

Toxicologic Study of Electromagnetic Radiation Emitted by Television and Video Display Screens and Cellular Telephones on Chickens and Mice

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Key Words

Electromagnetic radiation · Television screens · Video display units · Cellular telephone · Chickens · Mice

Abstract

The effects of continuous exposure of chick embryos and young chickens to the electromagnetic fields (EMFs) emitted by video display units (VDUs) and GSM cell phone radiation, either the whole spectrum emitted or attenuated by a copper gauze, were investigated. Permanent exposure to the EMFs radiated by a VDU was associated with significantly increased fetal loss (47–68%) and markedly depressed levels of circulating specific antibodies (IgG), corticosterone and melatonin. We have also shown that under chronic exposure conditions, GSM cell phone radiation was harmful to chick embryos, stressful for healthy mice and, in this species, synergistic with cancer insofar as it depleted stress hormones. The same pathological results were observed after substantial reduction of the microwaves radiated from the cell phone by attenuating them with a copper gauze.

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Introduction

A number of studies have suggested a possible bond between exposure to non-ionising electromagnetic fields (EMFs) and an increase in health risks. This concern relates equally to the microwaves emitted by cellular telephones and the extremely low frequency (ELF) waves emitted both by video display screens and by cellular telephones. Symptoms such as feelings of tiredness, high temperature, burning sensation of the skin and headaches have been reported [1] after use of cellular telephones, which could be related to local heating by the microwaves. Other effects which have been observed include disturbances of sleep [2], a deterioration of cerebral activity [3, 4], changes in the permeability of the meninges [5], an increase in blood pressure [6], induction of stress [7], hormonal disturbances [8–10] and carcinogenic changes [11–13]. Similarly, papers have been published which attracted the attention of scientists and the population in general and which purported to show a relationship between exposures to ELF and an increase in certain health risks such as cancer [14, 15], abortion [16, 17], suicide and depression [18, 19].

Various animal models have made it possible to show that exposure to ELF affects embryonic development

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[20–24], the immune system [25, 26] or the neuro-immuno-endocrine functions [27, 28]. In the present study, we have analysed the effects of the total EMFs produced by both a television and a computer, on embryonic mortality and neuro-immuno-endocrine changes in chickens. We also analysed the effects of the electromagnetic radiation emitted by a GSM¹ cellular telephone by considering firstly the electromagnetic emissions as a whole and then after eliminating a major part of the microwaves by interposition of a copper gauze. This second analysis used as end points the mortality of embryo chickens and variations in the stress hormones ACTH and corticosterone both in healthy mice and in mice with tumours (Lewis lung carcinoma or LLC1). Any increased development of metastases was also studied using this last model.

Under the experimental conditions used for the toxicology study, the embryos, young chickens or the two categories of mouse were irradiated continuously by the apparatus under study. This protocol was selected in order to determine the biological end points most sensitive to the EMFs, which would then make it possible to choose the best models to be used for determinations in an experimental design closer to reality.

We were able to show that the avian and murine models used were sensitive to the EMFs emitted both by the display screens and by the cellular telephones.

Methodology

Systems of Exposure

Video Display Screens

Emission sources were either a television (TV, Thompson, D 55 cm, 55MxP83, 75 W/50 Hz) or a microcomputer (Goupil G5 286 10, 220 V, 50 Hz). Both types of apparatus use cathode ray tubes as video displays. These emit a whole spectrum of electromagnetic radiation that includes a small amount at radio frequencies, some infrared, microwaves and X-rays. Most of the radiation is visible light, VLF and ELF radiation. These units are regulated with regard to some emissions, particularly X-rays. The major emissions were VLF and ELF [29].

Cellular Telephone

The emission source was a GSM digital telephone 900 MHz (SAGEM, France) emitting 2 W at maximum power. A series of experiments (mortality of the chicken embryos) was carried out with a mechanical system of automatic calling and reception. The me-

chanical system was placed at 1 m from the telephone whose face was turned towards incubating eggs. A second entirely automated system was used for exposure of the mice. In all the cases, calls were sent automatically every minute. An earthed metal grid (Soulas & Co., Montreuil Underwood, France) 0.47 m × 0.57 m and mesh diameter of 350 μm, interposed between eggs or the mice and the telephone, enabled us to stop the major part of the microwaves while letting the magnetic component of the ELF pass. This grid did not touch the animals but was placed 1 cm from the eggs or the mice.

