis levels between exposed and sham-exposed proliferating or differentiated cells		<a href="#">Moquet et al. (2008)</a>
Rat-embryo primary neurons	900 MHz (CW); SAR 2 W/kg; 24 h	Apoptosis assessed by condensation of DAPI-labelled nuclei, and TUNEL assay. Caspase-3 activity assessed by fluorimetry, and apoptosis-inducing factor (AIF) by immunofluorescence.	A highly significant increase in the percentage of apoptotic cells was seen at 24 h after exposure, compared with sham-exposed cells and cells incubated at 39 °C; no increase in caspase-3 activity, but increase in AIF labelling.	Results suggest caspase-independent mitochondrial apoptosis. Increase in temperature was 2 °C during exposure. Control experiments (no RF) with neurons at 39 °C did not show an increase in apoptosis	<a href="#">Joubert et al. (2008)</a>

8-OHdG, 8-hydroxy-2'-deoxyguanosine; Asc, apoptosis associated speck-like protein containing a CARD; BrdU, bromodeoxyuridine; CDMA, code-division multiple access; CAT, catalase; CW, continuous wave; d, day; ERK1/2, extracellular signal-regulated kinases; FDMA, frequency-division multiple access; FM, frequency-modulated; GSH-Px, glutathione peroxidase; GSM, Global Systems Mobile communications; GSSG, glutathione disulfide; h, hour; HSF, heat-shock transcription factor; HSP, heat-shock protein; IFN,  $\gamma$ -interferon; JNK1/2, c-Jun N-terminal protein kinases; LPS, lipopolysaccharide; NO, nitric oxide; RF, radiofrequency; ROS, reactive oxygen species; RT-PCR, reverse-transcriptase polymerase chain reaction; SOD, superoxide dismutase; *t*-BOOH, *tert*-butylhydroperoxide

(DCF-DA), was elevated by 16.6% and 14.6%, respectively, at these time-points. Exposure to RF radiation alone did not affect ROS production.

Exposure of mouse C3H 10T½ cells and hamster ovary HA-1 fibroblasts to RF radiation at 835.62 MHz as FMCW signal, or at 847.74 MHz as CDMA signal (SAR, 0.6 or 5 W/kg) for 1 or 24 hours did not increase the DNA-binding activity of heat-shock transcription factor ([Laszlo et al., 2005](#)).

Exposure of mouse L929 fibrosarcoma cells to continuous-wave or GSM-modulated RF radiation at 900 MHz (SAR, 0.3 or 1 W/kg) for 10 or 30 minutes, did not induce ROS formation by itself, or in combination with subtoxic concentrations of MX (3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, a by-product of water chlorination). In this study, MX strongly induced ROS formation ([Zeni et al., 2007b](#)).

[Höytö et al. \(2008b\)](#) exposed mouse L929 cells to continuous-wave or GSM-modulated RF radiation at 872 MHz (SAR, 5 W/kg), for 1 hour or 24 hours, under isothermal conditions. To investigate possible effects of co-exposure with other agents, menadione was used to induce ROS, and *tert*-butylhydroperoxide (*t*-BOOH) was used to induce lipid peroxidation. No effects were observed after exposure to RF radiation only. Menadione-induced caspase-3 activity was significantly increased (but not in human neuroblastoma cells used in the same experiments) only by exposure to the GSM-modulated signal; *t*-BOOH-induced lipid peroxidation was not modified by RF radiation.

[Lee et al. \(2006\)](#) exposed cultures of primary astrocytes from newborn rats (aged, 1–2 days) to RF radiation at 1763 MHz as CDMA signal (average SAR, 2 or 20 W/kg) for 30 minutes or 1 hour, under temperature-controlled conditions at  $37 \pm 0.2$  °C. RF radiation alone did not elicit a stress response and had no effect on TPA-induced MAPK phosphorylation.

[Campisi et al. \(2010\)](#) exposed cultures of primary astrocytes from newborn rats (age,

1–2 days) to continuous-wave or amplitude-modulated (50 Hz) RF radiation at 900 MHz (no SAR given; power density,  $0.26 \text{ W/m}^2$ ), for 5, 10 or 20 minutes. There was an increase in ROS levels and DNA fragmentation (measured with the comet assay) after an exposure of 20 minutes to the amplitude-modulated RF radiation. With regards to the temperature of the cells during the exposure, the authors note that low-intensity RF radiation caused a minimal increase ( $0.03$  °C) in temperature. [The publication gave few details about the experimental procedures.]

