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Marvin C. Ziskin & Joseph Morrissey

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RESEARCH ARTICLE

Thermal thresholds for teratogenicity, reproduction, and development

MARVIN C. ZISKIN[1] & JOSEPH MORRISSEY[2]

[1]*Temple University School of Medicine, Philadelphia, Pennsylvania and* [2]*Nova Southeastern University, Fort Lauderdale, Florida, USA*

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Abstract

The human embryo and foetus may be especially vulnerable to chemical and physical insults during defined stages of development. In particular, the scheduled processes of cell proliferation, cell migration, cell differentiation, and apoptosis that occur at different times for different organ structures can be susceptible to elevated temperatures. With limited ability to regulate temperature on its own, the developing embryo and foetus is entirely dependent upon the mother's thermoregulatory capacity. As a general rule, maternal core body temperature increases of ~2°C above normal for extended periods of time, 2–2.5°C above normal for 0.5–1 h, or ≥4°C above normal for 15 min have resulted in developmental abnormalities in animal models. Significant differences in thermoregulation and thermoneutral ambient temperatures make direct extrapolation of animal data to humans challenging, and the above temperatures may or may not be reasonable threshold predictions for adverse developmental effects in humans. Corresponding specific absorption rate (SAR) values that would be necessary to cause such temperature elevations in a healthy adult female would be in the range of ≥15 W/kg (whole body average or WBA), with ~4 W/kg required to increase core temperature 1°C. However, smaller levels of thermal stress in the mother that are asymptomatic might theoretically result in increased shunting of blood volume to the periphery as a heat dissipation mechanism. This could conceivably result in altered placental and umbilical blood perfusion and reduce heat exchange with the foetus. It is difficult to predict the magnitude and threshold for such an effect, as many factors are involved in the thermoregulatory response. However, a very conservative estimate of 1.5 W/kg WBA (1/10th the threshold to protect against measurable temperature increases) would seem sufficient to protect against any significant reduction in blood flow to the embryo or foetus in the pregnant mother. This is more than three times above the current WBA limit for occupational exposure (0.4 W/kg) as outlined in both IEEE C95.1-2005 and ICNIRP-1998 international safety standards for radiofrequency (RF) exposures. With regard to local RF exposure directly to the embryo or foetus, significant absorption by the mother as well as heat dissipation due to conductive and convective exchange would offer significant protection. However, a theoretical 1-W/kg exposure averaged over the entire 28-day embryo, or averaged over a 1-g volume in the foetus, should not elevate temperature more than 0.2°C. Because of safety standards, exposures to the foetus this great would not be attainable with the usual RF sources. Foetal exposures to ultrasound are limited by the US Food and Drug Administration (FDA) to a maximum spatial peak temporal average intensity of 720 mW/cm^2. Routine ultrasound scanning typically occurs at lower values and temperature elevations are negligible. However, some higher power Doppler ultrasound devices under some conditions are capable of raising foetal temperature several degrees and their use in examinations of the foetus should be minimised.

Keywords: *hyperthermia, human, radiofrequency, SAR, ultrasound*

Overview of thermoregulation in the mother

As outlined in Annex B of the IEEE C95.1 [1], section B.5.1.1 (Review of thermoregulation studies), human physiology is 'extremely efficient at maintaining optimal body temperatures in response to added thermal energy'. However, the absolute value of resting temperature among individuals can range from 36–38°C [2]. The average resting temperature for adult women (36.9°C) is slightly warmer than that for men (36.7°C) [3]. Humans also regularly experience 'normal' body temperature fluctuations between 35.5° and 40°C that can be

Correspondence: Marvin C. Ziskin, M.D. Center for Biomedical Physics, Temple University School of Medicine, 3420 N. Broad Street, Philadelphia, PA 19140, USA. Tel: (215) 707-4259. Fax: (215) 707-4324. E-mail: ziskin@temple.edu

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influenced by ambient conditions (e.g. temperature, humidity), circadian rhythm, exercise, food intake, menstrual variation, emotional state, the effects of drugs and alcohol, and other factors [4]. Individual susceptibility to elevated temperature can be influenced by age, sweating capacity, cardiac function and output, respiration rate, subcutaneous fat, pH, nutrition, and pressure effects [4, 5]. Women in general appear to experience slightly higher temperature increases in response to exertion than men [6], although this does not appear to be due to any fundamental difference in thermoregulatory response but rather to different physical characteristics. There is also a wide range of heat and cold tolerance between people from different regions of the world that may be due to cultural as well as genetic differences [7–10].

The average basal metabolic rate (BMR) in the human female (approximately equivalent to the rate during sleep) can fall between 1200 and 1500 kcal/day, and is probably a significant factor in the range of 'normal' human body temperatures and the different individual abilities to tolerate heat. Expressed as units that can be easily equated with radiofrequency (RF) energy absorption, the human female BMR is approximately 1-1.5 W/kg (whole body average or WBA) [11]. Climbing a flight of stairs can increase this metabolic rate to ~2.5 W/kg WBA, and sprint running can increase it to over 18 W/kg WBA. In some trained athletes, the metabolic rate can increase to almost 30 W/kg WBA [4, 11]. While much of this energy is lost in the process of performing work, a substantial amount of excess thermal energy is also produced and must be dissipated or hyperthermia will result.