Measurement of Microwave Emissions and ELF

The field strength of the ELF emitted during operation of the display screens lay between 135 and 270 nT from the TV screen (experiment 1) and 140 and 660 nT at the side of the computer from the screen (experiments 2 and 3). The eggs or the young chickens were located between 50 and 80 cm from the screen. VLF emissions were negligible (between 2 and 13 nT) [30]. The measurement of the microwaves emitted from the cellular telephone was carried out at many points, approximately 1 cm above the surface of eggs and 4 cm below the telephone. Microwaves were evaluated by their electric component with a probe PMM BA 05, Italy (measurements carried out by Montena Co., Rossens, Switzerland and Emitech, Vendargues, France) for a frequency of 900 MHz and are distributed as shown in figure 1a compared to the site of the telephone; the copper gauze modified the exposure of eggs as the microwave distribution in figure 1b shows. The ELF magnetic fields were measured with a probe HP 8568 (Hewlett Packard, Palo Alto, Calif., USA, measurements taken by Emitech Co. at a central frequency of 200 Hz; a sweep in frequency of 0 Hz; analysis bandwidth of 10 Hz; video filter 3 Hz; scanning rate 20 s). Measurements gave the values shown in figure 2a. The emissions were not stopped by the copper gauze as shown in figure 2b.

Biological Material

Eggs, Embryos and Chickens

The fertilised chicken eggs came from the stock Kabir Blache strain (Couvoir Cévennes Camargue, Lédénon, France). The young chickens used in the experiments carried out with the display screens came from surviving chicks irradiated during their embryonic life.

Mice

Four-week-old C57BL/6 j RJ mice (Janvier, Le Genes-St-Isle, France) were placed in non-metal cages (lighting 12 h/12 h, temperature 22 °C, food and water ad libitum). The tumour group of mice received cells of the Lewis pulmonary carcinoma (LLC1), which had been cultivated in medium RPMI 1640 (Eurobio, Ullis, France) supplemented with 10% of fetal calf serum (Gibco BRL, Germany), L-glutamine, penicillin, streptomycin at 37 °C in a humid atmosphere with 5% CO₂. LLC1 cells were injected into the muscle of the right hind leg at the beginning of the 12th week of irradiation.

Antigen Used

The young chickens (VDU experiments) were immunised by injecting subcutaneously porcine thyroglobulin at a rate of 125 μg/100 g of mouse. The first immunisation was carried out using Freund's complete adjuvant and the 2nd and 3rd injections with the addition of Freund's incomplete adjuvant.

¹ GSM = Global System for Mobile Communication, the cellular phone system currently used in Europe, Africa, Asia (not Japan) and some of the US.

