

This **special issue** of the BERENIS newsletter contains a detailed evaluation of two recent cancer studies investigating lifetime radiofrequency exposure of mice and rats:

- 1) The "NTP Study" conducted in the U.S. (NTP 2018a, 2018b, 2018c, 2018d, 2018e; Wyde *et al.* 2016, 2018a, 2018b)
- 2) The "Ramazzini Study" conducted in Italy (Falcioni *et al.*, 2018)

A short version of this evaluation is also included in [BERENIS Newsletter 15/2018](#).

Evaluation of the NTP and Ramazzini studies

Recently, the results of two comprehensive large-scale studies have been published. Both studies were performed with laboratory rats and mice roaming freely in cages to investigate the carcinogenic potential of lifetime exposure to radiofrequency electromagnetic fields (RF EMF). The focus of the study of the "U.S. National Toxicology Program" (NTP) was exposure associated with mobile phones (NTP 2018a, 2018b, 2018c, 2018d, 2018e; Wyde *et al.* 2018a, 2018b), while the study of the Ramazzini Institute in Italy focused on the far field of sources, i.e. exposure to mobile phone base stations or mobile phones of bystanders (Falcioni *et al.* 2018). In the following, these studies are referred to as "NTP study" and "Ramazzini Study", respectively. Compared to earlier studies with laboratory animals that evaluated carcinogenicity¹ or co-carcinogenicity of RF EMF, these studies are novel in that the animals were exposed roaming freely in their cages instead of being placed in narrow tubes for exposure. This reduced the stress caused by confinement and allowed for longer daily exposures.

NTP study: background and methods

In May and June 2016, first results of the largest animal study performed to date investigating whether mobile phone radiation increases cancer risk in rats and mice were published (Wyde *et al.* 2016) (see [BERENIS Newsletter 7/2016](#)), followed by the publication of the full preliminary project reports for the review panel in February 2018. Information on the entire process is transparent and publicly accessible. The experimental procedures and the analyses are described in detail in the preliminary technical reports provided by the NTP (NTP 2018b, 2018c), with all data and details being accessible to all. Comments from the public hearing have been posted on the NTP website as well. Although the study results are not yet published in a peer-reviewed scientific journal, the procedure and study results have been reviewed by an independent review panel consisting of experts from various areas including pathology and dosimetry in March 2018 (NTP 2018a). On 1 November 2018, after accounting for the reviewers' comments, the NTP has published the final technical reports (NTP 2018d, 2018e).

The study was conducted by the U.S. National Toxicology Program (NTP) on behalf of the U.S. Food and Drug Administration (FDA). The animals were exposed for their entire life². B6C3F₁³ mice of both

¹ Carcinogenicity is defined as occurrence of malignant tumors, combination of malignant/benign tumors and benign tumors known to potentially progress to a malignant tumor

² a so-called bioassay

sexes were exposed to 1900 MHz GSM- or CDMA⁴-modulated signals at whole-body specific absorption rates (SARs) of 2.5, 5 or 10 W/kg. Male and female Sprague Dawley rats were exposed to 900 MHz GSM- or CDMA-modulated signals at SARs of 1.5, 3 or 6 W/kg. Different frequencies have been chosen to achieve a similar distribution of EMF exposure within rats and mice. Both rats and mice were exposed in 10 min intervals (10 min on, 10 min off) for 18 hours per day and seven days per week. Thus, the daily cumulative exposure was 9 hours and 10 minutes. Importantly, the animals were exposed in cages in which they were able to roam freely. This is in contrast to many other studies using a so-called ‘Ferris wheel’ exposure, where the animals are restrained in narrow tubes for several hours per day⁵. In the NTP study, the animals were exposed life-long, starting already before birth (exposure of the dams) on day five after conception, i.e. 107 weeks for the rats and 106 and 108 weeks in male and female mice, respectively⁶. Sham exposure controls were run in parallel. The SAR was kept constant throughout the lifetime of the animals⁷. For each exposure condition, a total of 90 male and 90 female animals were used.