Homeostasis of body temperature (as well as blood pressure and fluid and electrolyte balance) is maintained by the hypothalamus [12]. The hypothalamus receives input from several sources including the nucleus of the solitary tract (blood pressure, digestive, and other visceral sensory information), reticular formations in the brainstem (skin temperature via peripheral thermoreceptors), retina and optic nerve (circadian and diurnal rhythms), circumventricular organs (osmolarity and toxins), and limbic and olfactory systems (eating, reproduction). In addition, input is received from core thermoreceptors located in the spinal cord, gut, veins, and within the hypothalamus itself. When an imbalance is detected, endocrine and autonomic signals are initiated by the hypothalamus to help re-balance the system. When temperatures in the adult human exceed the hypothalamic set point, a mechanism involving an osmotic balance between sodium and calcium ions in the posterior hypothalamic neurons [13] is activated and an efficient thermoregulatory system involving increased blood flow and evaporative cooling is set in place [14–19]. The set point for thermoregulation can vary between individuals and can be lowered during sleeping and inactive periods [20]. In the absence of any thermoregulatory response, the body would rapidly become hyperthermic, and prolonged exposure to moderate hyperthermia $\geq$41°C can cause cellular and organ damage [21].

The capacity of specific tissues to absorb RF energy as heat is defined by their dielectric properties [22, 23]. In terms of the ability to locally dissipate heat, most vascularised tissues (e.g. brain, muscle, skin) have resting blood perfusion rates of ~0.008 mL/g/s[A] [24], and this can be increased to as much as 1.5 times when the local temperature is elevated. A crude estimate of heating in a volume of tissue can be made assuming no heat dissipation (i.e. tissue temperature is directly proportional to the amount of RF energy it absorbs)[B] [25]. In this simplistic model, a SAR of 10 W/kg would produce a temperature increase of ~0.15°C per min [26], and could achieve local temperature elevations of 2°C above core temperature throughout the local target volume in approximately 13 min. While in reality both conduction and convection (via blood perfusion) dissipate heat very efficiently in living tissue (with conduction becoming more important for frequencies above 2–3 GHz), at volumes of $\geq$5–10 g the ability to dissipate heat becomes more challenged. Using a numerical analysis that considers the Pennes Bioheat Equation[C] [28] (i.e. accounting for the heat transfer between the target tissue and perfused blood), an SAR of 10 W/kg in a small (1-mm diameter) volume would result in a maximum steady state temperature elevation (above core temperature) of ~0.02°C and equilibrate in ~8 s. In a larger tissue volume (1 cm diameter, or ~5–7 g) this 10 W/kg rate of energy absorption would result in a maximum steady state temperature elevation of ~2°C and equilibrate in ~13 min (similar to the simplistic model with no heat dissipation). This level of thermal energy input, when averaged over the entire body weight of the human female, however, would be in the order of 4×10^4 W/kg. From the perspective of whole body RF exposure, several

[A] E.g. brain grey matter = 0.0155 mL/g/s, white matter = 0.0063 mL/g/s, whole brain = 0.0086 mL/g/s, muscle = 0.004 mL/g/s.

[B] T(tissue temp) = SAR/C (specific heat of tissue) × t(time of exposure).

[C] The Pennes equation is: $\kappa\Delta^2 T - \rho_b \rho_t C_b m_b T + \rho_t \, SAR = C_t r_t \, dT/dt$; where T = tissue temp in °C, κ = thermal conductivity of tissue (0.6 W/m°C), SAR = RF power deposition rate (W/kg), C_b = heat capacity of blood – energy required to raise 1 kg by 1°C (4000 W*s/kg°C), C_t = heat capacity of tissue (4000 W*s/kg°C), ρ_b = density of blood (1000 kg/m^3), ρ_t = density of tissue (1000 kg/m^3), m_b = volumetric perfusion rate of blood (~0.008 mL/g/s. ** for the present simple analysis; $r_b = r_t$ and $C_b = C_t$

studies have predicted that a whole body average SAR of 4.5 W/kg would be necessary to increase the core body temperature ~1°C [28].

