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Is there a Biological Basis for Therapeutic Applications of Millimetre Waves and THz Waves?

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Abstract Millimetre wave (MMW) and THz wave (THz) applications are already employed in certain industrial and medical environments for non-destructive quality control, and medical imaging, diagnosis, and therapy, respectively. The aim of the present study is to investigate if published experimental studies (in vivo and in vitro) provide evidence for "non-thermal" biological effects of MMW and THz. Such effects would occur in absence of tissue heating and associated damage and are the ones that can be exploited for therapeutic medical use. The investigated studies provide some evidence for both MMW and THz that can influence biological systems in a manner that is not obviously driven by tissue heating. However, the number of relevant studies is very limited which severely limits the drawing of any far-reaching conclusions. Furthermore, the studies have not addressed specific interaction mechanisms and do not provide hints for future mechanistic studies. Also, the studies do not indicate any specific importance regarding power density levels, frequencies, or exposure duration. It is also unclear if any specific biological endpoints are especially sensitive. Any therapeutic potential of MMW or THz has to be evaluated based on future high-quality studies dealing with physical, biophysical, and biological aspects that have specific health-related perspectives in mind.

Keywords Millimetre wave · THz wave · Non-thermal · In vivo · In vitro

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1 Introduction

A number of well-established and very useful tools based on various physical principles are available for both diagnostic and therapeutic purposes. These include X-ray, magnetic resonance imaging (MRI), acoustic millimetre waves (ultrasound), diathermy, neuromodulation, phototherapy, and computer tomography (CT) among others. Despite their obvious usefulness, each of the techniques also has drawbacks regarding, for example appropriateness for certain body locations, resolution, tissue-type preference, and costs. They may also cause serious side effects in the therapeutic situation.

It is thus still necessary to develop additional instruments that can improve on diagnosis and/or therapy, and at the same time being affordable to purchase, easy to use, and have reasonable running costs. From that perspective, significant interest has appeared for developing approaches that are based on the millimetre wave (MMW) and terahertz wave (THz) parts of the electromagnetic (EM) spectrum. Before such approaches will have any meaningful clinical impact, a number of requisites have to be fulfilled. These include to understand the interactions between MMW or THz on one hand and biological structures on the other; to understand how biological effects occur and which they are if a therapeutic use is intended; to have a relevant data base of experimental studies for design of further studies; and finally to perform controlled clinical studies of sufficient size.

Until presently, MMW, that lie in the frequency region from 30 to 300 GHz (wavelength, 1–10 mm), have been reported to produce therapeutic effects in certain conditions (see [1] for a recent review), whereas diagnostic use seems to be less well investigated. Although beneficial effects have been reported at low intensities (power densities of ca 10 mW/cm^2), the mechanism of action is nevertheless possibly mediated by small local temperature increases affecting thermo-sensitive cell populations [1].

THz, lying in the frequency region from 300 GHz to 10 or even 20 THz (wavelength, 15 μm to 1 mm), on the other hand, has received interest since it can be used for making imaging possible in locations not accessible to relevant investigations with conventional techniques, and with reasonable resolution. Suggested applications may include dermatological investigations to pick out cancerous lesions, wound assessment, and even dental investigations [2]. The case for cancer diagnostics has been recently reviewed by [3]. The therapeutic potential of THz waves is sparingly investigated, although for example certain skin conditions could be targets for THz treatment [4]. The potential therapeutic effects of THz exposure may be due to heating, which would be the case if exposures to high intensity continuous waves are used [5]. On the other hand, calculations and modelling suggest that if exposures are to short pulses, any intracellular heating is negligible and the effect would be mediated by non-thermal interactions between the THz irradiation and biomolecules [5, 6]. Alexandrov et al. [7] have furthermore argued that the main effect of THz radiation is to influence the stability of the DNA (and thus to influence gene expression). By means of modelling approaches, they found that spatial perturbation of DNA could occur above a certain threshold that is determined by intensity and frequency of the THz field, and by the exposure duration.

The primary requisite for any therapeutic action of MMW or THz waves is that exposure to these electromagnetic fields induces biological effects at exposure levels that are not damaging to tissues. Such detrimental effects will occur at high power density levels due to tissue heating, which may be desirable in certain clinical settings (e.g. killing of tumour cells). However, for the majority of clinical situations such drastic outcomes are not warranted. Thus, the question, which we address in this paper, is if experimental studies (in vivo and in vitro)

provide evidence for "non-thermal" biological effects. Such effects would not cause tissue heating and associated damage and are the ones that can be exploited for therapeutic medical use.

2 Medical Use of MMW and THz Waves

Russian scientists have pioneered MMWs therapy by employing low intensity millimetre waves, to reduce pain including headaches and joint pain [8]. Nowadays, such radiation alone or in combination with other treatment [9, 10] is extensively used, with a high success rate, throughout the former Soviet Union and some other eastern European countries as an alternative treatment for a variety of diseases like dermatitis, cardiovascular diseases, diabetes, gastrointestinal disorders, pain relief, and wound healing. The site of application varies with the disease being treated. The fields are also employed to reduce the toxic side effects of chemotherapy and radiotherapy in cancer patients [1]. MMW applications at frequencies of 42.2, 53.6, and 61.2 GHz are typically delivered at low intensity (power density, PD, of 10 mW/cm^2 and less), not inducing tissue heating, on a localized skin area during a 15 to 30-min daily treatment for 5 up to 15 days. The treated skin areas are the site of lesion in the case of surface wounds and skin diseases, whereas the affected joints are the targets in the case of arthritis [11].