Comparison of SAR values in animal studies with regulatory limits (log-scale)

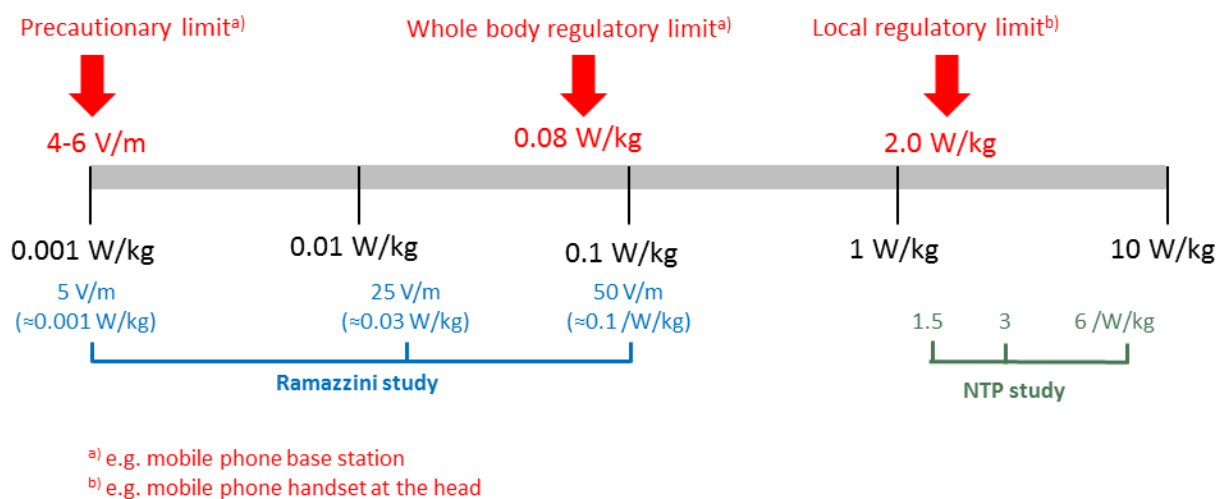


Figure 1: Comparison of SAR values in animal studies with regulatory limits (logarithmic scale)

³ This strain has been selected by the National Cancer Institute for cancer bioassays, and the NTP used this strain for conducting two-year bioassays since 1978

⁴ GSM=Global System for Mobile Communications (2G); CDMA=Code Division Multiple Access (3G)

⁵ An exception was also the PERFORM A study, a 2-year bioassay with whole-body exposure of the animals in narrow tubes for 2 h per day.

⁶ From day 21 to day 34 after birth, the pups were kept in groups of three animals (maximum), before they were separated by sex. Afterwards, the rats were kept individually.

⁷ The field strengths were adapted according to the growth of the animals, as they absorb the irradiation differently depending on their size.

NTP study results

The results are based on NTP's four categories of evidence that a substance may cause cancer: clear evidence (highest), some evidence, equivocal evidence, no evidence (lowest). The main findings of the NTP study (NTP 2018d, 2018e) were that high exposure to RF EMF used by cell phones was associated with

- *Clear evidence* of tumors in the hearts of male rats. The tumors were malignant schwannomas.
- *Some evidence* of tumors in the brains of male rats. The tumors were malignant gliomas.
- *Some evidence* of tumors in the adrenal glands of male rats. The tumors were benign, malignant, or complex combined pheochromocytoma.

The ratings presented in this chapter refer to the evaluation of the independent peer-review panel commissioned by the NTP⁸ in March 2018, following the preliminary NTP results published in February 2018. Regarding the main findings, however, the final evaluation of the NTP published in November 2018 did not differ from that of the independent peer-review panel.

GSM exposure: The number of heart schwannoma cases in male rats increased with increasing SAR levels in a dose dependent manner, and the effect was statistically significant for the highest exposure group when compared to sham-exposed animals (5 versus 0 cases) (Wyde 2018; NTP 2018a, 2018c). *Clear evidence* was concluded by the independent review panel and the final NTP report from November 2018 for the carcinogenicity of exposure towards malignant heart schwannomas. In male rats, *some evidence* of carcinogenicity was concluded for malignant gliomas in the brain and incidences of pheochromocytoma (benign, malignant or combined) in the adrenal medulla. *Equivocal evidence* of carcinogenicity in male rats was concluded for combined adenoma or carcinoma in the prostate gland, benign or malignant granular cell tumors in the brain, incidences of adenoma in the *pars distalis* of the pituitary gland, and incidences for cell adenomas or carcinoma (combined) of the pancreas.

Non-neoplastic lesions (hyperplasia) were increased in heart, brain, and prostate gland of male rats at exposure to GSM at 900 MHz. In GSM exposed female rats, increases in non-neoplastic lesions in the heart, thyroid gland, and adrenal gland were noted.