In terms of the whole body heat dissipation capacity in humans, for each extra L of blood shunted to the skin that returns 1°C cooler, heat can be dissipated at a rate equivalent to 1.16 W/h [29] (= ~1 kcal)[D,E]. The normal adult contains a blood volume of approximately 5 L that completely recirculates through the body once every minute. The large size of the skin organ (2 kg with a surface area of 1.8 m^2) can support a cutaneous circulation volume during thermoneutrality of 0.2–0.5 L of blood per min at an overall low linear flow velocity allowing efficient convective cooling. Blood flow changes in cutaneous tissue as detected by laser-Doppler flowmetry [30–32] have suggested a maximal volume under conditions of thermal stress and full vessel dilation of up to 7–8 L/min in some areas with a high density of arteriovenous anastomoses (which act to shunt blood to the peripheral tissues). By inference, such a rate of cutaneous circulation (8 kcal/min) could dissipate the equivalent of almost 8 W/kg WBA[F] of thermal energy. In addition, maximum sweating rates for humans [33, 34] (≥2–3 L/h) in combination with favourable air flow, low humidity, and given the latent heat of vaporisation (inversely related to temperature)[G] [35] can result in convective thermal loss at a rate of ~1335 W or ~19 W/kg in an average adult female. Calculated another way, a 350–675 W/m^2 rate of sweat across a total surface area of ~1.8 m^2 would be equal to an inferred thermal energy loss rate of ~17 W/kg[H,I]. However, a more realistic estimation of maximum average heat dissipation capacity in a human due to blood flow and sweating would be ~12–15 W/kg WBA [36]. This is in agreement with the most recent version of IEEE C95.1 [1] that states 'most young, healthy humans have the capacity to cope with thermal loads that are up to 15 times their resting metabolic rate of ~1.25 W/kg, even in thermally stressful environments'.

The thermoregulatory response in humans to RF energy absorption in deep tissues of the human body has been reported to be similar to that of exercise [37]. Numerical models have predicted that 100 W of RF power deposited into the head of a normal human for 30 min, or 5 W/kg into the body of an average 70 kg human for an indefinite duration would still not overcome normal healthy heat dissipation mechanisms [38–40]. A SAR (at 2450 MHz) of 65 W/kg over 1 g or 40 W/kg over 10 g into the brain has been estimated to be necessary to elevate the temperature of the healthy human to 3.5°C above normal [41] (a level in the literature associated with acute physiologic damage). Variables such as fever, however, can compromise heat loss mechanisms and increase heat production in the mother due to resetting of the hypothalamic set point [42–44]. The level of constant temperature increase at the skin to allow thermal sensation in the healthy adult has been predicted to be ~0.07°C [45] while that necessary to trigger pain is ~46.1 ± 1.0°C [46, 47].

Several researchers [48–50] have assigned a core body temperature of 39.2°C to represent the upper level of heat tolerance in adult humans. The Radiofrequency Radiation Dosimetry Handbook (their Figure 10.7) demonstrates that the ability of a normal adult to dissipate heat can be overwhelmed by conditions of ambient temperature and relative humidity of 42°C + 80%, 49°C + 50%, and 63°C + 20%. Under such conditions (without any RF irradiation), an average male human would achieve a core body temperature of 39.2°C within ~1 hour.

Overview of thermoregulation in the embryo and foetus

The foetus may be more vulnerable to temperature elevations due to MRI [51, 52] or other energy input sources. Animal models have demonstrated equivalent effects of RF and water bath emersion (resulting in the same core body temperature) on teratogenic and physiologic endpoints [53]. The developing foetus, in contrast to the adult, has a higher basal metabolic rate equivalent to ~3–4 W/kg WBA [54, 55] or about three times that of the adult female. The foetus also has more limited options with regard to heat dissipation, and a temperature difference of ~0.5°C between the foetus and mother is required for sufficient conductive cooling (~15% of heat dissipation [56]) via amniotic/allantoic fluids as well as convective cooling (~85% heat dissipation [56]) via umbilical and placental circulation [55–57]. The foetus is therefore entirely dependent upon

[D] [1 W*h = 3.6 kJ]; for local 1 g perfusion, 0.01 W*h (*3.6) = 0.036 kcal (*4.2) = 0.15 kJ(/g/s) = ~15 W/kg. For skin perfusion, 1.16 W*h = ~4.2 kJ (4200 J) or (/4.2) = ~1 kcal.

[E] Assuming a perfusion rate of 0.008 mL (cm^3)/g/s of tissue, a blood mass density of 1058 g/cm^3, and a specific heat of muscle tissue of 3550 W/kg.

[F] Assuming 8 L of blood/min (= 8 kcal/min) (*4.2) = 33.6 kJ/60 s, or 560 J/s, or 560 W in a 70 kg female = 8 W/kg.

[G] $\lambda = 2{,}490.0 - 2.34 \times t$, or at 37°C the vaporisation of 1 mL (=1 g) H_2O absorbs 2.403 J of heat energy, given 2000 mL sweat/h = 0.55 mL sweat/s × 2,403 J = ~1335 J/s or W, and in a 70 kg female = 19 W/kg.

[H] A total human surface area of 1.8 m^2 is assumed for calculations from Bender AE, Bender DA. Body Surface Area: A Dictionary of Food and Nutrition. Oxford: Oxford University Press, 1995.

