Tumor promotion by exposure to radiofrequency electromagnetic fields below exposure limits for humans

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ABSTRACT

The vast majority of in *vitro* and *in vivo* studies did not find cancerogenic effects of exposure to electromagnetic fields (RF-EMF). i.e. emitted by mobile phones and base stations. Previously published results from a pilot study with carcinogen-treated mice, however, suggested tumor-promoting effects of RF-EMF (Tillmann et al.. 2010). We have performed a replication study using higher numbers of animals per group and including two additional exposure levels (0 (sham), 0.04. 0.4 and 2 W/kg SAR). We could confirm and extend the originally reported findings. Numbers of tumors of the lungs and livers in exposed animals were significantly higher than in sham-exposed controls. In addition. lymphomas were also found to be significantly elevated by exposure. A clear dose—response effect is absent. We hypothesize that these tumor-promoting effects may be caused by metabolic changes due to exposure. Since many of the tumor-promoting effects in our study were seen at low to moderate exposure levels (0.04 and 0.4 W/kg SAR), thus well below exposure limits for the users of mobile phones. further studies are warranted to investigate the underlying mechanisms. Our findings may help to understand the repeatedly reported increased incidences of brain tumors in heavy users of mobile phones.

1. Introduction

The increased use of mobile phones during the last two decades was accompanied with fears that their emission of radiofrequency electromagnetic fields (RF-EMF), sometimes also called "radiation", may have adverse health effects. So far, no biophysical mechanism has been identified which would speak in favor of such effects since the quantum energy in the frequency range used for mobile communication is far too low to break chemical bonds. The only accepted mechanism by which RF-EMF could be harmful is heating which is prevented at the current exposure limits for the general population (specific absorption rate (SAR) 0.08 W/kg whole body; 2 W/kg local exposure)111. Some epidemiological studies, however, have found increased incidences of brain tumors in heavy users of mobile phones 12,31.

In 2010, a study was published 141 showing tumor-promoting effects of life-long exposure to RF-EMF (Universal Mobile Telecommunication System, UMTS) at moderate exposure levels in mice treated with a carcinogen (ethylnitrosourea, ENU) *in utero.* Those results were potentially influenced by an unexpected infection with *Helicobacter hepoticus* (which may have had an influence on the pathological findings in the liver, as suggested by the authors). Nevertheless the data showed clear effects of RF-EMF exposure on the incidences of lung and liver tumors. We have replicated this study with higher numbers of animals per group, but otherwise under similar conditions, in order to clarify whether the previously reported results could be confirmed. In addition, two additional SAR levels of exposure (low and high) were included in order to investigate possible dose—response relationships.

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Furthermore, we ensured that we did not have any infection with Helicobacter species in our animals.

2. Materials and methods

2.1. Experimental design

The experiment was performed according to the German Animal Welfare Act and approved by the local authorities (city state of Bremen). Special care was taken to repeat the study by Tillmann et al. [41 as accurately as possible. Male C3H/HeNCrl (n = 43) and female C57B1i6N (n = 290) mice were purchased in a staggered design from Charles River Germany, Sulzfeld, Germany, at an age of 8-9 weeks. After acclimatization, at the age of 12 weeks (females), the males and 128 females were mated for one week (ratio 3 females: 1 male) in two rounds, thus a total of 256 potentially pregnant females were obtained. They were distributed to the 128 cages of the exposure devices, two animals per cage. Exposure or sham-exposure of the pregnant females thus started at day 6 p.c. (post conception). All females were weighted at day 13 p.c., and the ones with the highest weight gains remained in the exposure devices while the others were sacrificed (CO_2 overdose). The remaining 34 females, age 12 weeks, were mated with the males, and the female offspring served as the untreated, unexposed cage control (n = 96, three animals per cage). At day 14 p.c., the females in the exposure devices were injected (i.p.) with ethylnitrosourea

(ENU; Sigma—Aldrich, Taufkirchen, Germany) at a dose of 40 mg/kg in saline. Six days after birth, after sexing three female Fl animals were left with their mothers, while the surplus females and the males were removed and sacrificed. Litters with too few female pups were filled up with surplus females from other litters of the same exposure group. In total, four groups of female Fl mice were obtained, 96 animals per group. At day 21, pups were weaned, and the dams were sacrificed.