[Xu et al. \(2010\)](#) exposed primary cortical neurons from newborn rats to intermittent (5 minutes on, 10 minutes off) GSM-modulated RF radiation at 1800 MHz (average SAR, 2 W/kg) for 24 hours, and found significant increases ( $P < 0.01$ ) in ROS production and in mitochondrial concentrations of 8-OHdG, and a reduction in copy numbers of mitochondrial DNA and mitochondrial RNA transcripts. These effects were partly reversed by treatment with melatonin 4 hours before exposure to RF radiation.

[The Working Group concluded that there was weak evidence that exposure to RF radiation activates stress response or ROS production in a variety of rodent cells *in vitro* under conditions not confounded by thermal effects.]

#### (ii) Cell proliferation and cell cycle

Exposure of Chinese hamster ovary (CHO) cells to pulsed-wave RF radiation at 2450 MHz (SAR, 33.8 W/kg) for 2 hours, did not affect cell-cycle progression, measured by analysis of first- and second-division mitotic cells after incorporation of bromodeoxyuridine. In the presence of adriamycin (given immediately before the exposure) RF radiation did not affect the cell-cycle progression induced by this drug ([Ciaravino et al., 1991](#)).

[Huang et al. \(2008b\)](#) did not find evidence for the induction of cellular responses, including cell-cycle distribution, DNA-damage induction,

stress response and altered gene expression, in immortalized HEI-OC1 mouse auditory hair cells exposed to RF radiation 1763 MHz (CDMA; SAR, 20 W/kg) for 24 or 48 hours. [The Working Group noted that the choice of auditory hair cells was justified by the fact that auditory cells may be exposed to radiation from mobile phones.]

In V79 Chinese hamster lung fibroblasts, microtubule morphology – analysed by use of immunocytochemical methods – appeared modified following a 3-hour exposure to continuous-wave RF radiation at 935 MHz (SAR, 0.12 W/kg). No changes were noted after exposure for 1 or 2 hours ([Pavlic & Trosic, 2008](#)).

In V79 Chinese hamster cells exposed to continuous RF radiation at 7.7 GHz (SAR not given; power density, 30 mW/cm<sup>2</sup>) for 15, 30, or 60 minutes, the incorporation of [<sup>3</sup>H]thymidine decreased immediately after exposure. At longer time intervals after exposure, the incorporation of [<sup>3</sup>H]thymidine increased and it returned to control values by 24 hours ([Garaj-Vrhovac et al., 1990b](#)). In the same cells exposed to RF radiation under the same conditions with power densities of 0.5, 10, 30 mW/cm<sup>2</sup>, the surviving fraction – assessed by colony-forming ability – was reduced in a time- and energy dependent manner ([Garaj-Vrhovac et al., 1991](#)).

[Cao et al. \(1995\)](#) exposed CHO cells in different phases of the cell cycle to continuous-wave RF radiation at 27 MHz (SAR, 5 or 25 W/kg), for 2 hours. The cells were followed at sampling time-points up to 96 hours after exposure. Significant SAR-dependent changes in cell-cycle progression were observed, with the maximum change occurring at 3 days after exposure.

[Cleary et al. \(1996\)](#) exposed CTLL-2 mouse cytolytic cells to continuous-wave RF radiation at 2450 MHz (SAR, 5–50 W/kg), or to pulsed-wave RF radiation at 2450 MHz (SAR, 5 W/kg) for 2 hours. There was a decrease in cell proliferation (assessed by means of [<sup>3</sup>H]thymidine incorporation) with continuous-wave, and an increase with pulsed-wave radiation. The effects

were dependent upon the IL2 concentrations in the culture and the stage of the cell cycle.

[Donnellan et al. \(1997\)](#) exposed rat RBL-2H3 mast cells to RF radiation at 835 MHz (estimated power density, 81 W/m<sup>2</sup>) for 20 minutes, three times per day for 7 days. Increased uptake of [<sup>3</sup>H]thymidine and increased cell counts were observed at days 6 and 7, and an increase in the release of calcium was detected in the exposed group. [The exposure was variable across the exposure chamber based on temperature variations; eight samples were used for each group for analysis.]