[I] 675 W/m^2* 1.8 total m^2 surface area = 1215 W/70 kg female = 17 W/kg.

maternal temperature as well as circulation (convective cooling flows) to avoid hyperthermia. In fact there may be physiologic inhibitors (e.g. prostaglandins, adenosine) active in the foetus to make it entirely ectothermic and block any attempt to thermoregulate independently of the mother [55, 58]. Thermal stress in the mother from vigorous exercise [59, 60] or hyperthermia [61]can reduce blood flow to the uterus and placenta and may also contribute to the finding of placenta and foetal weight reduction under extreme conditions [62, 63]. With limited ability to regulate its own temperature [55], spontaneous abortion and malformations in the foetus have been reported as a result of maternal fever ($\geq$37.8°C) [64–71], hot tub use [72], and other situations associated with elevated maternal temperature [73–77]. However, these are hypothetical possibilities but have not been proven in humans. For example, spontaneous abortions and malformations may be due to the disease causing the fever rather than the temperature *per se*. Unfortunately, the effects of long-term low-level thermal stress on the foetus have not been well documented.

Human development and susceptibility to teratogenesis

A review of the relevant literature shows that absolute thresholds for hyperthermia differ depending upon the animal model used (including strain differences), the specific developmental stage exposed, and the organ system or malformation being evaluated. As a general rule, however, maternal temperature increases of ~2°C above normal for extended periods [78], or 2–2.5°C above normal for 0.5–1 hour [54, 79] are indicated in the literature as necessary for heat-induced abnormalities in the developing mammalian foetus [90]. Such abnormalities include death, abortion, growth retardation, microencephaly, cataract, hypoplasia of digits and incisors, talipes, exomphalos, and other malformations. In general, the incidences of these abnormalities increase with increasing temperature elevation and exposure duration. Although the occurrence of developmental abnormalities due to hyperthermia has been observed in virtually all laboratory animal species tested, it has not been confirmed in humans.

For exposures of 5–10 min or less, the threshold may be as much as 4°C above normal core temperature. Cell proliferation, cell migration, cell differentiation, and scheduled apoptosis seem to be the essential processes that occur during foetal and post-natal development that are specifically vulnerable to elevated temperatures, especially in the developing nervous system [80–82]. Damage to membranes [83] and the cytoskeleton [84, 85] may also be involved. The influence of thermal dose and time course of thermal exposure [86, 87] as well as thermoprotection due to an initial moderate hyperthermic exposure prior to a significantly higher thermal dose [88, 89] is also a factor that has been well characterised.

In general, elevated temperatures that occur before or during implantation of the embryo into the uterine wall do not present as subsequent developmental abnormalities. In such cases, if the blastocyst is sufficiently damaged it tends not to successfully implant or progress further. This phenomenon in which the embryo during this period is quite resistant to the teratologic effects of most teratogens but very susceptible to the lethal effects is called the 'all or none' effect [90]. Hyperthermia that occurs during the period of organogenesis following implantation can result in a variety of developmental malformations, and animal studies demonstrate different threshold temperatures and different windows during development can give rise to different malformations [91]. In general, the earlier the injury to the developing embryo, the more severe the malformation will be. During the period of later foetal development, animal models have shown that heat mainly reduces growth rate. The fundamental development of most structures at this stage is not affected to the same extent as in embryonic stages, although the nervous system can still be significantly affected.

During organogenesis in the human embryo, the ectoderm, endoderm, and mesoderm layers begin to develop into more complex organ systems. This process occurs in humans between the third and eighth week post-conception [92] and results in limb buds as well as structures that will eventually become the skeleton, nervous system, and circulatory system (including blood vessels and blood cells), and organs such as the digestive system, liver, pancreas, and other endocrine organs. The window of time when organs are most susceptible to hyperthermia generally corresponds to periods of rapid cell proliferation involved in the development of the organ. This window can vary in length and temporal sequence for different organ systems. The embryo remains generally resistant to elevated temperature outside of these periods of rapid cell proliferation.

The developing brain and central nervous system seems particularly vulnerable to malformations that may be associated with hyperthermia, although the mammalian embryo is also susceptible to craniofacial, skeletal, and cardio malformations. At ~day 20 to day 28 of human development, as neural plate cells migrate to form neural folds and eventually the neural tube, the human embryo is most susceptible to gross malformations of the brain (anencephaly, spina bifida, encephalocele), eyes (anophthalmia, microphthalmia, defects of the iris), face (small

upper or lower jaw, cleft palate) and heart. When neuronal cells that form the brain proliferate and make connections during the mid to late embryonic stages, the embryo is also susceptible to microencephaly and subsequent learning deficits [93]. With regard to RF exposure, the embryo at day 28 has dimensions of $\sim 0.5 \times 0.5 \times 1.0$ cm, and from tissue modelling studies of Foster and Glaser [94] such a size exposed to RF at a constant rate of 1 W/kg would have a thermal equilibration time of ~13 min and a maximal expected temperature increase of 0.2°C (given normal bioheat dissipation). Although it is difficult to imagine how such an exposure could be concentrated to the embryo without having a significant portion absorbed by the mother, this estimate offers an approximation for local exposures that should not elevate temperature to an adverse level.