22. Exposure to electromagnetic fields

The exposure devices consisted of eight radial waveguides with 16 cages each, arranged in stacks of two and connected to power amplifiers and RF-generators. Details have been published earlier [5]. Extensive numerical calculations of the field distributions and the corresponding SAR values revealed unavoidable substantial variations for animals in different positions and within animals (local maximum SAR values) which could be as much as 3-5 times higher than the whole-body SAR. Two waveguides per exposure group with 16 cages each (32 cages in total, 96 animals) were one out of four groups with the following nominal whole-body SAR levels: sham-exposed (0 W/kg), 0.04 W/kg (low), 0.4 W/kg (moderate) and 2 W/kg (high) for a reference configuration of three mice (body weight 20 g each) per cage, with a standard deviation for this configuration of around 36%. The exposure was comparably homogeneous with standard deviations of the whole body SAR within
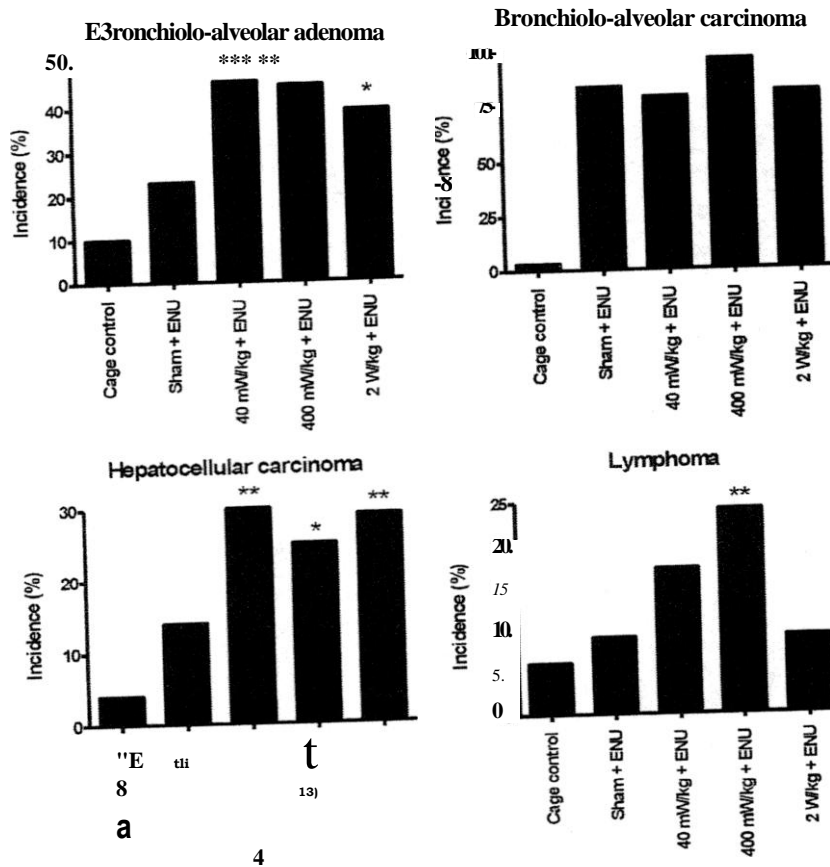

Fig.l. The effects of life-long exposure to RF-EMF in mice treated with ENU *in utero.* Shown are the tumor incidences as percentages of animals, based on histopathological analyses of 93-96 animals per group. Significant differences are indicated by asterisks (Fisher's one-tailed exact test): 4. p < 0.05; **. p < 0.01; ***. p < 0.001.

the cages between 30% (adult animals) and 91% (pups) due to spatial electric field variations and movement of the groups of animals. In order to equalize the exposure between the 16 cages of each waveguide (max. 12% variation of the cages mean electric field strengths), the cages were permuted every second day by one exposure section. Exposure was 24/7 for the entire period (72 weeks) with the exception of half an hour each night at 3 a.m. in order to shut down the generators and to reboot the system. It should be noted that exposure was interrupted for 4 h daily in the previous study [41 for animal caretaking. Another difference was due to different geometries of the radial waveguides used in the previous study and in the present one (diameter of the three waveguides with 20 cages each were 1.9 m in Ref. [4]. and 2.2 m in the present study). Thus, the ratios of whole body SAR values to the respective incident field strengths were different. This difference, however, is not biologically important but only relevant for the SAR calculations.