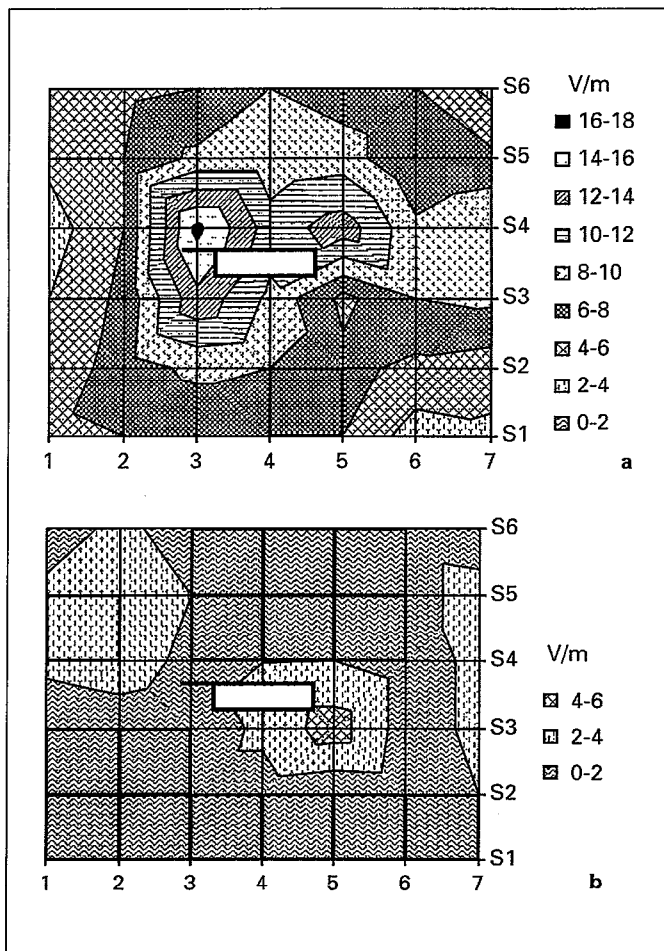


Fig. 1. Value of microwaves without (a) and with (b) the copper gauze (the position of the telephone is noted schematically).

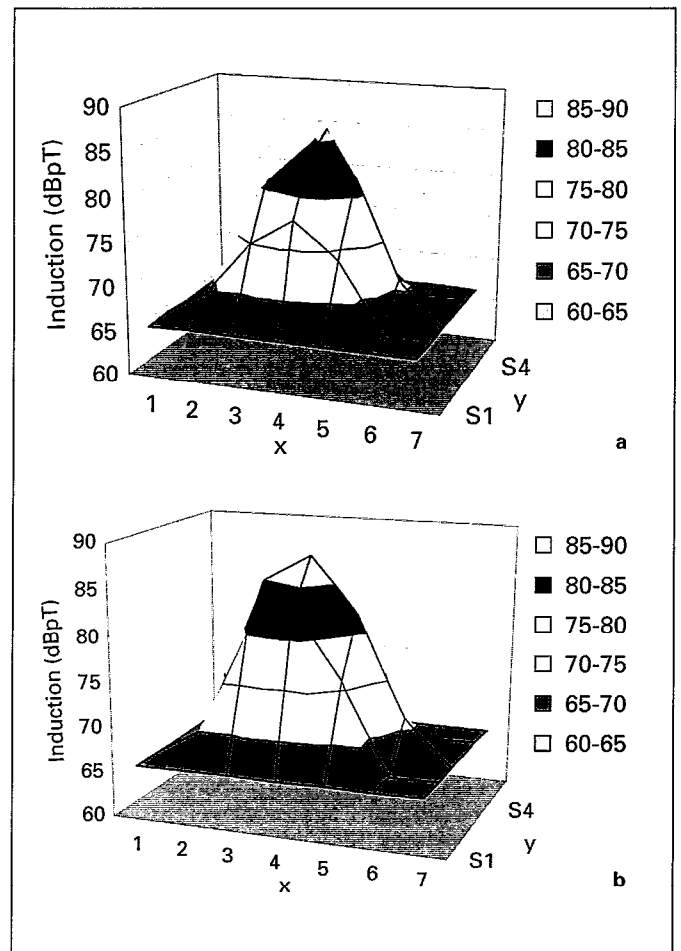


Fig. 2. Representation of the values of the ELF magnetic fields without (a) and with (b) the copper gauze.

Dosage

Hormone Levels in Young Chickens

Plasma corticosterone and melatonin were titrated by protein-binding competition and radio-immunological assay, respectively [30–33].

Stress Hormone Levels in the Mouse

The levels of corticosterone and plasma ACTH were measured by radioimmunological assay (ICN Pharmaceutical Inc., Costa Mesa, Calif., USA). For ACTH, the coefficients of variation for intra- and inter-comparisons were 5 and 7.5%, respectively, with a detection limit of 10 pg/ml, and for corticosterone 7% with a detection limit of 25 ng/ml.

Antibody Levels in Young Chickens

The level of antibodies of porcine IgG-type antithyroglobulin was determined by immuno-enzymatic technique (ELISA) in chicken serum according to the method described by Youbicier-Simo et al. [31]. The antibody titre was defined as the inverse of the plasma dilu-

tion having an absorbance equal to 1. The results are given in the form of the decimal logarithm of the serum titre.

Statistical Analysis

All the data (mean \pm SEM) were analysed by the Mann-Whitney U test.