Internal diseases are treated at a number of anatomic or acupuncture points such as the lower end of the sternum. Following local exposure, the human skin, which is made up by 70–80% of water, is clearly the route by which the MMWs are absorbed with a penetration depth of a few tenths of a millimetre. However, the mechanisms by which MMWs can produce systemic whole-body effects are not well understood. Among the proposed mechanisms, the stimulation of the nervous system [12] and the modulation of the immune system seem the most plausible [1, 13], since components of these systems are present in the dermis part of the skin and thus accessible for the MMW, at least in certain locations (such as where the epidermis is thin and subcutaneous fat is sparse). Despite the large number of patients treated in Eastern Europe, Western physicians have not accepted this technique and scientists due to the lack of well-controlled, double blind clinical trials, and the lack of an accepted mechanism explaining how a localized MMWs exposure on the skin can be therapeutic for generalized pathologies. However, some reproducible evidence of hypoalgesic effects in animal models or in human volunteers has been gained using "blinded" experimental conditions [11, 14–16].

Diagnostic use of MMWs is less investigated. Recently, the possibility to detect different water content in skin samples associated with psoriasis, eczema, malignancy, and thermal burn wounds are being explored by using the signatures of the human skin in the millimetre wave band [17].

In contrast to MMWs, THz radiation has been investigated much more for diagnostic than therapeutic purposes. Differences in water content in the tissues make THz biomedical imaging in different application fields possible. The most investigated areas pertain to the assessment of burn injuries and cancer detection. Dynamic changes in rat skin burns can be observed by in vivo THz imaging. Thus, Tewari et al. [18] reported that the initial post-damage oedema onset showed a highly reflected signal; subsequently, the oedematous response began to organize making the shape of the burn visible. Finally, the zone of stasis containing the dehydrated tissue became discernible in THz images due to structural variations.

Cancerous or otherwise diseased tissues may contain more interstitial water due to excess vascularization or oedema [19] which yield different THz absorption spectra compared to the ones of normal tissues. Not only differences in the water content but also differences in the structure of the tissues can be detected by THz imaging. As an example, Sy et al. [20] demonstrated that cirrhotic liver tissue has a higher water content and absorption coefficient than the normal tissue and with that even after formalin fixation, there are significant differences between the normal and cirrhotic tissues' terahertz properties. Oh et al. [21], who studied whole rat brain tissues with and without glioma, demonstrated that THz imaging can distinguish between grey and white matter regions and the cell density when water is removed. A summary of the studies available so far is presented in a recent review paper, in which the need for standardized and comparable investigations to have repeatable and unbiased results is also highlighted [22].

Regarding therapeutic applications of THz radiation, it is mainly the potentials and prospects for cancer treatment that have been highlighted in sporadic investigations which rely on certain non-thermal cellular effects under THz exposure (see [23] for a recent overview including studies related to medical use from the former Soviet Union). Sporadic evidence has been provided that THz radiation interacts with cellular components at multiple levels including chromosomes, DNA, genes, and proteins (reviewed in [24]). Moreover, THz radiation has been hypothesized to be a useful and non-contact tool for the selective control of specific genes and cellular processes. As a matter of fact, continuous wave 2.45 THz radiation in human cells has been demonstrated to trigger significant changes in the expression of numerous mRNAs and microRNAs affecting specific intracellular pathways that are not affected in thermally matched bulk-heating exposed cells [25].

3 Biological Evidence for Health-Relevant Effects of Exposures

An analysis of the in vitro and in vivo experimental studies investigating the effects of MMWs and THz waves is presented in this section, with the aim to find if there is any evidence for robust, reproducible, and reliable biological responses under non-thermal conditions. In this respect, the quality of the studies was considered, which enables a more accurate analysis of the available data. The presence of sham-exposure, appropriate dosimetry, use of positive control, blinded analysis, and temperature control were defined as "quality criteria" for the in vitro experimental work to be adequately carried out from a biological and electromagnetic point of view (cf. [26]). Regarding in vivo studies, the inclusion of such quality criteria is only partially possible, thus only sham-exposure, dosimetry, and temperature control were considered for these studies. Several recent papers have addressed these issues [27–31], which have also been demonstrated to have a critical role in the outcome of the studies [26].

Here, we focus on health-relevant biological endpoints found in original scientific reports in the English language that were obtained after searches made on the PubMed (www.ncbi.nlm.nih.gov/pubmed) and the EMF-Portal websites (www.emf-portal.org/). The searches used the various relevant forms of "millimetre wave" and "terahertz" in combination with terms relevant for experimental biomedical studies (i.e. search terms helping us to identify cellular studies and animal studies). A large number of studies were identified dealing with either MMW or THz waves. The studies were further screened individually for appropriateness, whereby a large number was excluded due to irrelevant focus (purely technical nature, not health-relevant biological studies, medical application studies such as hyperthermia use,

method development, etc.). A further screening for study quality was subsequently done, where studies lacking with any information regarding exposure conditions were sorted out. The literature search provided identification of a number of studies where only the title, and in some cases title and abstract, were in the English language. Mostly, these studies were in Russian and have not been included in our paper.