Electrical fields inside the waveguides as well as temperatures were measured automatically. One power amplifier in the highest exposure group malfunctioned for 20 days (weeks 33-35), producing only one fourth of the SAR during this period, affecting half of this exposure group. All other generators and amplifiers worked without failure so that the total time without exposure was minimal (0.5%). Exposure conditions were not known to the persons handling the animals or otherwise being involved in the experiment. Only after all data have been analyzed, they were sent to the cooperating partners (University of Wuppertal) in exchange with the exposure codes.

2.3. Procedures during and at the end of the experiment

The mice were routinely inspected visually, and their body weights were recorded weekly during the first 14 weeks, and thereafter every two weeks. After one year (week 52), pooled feces

samples alai' cages were collected and checked for the presence of *Helicobacter ssp.* (PCR analysis, Charles River). None of the samples was positive. When signs of disease were noted, or when the body weight of an animal showed a sudden drop, these animals were removed from their cages, sacrificed by CO_2, and immediately dissected. All surviving animals, including the cage controls, were sacrificed when survival rates of the ENU-treated animals dropped below 25% (OECD guideline No. 451 'Carcinogenicity Studies'). Due to a technical mishap, sham-exposed and exposed (2 W/kg) animals were sacrificed 1-2 weeks too early or too late, respectively. Gross morphological abnormalities were noted, and the following organs were immersion-fixed: brain, kidneys, spleen, liver, and lymph nodes. Lungs were immersion-fixed after intratracheal instillation of formalin (37%). Tissues (except lymph nodes) were embedded in paraffin, and sections of 4 um were stained with hematoxylin/eosin. Histopathological examination was done according to international standards [61. To ensure that our diagnoses were correct, a set of 107 sections with different tumors were cross-checked by a professional pathologist No deviations from our diagnoses were found.

2.4. Statistical analysis

Comparisons of body weights was done by parametric analysis of variance, followed by repeated measures post-hoc test. Survival times were compared by log-rank test (SPSS v. 22, IBM). Incidences of tumors were compared by Fisher's exact test (one-tailed) using the program GraphPad Prism (GraphPad Software, San Diego, CA, U.S.A.). For the Bayesian analysis, a script was programmed in R 171.

3. Results

Our study confirms and extends the previously published observations of tumor-promoting effects of life-long RF-EMF

Table 1
Incidences of neoplastic and pre-neoplastic tumors.

