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Basic principles of thermal dosimetry and thermal thresholds for tissue damage from hyperthermia

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This paper is one of several in this Special Issue of the *International Journal of Hyperthermia* that discusses the current state of knowledge about the human health risks of hyperthermia. This special issue emanated from a workshop sponsored by the World Health Organization in the Spring of 2002 on this topic. It is anticipated that these papers will help to establish guidelines for human exposure to conditions leading to hyperthermia. This comprehensive review of the literature makes it clear that much more work needs to be done to clarify what the thresholds for thermal damage are in humans. This review summarizes the basic principles that govern the relationships between thermal exposure (temperature and time of exposure) and thermal damage, with an emphasis on normal tissue effects. Methods for converting one time-temperature combination to a time at a standardized temperature are provided as well as a detailed discussion about the underlying assumptions that go into these calculations. There are few *in vivo* papers examining the type and extent of damage that occurs in the lower temperature range for hyperthermic exposures (e.g. 39–42°C). Therefore, it is clear that estimation of thermal dose to effect at these thermal exposures is less precise in that temperature range. In addition, there are virtually no data that directly relate to the thermal sensitivity of human tissues. Thus, establishment of guidelines for human exposure based on the data provided must be done with significant caution. There is detailed review and presentation of thermal thresholds for tissue damage (based on what is detectable *in vivo*). The data are normalized using thermal dosimetric concepts. Tables are included in an Appendix Database which compile published data for thresholds of thermal damage in a variety of tissues and species. This database is available by request (contact MWD or PJH), but not included in this manuscript for brevity. All of the studies reported are for single acute thermal exposures. Except for brain function and physiology (as detailed in this issue by Sharma *et al*) one notes the critical lack of publications examining effects of chronic thermal exposures as might be encountered in occupational hazards. This review also does not include information on the embryo, which is covered in detail elsewhere in this volume (see article by Edwards *et al*.) as well as in a recent review on this subject, which focuses on thermal dose.

Key words: Thermal dosimetry, hyperthermia, damage threshold, Arrhenius analysis, isoeffect, normal tissue tolerance.

1. Introduction

The purpose of this review is to present basic concepts relating temperature and time-at-temperature to cell killing and tissue damage. These concepts can be used as a framework to develop regulatory guidelines for occupational and incidental

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population exposures to hyperthermia that might occur as a result of accidental or repeated exposures to radiofrequency fields (RF). Factors influencing the time-temperature-damage relationships for thermal damage will also be discussed. Thresholds for thermal damage to a range of tissues are summarized.

2. Kinetics of cell killing by hyperthermia: the arrhenius relationship

2.1. In vitro *studies*

An excellent review on this subject has been published previously and readers are encouraged to examine this review, if additional details are required[1]. Numerous *in vitro* studies show that the rate of cell killing during exposure to heat is exponential and dependent on the temperature and length of exposure. Figure 1(*a*) shows a family of survival curves for Chinese hampster ovary (CHO) cells covering the range from 42–45°C with heating times up to 5 h[2]. These survival curves typically