All studies selected for the final assessments are presented in the Supplementary material Tables 1 and 2, focusing on each frequency range. Furthermore, these tables provide information regarding study model (e.g. cell line, animal species), exposure characteristics, study endpoint, and outcome (in the case of observed effects of exposure, those that were considered as "non-thermal" by the authors). The numbers of studies were extracted, as well as the applied frequencies, power densities, exposure durations, and biological endpoints. Furthermore, the data were stratified into specific groups for frequency, power density, and exposure duration. Power density (W/m^2) was selected as measure of exposure intensity since this metric was most often given and thus providing comparable information.

Some publications have presented their exposure conditions as belonging to the THz frequency band, although the studies are actually employing MMWs at the upper frequency border according to accepted definitions [32]. However, we have in these few cases followed the terminology of the authors. Unless otherwise noted, the main body of the text refers to the overall database obtained from the studies and does not provide discussions of every single paper. We refer to Tables S1 and S2 for further details regarding individual studies.

3.1 MMW Studies

Regarding exposure to MMW, we identified 81 publications of which some performed both in vivo and in vitro experiments. The majority of the studies (52 publications, 64.2% of the total number) were performed in vivo and in 38 of these (73.1%), biological responses were detected (i.e. "effects" occurred). The MMW exposures caused responses at all investigated frequencies, power densities, and exposure durations. In vitro methods were used in 37

Table 1 Summary of MMW studies

	All	In vitro	In vitro response	In vivo	In vivo response	% response	In vivo/in vitro
Frequency (GHz)							
Below 10	4	0	0	4	1	25.0	
30.0–40.0	22	7	7	15	12	86.4	2.1
40.1–50.0	22	9	7	13	9	72.7	1.4
50.1–60.0	14	9	4	5	4	57.1	0.6
60.1–65.0	21	11	5	10	8	61.9	0.9
Above 65.0	14	9	6	5	4	71.4	0.6
Unknown	1	0	0	1	1		
Power density (mW/cm^2)							
Below 1	20	11	8	9	9	85.0	0.8
1.0–10.0	25	15	8	10	7	60.0	0.7
10.1–50.0	21	6	2	15	12	66.7	2.5
Above 50	13	2	2	11	5	53.8	5.5
Exposure duration							
Sec–9 min 59 s	19	6	5	13	10	78.9	2.2
10–60 min	39	16	11	23	16	69.2	1.4
1.1–24 h	14	10	1	4	1	14.3	0.4
Intermittent	22	5	5	17	12	77.3	3.4

Table 2 Quality criteria in the MMW studies

	In vivo		In vitro	
MMW studies in the presence of	No response	Response	No response	Response
Sham	11	22	13	11
Dosimetry	13	22	13	11
Temperature control	8	26	12	13
Positive control	–	–	11	8
Blind	–	–	0	1

publications and in 23 of these (62.2%), biological responses were detected. The ratio between in vivo and in vitro studies was 1.4. (See Table 1 for a summary of the MMW studies).

The "biological endpoints" that were studied range over the entire spectrum and with only a single or a few studies focusing on each of these endpoints. As can be seen in Table S1a, the endpoints in the in vitro studies include studies related to cell proliferation and cell cycle progression, cell viability, genotoxicity, gene and protein expression, protein function, metabolism, oxidative stress, cell morphology, nerve cell function, and cell signalling and signal transduction.

Similarly, the in vivo studies (Table S1b) comprise work related to most organ systems (the nervous system, circulatory system, immune system, reproduction and development, gastrointestinal tract, metabolism, and locomotor system) and also to cancer and pathology in general as well as toxicology, cellular effects, and eye integrity.

Even in the cases where several studies have addressed the same endpoint, the studies are measuring different specific outcomes. Thus, for example gene expression and protein expression studies are not necessarily investigating the same genes/proteins. This makes comparisons between studies very difficult when it comes to more specific effects of exposures.

The applied "frequencies" range from 5 to 160 GHz. Few studies were completed below 10 GHz (see Table 1, Fig. 1a), and all of these were in vivo studies. At least 89 investigations (a single study can employ several exposure conditions and thus provide > 1 investigation) were performed between 30 and 160 GHz and 66 of these (74%) showed biological responses to the applied exposure. The responses ranged from cellular effects on membrane function, DNA integrity, gene expression, etc. to in vivo effects on organ function, disease development, and behaviour. An extensive overview of effects is provided in Supplementary Tables S1a and S1b. The ratio between in vivo and in vitro studies amounts to1.1. Interestingly, it seems that there is no frequency dependency regarding biological responses. All frequency ranges display more responding than non-responding results (22 studies at 30–40 GHz with 86.4% showing biological responses; 22 at 40.1–50 GHz with 72.7%; 14 at 50.1–60 GHz with 57.1%; 21 at 60.1–65 GHz with 61.9%; 14 at above 65 GHz with 71.4% response rate, with a ratio of in vivo/in vitro of 2.1, 1.4, 0.6, 0.9, and 0.6, respectively).