Lesions	Cage control	0 W/kg	0.04 Wikg	0.4 W/Icg	2 W/kg
Cerebrum	[96]	196j	[92]	[96]	0 (0 %)
Mixed Glioma [TA!	0(0%)	0(0%)	0 (0%)	1 (1%)	1 9 6 I
Oligodendroglioma [MI	0(0%)	1(1%)	0(0%)	1(1%)	1 9 6 1
Oligodendroglioma [B]	0(0%)	1(1%)	0(Q%)		[961
Astrocytoma IBI	0(0%)	1(1%)	0 ( )	0:0%;	0 (0%)
IvIeningioma [B]	0(0%)	I (1%)	0(0%)	22%)	1(1%)
Lungs	[961	[96]	194i		O -: -
Bronchiolo-Alveolar Carcinoma [M]	3(3%)	81 (84%)	74(79%)	g2:96t·	O
Bronchiolo-Alveolar Adenoma [B]	10(10%)	22(23%)		4 3 oar —	77,1:1:1;l;
Bronchiolo-Alveolar hyperplasia	3(3%)	7(7%)	7(7%)	9 (9%3	
Liver	[96]	(96)	[93j		31.,3V
Hepatocellular Carcinoma [MI	4(4%)	13(14%)	28(30%)'	2425%T	211.
Hepatocellular Adenoma 1111	37(39%)	33 (34%)	37 (40%)	34 :35%1	33 .43W
Hepatoblastoma [MI	0(0%)	3(3%)	3(3%)	1(1%)	1f111)
Hemangiosarcorna [M]	0(0%)	2(2%)	3(3%)	3(3%)	3 3 ;
Hemangioma (BI	3(3%)	4(4%)	1(1%)	2(2%)	2
Focus/Foci of hepatocellular alteration	9(9%)	14(15%)	15(16%)	1 8 (1 9 %)	
Bile duct hyperplasia	0(0%)	2(2%)	0(0%)	1 (1 %)	
Kidneys	[961	[96]	[91]	[96]	1961
Renal Tubule Carcinoma 'MI	0(0%)	2(2%)	3(3%)	7(7%)	5t5%
Renal Tubule Adenoma [B]	2(2%)	3(3%)	2(2%)	5(5%)	2f2%
Renal Tubular hyperplasia	0(0%)	5(5%)	3(3%)	5(5%)	0 (0 %
Spleen	[96]	[96]	(93)	1961	1961
Hemangiosarcorna [M]	2(2%)	3(3%)	1(1%)	0(0%)	0 (0 %
Hemangiorna [B]	0(0%)	1 (1%)	1(1%)	2 (2 %)	
Stromal hyperplasia	1 (1%)	7(7%)	8(8%)	8(8%)	5(5%)
Hematopaymphorel Tissue	[96]	(96)	[93]	[96]	1⁹61
Lymphoma [M]	6(6%)	9(9%)	16(17%)	23(24%)'	9(9%)
Histiocytic Sarcoma [M]	0(0%)	4(4%)	2(2%)	1(1%)	2(2%)

Numbers in squared brackets represent the numbers of animals from which the respective organs were examined.
(15) Benign neoplasia/1M] Malign neoplasia.
1",v < 0,05; '"sp < 0,01; `"p < 0.001 vs. sham (Fisher's exact test. one-tailed).

exposure. The numbers of both adenomas and carcinomas were significantly increased in the lungs, and carcinomas were significantly elevated in the livers of RF-EMF exposed animals (Fig. I. Table 1). As compared to the sham-exposed control mice, numbers of animals with bronchiolo-alveolar adenomas (lungs) were doubled at low and moderate SAR levels, and hepatocellular carcinomas were nearly or more than doubled at low, moderate, and high SAR levels, respectively. The numbers of multiple tumors were found to be significantly elevated at 0.04 W/kg (bronchiolo-alveolar adenomas, Table S1). The numbers of animals with lymphomas were increased 2.5 fold at moderate SAR levels (Fig. 1, Table 1). No increased tumor numbers were found in the brains, kidneys, and spleens of the exposed animals. Here the tumor rates were well below 10%. As expected, survival times in all ENU-treated animals were much lower than in cage controls, but not affected by exposure (Fig. S1). Body weights of (sham-) exposed animals were only slightly different from untreated, unexposed cage-control mice (Fig. S2).

Fig. 2 shows a comparison of the findings of the previous and the present study for the lung tumors due to exposure for a nominal SAR level of 0.4 W/kg (moderate) since this one was used in both studies. It is obvious that both studies are in good agreement.

To address the debates about both the usefulness of null hypothesis significance testing (NHST) in general 18-101, and the proper statistical analysis of replication studies 01-131, we additionally performed a Bayesian analysis. Exemplary analysis results for the lung tumors due to exposure at moderate levels as compared to sham-exposure are presented in Fig. 3.The hypothesis of no difference between the exposed and the sham-exposed animals is outside the 99% prediction interval for all analyses no matter whether an uninformative prior is used or an informative one based on the results from the previous study. As can be

expected from the significance of the results in Ref. 141, the posterior distribution derived from the informative prior is shifted towards the right and the prediction intervals are further away from the hypothesis of no difference.