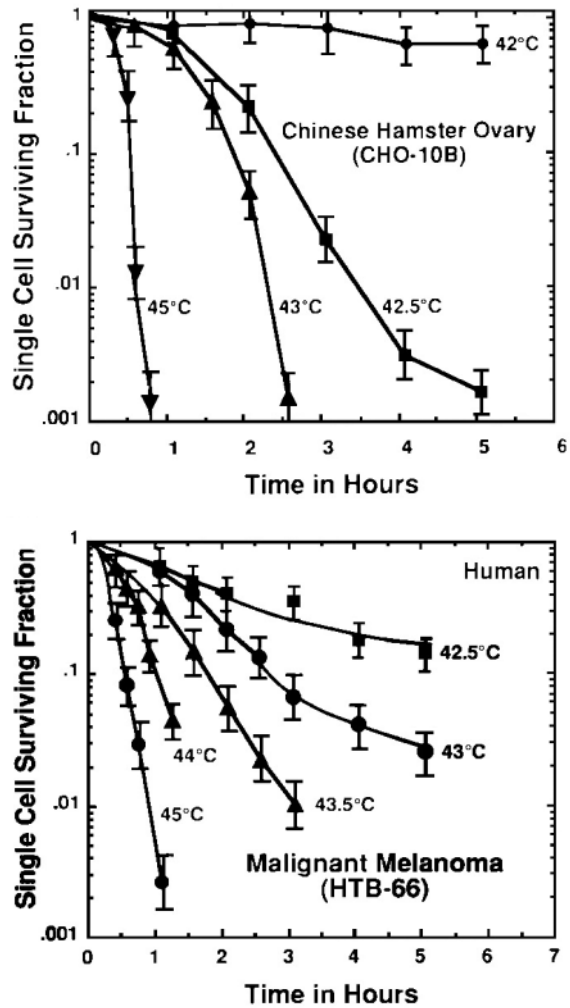


Figure 1. (*a*) Survival of Chinese hampster ovary cells, heated over a range of temperatures. (*b*) Survival of a human melanoma cells, heated to similar ranges of temperatures. (Roizin-Towle and Pirro[2], reproduced with permission of the publisher.)

have a shoulder. The width of the shoulder region varies with cell line and is also dependent upon temperature. The shoulder region shows that there is a threshold for thermal damage to cells. Once cytotoxicity starts to occur, the rate of cell killing, which is exponential with time of heating, is dependent on temperature. For the example provided, there is very little cytotoxicity for up to 5 h of heating at 42°C. At 42.5°C, however, three logs of cell kill are achieved after five hours of heating. For comparison purposes, a similar family of survival curves is plotted for a human tumour cell line (HTB-66; figure 1b). The CHO and HTB-66 cell lines show a reduction in slope of the cytotoxicity curve after 4 h of heating at 42.5°C and 3 h of heating at either 42.5 or 43°C, respectively. This reduction in slope is due to acquired resistance to heating or thermotolerance.

A number of authors have used data such as those shown above to determine the heat of inactivation of cells, by using an Arrhenius analysis (figure 2). This analysis is done by plotting the rate of cell killing ($1/D_o$; where D_o is defined at the number of minutes to reduce survival by 63% on the exponential portion of the survival curve) vs 1/temperature (°K). Using equation (1), the heat of inactivation can be calculated.

$$K = Ae^{(-E/RT)} \tag{1}$$

where $E =$ heat of inactivation in kcal/mole, A is a constant that is assumed to be unchanged over the temperature range studied, $R =$ molar gas constant (1.987×10^{-3} Kcal/mole-°K) and T is the absolute temperature in °K.

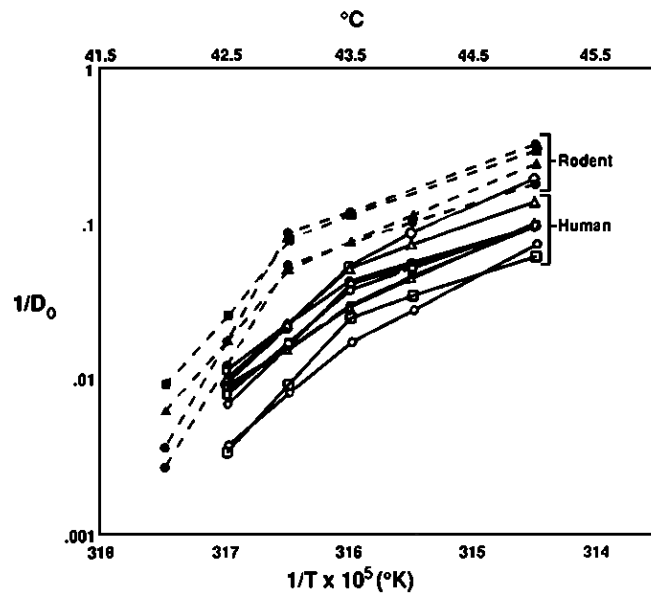


Figure 2. Comparison of Arrhenius plots for a series of rodent and human cell lines, derived from cell survival curve data, as shown in figure 1. The Arrhenius plots consider the rate of cell killing on the exponential portion of the curves. The size of the shoulder of the survival curve is not included in this analysis. The rodent cell lines included in this figure are CHO, AD-%, C3H 10T-1/2: Human cell lines are KB7, MIA-PACA2, glioblastoma (not otherwise defined), WiDR, AG-1522, HTB66, HTB72, KB8 and A549. Data from a paper by Roizin-Towle and Pirro[2], reproduced with permission of the publisher.