Experimental Protocols

Experiments Analysing the Effect of the Display Screens (VDU) on Embryonic Mortality and Neuro-Immuno-Endocrine Behaviour of Young Chickens [30]

Experiment 1. The egg embryos were placed in front of the TV, which was turned on but covered with a black screen to cut off the light. The 'exposed' batch of 30 eggs were placed between 50 and 80 cm from the screen in the incubation room and were compared with a control batch of 30 eggs similarly placed but with the TV off. The eggs were irradiated continuously until hatching. The eggs were turned manually and candled during incubation in order to follow

the kinetics of mortality of the embryos (this observation is impossible from day 13 of incubation until hatching because of the opacity of the eggs). The young chickens resulting from the two groups (control and radiation-exposed) were again exposed under the same conditions as the eggs until the end of the experiment. They were immunised with porcine thyroglobulin at the age of 21, 30 and 39 days. Blood was taken for analysis the day preceding the immunisation and also on the 47th day, at the end of the experiment.

Experiment 2. This experiment was similar in all respects to experiment 1 with three modifications: the display screen belonged to a computer (see 'Systems of Exposure' above); the eggs were placed at the same distance but to the side of the apparatus; blood was taken from the chickens on the day preceding the immunisation and on day 38 to measure the maximum hormone levels.

Experiment 3. The two preceding experiments had shown a significant mortality in the exposed embryos. Therefore, a third experiment with batches of 60 eggs, controls and exposed, was carried out in order to check the validity of the observation. The experiment was not continued with the hatched chicks.

Experiments Analysing the Effect of Cellular Telephones on the Mortality of Chicken Embryos

The embryos used in these experiments were exposed in three treatment categories: one group received the totality of the telephone emissions (telephone group); a second group received the emissions filtered by the copper gauze whose mesh was supposed to stop the microwaves while letting ELF pass (telephone + gauze group), and a third group which was not exposed to the telephone (control group). As two groups could be incubated at a time it was possible to expose the eggs simultaneously and the experiments were performed in pairs: control vs. telephone and control vs. telephone + gauze. The two rooms in which the experiments were conducted were examined beforehand to check that the conditions in each were the same.

Experiments 1, 2 and 3: Control vs. Telephone. The protocol was the same as for experiment 3 with the VDU in which 60 fertilised eggs were subjected to the permanent radiation from the telephone which had a mechanical system arranged to automatically call every minute. The telephone was placed in the centre of the plate supporting the eggs at a height of 10 cm and 5 cm from the surface of the eggs. Using the protocol made it possible to count the mortality kinetics until the 13th day as well as total mortality at 21 days. Three identical experiments were carried out.

Experiments 3 and 4: Control vs. Telephone + Gauze. The protocol used was identical to that for the three preceding experiments with the difference that a copper gauze of 350 µm mesh was interposed between the eggs and the telephone located at 5 cm from the top of the eggs without touching them. The conditions of incubation and the reading of the mortality of the embryos were as before. Two identical experiments were carried out.

Experiments Analysing the Effect of the Cellular Telephone on Healthy Mice and on Mice Carrying a Lewis Tumour

The aim of these experiments was to measure the influence of exposure to the radiation from the cellular telephone on the plasma levels of the stress hormones (ACTH and corticosterone) in the mice, either in healthy mice or in mice carrying the Lewis tumour (pulmonary carcinoma). This tumour readily develops metastases, and a search was made for any possible influence of the electromagnetic radiation on the progress of the tumour.

Experiment 1 (Mice with Tumour). Sixty 4-week-old C57BL/6j RJ mice were randomised into four groups of 15 mice each. The mice were placed in non-metal cages. After 1 week of acclimatisation and under living conditions which conformed with the protocol for experimentation on mice (see 'Biological Material' above), the mice were subjected to the radiation produced by the telephone placed 4 cm below their cage, which continuously received an automatically generated call every minute. The irradiation lasted for 15 weeks, from the age of 5 weeks up to 20 weeks. Lewis tumour (LLC1) cells were injected into the left hind leg of the mice at 17 weeks of age (after 12 weeks of irradiation). Four groups of mice were studied: (1) a control group without tumour, not exposed to radiation but in the presence of a telephone which was turned off, and which received an injection of physiological saline; (2) a group with the tumour, not exposed to radiation but in the presence of a telephone which was turned off; (3) a group telephone + tumour which received both the tumour and exposure to radiation from the telephone, and (4) a telephone + gauze + tumour group which received the tumour but was protected from the microwaves of the telephone by the copper mesh. After 15 weeks of irradiation (age of the mice 20 weeks), retro-orbital blood samples were taken from the mice between 10.00 and 12.00 h for measuring the stress hormones and the mice were sacrificed for examination of the tumours and any metastases.