4. Discussion

The fact that both studies found basically *the* same tumor-promoting effects at levels below the accepted (and in most countries legally defined) exposure limits for humans is worrying. Although animal experiments are generally not easily transferable to the situation in humans, the findings are a very clear indication that — in principal — tumor-promoting effects of life-long RF-EMF exposure may OCCUT at levels supposedly too low to cause thermal effects. The basis for defining safety guidelines regarding RF-EMF exposure by mobile phones and other RF-EMF emitting devices relies on the assumption that increases in temperature above a certain threshold are the only way how exposure can cause damage (thermal effects). These are clearly prevented by the exposure limits. However, the RF-EMF energy absorbed by the tissues or organisms, respectively, is converted to thermal energy regardless the exposure dose. As a consequence, this thermal energy influences to some extent the energy balance of tissue and the entire organism. It was shown that RF-EMF exposure at low levels (0.08 W/kg) causes increased body weights in hamsters which indicates a shift in metabolism of food 1141. Other experiments in hamsters have shown that the consumption of food and the production of CO_2 is decreased by RF-EMF exposure, albeit only at relatively high SAR-levels [15]. It is therefore plausible to assume that RF-EMF energy, when absorbed and converted into thermal energy, influences metabolism and energy balance to some extent which may play a role for the observed tumor-promoting effects.
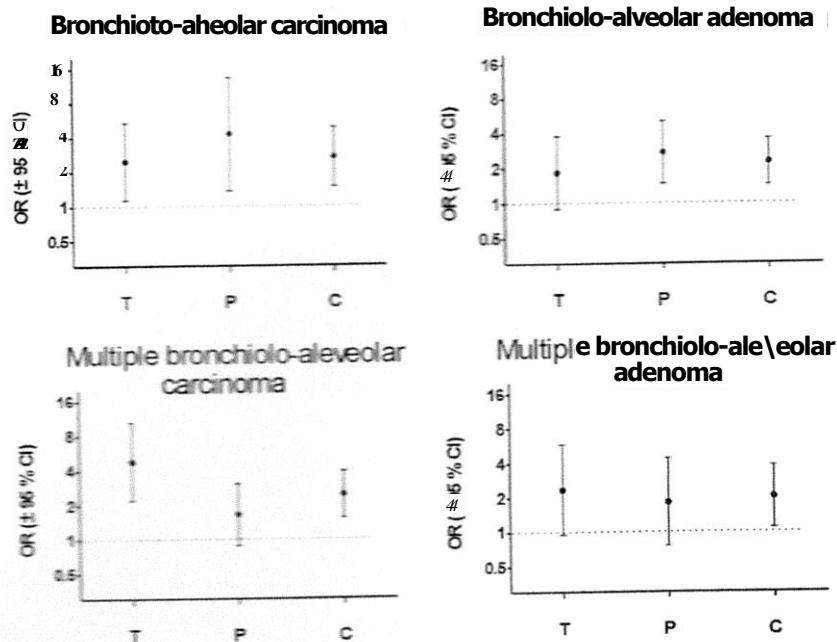