In male mice, *equivocal evidence* of carcinogenicity was concluded for combined incidences of fibrosarcoma, sarcoma or malignant fibrous histiocytoma in the skin, alveolar and bronchiolar adenoma or carcinoma (combined) in the lung. In female mice, *equivocal evidence* was stated for malignant lymphoma in all organs (NTP 2018a, 2018b).

CDMA exposure: *Clear evidence* of carcinogenicity was concluded in male rats for malignant schwannomas in the heart. Again, the increase in case numbers in RF EMF exposed male rats was statistically significant when compared to the sham-exposed rats (6 versus 0 cases). Similar to the results obtained in male rats exposed to GSM, a dose-response trend was observed. *Some evidence* was noted for malignant gliomas in the brain in male rats. *Equivocal evidence* of carcinogenic activity in male rats was concluded for adenoma in the *pars distalis* of the pituitary gland, adenoma or carcinoma (combined) in the liver. In female rats, *equivocal evidence* of carcinogenicity was concluded for malignant gliomas in the brain and pheochromocytoma (malignant, benign or a combination) in the adrenal medulla and incidences of malignant schwannomas in the heart (NTP 2018a, 2018c).

⁸ Sub-committee of the NTP's scientific advisory board

Increases in non-neoplastic lesions of the heart, brain, and prostate gland were observed in male rats. In female rats, increases in non-neoplastic lesions of the brain were noted for the exposure to CDMA at 900 MHz. In male mice, *equivocal evidence* was noted for hepatoblastoma in the liver. In female mice, *equivocal incidence* of malignant lymphoma was concluded by the review panel in all organs (NTP 2018a, NTP 2018b). Male rats in the control group showed significantly shorter survival times compared to almost all exposed groups, for both modulations. This was taken into account in the statistical analysis.

Ramazzini Study: Methods and results

In the study of the Ramazzini foundation in Italy (Falcioni *et al.* 2018), rats of both sexes were exposed to a 1.8 GHz signal, at intensities (field strengths) of 0, 5, 25 und 50 V/m. This exposure resembles the EMF emitted by GSM base stations, and corresponds to estimated averaged whole-body SARs of 0.001, 0.03, and 0.1 W/kg⁹. The animals were exposed in their cage for 19 hours per day beginning in utero at gestation day 12 until their natural death. Compared to the NTP study, the groups were larger (200-400 animals), and a total of 2248 animals were used.

The publication reports about the incidence of gliomas and heart schwannomas. Regarding food consumption and survival, which are important indicators for the health of the animals, no differences were observed between RF EMF-exposed and sham-exposed animals. In male rats exposed to RF EMF at the highest dose (50 V/m), a significant increase of 1.4% in the tumor incidence of heart schwannomas was found. In the concurrent control group, no schwannomas occurred in the male rats (tumor incidence: 0.0%), and four schwannoma cases occurred in the female rats (tumor incidence: 1.0%)¹⁰. In both sexes, a dose-dependent trend (i.e., not significant) in Schwann cell hyperplasia was found. In addition, a more frequent occurrence of malignant brain tumors (gliomas) was observed in female rats, although not statistically significant war (Falcioni *et al.* 2018).

Comparison and evaluation of the two studies (with a focus on schwannomas and gliomas)

The NTP and the Ramazzini study are state-of-the-art studies applying both strict guidelines in good laboratory practice (GLP) throughout the experimental procedure, and advanced procedures in pathology and statistics. Furthermore, different dose groups (SAR or incident field) were used in both studies allowing for the evaluation of dose-response trends. Dosimetry has been well characterized in the NTP study but is not available for the Ramazzini study. The group size used in the NTP study (n=90) was almost twice of what is commonly used in bioassays (on average 50 animals per group), whereas the number of animals used in the Ramazzini study was exceptionally high.

The stress the animals experienced during RF EMF and/or sham-exposure in their home cages (NTP and Ramazzini study) is certainly less compared to a constraint exposure in tubes in the so-called carousel exposure setup. Hence, such stress effects can be ruled out in both studies.

Despite the dose difference (Figure 1), a significant increase in heart schwannomas has been found

⁹ These whole body SARs were correct for a certain weight of the animals only; as the SAR changed with body weight and no SAR adjustments were performed considering the weight gain of the animals over their lifetime.

¹⁰ In the corresponding historical controls, a tumor incidence of 0.6% was observed in male rats and 0.3% in female rats.

in male Sprague Dawley¹¹ rats in both studies.