When we analysed the "power densities" employed in the studies, we found that a total of 79 investigations covered different power density ranges from below 1 to above 50 mW/cm^2 (Fig. 1b). It has to be pointed out that here only those publications were considered; where exposure intensity with the metric power density (W/m^2) was provided. The response frequency was above 50% at all exposure levels. In the group below 1 mW/cm^2, 20 investigations were performed of which 17 were showing biological responses (85%). The largest number of investigations (25) was performed in the range of 1–10 mW/cm^2, of which 60% (n=18)

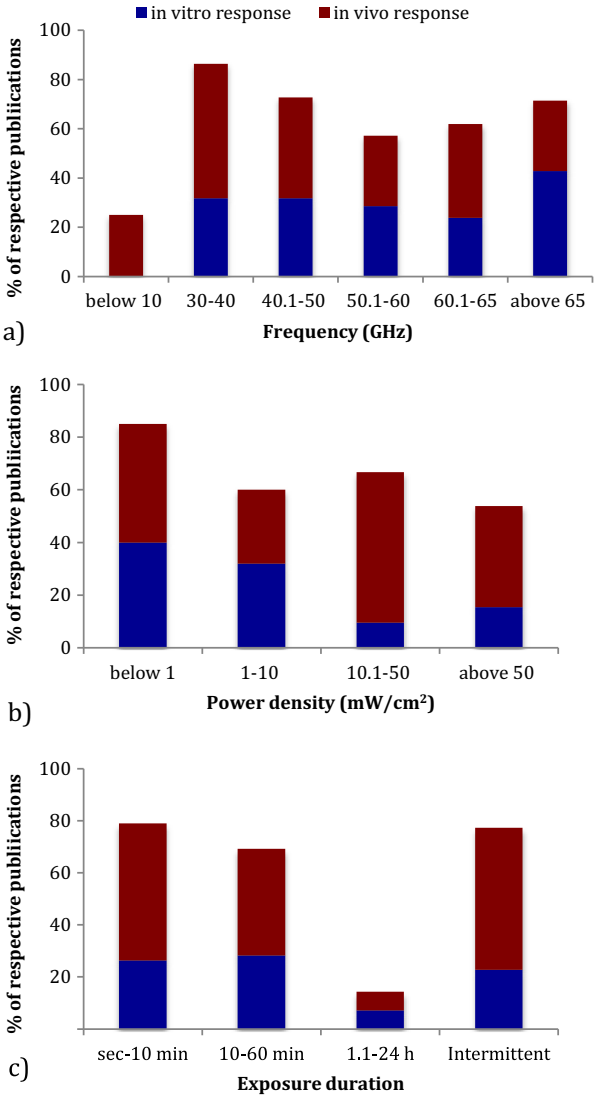

Fig. 1 The bars describe the percentage of studies reporting in vitro (red) and in vivo responses (blue) after MMW exposure. Data were stratified into specific groups for **a** frequency, **b** power density, and **c** exposure duration

showed biological responses. In the next range, 10.1–50 mW/cm^2, 21 investigations were performed of which at least 14 (66.7%) showed biological responses. At power densities higher than 50 mW/cm^2, 53.8% of the 13 investigations are showing non-thermal biological responses with a ratio of in vivo/in vitro of 5.5.

We divided the "exposure durations" into four ranges from seconds to at least 24 h, and also intermittent exposures over days (Fig. 1c). A total of 94 studies were analysed. The short exposure duration (from seconds to 10 min) shows that 78.9% (19 investigations) were responding to the MMW exposure. In the group of 10 to 60-min exposure duration, 69.2% were responding (in total 39 investigations). In the exposure group of 1.1 to 24 h, 14 investigations were found of which only two (14.3%) showed responses. Here, it should be mentioned that few in vivo studies were performed, with the ratio of in vivo/in vitro = 0.4.

However, the corresponding ratio for the intermittent exposure durations shows 22 positive responses representing 77.3% of the investigations. The question arises what causes that much fewer studies showed responses at 1.1-to 24 h exposure duration? Is it the applied exposure duration, the experimental model (in vivo, in vitro), or other parameters that are responsible for this phenomenon? In in vitro studies thermal effects are easier to identify by temperature-controlled exposure setups, thus, increased temperature effects are faster to detect.

We also investigated the "quality characteristics" of the studies. In case of in vivo studies, we considered the presence of sham/control, dosimetry, and temperature control. In case of in vitro studies, these criteria were completed with positive control and blinded experimental manner. Proper blinding is accomplished when the operator who is performing collection and analysis of data is unaware of presence or absence of exposure. Sham/control, dosimetry, and temperature control were frequently included (in about 2/3 of the studies in both in vivo and in vitro conditions). Furthermore, the quality criteria were present in both response and no response outcomes, and prominently present in the in vivo positive studies.