[Stagg et al. \(1997\)](#) exposed rat primary glial cells and C6 glioma cells to RF radiation at 836.55 MHz as TDMA signal (SAR, 0.59, 5.9, 59 mW/kg) for 4 or 24 hours. A small but significant increase ( $P = 0.026$ ) in the uptake of [<sup>3</sup>H]thymidine was detected in C6 glioma cells at 5.9 mW/kg. In the other exposure groups no effects from exposure to RF radiation were observed ([<sup>3</sup>H]thymidine uptake, cell growth).

[Higashikubo et al. \(2001\)](#) exposed mouse fibroblast (C3H 10T<sup>1/2</sup>) and human glioblastoma (U87MG) cells to RF radiation at 847.74 MHz as CDMA signal or at 835.62 MHz as TDMA signal (SAR, 0.6 W/kg) for up to 100 hours. No significant effects were found on cellular replication, as measured with the bromodeoxyuridine pulse-chase flow-cytometry method.

[Takashima et al. \(2006\)](#) exposed Chinese hamster ovary CHO-K1 cells to continuous-wave RF radiation at 2450 MHz (SAR, 0.05–200 W/kg) for 2 hours, or to intermittent RF radiation at 2450 MHz (average SAR, 50 or 100 W/kg) for 2 hours. Continuous-wave RF radiation at 200 W/kg decreased cell replication and cell survival. None of the other exposures showed an effect. [The temperature of the medium increased to 44.1 °C during exposure at a SAR of 200 W/kg.]

[Yao et al. \(2004\)](#) exposed replicates of rabbit-lens epithelial cells to continuous-wave RF radiation at 2450 MHz (no SAR given; power density,

0.1–2 mW/cm<sup>2</sup>, for 8 hours at 25 °C. Cell viability was significantly reduced at power densities of 0.5 mW/cm<sup>2</sup> and higher. The numbers of cells in S-phase decreased and that of cells in G<sub>0</sub>/G<sub>1</sub> phase increased – both significantly – at power densities ≥ 0.5 W/m<sup>2</sup>. [The Working Group had some difficulty in understanding the description of the exposure conditions in this study.]

[Nikolova et al. \(2005\)](#) exposed mouse embryonic stem cells to intermittent (5 minutes on, 30 minutes off) RF radiation at 1720 MHz (time-averaged SAR, 1.5 W/kg; during actual exposure, 12 W/kg) for 6 or 48 hours. No effects on the incorporation of bromodeoxyuridine were observed.

[Sylvester et al. \(2005\)](#) exposed mouse pre-neoplastic CL-S1 mammary epithelial cells to RF radiation as ultra-wide band pulses with an electric-field strength of 18 kV/m and a repetition rate in the range of 1–1000 kHz for up to 6 hours. No effect on CL-S1 cell growth or viability was observed after exposures of 0.25–3 hours. Exposure for 4 hours resulted in a significant increase in cell proliferation compared with untreated controls. There was no further increase at 5 or 6 hours.

[The Working Group concluded that the evidence that RF radiation has an effect on cell proliferation and cell cycle was weak.]

### (iii) *Ornithine decarboxylase activity (rodent and human cells)*

Ornithine decarboxylase (ODC) is the first and rate-limiting enzyme in the polyamine biosynthesis pathway. Because polyamines are involved in the control of cell replication and differentiation, a change in cellular ODC activity is relevant to carcinogenesis. Tumour promoters such as TPA induce ODC activity, and a high level of ODC activity has been found in several premalignant conditions.

[Byus et al. \(1988\)](#) exposed Reuber H35 hepatoma, Chinese hamster ovary (CHO), and human 294 T melanoma cells to

amplitude-modulated RF radiation at 450 MHz (SAR not given; power density, 1.0 mW/cm<sup>2</sup>) for 1 hour. A 50% increase in ODC activity was observed after exposure to RF radiation alone. In addition, ODC activity induced by TPA was further enhanced by exposure to RF radiation in H35 and CHO cells.

[Litovitz et al. \(1993\)](#) reported a 90% increase in ODC activity in murine L929 fibroblasts exposed to RF radiation at 915 MHz (SAR, 2.5 W/kg; amplitude-modulated at 55, 60, or 65 Hz) for 8 hours. A continuous-wave signal did not affect cellular ODC activity. Subsequent findings from the same laboratory ([Litovitz et al., 1997](#); [Penafiel et al., 1997](#)) showed increased ODC activity in L929 cells exposed at 840 MHz (SAR, 2.5 W/kg) as a TDMA mobile-phone signal (burst-modulated at 50 Hz, with 33% duty cycle) for 2–24 hours. Also, signals with amplitude modulation at 60 Hz or 50 Hz induced ODC activity, whereas a signal modulated with speech, the signal of an analogue mobile phone, or a signal frequency modulated at 60 Hz, did not affect ODC activity. Various exposure times between 2 hours and 24 hours were used and the effect was most pronounced after exposure for 8 hours.