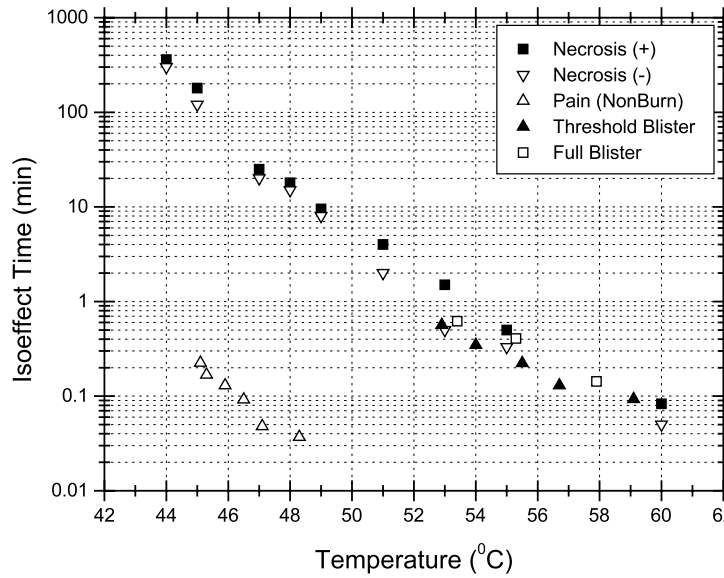
The slope of the Arrhenius plot is typically biphasic. The curves typically have a 'break point' and the slope tends to be steeper below the break than above it. The inactivation energy for the temperature range above the 'break point' is typically around 120–150 kcal/mole, which is consistent with the heat of inactivation of proteins and enzymes[3] (also, see article by Lepock in this Special Issue). The change in slope of the Arrhenius plot below the breakpoint is generally thought to be related to the development of thermotolerance (acquired thermal resistance) during heating[4]. When heating is delivered at temperatures above the breakpoint, thermotolerance does not occur *during* the heating period. It should be kept in mind, however, that thermotolerance does develop *after* heating at temperatures above and below the breakpoint.

Arrhenius plots for human and rodent cells are different in two key aspects (figure 2). For human cells, the breakpoint appears to be $\sim$43.5°C whereas it is nearer 43°C for rodent cells. Secondly, the slopes of the survival curves for human cells tend to be shallower (smaller) than they are for rodent cells, at any defined temperature. This means that more time is needed to kill human cells at a defined temperature. The higher breakpoint for human cells is interpreted to mean that human cells develop thermotolerance at a higher temperature than rodent cells. This is verified by comparing the data in figure 1(*a*) to that in 1(*b*). Note that CHO cells show no evidence for thermotolerance at 43°C (i.e. change to shallower slope after several hours of heating), whereas there is clear evidence for thermotolerance for human cells at that temperature. These characteristic differences in the Arrhenius plot between human and rodent cells indicate that human cells are more thermally resistant than rodent cells. As a qualitative validation, the difference in thermal sensitivity of the CHO and human tumour cell lines at 42.5°C is quite apparent (figures 1(*a*) and (*b*).

Above the breakpoint of the Arrhenius plot, the rate of cell killing essentially doubles for every degree increase in temperature. This means, for a given isoeffect, such as a defined level of survival, the time at temperature needed to achieve that isoeffect is halved for each degree increase in temperature. At temperatures below the breakpoint the rate of cell killing decreases by a factor of 4–6 for every degree decrease in temperature[4]. As will be discussed below, there are essentially no human data to establish whether these guidelines relating the rate of cell killing vs temperature are valid for human tissues, particularly below the breakpoint. Acquisition of more definitive data may be important for establishing guidelines for human thermal exposure.

2.2. In vivo *studies*

Arrhenius data have also been derived from a large number of *in vivo* studies. In this case, the endpoint is usually the time to reach an isoeffect, at a defined temperature. One of the first attempts to do this type of study was performed by Moritz and Henriques[5] in 1947. They used a hot water applicator to test the time–temperature relationships needed to create 2 and 3° burns in human and pig skin, over a wide range of temperatures (44–70°C). In a subsequent paper, Stoll and Greene[6] obtained very similar results using more precise thermometry, but over a more narrow temperature range. An important point from the latter data is that the threshold for pain is much lower than the threshold for grossly detectable physical injury. However, the isoeffect relationships appear to have the same slope, regardless of the endpoint (pain, blister, full necrosis) (figure 3(*a*)). Inactivation energies for these tissues are