Although heart schwannomas do virtually not occur in humans, the outcome is of interest because acoustic neuroma have been linked to mobile phone use in epidemiological studies (acoustic neuroma are also schwannomas, so-called vestibular schwannomas)¹². The increase in heart schwannomas was seen in both male and female rats, but only reached statistical significance in the male animals. One might argue that this might point to random findings. However, differences between gender, and especially an increase in tumors only in male animals are frequently reported in toxicological animal studies (Kadekar *et al.* 2012). Therefore, the fact that the statistically significant increase in schwannomas was only found in male rats is not a reason to question this result *per se*.

The NTP study has been criticized for having included only one concurrent control group per species, used to compare with the GSM and CDMA exposed groups. The results of both exposed groups were compared with this control group and are therefore not independent from a statistical point of view. It has been argued that the absence of any schwannoma cases in the control group of male rats has driven the statistical analyses. However, historical controls of the specific strain of rats used in the NTP study showed a tumor incidence for heart schwannomas of 0.8% for male and 0.3% for female rats¹³.

In the NTP study, 5.6% and 6.7% (5/90 and 6/90) of male rats exposed to the highest dose (6 W/kg) of GSM and CDMA developed malignant heart schwannomas¹⁴. Based on historical controls, one would have expected to observe zero to maximum one case in the control group of male rats. However, even if such a case had occurred, the observed number of 5 and 6 cases in the highest GSM and CDMA exposure group is substantially higher compared to the historical incidence¹⁵. It also needs to be stressed that the concurrent control is always the preferred control group for such kind of comparisons.

No cage controls¹⁶ were used in the NTP study as the animals would not be exposed to the same conditions as the treated animals, which is in line with the OECD Guideline 451 (2009) and therefore a state-of-the-art procedure.

In comparison, the Ramazzini study found a 1.4% incidence of heart schwannomas in male rats exposed to RF EMF at the highest dose (50 V/m), with no cases observed in the sham control. In the

¹¹ Sprague Dawley rats belong to an outbred strain that is effectively wildtype in nature and thus not genetically uniform compared to an inbred strain.

¹² Schwann cells are glial cells of the peripheral nervous system, which also comprises the acoustic nerve. From a biological point, they are thus relevant with respect to gliomas formed from the same brain cell type.

¹³ For gliomas, tumor incidence in historical controls of the specific strain of male rats used by the NTP was 1.3% (2/190, range of 0-4%) and spontaneous formation of malignant gliomas was twice as high in male rats than in female rats. It should be noted that this number in the NTP report is probably derived from four control groups, three groups with 0 tumors in 50 animals and one group with two tumors in 50 animals.

¹⁴ The exposure values for the heart at the lowest exposure dose used in the NTP study (i.e. 1.5 W/kg whole-body exposure) were found to be 2.7 W/kg

¹⁵ The spontaneous incidence of cancer in female rats is very low for rare tumors (defined by an incidence of <1% occurrence in the lifetime of the specific rat or mouse strain) such as the heart schwannoma. Hence, the finding in the control group (0/90) is a result that can be expected. There are formal statistical methods that could be used to include these historical controls in the analysis and this would be more appropriate than an *ad-hoc* argument about what could have been.

¹⁶ A cage control refers to a group of animals living in the same environmental conditions but is constantly kept in the cage (without any sham or real exposure intervention).

historical controls, the respective values were 0.6% in male rats and 0.3% in female rats. Furthermore, in both sexes, a dose-dependent trend (not significant) was found for Schwann cell precancerous lesions (hyperplasia).

The EMF exposure in the highest dose group of the Ramazzini study was comparable to the exposure limits for the general public recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP). In the NTP study, the whole-body SAR was considerably higher than the exposure limit of the general public. In male rats, the highest increase in body core temperature was found in the highest EMF dose group (6 W/kg) with the temperature being less than 1° Celsius higher compared to the sham-exposed rats. The significant increase in heart schwannomas and the non-significant increase in gliomas are unlikely to be related to this temperature increase because it is not known that a temperature increase of this magnitude affects the tumor incidence¹⁷. If a thermal effect is considered, the thermal threshold of 1° Celsius on which ICNIRP has based its guidelines so far has to be questioned.

As pointed out in [Newsletter 7/2016](#), the longer survival time of the exposed rats of the highest exposure group (SAR = 6 W/kg) in the NTP study raises the question whether the animals in the control group had a smaller chance to develop tumors because of their shorter life span. If this was the case, one would expect to see an increase in hyperplasia (preliminary stage of a tumor) in the control group, which was not observed. In addition, the survival differences were appropriately considered and corrected for in the statistical evaluation. In fact, the male rats of the control group died from nephropathy, and no difference in body weight (an important measure in bioassays) was found between controls and RF EMF-exposed groups.