Experiment 2 (Healthy Mice Given the Tumour at 17 Weeks). The protocol was exactly the same as in the preceding experiment. However, two additional measurements of stress hormones were carried out to identify possible variations in mice not yet injected with the tumour after 2 and 5 weeks of exposure (when the mice were 7 and 10 weeks old, respectively). This was done in order to evaluate the impact of the effect of the telephone on healthy mice and after shorter exposures. The experiment continued as previously with the tumour given after 12 weeks of irradiation (at age 17 weeks) and evaluation of the results after 15 weeks of irradiation including three in the presence of the tumour (age of the mice 20 weeks).

Results

Experiments to Analyse the Effect of the Display Screens (VDU) on Embryonic Mortality and the Neuro-Immuno-Endocrine Behaviour of Young Chickens

Mortality of the Embryos in Experiments 1, 2 and 3. The results from the exposed groups showed mortality rates of 57, 47 and 68%, respectively in the exposed groups for experiments 1, 2 and 3 with levels in the corresponding control groups at 27, 33 and 10%, respectively. These experiments were carried out in different seasons which could explain the variations observed by the experimenters. A kinetic study of the mortalities showed that a greater number of embryos died at the end of their embryonic development in the controls, but deaths were spread throughout their fetal life for the exposed batches.

Immune Response and Hormone Levels of Young Exposed Chickens. The young chickens hatched from the

Table 1. Effect of the EMFs emitted by the VDU on the blood levels of corticosterone, melatonin and IgG class antibody in young chickens immunised with pig thyroglobulin

Dosages	Experiment	Groups	Day 20	Day 29	Day 35	Day 38	Day 47
Corticosterone, ng/ml	1	control (n = 10)	3.0±0.1	3.4±0.1	–	6.0±0.2	2.6±0.1
		exposed (n = 10)	2.3±0.2	2.0±0.2	–	2.5±0.1	2.4±0.2
	2	control (n = 17)	2.4±0.1	3.5±0.1	4.5±0.1	8.6±0.4	–
		exposed (n = 10)	2.5±0.1	3.0±0.1	3.4±0.1	4.0±0.1	–
Antibody IgG, log of titre	1	control (n = 10)	1.6±0.1	3.4±0.2	–	4.0±0.1	3.7±0.7 ^{a,b}
		exposed (n = 10)	1.5±0.1	2.0±0.2	–	2.7±0.3	2.7±0.4
	2	control (n = 17)	1.3±0.0	3.8±0.2	4.8±0.5 ^{a,b}	5.0±0.3 ^{a,b}	–
		experiments (n = 10)	1.5±0.9	2.2±0.4	3.8±0.2 ^a	2.8±0.2	–
Melatonin, pg/ml	1	control (n = 10)	–	–	–	–	–
		exposed (n = 10)	–	–	–	–	–
	2	control (n = 17)	8.5±1.5	36.0±8.0 ^{a,b}	66.5±8.8 ^{a,b}	49.0±2.0 ^{a,b}	–
		exposed (n = 10)	7.0±2.0	3.5±2.0	0.5±0.5	4.0±0.5	–

In experiment 1, the chickens were immunised with the porcine thyroglobulin at the age of 21, 30 and 39 days and blood samples were taken on days 20, 29, and 35 and 47 so that the corticosterone and the antibodies could be determined.

In experiment 2, the immunisations were carried out at the age of 21, 30, and 36 days and blood samples were taken on days 20, 29, and 35 and 38. Melatonin was also determined.