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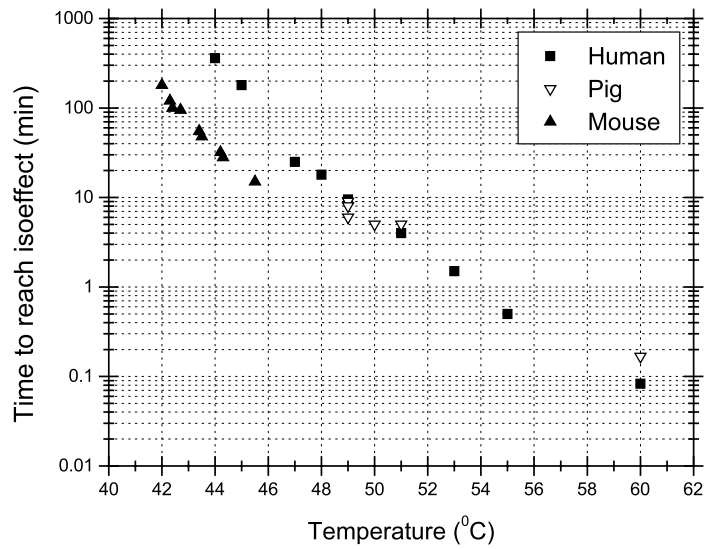

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Figure 3. (*a*). Time–temperature combinations to achieve varying thresholds of thermal damage to human skin. Data obtained from Moritz and Henriques[5] and Stoll and Greene[6]. Note that the isoeffect curves are parallel for the various forms of injury. Also, the pain threshold is significantly lower than the threshold for significant injury, indicating that pain avoidance effectively minimizes significant injury to skin. Necrosis (+) refers to full thickness, or third degree burn. Necrosis (–) refers to less serious injury or no injury observed at all. (*b*) Time to reach epidermal necrosis as a function of temperature for mouse, human and pig skin. The data for mouse ear skin necrosis were derived from a paper by Law[7]. The human and porcine data come from a paper published by Moritz and Henriques[5]. Note that the thermal sensitivities of pig and human skin are virtually identical and both appear to be more resistant to thermal damage than mouse skin.

Table 1. Activation energies calculated from double exponential fits to data from figure 3. The breakpoints were 47°C for man and pig and 42.5°C for mouse.

Species	Temperature range (°C)	Activation energy (kcal/mole)
Man	44–47	182.2
	47–60	95.78
Pig	44–47	150.75
	48–56	106.38
Mouse	41.5–42.5	273.89
	42.5–44.5	138.26

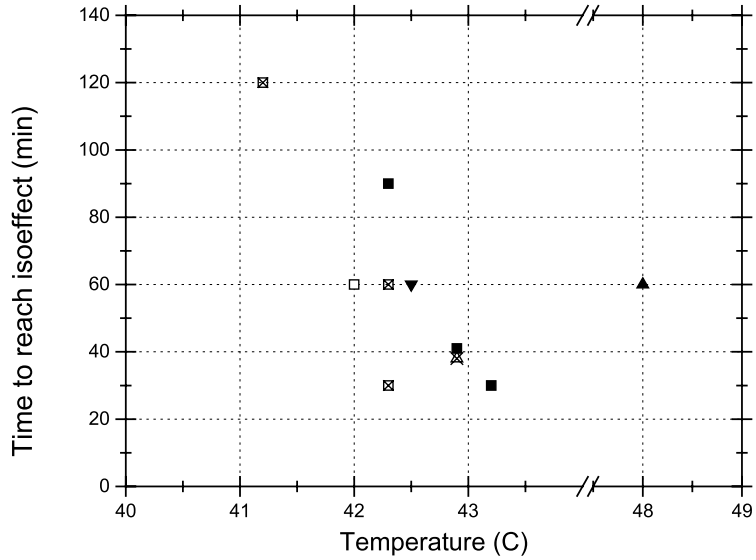
shown in table 1. The human data are compared with porcine studies of skin tolerance which had the same endpoint as the human results (figure 3(*b*)). Data derived for mouse ear skin necrosis are also included[7]. Note that there is near perfect agreement between the human and porcine data, indicating that the thermal sensitivities of human and pig skin are similar. The data for mouse ear necrosis are quite different, however. The most obvious difference is that the time–temperature relationships to achieve ear necrosis are far lower for mouse ear than for the skin of humans/pigs. Secondly, determination of the breakpoint for the human data is difficult because the curve appears to be continously bending. However, good linear fits to the data can be obtained for temperatures between 44–47°C and for temperatures between 47–55°C. It is not known if there is a breakpoint near 43°C, because there are no data in that temperature range. Moritz and Henriques[5] reported that heating of human skin to a temperature of 44°C for 5 h (600 CEM 43°C) resulted only in mild hyperemia in two subjects. By comparison, heating at the same temperature for 6 h (720 CEM 43°C) resulted in complete epidermal necrosis. To test whether 43°C heating could cause thermal damage to human skin, they would have had to heat the subjects for periods between 600–720 min (10–12 h), which probably explains why it was not done. Thus, on the surface these *in vivo* data tend to corroborate the *in vitro* studies suggesting that human tissues are more thermally resistant than rodent tissues.