During the period of foetal development when granule cells of the cerebellum, olfactory bulb, and dentate gyrus show late proliferative burst, exposure to hyperthermia may result in decreased spatial memory behaviour similar to ionising radiation exposure [95–98]. Brain development in the human continues after birth into the infant and juvenile periods [99] suggesting additional periods of vulnerability to elevated temperatures.

Studies of teratogenicity in animal models

Experimental models of hyperthermia and teratogenesis have been predominantly performed in animal models including guinea pigs [100, 101], pigs [102], rats [103], mice [104–106], and other species [107], and have used radiofrequency (RF) radiation [108–111], water baths [91, 112], warm air [113–115], surgically exteriorised uterine horns heated in warm water [116], and embryos heated in culture [117, 118]. Comparison of studies that have incubated animal embryos in vitro with the findings from in utero exposure studies [117–119] suggest hyperthermia acts predominantly on the developing embryo itself as opposed to having any primary effect on the maternal factors supporting embryonic growth.

A collective assessment of animal results suggests unique windows of vulnerability for neurological as well as craniofacial and skeletal defects that predominantly occur within the early stages of organogenesis during the embryonic period equivalent to human gestation week 3 through 7. The required maternal temperature elevations fall between 2–5°C above normal and show a clear temperature–time relationship (i.e. longer times of exposure requiring lower absolute temperature increases). By inference from animal studies, and for time periods that can be compared with the current time averaging periods for general public exposure levels to RF energy per the current IEEE C95.1 standard (i.e. 30 min [1]) in the range of wireless communication systems (100 MHz–5 GHz), elevations above maternal core body temperature in the order of $\geq$2–2.5°C would be required. For time periods that can be compared with the current time averaging periods for occupational exposure per the current IEEE C95.1 standard (i.e. 6 min [1]), temperature elevations above maternal core body temperature in the order of $\geq$4°C would be required.

In support of these observations, the majority of rodent studies in the IEEE EMF literature database [120] suggest that levels of RF exposure $\geq$9 W/kg to pregnant dams sufficient to elevate maternal core temperature $\geq$2°C during the entire period of gestation are required to observe significant foetal malformations [1,108–111, 121–126]. These RF studies also confirm a clear dose response relationship between temperature elevation and malformations. In contrast to long-term hyperthermia induced by RF, brief periods of elevated maternal temperature created with short duration RF exposures have not produced significant foetal malformations [127, 128]. Synergy with known teratogens such as ionising radiation [129], 2-methoxyethanol [130–132], glycol ethers [133], salicylic acid [134], and arabinoside [135] has also been reported to require levels of RF exposure sufficient to elevate the core temperature of pregnant rodents $\geq$1–2°C. A multi-generation study funded by the National Institute of Environmental Health Services and performed at the Illinois Institute of Technology Research Institute [120] is currently ongoing. This study will expose mice and rats to levels of RF energy at the maximal tolerated dose that will not overwhelm the basal metabolic rate, and should provide important information with regard to the threshold levels for long-term thermal stress and teratogenesis.

Extrapolating animal data to humans

It is difficult to directly extrapolate the findings of animal studies to humans in terms of absolute temperature because the normal body temperature of animals is often not the same as the ~36.8°C of a normal healthy human (e.g. guinea pigs = 39.1°C [136], pigs = 39°C, sheep = 39°C [79], birds = 40–42°C [137], mice = 37°C [138], rats = 38.5°C [139]). In addition, a consistent shortfall in these studies, as well as almost all of the historical animal studies in the RF bioeffects literature, is the housing of small animals in vivaria that are normally set to 'room temperature', or ~22–25°C. Rodents in particular are much smaller

in size than humans (i.e. larger surface to volume ratio) and require a higher basal metabolic rate to maintain their core temperature of 36–37.5°C. They also have a much tighter thermoneutral zone (TNZ) (28–34°C in rats and hamsters, 30–31°C in guinea pigs, 26–34°C in mice) than humans (TNZ = 24–31°C). Outside of the TNZ, increased metabolic energy must be spent to activate various thermoeffectors to maintain temperature [35]. When housed at temperatures up to ~10°C below the lower critical limit of the TNZ, small rodents depend much more heavily on increased metabolic rate than humans who rely initially on vasomotor control of peripheral circulation. As such, many of the existing studies on teratogenesis in small rodents have presumably used controls that are 'chilly' or mildly cold-stressed with a heart rate that may be increased >15% [140] and a metabolic rate that may be increased up to 25% [141]. While housing both control and exposed animals under mild cold-stressed conditions may not significantly influence the comparison of many chemical or physical agents, the nature of RF energy is to offer the cold-stressed animal a source of thermal energy. In doing so, low levels of RF exposure (≤2–3 W/kg) may actually help bring the animals into thermoneutrality and lower metabolism, perhaps significantly. While higher exposures of RF energy may drive the animal above the TNZ and initiate an opposing set of thermoeffector responses, many animal studies using exposures ≥4 W/kg have employed forced air cooling systems to assist the animal in maintaining core temperature. In all, it is difficult to assess what effect using mildly cold-stressed rodents and comparing them to either thermoneutral or mildly heat-stressed rodents may have had on the observed outcomes.