[Desta et al. \(2003\)](#), in an attempt to replicate the study of [Penafiel et al. \(1997\)](#), did not find any increase in ODC activity in murine L929 cells exposed to RF radiation at 835 MHz (SAR, < 1 W/kg; TDMA modulated) for 8 hours. In contrast, a decrease in ODC activity was observed at SARs of 1–5 W/kg. This decrease became statistically significant at SAR values > 6 W/kg, associated with a temperature increase of > 1 °C in the cell-culture medium.

In another replication study, [Höytö et al. \(2007\)](#) found no increase in ODC activity in L929 cells from two different sources using the same exposure system as [Penafiel et al. \(1997\)](#): a decrease in ODC activity was observed at the highest SAR used (6 W/kg). With a different exposure system and better temperature control

was used, a small increase in ODC activity was observed after 8 hours of exposure at 6 W/kg. This increase could be related to the temperature-control system, creating a temperature gradient in the cell cultures (lower temperature at the bottom of the cell culture). [Höytö et al. \(2006\)](#) reported no effects on ODC activity in L929 cells exposed to continuous-wave or GSM-modulated RF radiation at 900 MHz (SAR, 0.2 or 0.4 W/kg) for 2, 8, or 24 hours. ODC activity decreased after conventional heating (without exposure to RF radiation), consistent with the findings of [Desta et al. \(2003\)](#). Apparently, temperature differences of < 1 °C are sufficient to influence ODC activity.

[Höytö et al. \(2007b\)](#) also exposed L929 murine fibroblasts, rat C6 glioblastoma cells, human SH-SY5Y neuroblastoma cells, and rat primary astrocytes to continuous-wave and GSM-modulated RF radiation at 815 MHz (SAR, 1.5, 2.5 or 6 W/kg) for 2, 8 or 24 hours. A significant decrease in ODC activity was consistently observed in all experiments with rat primary astrocytes exposed to GSM-modulated or continuous-wave RF radiation at SARs of 1.5 or 6.0 W/kg. No effects were seen in the other cell lines.

[Billaudel et al. \(2009a\)](#) found no effects on ODC activity in L929 mouse fibroblasts exposed to RF radiation at 835 MHz, 900 MHz, or 1800 MHz as GSM or DAMPS-modulated signals (SAR, 0.5–2.5 W/kg) for 2–24 hours. The same authors reported that – consistent with the findings in murine cells – ODC activity was unaffected in human SH-SY5Y neuroblastoma cells exposed to GSM-modulated RF radiation at 1800 MHz, or DAMPS-modulated RF radiation at 835 MHz (SAR for both, 1 or 2.5 W/kg) for 8 or 24 hours ([Billaudel et al., 2009b](#)).

[The Working Group concluded that there was moderate evidence that RF radiation alters ODC activity.]

#### (iv) Apoptosis

Rat embryo primary neurons were exposed to continuous-wave RF radiation at 900 MHz (SAR, 2 W/Kg) for 24 hours. Because the temperature increased by 2 °C during the exposure, a control experiment at 39 °C was included (without RF radiation). Apoptosis was measured with two different methods (staining of nuclei with 4',6-diamino-2-phenylindole (DAPI) and analysis of DNA fragmentation with TUNEL-flow cytometry). With both techniques, a highly significant increase in the percentage of apoptotic cells was seen at 24 hours after exposure, compared with the sham-exposed cells and the cells incubated at 39 °C ([Joubert et al. \(2008\)](#)).

[Nikolova et al. \(2005\)](#) exposed mouse embryonic stem cell-derived neural progenitor cells to intermittent (5 minutes on, 30 minutes off) GSM-modulated RF radiation at 1710 MHz (time-averaged SAR, 1.5 W/kg; during actual exposure, 12 W/kg) for 6 or 48 hours. No effects on apoptosis or on mitochondrial membrane potential were found.