Bronchioto-aheolar carcinoma

Bronchiolo-alveolar adenoma

Multiple bronchiolo-aleveolar carcinoma

Multiple bronchiolo-ale\eolar adenoma

Fig. Z. ... by atimar.-- (2010) |4| and the present data for lung tumors at moderate exposure levels. Since both studies followed the ... sam ... Wel:h overlapping SAR ranges. the results were combined_ Data are expressed as odds ratios (OR) with 95% con 3eate 3. ... 77'7 !be ... 1.7- ... with OR — 1 (dashed line) indica US significantly elevated ORs. T. iillmann et al (2010): P. present study; G con4: 3 Data
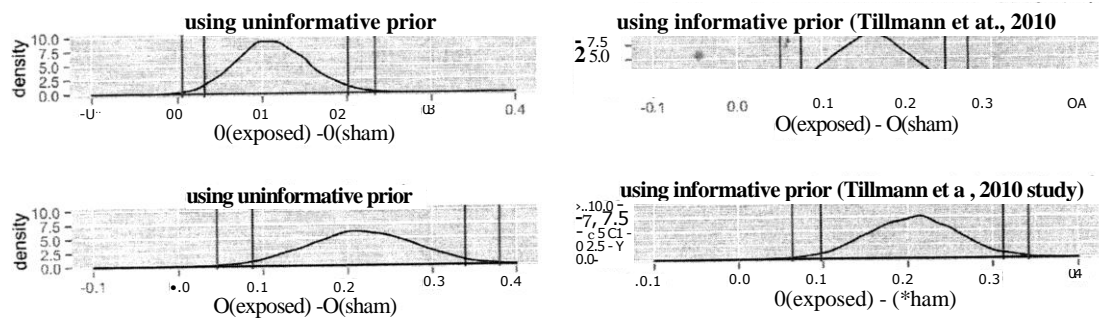
Posterior distributions
Bronchiolo-alveolar

Fig. 3. Results from a Bayesian analysis showing the posterior probability distributions for the parameter differences between moderate exposure levels and sham exposure for lung tumors. The upper panels show the results for the bronchiolo-alveolar carcinoma, the lower panels show the results for the bronchiolo-alveolar adenoma. The plots on the left show the posterior distributions derived from an uninformative prior (a uniform distribution over the parameter space), while the distributions on the right are derived using the results from the study by Tillmann et al. (2010)141 to define the prior distribution. The vertical lines indicate the boundaries of the 95% (blue) and 99% (red) prediction intervals. The peaks of the distributions indicate that there is an expected increase in lung cancer of more than 10 percentage points for the moderate exposure level in contrast to sham exposure, and an increase of about 20 percentage points for the adenoma. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In this context it is important that the carcinogen ENU was administered to the pregnant mice at day 14 of pregnancy. We do not know at which time periods after the treatment with the carcinogen the tumor-promoting effects occurred. Early studies [iάj clearly demonstrated that the prenatal time point of ENU-administration is crucial for the development of tumors in the adult. Since the cardnogen was administered to the pregnant females while being already exposed to RF-EME it is possible that immediately after ENU-treatment the promoting effects happened. Alternatively, they occurred durum' the later stages of development. Another possibility why tumor-promoting effects were seen in both studies is that the uptake of the carcinogen by the fetuses was higher in the exposed animals *due* to elevated metabolism. Studies addressing this cr..:-H·Jon ⁻aᵉ dearly nee&d.

Another point of the absorption of RF-EMF by tissue in relation to blood flow. V. z2Iculations are based on the electric properties of the ₅₅_ₛ tlectric conductivity), blood flow is rr:⁻ 7775: 2: ·was not considered for our SAR - De p055il⁻-2. by computing the local heat a·,..⁻.7usst ti⁻ics of bkxxl flow. It is known. however. thzt hirtmti II.: A ;I/ 1e:7_5 .-s as as in tumors is considerably it sitir lower. iv.t compared to other tissues F -)(bed RF-EMI energy may lead to differe, _ -Zr: effeas which, in turn, may help _ 7-p::,..moting effects as seen here. - S 01 ia 5fl.K 5O StiSS the importance of exposure are unfortunately often S . :r .7f:a y different studies. For promoting effe in transgenk mice prone for developing lyrn-phomas [18]. T wo replication studies did not confirm these ef fects [19] 3oth replication studies, however, deviated from original st the : in several ways. Not only were *the* exposure different, b z also were the mice in the replication studies exposed wf restrained (in tikes). whereas in the original study the mice were non-restrained. While restrained animals avow exposure at comparably low SAR variations, the physio- and metabolic situations are fundamentally different in ie.ampanlion to freely moving animals 1211. In fact, the able SAR variations in non-restrained. freelv moving animals mav turn out tc be of key importance for the understanding of tumor-promoting effects.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.1.51.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http:// dx.doi.org/10.1016/j.bbrc.2015.02.151.