Of the 52 in vivo studies, 38 detected non-thermal biological responses; of these, 22 used sham/controls, 22 applied dosimetry, and 26 studies were performed in the presence of temperature-controlled conditions (Table 2 and Fig. 2). On the other hand, 11 (sham/control), 13 (dosimetry), and 8 (temperature control) studies using the same quality characteristics did not detect responses to the applied MMW. Furthermore, there are 16 studies presenting biological responses in the absence of sham/control, 14 without any dosimetry and 12 that were not using temperature controls (Table S1).

Regarding in vitro studies, 37 publications presented 23 non-thermal biological responses. Again, we detected that 2/3 (65.8%) of the studies employed the same three quality

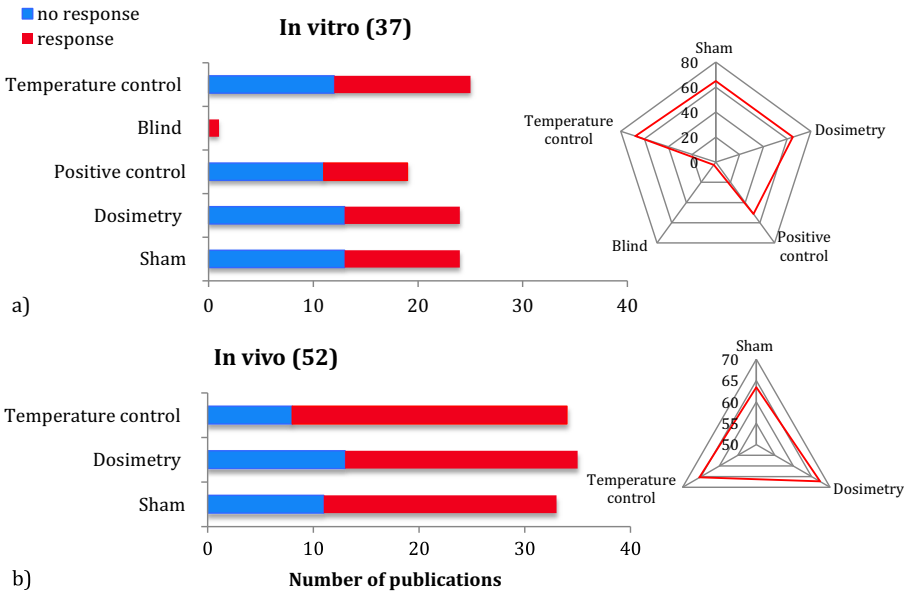

Fig. 2 The quality of the MMW-related data read out from 89 investigations. The number of **a** in vitro and **b** in vivo experiments (blue: no response, red: response) that employed the listed quality characteristics (*y*-axis) is shown. The spider net diagrams present the percentage of the investigations as a function of the quality characteristics

characteristics as the in vivo studies. In addition, positive controls were used in 51.4% of the studies and a blinded protocol was applied in just 2.7% (that is only one publication, Fig. 2a).

In summary, a majority of studies using MMW exposures display biological responses during conditions that are not causing general tissue heating. The responses occur in vivo as well as in vitro, and concern all investigated biological endpoints. Furthermore, there does not seem to be any consistent relationship between intensity, duration, or frequency and effects of exposure. Most studies have employed essential quality criteria such as sham conditions, dosimetry, and temperature controls. In in vitro studies, positive controls were used in half of the studies, whereas blinded protocols were virtually absent. Due to the disparity and the low number of studies, any further correlation analysis was not meaningful to perform.

3.2 THz Studies

Regarding THz, we identified 36 publications including 4 in vivo studies and 32 in vitro investigations. The four in vivo studies are not representing a coherent group feasible for any kind of analysis. However, in Table 3, the available data are presented.

The 32 in vitro studies investigated different biological endpoints from the cellular to the molecular level (Table S2a; cell proliferation and cell cycle regulation, cell viability, genotoxicity, gene and protein expression, nerve cell function, morphology, oxidative stress, and differentiation). Regarding "exposure frequency" (Fig. 3a), 17 investigations were performed below 1 THz with 8 (41.7%) showing biological responses. The next range of 1–4.9 THz showed 11 responses (81.8%), whereas the group of 5–10 THz contains only 3 investigations without any biological response. Only one in vitro study, which showed responses to exposure, was performed in the frequency range above 10 THz.

The grouping based on "power density" is shown in Fig. 3b. The group below 1 mW/cm^2 included 12 investigations, of which at least 5 (41.7%) showed biological responses. In the range 1–10 mW/cm^2, 55.6% (five studies), were responding to the THz exposure. Fourteen in vitro studies were performed above 10 mW/cm^2, of which 11 (78.6%) displayed responses. This tendency to a dose-response relationship with the highest fraction of responses to exposure at the highest power density is intriguing, if the effects are non-thermal and due to

Table 3 Summary of THz studies

	All	In vitro	In vitro response	In vivo	In vivo response	% response
Frequency (THz)						
Below 1	17	17	8	0	0	47.1
1.0–4.99	13	11	9	2	2	84.6
5.0–10.0	3	3	0	0	0	0.0
Above 10	3	1	1	2	2	100.0
	36	32	18	4	4	61.1
Power density (mW/cm2)						
Below 1	12	12	5	0	0	41.7
1.0–10.0	9	9	5	0	0	55.6
Above 10	15	14	11	1	1	80.0
Exposure duration						
Sec–9 min 59 s	8	8	0	0	0	0.0
10–60 min	15	13	0	2	2	13.3
1.1–24 h	17	17	11	0	0	64.7