[Höytö et al. \(2008a\)](#) exposed mouse L929 cells to 872 MHz continuous-wave or GSM-modulated RF radiation (SAR of 5 W/kg) for 1 or 24 hours under isothermal conditions. Menadione-induced apoptosis (tested by measuring caspase-3 activity) was increased in cells exposed to the GSM-modulated signal, but not in cells exposed to the continuous-wave signal. No effects were seen from RF radiation in the absence of menadione. As described earlier, no effects or RF radiation on apoptosis were observed in human cells in this same study.

[Höytö et al. \(2008b\)](#) exposed mouse L929 fibroblasts that had been stimulated with fresh medium, stressed by serum deprivation, or not subjected to stimulation or stress, to continuous-wave or GSM-modulated RF radiation at 872 MHz (SAR, 5 W/kg) for 1 hour under isothermal conditions. Increased apoptosis (tested by measuring caspase-3 activity) was

seen as a response to serum deprivation, but no consistent effects of exposure to RF radiation were found.

[Joubert et al. \(2007\)](#) studied apoptosis in rat primary cortical neurons exposed to GSM-modulated RF radiation at 900 MHz (SAR, 0.25 W/kg), or continuous-wave at 900 MHz (SAR, 2 W/kg) for 24 hours. No effects on apoptosis were detected, either just after the exposure or 24 hours later, with three different techniques, *viz.* 4',6-diamino-2-phenylindole (DAPI) staining, flow cytometry with double staining (TUNEL and propidium iodide), or measurement of caspase-3 activity by fluorometry.

[Zhao et al. \(2007a\)](#) exposed cultured primary mouse embryonal neurons and astrocytes to 1900 MHz RF radiation from a working mobile phone (SAR not given) for 2 hours. The phone was placed with its antenna over the centre of the culture dish. During sham-exposures the phone was on “stand-by.” Three apoptosis-associated genes (*Pycard*, encoding the Asc protein – apoptosis-associated speck-like protein containing a caspase-recruitment domain – *Casp2*, and *Casp6*) were upregulated in neurons, both after exposure and sham-exposure. In astrocytes the upregulation was observed in exposed cells only. In addition, the astrocytes – not the neurons – showed RF radiation-dependent upregulation of the *Bax* gene. [The Working Group noted the ill-defined exposure conditions in this study; see above.]

[Moquet et al. \(2008\)](#) exposed mouse neuroblastoma N2a cells to RF radiation at 935 MHz (SAR, 2 W/kg) for 24 hours, as GSM basic (amplitude-modulated), GSM “talk,” and continuous-wave signal. No significant differences in levels of apoptosis were observed between exposed and sham-exposed cells.

[The Working Group concluded that there is weak evidence that RF radiation affects apoptosis in mammalian cells.]

## 4.5 Physical factors that affect interpretation of study results

### 4.5.1 Effects of critical RF-field parameters

#### (a) Modulation

There is evidence that modulation of the carrier waves of RF radiation can cause changes in biological processes that do not occur when the waves are not modulated. Examples of biological reactions to modulated RF radiation were clearly shown by [Bawin et al. \(1975\)](#), replicated by [Blackman et al. \(1979\)](#). For more examples and details, see the reviews by [Blackman \(2009\)](#) and [Juutilainen et al. \(2011\)](#).

#### (b) Power-intensity “windows”

Studies by [Bawin et al. \(1975, 1978\)](#) and [Blackman et al. \(1980\)](#) have characterized the power-density response in detail for the RF radiation-induced release of calcium ions from the chick brain *ex vivo*. Both groups observed regions of power density, termed “windows,” in which the release of calcium ions was exposure-dependent, separated by regions that did not respond as a function of the power density of incident radiation. Subsequent reports by [Dutta et al. \(1984, 1989\)](#) revealed similar power-density windows of induced response in nervous system-derived cultures of human and animal cells, and [Schwartz et al. \(1990\)](#) observed windows of calcium-ion release from the frog heart *ex vivo*. This phenomenon appeared to be caused by the response characteristics of the particular biological preparations. The extensive characterization of exposure–response at 50, 147 and 450 MHz (amplitude-modulated, 16 Hz) in the chick brain showed that the windows could be aligned across carrier frequencies if one used the calculated electric-field strength at the tissue surface, rather than the incident power density ([Joines & Blackman, 1980, 1981](#); [Joines et al., 1981](#); [Blackman et al., 1981, 1989](#)). See reviews by [Blackman \(2009\)](#) and [Belyaev \(2010\)](#).