References

[1] A. Ahlbom. U. Bergqvist, J.H. Bernhardt, et al.. **ICNIRP guidelines for limiting** exposure to time-varying electric. magnetic, and electromagnetic **fields (up to** 300 Gliz). Health Phys. 74 (1998) 494-522.
[2] **C.** Coureau, G. Bouvier, P. Lebailly, et al.. Mobile phone **use and brain tu-** mours in the CERENAT case-control study, Occup. Environ. Med. **71 (2054)** 514- 522.
[3] Interphone Study Group. Brain tumour risk in relation to mobile telephone use: results of the INTERPHONE international case-control study, **mt.**J. Epi-derniol. 39 (2010) 675-694.
[4] T. Tillmann. H. Ernst, J. Streckert. et al.. Indication of cocarcinogenic **potential** of chronic UMTS-modulated radiofrequency exposure in an ethylnitrosourea mouse model. Int. J. Radiat Biol. 86 (2010) 529-541.
[5] T. Reinhardt, A. Bitz, A. El Ouardi, et al. Exposure set-ups for in vivo experiments using radial waveguides. Radiat. Prot. Dosim. 124 (2007) 21-26.
[6] World Health Organisationilmemational Agency for Research on Cancer (WHOilARC). International classification of rodent tumors. in: U. Mohr (Ed.), The Mouse. Springer, Heidelberg, 2001.
[7] The Core Team. R: a Language and Environment for Statistical computing., R Foundation for Statistical Computing. 2012. Vienna.
[8] P. Dixon. Why scientists value p values, Psychon. B. Rev. 5 (1998) 390-396.
[9] T. Johansson. Hail the impossible: p-values. evidence. and likelihood. Scand. J. Psycho'. 52 (2011) 113-125.
[10] R. Nickerson, Null-hypothesis significance testing: misconceptions, **in:** M. LOVTiC (Ed.), International Enzydopedia of Statistical Sciences, Springer. Berlin. Heidelberg. 2014. pp. 1003-1006.

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[ill J.B. Asendorpf, M. Conner, F. De Fruyt, et al., Recommendations for increasing replicability in psychology. Eur. J. Pers. 27 (2013) 108-119.

1121 J.C. Valentine, A. BigIan, R.F. Boruch, e.t al.. Replication in prevention science, Prev. Sci. 12 (20H) ·103-117.

1131 E. Yong. Replication stu(Iies: bad copy. Nature 485 (2012) 298-30(J.

[141 A. Lerchl. H. Kruger, M. Niehaus, et al.. Effects of mobile phone electromagnetic fields at nonthermal SAR. values on melatonin and body weight of djungarian hamsters (Phodopits sungortts)..1. Pineal Res. 44 (2008) 267-272.

1151 K. Taberski, M. Klose. K. Grote, et al. Noninvasive assessment of metabolic effects of exposure to 900 MHz electromagnetic fields on djungarian hamsters (·Phodopü,s sungorus), Radiat. Res. '181 (2014) 617-622.

1161 B.A. 'Diwan, H. Meier. Strain- and age-dependent transplacental carcinogenesis by 'I -ethyl-1 -nitrosourea in inbred strains of MiCe. Cancer Res. 34 (1974) 764-770.

1171 S. Fiocchi, M. Parazzini, I. Liorni, et al,, Temperature increase in the fetus exposed to UHF RFID readers, IEEE Trans. Biomeci. En,g 61 (2014) 2011-2019.

1181 M.H. Repacholi, A. Bastes V. Gebski, et al., Lymphomas in *E I* trans-genic mice exposed to pulsed 900 MHZ electromagnetic fields, Radiat Res. 147 (1997) 631-640.

1191 G. °berm, K. Rolf°, P. Yu, et at.. Carcinogenicity study of 217 Hz pulsed 900 MHz electromagnetic filds in Mini transgenic mice, Radiat. Res. 168 2007) 3·16-326.

1201 T.D. Utteridge, V. Gebski, J.W. Finnic, et al., Long-term exposure of E-mu-Pinif transgenic mice to 898.4 MHz microwaves does not increase lymphoma incidence. Radiat. Res. 158 (2002) 357-354.

[211 A, Lerchl. To restrain or not to restrain animals in RF-EMF exposure settings. Frequent 63 (·2009; 144-146.