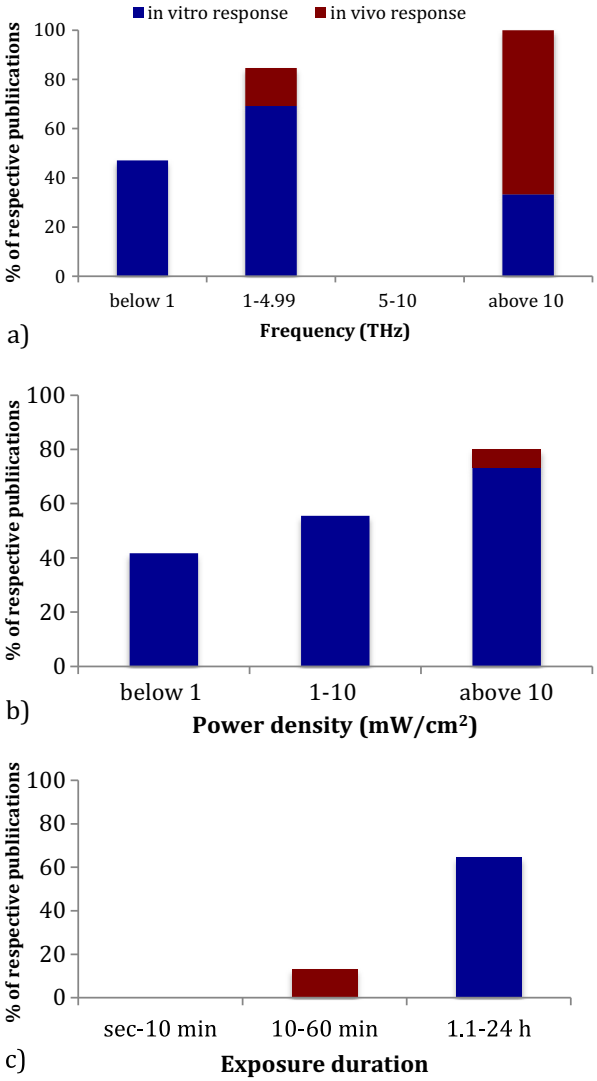

Fig. 3 The bars describe the percentage of studies reporting in vitro (red) and in vivo responses (blue) after THz exposure. Data were stratified into specific groups for **a** frequency, **b** power density, and **c** exposure duration

hitherto unknown mechanisms. However, this tendency is seen among a low number of studies, which precludes any further conclusions.

Concerning the "exposure duration" in the investigated studies, we identified eight data points in the seconds to 10-min range of which no one showed any non-thermal responses. A similar outcome was recorded for the group of 10–60 min (13 studies; Fig. 3c). Interestingly, in the exposure duration group that represents a longer time period (1.1–24 h), 17 in vitro studies were performed with 11 (64.7%) exhibiting non-thermal responses. The lack of effect at the shortest exposure duration is once again intriguing, but should be treated with caution based on the low number of studies.

As with the studies employing MMW exposures, the numbers of similarly performed studies are not sufficient for any further statistical analysis. In addition, the "quality characteristics" of the 33 in vitro studies make the findings questionable (Table 4). Even if 87.9%

Table 4 Quality criteria in the THz studies

	In vivo		In vitro	
THz studies in the presence of	No response	Response	No response	Response
Sham	0	4	13	16
Dosimetry	0	1	13	9
Temperature control	0	1	8	10
Positive control			11	7
Blind			0	0

employed sham/control conditions, 66.7% applied dosimetry, only 54.5% of the studies used positive controls, and the same percentage performed the experiments under temperature-controlled condition. This significantly limits the interpretation of the results of the studies. None of the investigations used a blinded protocol (Fig. 4a).

Out of the four in vivo studies only one used temperature control and one performed dosimetry, whereas all four applied sham/control conditions (Fig. 4b).

In summary, the available studies regarding biological responses to THz waves are few and scattered regarding all investigated parameters (biological endpoints, frequencies, power density, and exposure duration). Although quite many studies show responses, there is no obvious pattern regarding dependency on the physical factors. Furthermore, the quality criteria do not provide information regarding the plausibility of "true" non-thermal effects.