However, general corroboration of relative temperature increase above normal maternal body temperature can be seen from the epidemiological studies reporting an elevation of ~2°C for at least 24 h (due to fever or other sources) is necessary to correlate with the increase in developmental abnormalities [64–77]. Although many of these studies focus on the effects of fever due to illness where other disease confounders may play a role, there are studies that have followed non-fever sources of thermal stress on pregnancy outcome [73, 78]. The exact stage of gestation during which the episode of fever occurred in many of these studies is also not always well documented. Little information exists, however, on thresholds for shorter exposures.

The principal molecular mechanism responsible for the adverse effects on the foetus from hyperthermia is heat-induced protein denaturation. Enzymes are proteins, and when a temperature elevation exceeds a threshold the physical structure of an enzyme is altered. This leads to enzymatic inactivation and subsequent cell death. The time required to cause cell death depends on the magnitude of the temperature elevation – the higher the temperature, the shorter is the time required. Vulnerability to thermal damage varies with cell type. Rapidly dividing cells are most sensitive, and this explains why the embryo is so vulnerable to the effects of hyperthermia. The embryonic structures most affected by hyperthermia depend on which structures are most rapidly dividing at the time of exposure. Very early in gestation, the brain and neural tube are most sensitive; later on the upper extremity is most vulnerable, and later still the lower extremity is most vulnerable [142].

Recent (since January 2004) RF bioeffect studies on teratogenesis

Since the last revision of IEEE C95.1 (containing a review of literature through December 2003), there have been seven reports of RF exposure and teratogenesis. Two studies reported exposures to *Drosophila melanogaster* that resulted in a decreased number of offspring and delays in pupation. The first from Turkey exposed *Drosophila* larvae to RF energy at an estimated SAR of 9.8 mW/kg [143], and the second from Greece exposed adults to an average power density of 0.618 mW/cm^2 for 6 min per day for the first 2–5 days of life [144]. Although the authors report no significant temperature increase, the exposure systems were not well characterised and dosimetric evaluation was lacking from both studies. A study from Belgium reported house sparrow breeding populations were significantly decreased in the vicinity of mobile phone base station transmitters, with the decrease having an inverse correlation with measured field strengths [145]. In contrast, each of three studies using animal models reported no effects on survival, teratogenicity, or development using exposures close to the thermal threshold. The first from Japan exposed pregnant Sprague Dawley rats to 2 GHz W-CDMA for 90 min/day at a SAR of up to 2 W/kg brain average (0.4 W/kg whole body including the foetus) from gestation day 7 to 17 [146]. The second, from Korea, exposed pregnant ICR mice to 20 kHz at 30 uT for 8 h/day, 5 days/week until day 18 of gestation [147]. Finally, a multigeneration study from Germany exposed mice continuously over four generations to 1966 MHz (UMTS) at up to 1.3 W/kg WBA [148]. Two studies of human populations were reported. The first, from Denmark, involved a study of mobile phone use in pregnant mothers [149]. The authors did report a correlation with behavioural problems in the children by age 7, although they suggest this correlation, if real, is not likely due to the effects of RF exposure.

A pair of studies on Navy personnel in Norway reported a higher rate of infertility and offspring with congenital malformations with occupations having significant exposure to RF, although the data were based on small numbers [150, 151]. Overall, recent studies since January 2004 do not significantly change the larger weight of evidence or support the need to alter the conclusions from IEEE C95.1-2005 regarding teratogenesis.

Human reproductive tissues

In the adult male human, the testes generally resides outside the body at a resting temperature of ~34–35°C – a temperature necessary to promote optimal spermatogenesis [152]. Under periods of cold (for warmth) or stress (for protection), the cremasteric muscle can contract and move the testes upwards into the urogenital ridge of the body, thereby increasing the temperature naturally. Decreased sperm production and eventual morphological changes within Leydig cells at higher temperatures is well documented in the literature [153]. Other effects such as decreased androgen production can also occur with prolonged temperature elevation [154]. If temperature elevations are transient (e.g. 43°C for 15 min), however, these changes have been observed to be completely reversible [155]. Further, if the temperature in the male testes did ever exceed core body temperature, active convective cooling through the testicular artery and vein would limit the magnitude of temperature rise [156]. In the adult female, there is evidence that hyperthermia can disrupt oocyte development, especially in sensitive subpopulations, prior to release from ovarian follicles [157]. The mechanism for this appears to be associated with alterations in membrane lipid composition [158].

RF bioeffect studies (reproduction)

Since the last revision of IEEE C95.1 (containing a review of literature through December 2003), there have been 10 reports on RF exposure and reproduction. Several studies have reported decreases in human male fertility and sperm motility that correlated with mobile phone use or storage of the phone in one's pocket [159–162]. However, accurate exposure assessment and dose response analysis in these studies is missing.