Significant differences in results, compared to day 20: ^a p < 0.01; compared to exposed group: ^b p < 0.01.

surviving irradiated embryos were immunised by a xeno-antigen (porcine thyroglobulin) so that they produced a humoral response. The results of measurements of their antibody titre are given in table 1. One notes a reduction of the titre of almost 50% by the end of the immunisation in the exposed animals compared with controls. The hormones studied (corticosterone and melatonin) show a decrease in corticosterone greater than 50% on the 38th day (peak of regulation of the secondary humoral response) and a collapse in melatonin levels in the exposed group (table 1).

Experiments Analysing the Effect of the Cellular Telephones on the Mortality of the Chicken Embryos
Mortality of the Embryos in Controls vs. Telephone Group. The embryos exposed to the electromagnetic fields emitted by the cellular telephone were sensitive to the total radiation (microwaves and ELF) given out by the telephone (experiments 1, 2 and 3, table 2). The death rates were fairly similar in the three experiments undertaken on groups of 60 eggs (table 2) with a rather constant progression of mortality over the course of time. Death was noted when candling during the incubation of the eggs and the date verified at the time of hatching. There is no bias in this type of model. Total mortality was on average 11% of control embryos in the first three experiments

and 64% in those eggs exposed to radiation, which corresponds to a specific mortality due to EMFs of approximately 52%. The maximum level of embryonic mortality was observed in those eggs in the vicinity of the telephone and its antenna.

Mortality of the Embryos in Controls vs. Telephone + Gauze Group. The copper grid interposed between the telephone and the eggs in experiments 4 and 5 did not appreciably modify the results of the previous experiments (table 2). The batches of eggs used in these experiments were apparently more fragile than those used formerly since spontaneous mortality rose to 25% in the two experiments while the mortality of the exposed batches protected by the gauze rose to 75% on average (mortality specific to EMFs was always 54% on average). The kinetics of embryonic mortality over the time course of the experiment was practically always the same. The pattern of embryonic mortality was more homogeneous when the copper gauze was present.

Experiments Analysing the Effect of the Cellular Telephone on Healthy Mice and Those Carrying a Lewis Tumour

The results obtained with the mice are divided into two parts: the first piece of work relates to mice intended to develop cancer with a Lewis tumour (murine pulmo-

Table 2. Effect of exposure to the EMF emitted by a cellular telephone on the survival of chick embryos

Experiment	Date	Groups	Eggs incubated day 1	Fertile eggs day 3	Embryos dead (cumulative mortality)							Total mortality, % day 21	Mortality due to EMF, % day 21
					day 3	day 5	day 7	day 9	day 11	day 13	day 21		
1	June	control	60	59	1	1	1	2	2	2	7	12	47
		telephone	60	59	6	8	10	15	17	18	35	59	
2	July	control	60	55	0	0	0	0	0	0	6	11	47
		telephone	60	59	7	8	8	10	17	17	34	58	
3	March	control	60	56	0	0	0	0	0	0	6	11	61
		telephone	60	56	3	3	3	5	8	12	40	71	
4	April	control	60	57	2	4	5	5	6	6	14	25	50
		telephone + gauze	60	58	2	4	8	16	19	19	43	74	
5	May	control	60	58	3	4	4	4	5	5	11	19	59
		telephone + gauze	60	58	3	4	7	15	25	28	45	78	

Table 3. Plasma levels of ACTH and corticosterone in mice with tumours exposed continuously to a cellular telephone for 15 weeks with or without copper gauze

Hormones	Experimental groups			
	control	with tumours	telephone + tumour	telephone + copper gauze + tumour
First experiment				
ACTH, pg/ml	244 ± 12	234 ± 15	92 ± 6 ^c	92 ± 7 ^c
Corticosterone, ng/ml	282 ± 13	156 ± 9 ^a	107 ± 7 ^d	113 ± 9 ^d
Second experiment				
ACTH, pg/ml	236 ± 23	223 ± 11	117 ± 9 ^d	113 ± 9 ^d
Corticosterone, ng/ml	234 ± 10	138 ± 9 ^b	110 ± 10 ^d	95 ± 10 ^d

Statistical significance vs. control: ^a p < 0.01, ^b p < 0.05; vs. tumour: ^c p < 0.01, ^d p < 0.05.

nary carcinoma) that metastasises very easily. The animals were continuously exposed, as in a conventional toxicology study of a physical agent, to irradiation for the 15 weeks that the experiment lasted. The results were negative and no really significant effect from this continuous exposure to the telephone was observed either on the dimensions of the induced tumours or the number of pulmonary metastases.