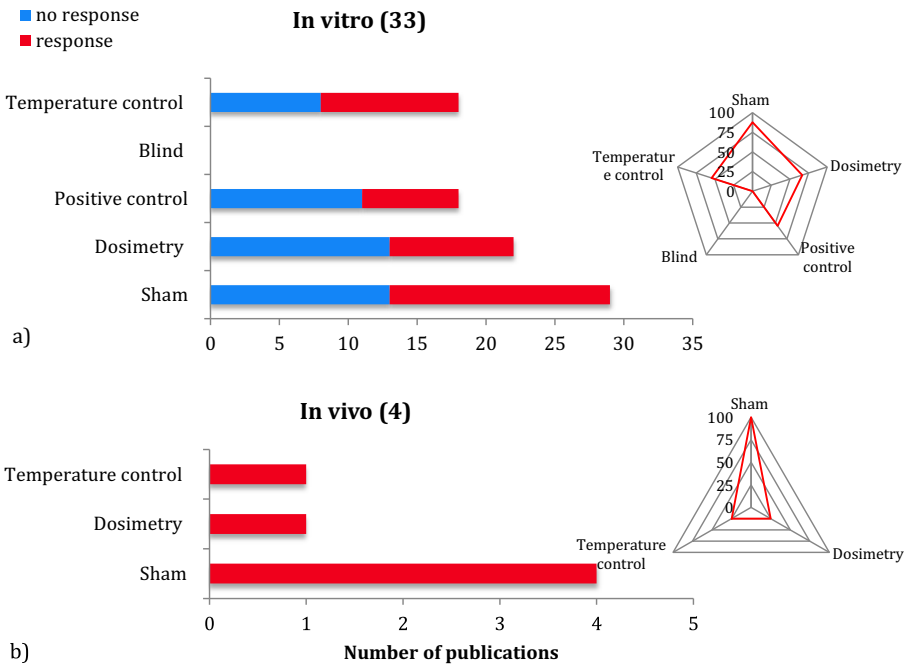

Fig. 4 The quality of the THz-related data read out from 36 investigations. The number of **a** in vitro and **b** in vivo experiments (blue: no response, red: response) that employed the listed quality characteristics (*y*-axis) is shown. The spider net diagrams present the percentage of the investigations as a function of the quality characteristics

4 Safety Issues Associated with Exposures to MMW and THz

Beside the scenario in which the use of MMWs and THz is envisaged in a clinical setting, general public and occupational exposure will also take place in the near feature due to security and telecommunication applications operating at this frequency [33, 34]. In the transition period until this is achieved, a basic understanding of the interaction between MMW and THz waves with living matter needs to be gained. The study of biological effects, which lays the foundation for the development of clinical applications, is a valuable tool to increase the understanding of the mechanisms governing the interactions with biological systems and is crucial for health risk evaluation and thus for the development of empirically based safety standards [35].

An important issue concerns the safety thresholds related to the specific frequency ranges. At lower frequencies, the safety thresholds have been set to prevent the occurrence of immediate to short-term effects that are scientifically justified and well recognized, such as stimulation of excitable tissue or temperature rise in the tissues. The dosimetry metrics i.e. the metrics that are more directly related to the observed health effects, vary with frequency and include the current density (up to 10 MHz), specific energy absorption rate, SAR (100 kHz–10 GHz), and incident power density (10–300 GHz). The choice of a proper dosimetry metric for THz frequencies is still under debate [36]. Furthermore, the current recommendations for safety limits have been determined using extrapolated estimates from the neighbouring spectral regions of millimetre waves on the lower frequency side, and optical radiation on the upper frequency side [32, 37]. There are thus no specific guidelines generated for the THz frequency range. In addition, only a few studies have collected experimental data to support any drafting of specific standards.

5 Discussion and Concluding Remarks

The present and future use of applications using and emitting EM fields in the two investigated frequency ranges—MMW and THz—justify studies investigating biological effects and health effects of these technologies. Studies investigating biological effects aim to identify consistent modifications of the physiological conditions of an isolated biological system (molecule, cell, etc.), and allow the understanding of the mechanisms governing interactions between EM fields and biological tissue. Studies investigating health effects are carried out on entire organisms (animals/humans) since a health effect takes place when the induced biological effect exceeds the capability of an entire organism to compensate the modifications. The latter studies allow to determine the safe levels of exposure.

This paper is addressing the question if MMW and THz exposures can provide “non-thermal” biological effects, which would be a prerequisite for many of the suggested therapeutic applications based on these parts of the EM spectrum. Our approach has been to scrutinize published in vivo and in vitro studies for evidence of effects, taking also study quality criteria into consideration. Epidemiological studies were not considered since they are presently not available.

In total, less than 120 relevant papers were identified and analysed regarding exposure conditions, biological endpoints and outcomes, and study quality. Importantly, analysed papers were considered to be performed under conditions that would allow “non-thermal” effects to

occur, either according to the data provided in the description of the study or according to a statement to that effect by the study authors.

When analysing the details in the studies, it is apparent that the relatively modest number of identified papers cover many different exposure conditions and endpoints. With a few exceptions (discussed below in the specific settings), almost no studies are comparable to any other. Thus, any conclusions regarding possible health-relevant effects are not possible to draw based on independent replications or strong similarity between studies.

Our intention has not been to produce an all-inclusive comprehensive review, but to obtain an understanding of whether performed studies are relevant, performed according to accepted standards, and if they provide evidence for any conclusions regarding possible health-relevant effects.

5.1 MMW Studies

The area of millimetre waves has the highest number of publications in our study. These studies investigate a wide band of biological endpoints by using different exposure conditions. In this frequency area, the temperature increase is relatively fast within water-containing solutions as a function of the applied power density. Therefore, we prioritized as a relevant quality characteristic of the studies the presence or absence of controlled temperature conditions.