#### 4.5.2 Frequency dependence and frequency windows

Effects of RF radiation are dependent on the frequency of the carrier wave. Differences in the response of human cells to GSM-type RF radiation were observed at frequency channels of 905 and 915 MHz, where the other conditions of exposure were the same ([Belyaev et al., 2009](#); [Markovà et al., 2010](#)). Thus, it is important to know which difference in carrier frequency is acceptable to compare results from different studies.

The frequency-dependence of the effects of microwave radiation in different model systems and with different end-points measured has been reviewed ([Grundler, 1992](#); [Grundler et al., 1992](#); [Belyaev et al., 2000](#); [Belyaev, 2005, 2010](#)). The effects of resonance-type microwave radiation were observed within multiple frequency-windows at intensity values well below those at which any thermal effects had been observed. The half-width of resonances and distance between them varied in dependence on the intensity of the RF radiation. Sharper and narrower resonances, and half-widths reaching at least 2 MHz were observed at the lower intensities.

#### 4.5.3 Polarization

Different kinds of polarization were applied in the experimental studies discussed above: linear, left-handed circular, and right-handed circular polarization. It has been shown in many studies that biological effects are dependent upon polarization ([Belyaev et al., 1992a, c, d, 1993a, b](#); [Shcheglov et al., 1997](#); [Ushakov et al., 1999, 2006a](#); [Belyaev & Kravchenko, 1994](#); [Belyaev, 2010](#)). For example, polarization should be taken into account when attempting to replicate the results of previous studies. For example, [Lai & Singh \(1996\)](#) used circular polarization, whereas linear polarization was applied in subsequent studies aimed at replicating their results, thus reducing sensitivity.

#### 4.5.4 Dose and duration of exposure

While accumulated absorbed energy is measured as “dose” (dose rate multiplied by exposure time) in radiobiology, guidelines for exposures to RF radiation usually state power density or SAR (dose rate analogue) to define exposure. Several studies have analysed the relationship between dose and duration of exposure, with results suggesting that duration of exposure and dose may be important for cancer-relevant effects. In particular, prolonging the duration of exposure could compensate for the effects of a reduction in intensity.

[Kwee & Raskmark \(1998\)](#) analysed proliferation of human epithelial amnion cells exposed to RF radiation at 960 MHz, with SARs of 0.021, 0.21, or 2.1 mW/kg. These authors reported linear correlations between duration of exposure at 0.021 and 2.1 mW/kg and changes in cell proliferation, although no clear correlation was seen at 0.21 mW/kg.

Exposure of *E. coli* and rat thymocytes to RF radiation at power densities 0.01–1 mW/cm<sup>2</sup> resulted in significant changes in chromatin conformational state, if exposure was performed at resonance frequencies for 5–10 minutes ([Belyaev et al., 1992a, b](#); [Belyaev & Kravchenko, 1994](#)). Decreases in these effects caused by lowering the power density by an order of magnitude could be compensated for by a several-fold increase in the duration of exposure. At exposures longer than 1 hour, the same effect could be observed even at the lowest power density ([Belyaev et al., 1994](#)).

#### 4.5.5 Background fields of extremely low frequency (ELF)

Background ELF (1–300 Hz) fields vary between laboratories. Even within the same laboratory or the same RF exposure system, variations of up to 5 µT are not uncommon. Four studies investigated the influence of background

ELF fields on the effects of exposure to RF radiation: ODC activity in L929 cells (Litovitz *et al.*, 1997), hypoxia sensitization caused by long-term repeated exposures of chick embryos (Di Carlo *et al.*, 2002), spatial learning deficits in rats induced by microwave radiation (Lai, 2004), and DNA-damage induction in rat brain cells (Lai & Singh, 2005). In these studies, the effects caused by RF radiation were significantly reduced by imposing an ELF field of up to 5  $\mu$ T.

#### 4.5.6 Net static geomagnetic field

The static geomagnetic field (30–70  $\mu$ T, depending on the location) may alter the cellular response to RF radiation (Belyaev *et al.*, 1994; Ushakov *et al.*, 2006b). Net static magnetic fields vary by location, even within the same laboratory and with the same exposure system, due to the ferromagnetic properties of laboratory equipment. For example, the resonance effects of microwave radiation on DNA repair and chromatin conformation in *E. coli* depend on the magnitude of the net static geomagnetic field at the site of exposure (Belyaev *et al.*, 1994; Ushakov *et al.*, 2006b).

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