Four animal studies reported using a mobile phone hooked to the local network as an exposure source, which prevents accurate assessment of exposure and dose response analysis. The first, a study from Brazil [163], exposed Wistar rats for 1 h/day for 11 weeks and reported no effect on body weight, testicular or epididymal weight, sperm count, lipid peroxidation in the testes and epididymis, serum testosterone level, or general histology. The second study from the Medical College of Wisconsin [164] exposed Sprague Dawley rats for 6 h/day for 18 weeks at estimated SARs of 1.8 W/kg (AMPS), 0.9 W/kg (GSM 850 MHz) or 1.18 W/kg (GSM 1900 MHz) and reported changes in sperm cell viability (60% increase in death) and abnormal clumping. The third, a study from Hungary [165], exposed male mice at SARs of 0.018 to 0.023 W/kg for 2 h/day for 5 days. The authors reported a slight increase in serum testosterone but no histological change in body weight, Leydig cells, epididymis, or other histological parameters of male reproduction. The fourth, a study from Turkey [166], exposed pregnant mice for 11 h 15 min in standby mode and an additional 15 min in talk mode each day for 21 days during pregnancy. The authors report exposure was associated with a significant decrease in the number of pups per delivery but not average pup weight. There was also a significant decrease in the volume and number of follicles in maternal ovarian sections.

Three studies also used mobile phones as an exposure source, although in the first two the phone was hooked to a base station simulator as opposed to the local network allowing some control of transmit power level. A study in Sprague Dawley rats from Turkey [167] saw no effect on spermatogenesis or sperm apoptosis following exposure at a calculated SAR of 0.07–0.57 W/kg in the testes for 2 h/day, 7 days/week, for 10 months. A study from Japan [168] exposed male New Zealand rabbits for 8 h/day for 12 weeks by placing the mobile phone directly under the cage beneath the area of the testes. The authors approximated a whole body average exposure level of 0.43 W/kg (but did not provide a local SAR in the testes) and reported a significant drop in sperm concentration and motility after the eighth week of exposure. There was no change in core temperature, body weight, weight of the testicular tissue, morphology of sperm, or testosterone levels. In the third study from South Africa [169], the mobile phone was used as a signal source to drive a TEM cell and expose sperm samples from healthy volunteers for 1 h at 2 or 5.7 W/kg in a Petri dish. The authors report no effect on apoptosis, mitochondrial membrane potential, reactive oxygen species (ROS), but do report a progressive decrease in motility at the highest exposure level (5.7 W/kg). In all, the recent studies since January 2004 report several effects on sperm function and fertility. However, the lack of exposure assessment associated with the majority of these studies makes interpretation difficult. Further, these changes in reproductive endpoints do not seem to translate into effects seen in multiple animal studies exposed to similar levels of RF energy.

Table I. Common sources of RF radiation and potential for significant heating of foetus.

RF source	Frequency	Penetration (cm)	Significant heating of foetus
AM radio	0.6 MHz	>100	No
Short wave diathermy	27.2 MHz	14.3	Yes
TV	100.0 MHz	6.7	No
Analogue cell phone	900.0 MHz	3.1	No
Radar	1.0 GHz	2.5	No
Digital cell phone	2.0 GHz	2.0	No
Microwave oven	2.45 GHz	1.7	No
Microwave diathermy	2.45 GHz	1.7	Yes
Airport scanners	40.0 GHz	0.06	No

Note: Frequencies are typical values for each source. Penetration is the distance travelled into the skin for the RF amplitude to decrease to 36.8% of its initial value. Significant heating of foetus refers to whether or not the source has sufficient energy or penetration to cause a 1°C temperature elevation.

Table II. Maximum temperature elevations from MRI examinations.

MRI scanning level	Max SAR	Max temperature rise
For whole body scans		
Normal level	2 W/kg	0.5°C
First level	4 W/kg	1.0°C
Second level	>4 W/kg	>1.0°C
For partial body scans (<25% of body)		
Normal levelNormal level	8 W/kg	1.1°C

Table III. Maximum allowed intensities for ultrasound applications.

Application	Max intensity (mw/cm^2)*
Foetal and general imaging	720
Cardiac	720
Peripheral vascular	720
Ophthalmologic	50

*Spatial-peak, temporal-average intensity measured in water and derated by 0.3 dB cm^{-1} MHz^{-1}.

Overall, the studies on RF exposure and reproduction since 2004 do not significantly change the larger weight of evidence or support the need to alter the conclusions in IEEE C95.1-2005.

Temperature elevations in RF, MRI and diagnostic ultrasound

Radiofrequency (RF) radiation

Humans are constantly exposed to RF radiation from many sources, both in and outside of the home. RF radiation refers to electromagnetic waves from 300 kHz to 300 GHz. Table I provides a list of common RF sources to which humans are exposed.

Both the International Commission on Non-Ionizing Radiation Protection (ICNIRP) and the IEEE International Committee on Electromagnetic Safety (ICES) have set the basic restriction for RF radiation (3 kHz to 300 GHz) for Occupational Workers at 0.4 W/kg for unlimited exposure duration [170, 171]. This level is one tenth the level that would produce a 1.0°C rise in core temperature after 1 h of exposure.