On the other hand, the results obtained from the chickens exposed to the display screens which showed neuro-immuno-endocrine modification [30] had demonstrated that an effect of this type could occur. We thus took blood samples from the mice at the time of the sacrifice before the lethal anaesthesia to measure the levels of stress hormones (corticosterone and ACTH). Identical results were obtained in the two experiments carried out (table 3). We

noted a fall in corticosterone levels due to the presence of the tumour, which was accentuated by irradiation both without (telephone + tumour group) or with the copper gauze (telephone + gauze + tumour group). The tumour did not affect the plasma level of ACTH whereas the irradiation did, in the same way to that found previously. Irradiation caused a significant fall in the plasma level of this hormone in the mice exposed without (telephone + tumour batches) or with the copper gauze (telephone + gauze + tumour group).

During the second experiment and before the implantation of the tumour, which occurred in the 12th week of irradiation, we checked whether early on in the irradiation it could already have an effect on the plasma levels of corticosterone and ACTH in the young but still healthy mice. Measurements were carried out at two time points,

Table 4. Plasma levels of ACTH and corticosterone in healthy mice irradiated continuously by the cellular telephone in the presence or not of copper gauze

	Experimental groups		
	control	telephone	telephone + gauze
After 2 weeks of irradiation			
ACTH, pg/ml	252 ± 32	144 ± 21*	98 ± 14*
Corticosterone, ng/ml	253 ± 37	146 ± 11*	178 ± 5*
After 5 weeks of irradiation			
ACTH, pg/ml	245 ± 18	138 ± 18*	126 ± 27*
Corticosterone, ng/ml	228 ± 11	168 ± 16*	149 ± 16*

* p < 0.05.

one after 2 weeks of irradiation (7-week-old mice), the other after 5 weeks (10-week-old mice). The results presented in table 4 show a significant fall of 42% in corticosterone in the 2nd week for those exposed to the total emissions from the telephone (telephone group) and of 30% with the copper gauze in place (telephone + gauze group), with a slight increase (to 26% reduction) in the 5th week for the telephone group. Levels in the group exposed through the copper gauze continued to drop (35%). ACTH dropped to nearly half (43% at 2 weeks) where it remained (44% at 5 weeks) for the group exposed to the total emissions from the telephone (telephone group). The effect was less with the copper gauze in place, but there was still a drop in the telephone + gauze group (61 and 49%, respectively, for 2 and 5 weeks of exposure).

Conclusion

The results obtained with the three pieces of apparatus and the various models studied *in vivo* have enabled us to conclude that the electromagnetic radiation emitted by the equipment is harmful to biological systems. Under the experimental condition of continuous emission used it was possible for us to determine those biological end points that could be used to highlight any effect related to more traditional (human) use of the apparatus. The results suggest that it would be better to restrict the time spent close to the screens of computers and to cellular telephones.

The phenomena observed following exposure to the total radiation emitted by the apparatus do not define precisely the frequencies responsible for the effects. Our experiments only enable us to identify the spectral bands (ELF, VLF for the VDU screens; ELF, MW for the cellu-

lar telephones). Using copper gauze with a mesh size of 350 µm leads us to suggest that the damaging radiation is either the residual microwaves whose maximum intensity does not exceed 3–4 V/m, or the magnetic component of the ELF, or a combination of the two types of waves. However, the biological effects caused by exposure to the radiation emitted by the VDU screens suggest that the ELF is the most important. Measurements of the ELF of 200 Hz emitted by the cellular telephone showed that they attenuated quickly and were negligible only a few centimetres from the telephone (fig. 2). We would like to propose, therefore, another hypothesis that implicates the very low frequency ELF (8 Hz) emitted by the telephone and suggest that these interfere either with endogenous ELF of similar frequency (for example alpha waves of the brain, etc.) or with environmental ELF (waves of Schumann) [34].

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