The fact that the animals in both studies were exposed for their entire lifetime is relevant as it mimics long-time exposure in humans. On the other hand, the animals were exposed or sham-exposed under controlled conditions, and they were not exposed to other carcinogenic agents so that co-carcinogenicity and tumor promotion could not be evaluated. In a study published by Tillmann *et al.* (2010) that has later been replicated by Lerchl *et al.* (2015), mice were exposed to RF EMF at SAR levels of 0.04, 0.4 and 2 W/kg. A tumor-promoting effect was found in the lung (significantly more lung carcinoma; Tillmann and Lerchl) and the liver (Lerchl) and for lymphoma (Lerchl) if the mice were concurrently exposed to the carcinogen ethylnitrosourea during the prenatal phase (Tillmann *et al.* 2010, Lerchl *et al.* 2015). However, a clear dose-response effect was not seen. Such kind of study protocols may be of relevance since humans are not only exposed to RF EMF, but also other carcinogenic or co-carcinogenic agents, e.g. cigarette smoke.

Conclusions

The NTP and Ramazzini studies are most comprehensive animal studies with regard to cancer and exposure to mobile phone and base station signals that have been conducted to date. The scientific quality and standard of laboratory techniques are high, especially in the NTP study. Compared to earlier studies with laboratory animals that evaluated carcinogenicity or co-carcinogenicity of RF EMF, these studies are novel in that the animals were exposed roaming freely in their cages instead of being placed in narrow tubes for exposure. This reduced the stress caused by confinement and allowed for longer daily exposures.

¹⁷ The whole-body temperature was determined, whereas temperature in the organs with increased cancer rates, namely the brain and the heart, was not measured and therefore remains unknown. The increase in temperature can be judged as an undefined confounder.

The results of these two animal studies are of great scientific relevance and importance for health policy because according to the International Agency for Research on Cancer (IARC), positive results from animal studies with lifetime exposure are very important with regard to the classification of cancer risk of an agent, together with data from epidemiological and mechanistic studies. Based on the observed evidence regarding a correlation between mobile phone use and gliomas as well as acoustic neuroma, the latter data led to the IARC classification of mobile phone radiation as ‘possibly carcinogenic’ (group 2B) in 2011. The IARC classifies the risk that an agent or environmental toxin causes cancer in humans into five groups, namely ‘probably not carcinogenic’ (group 4), ‘not classifiable’ (3), ‘possibly carcinogenic’ (2B), ‘probably carcinogenic’ (2A) or ‘carcinogenic’ (1).

Despite the methodological differences, both new animal studies showed relatively consistent results in schwannomas and gliomas, as well as a dose-dependent trend to an increase in the carcinogenicity of these tumors. The NTP study used high whole-body doses (SAR – specific absorption rates) as compared to the regulatory limits for whole-body exposure recommended by ICNIRP. For the general public, this limit is 0.08 W/kg, with Switzerland additionally having introduced lower precautionary limits. The question arises of how transferable the NTP study results are to real-life exposure of the public, considering that mobile phone use exposes only parts of the body to EMF levels comparable to the ones applied to the whole animal by the NTP study. First, it is common practice in toxicology to study higher doses to evaluate possible hazards of an agent. Second, the NTP study found an increase in carcinogenicity for GSM and CDMA exposure conditions. Since the findings are similar for both types of exposure, they indicate that the modulation of the signals does not seem to be relevant. Third, mobile phone use can cause local SAR values up to 2 W/kg, averaged over a cube of 21 mm side length in the closest proximity of the phone (e.g. at the ear, cheeks, hand, pocket locations, etc.). Thus, the results of the NTP study are mostly relevant for the exposure situation when using a mobile phone close to the body. In contrast, the Ramazzini study observed carcinogenicity at levels as high as the environmental exposure limits, with no statistically significant effect at lower doses. However, a dose-dependent trend was found for malignant heart schwannomas, which is consistent with the findings of the NTP study. This may indicate that the non-significant increase in case numbers at lower exposure levels represents a true effect that has not reached statistical significance due to the given sample size.

In summary, BERENIS supports a precautionary approach for regulating RF EMF based on the findings and their evaluation. A full risk assessment analysis taking into account all available studies (animal studies and epidemiological studies) is necessary to assess whether the current standards should be changed.

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Additional information:

[BERENIS - Swiss expert group on electromagnetic fields and non-ionising radiation](#)

[List of abbreviations \(pdf\)](#)