Magnetic resonance imaging (MRI)

MRI has become a major diagnostic imaging procedure. The images provide excellent depiction of internal anatomy. MRI exposes patients and health professionals to strong stationary magnetic fields as well as RF radiation.

The FDA requires that all MRI scanners specify the SAR for each pulse sequence and scanning procedure that is available to users [172]. The International Electrotechnical Commission (IEC) has defined three levels of operation: Normal, First Level, and Second Level [173]. The maximum expected temperatures are shown in Table II.

The FDA limits the average whole body SAR during MRI exams to 4 W/kg for any 15-min period [174].

Ultrasound imaging

Ultrasound has become an indispensable imaging modality, especially for foetal diagnosis. There has been constant improvement in obstetrical imaging over the past 45 years and the ability to see anatomical detail in the embryo and foetus is truly remarkable. This has led to the nearly universal exposure for the foetus to ultrasound at least once and usually more times during a pregnancy. Because of this widespread exposure to ultrasound, even a small risk of harm has a great impact on public health. Although diagnostic ultrasound has had an excellent safety record – there have been exceedingly few adverse effects reported – laboratory studies in animals have shown serious harm if the intensity is sufficiently high. To minimise the possibility of harm, the FDA has established the following limits

Table IV. Diagnostic ultrasound instruments.

Ultrasound sources	Acoustic output mW/cm^2	Temperature elevation
Doppler foetal heart monitor	20	<1°C
B-scanner	35	1°C
Colour flow Doppler	450	3°C
Duplex Doppler	400	2°C
Ophthalmic scanner	40	<1°C

Note: Acoustic outputs are typical values of derated spatial-peak temporal-average intensity values. Also provided are possible temperature elevations in the foetus.

to permit an ultrasound imaging device to gain marketing approval under the 510 K process [175].

The temperature elevations that occur when internal organs are exposed to ultrasound beams depend on many physical factors such as beam size, ultrasonic frequency, and intensity as well as differing tissue properties [176]. The most important tissue factor is the presence or absence of bone, because of its high absorption of ultrasound. In routine scanning of the foetus in the first trimester where there is an insignificant amount of bone, the intra-uterine temperature rise would not be expected to exceed 1°C. In late pregnancy where there is substantial bone present, temperatures close to bone could rise several degrees. This is also true for Doppler applications where the acoustic power output is typically higher than that used in routine scanning. Table IV lists various diagnostic ultrasound instruments and the anticipated temperature elevations.

As an aid to minimise the temperature elevation during a clinical ultrasound examination, the thermal index (TI), has been incorporated into modern ultrasound scanners. This dimensionless index, using instrument settings and reasonable tissue models, estimates the maximal anticipated temperature elevation during an examination. The continually updated TI is displayed on the image and provides the ultrasonographer with guidance on how changes in the instrument control settings affect internal temperature. Ultrasonographers are trained to keep the TI value low during foetal examinations.

Conclusions

Temperature thresholds for teratogenicity in the developing human embryo and foetus are difficult to determine since different time–temperature windows and thresholds apply for different endpoints. In addition, animal studies of RF exposure have generally not taken into account significant differences in thermoregulatory behaviour and thermoneutral ambient temperatures as compared with humans that may represent a potential confounder when thermal energy is applied. What is constant, however, is the dependence of the developing embryo and foetus on the mother for thermoregulation. As a general rule, maternal temperature increases of ~2.0°C above normal for extended periods of time, or 2–2.5°C above normal for 0.5–1 h, or ≥4°C above normal for 15 min have been required to observe developmental abnormalities in animal models, and these are the best available threshold predictions for adverse developmental effects in humans. However, it must be remembered that extrapolation from animal models to humans is very difficult because the normal body temperature varies by more than several degrees across species and that the same temperature increment may pose a different thermal stress in different species because of their differing thermoregulatory capabilities. Corresponding SAR values that would be necessary to cause such temperature elevations in the healthy adult female are probably in the range of ≥15 W/kg WBA. However, thermal stress in the mother (even if asymptomatic) could theoretically result in altered placental and umbilical blood perfusion and subsequently decrease the only heat dissipation mechanism available to the embryo or foetus. To address this possibility, a very conservative estimate of 1.5 W/kg WBA (1/10th the threshold to protect against measured temperature increases) would seem sufficient to protect against any such effect of reduced blood flow to the embryo or foetus in the exposed pregnant mother. This is >3 times above the current WBA limit for occupational exposure (0.4 W/kg) as outlined in both IEEE C95.1-2005[1] and ICNIRP-1998[181]. With regard to local RF exposure directly to the embryo or foetus, significant absorption by the mother as well as heat dissipation due to conductive and convective exchange would offer significant protection. However, a theoretical 1 W/kg exposure averaged over the entire 28-day embryo, or averaged over a 1 g volume in the foetus, should not elevate temperature more than 0.2°C.

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