The main finding is that two thirds of all studies found biological responses after the applied exposure, during conditions that were considered non-thermal by the authors themselves. As shown in Fig. 1, we did not detect any clear frequency, power density, or exposure duration dependency for non-thermal biological responses. Critically, only 68% of the studies used temperature controls. There is thus a remaining question mark regarding the possibility for heating to be the principal agent in causing the MMW effects in a number of the studies.

Our analysis presents an average "picture" of the performed investigations. We have not proceeded to further divide, group, and analyse the performed experiments by statistical means for two reasons:

- because of the heterogeneity of the investigated endpoints and
- because of the presence of a few strong research groups with high number of publications that provide a substantial part of all the available studies

As [38] presented and also in a recent publication [26], a grouping of results is possible only if there is an appropriate number of studies available that are investigating similar biological endpoints. Since this is not the case for MMW studies, a detailed analysis of the performed experiments is not feasible. Moreover, regarding MMW papers, there are two research groups, namely around Dr. Ziskin and around Dr. Zhadobov whose publication numbers are dominating the available data set. These two groups presented 30 publications out of the 81, using a high number of experiments. Thus, a detailed analysis of the entire dataset would possibly be very much biased by the endpoints and experimental approaches used by the two groups and lead to a distorted outcome interpretation.

However, Dr. Ziskin's group presented 21 publications investigating responses in a variety of in vivo and in vitro models. The main used exposure frequencies were 42.25 and 61.82 GHz (from 10 to 30 mW/cm^2, and different exposure durations). In 13 of the studies, non-thermal responses were detected, and in the 8 "no response" studies, four did not use temperature

control. The majority of the studies applied sham/control, dosimetry, and positive controls; only three used blinded protocol. Dr. Zhadobov's group investigated mainly gene and protein expressions after 60 GHz exposures from 5.4 μW/cm^2 to 20 mW/cm^2 and with different exposure durations (from minutes to days) and detected no responses. Beside the absence of blinded experimental performance all quality characteristics were present. Despite the similarities regarding physical parameters in the studies and that both groups consistently have used most of the quality criteria, the main outcomes are different. Thus, Dr. Ziskin's group consistently reported exposure effects, which Dr. Zhadobov's group did not. There is no obvious explanation for the different results, although differences regarding both biological and physical/engineering aspects of the studies are present and can be of importance.

Basic exposure-related quality characteristics such as the presence of sham/control and temperature controls have to be used in future studies. By scrutinizing the overall performance of the in vitro studies, as shown in Fig. 2, it is clear that the quality of the experimental controls need improvement. The almost complete absence of blinded protocols, the absence of positive controls in 50% of the studies, and the absence of dosimetry, sham/control, and temperature control in 30% of the studies needs amendments. In a similar manner, in vivo studies need corresponding improvements.

5.2 THz Studies

Similar to the MMW studies, studies with reported effects of exposure are a majority of the studies at hand. One has to keep in mind, however, that very few studies have actually been published. Thus, the very few in vivo studies that have been identified are too diverse to allow any kind of conclusion regarding possible effects of exposure. The in vitro studies ($n = 33$) are also diverse, including numerous frequencies and power density levels, as well as both short-term (minutes) and medium-term (several hours) exposure durations.

The authors of the in vitro studies declare occurring responses to the exposure to be non-thermal. However, the absence of one or more of the quality criteria in both studies with or without positive outcome makes conclusions difficult to draw. The interpretation of the studies in view of endpoints is also difficult. Although many studies have focused on genotoxicity, gene and protein expression, or cell proliferation, the studies are still too diverse to allow conclusions regarding possible effects.

5.3 Overall Conclusions

The investigated studies provide some evidence for both MMW and THz waves that can influence biological systems in a manner that is not obviously driven by tissue heating. However, the number of relevant studies are limited regarding MMW, and very few regarding THz exposures, which severely limits the drawing of any far-reaching conclusions. Furthermore, the studies have not addressed specific interaction mechanisms and do not provide hints in what direction such studies should go. It is thus probably too early to formulate and test specific hypotheses, but the need to continue with discovery science approaches and adopt e.g. high-throughput-screening and other screening methods is obvious. Tellingly, the studies do not indicate any specific importance regarding power density levels (although the THz in vitro studies could suggest a classic dose-response relationship), frequencies, or exposure duration. It is also unclear if any specific biological endpoints are especially sensitive.

A key feature of establishing any effect is that studies are possible to independently replicate and that they are done in a reliable and relevant way. We have adopted a set of quality criteria in order to ascertain that studies are performed according to such standards. These quality criteria are only partly fulfilled in the studies we have investigated here. This diminish the credibility of findings reporting effects of exposure, but strengthens the argument that fundamental exposure-related quality characteristics such as at least the presence of sham/control and temperature controls have to be used in future studies.

At present, the available knowledge based from experimental studies on biological effects of MMW and THz does not disprove that non-thermal effects can occur. However, performed studies raise many questions and do not provide any mechanistic explanations. Any therapeutic potential has to be evaluated based on future studies dealing with physical, bio-physical, and biological aspects that have specific health-related perspectives in mind.

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