However, there is a fundamental weakness to the human and porcine data. The investigators only measured surface temperature, which is not an accurate estimate of what the temperature was at the level of the basal layer of the epithelium, where the stem cells reside. Buettner[8] examined human skin surface and sub-surface temperatures (0.2 mm depth, using thermocouples) during heating with a radiant heating device. Depending upon the applied power and the time of measurement after heating device was turned on, the temperature at 0.2 mm depth could be as much as 1–6°C lower than the surface temperature. Also, it takes a few seconds to reach thermal equilibrium. Thus, for short heating times, the average temperature of the skin surface would have been much lower than the final target temperature, which is the temperature reported for the papers by Moritz and Henriques[5] and Stoll and Greene[6]. Thus, heat up time and temperature difference at depth were not considered in the work of Moritz and Henriques[5] or Stoll and Greene[6]. For temperatures >50°C, heat up time could have been a large proportion of the total thermal exposure time to achieve a thermal injury. Additional theoretical and experimental studies have been performed to examine heat transfer in limbs. In rabbit hind limb, a 1°C temperature gradient has been seen between the skin surface

and the basal layer of the epithelium[9] under ambient conditions. A plexus of highly ordered vasculature is seen immediately beneath the epidermis that is capable of very efficient heat transfer. Perfusion of the skin (including studies of human skin) has been measured under conditions of local thermal exposure and has been found to increase by a factor of 10–20[10, 11]. Therefore, when the epidermal surface is being heated, the heat transfer capability of the skin will increase, thereby effectively increasing the magnitude of the thermal gradient across the epidermal layer. Given these considerations, it is likely that the temperatures to achieve injury in human skin were significantly lower than reported and that the heating times to achieve isoeffect injuries would have been shorter than reported for temperatures in excess of 50°C, where the total heating times are relatively short (a few seconds). This means that the slopes of the Arrhenius plots as well as the breakpoint temperatures for both human and porcine skin, that are derived from the data of Moritz and Henriques[5] and Stoll and Greene[6] are very likely inaccurate.

There are reports across multiple species for brain and spinal cord injury as well. Figure 4(*a*) compares time–temperature thresholds in three species for a range of damage endpoints following heating of the spinal cord. Note that the thresholds for avoidance of or minor damage are lower than for more severe damage. The slopes for less severe damage are parallel to those for more severe damage. From these data, it can be concluded that the thresholds for thermal damage to spinal cord are similar across species. Figure 4(*b*) compares time–temperature thresholds for thermal damage to brain, again across three species. There appears to be a clear temperature threshold for significant thermal damage, but it is not clear whether there is a definable slope to the data that would allow for determination of an Arrhenius relationship. Additional studies, in which both time and temperature were varied over a broader range, might help to define this relationship more clearly. It is important to note that the late Dr Lele[12] reported damage threshold data for necrosis in human brain tissue in a book chapter in 1983. During that time period, ultrasound had been used in some patients to ablate epileptic foci and presumably this is where the data came from. However, no details were provided in that paper on where the data were obtained. For this reason, the human data was not included in this summary review. More detail on the parallel data in the cat was provided in an earlier paper, and thus, those data are included[13]. It should be noted that his analysis suggested that human brain thresholds for necrosis did not deviate at all from the threshold data obtained for the cat. It is unfortunate that what might be the most important data ever generated on thermal thresholds for damage to a vital human tissue is not available in a form that can be critically reviewed.

The relative resistance of human tissues to thermal damage is a significant issue when it comes to considering issues related to regulation of thermal exposure because direct data relating thermal exposure to injury in humans are virtually non-existant aside from the skin data shown above. So, what can be done to estimate the thermal sensitivity of organs in humans? If all organs in animal models had the same thermal sensitivity, one could speculate that this might be true for humans also. This is not the case, however. Figure 5 compares thermal data for four thermal damage endpoints in murine tissues; namely foot necrosis, ear skin necrosis, testis weight and intestinal damage. Note that, while the curves tend to be parallel, the exact time–temperature combinations needed to achieve injury vary. Part of the difference could be due to the type of endpoint used. The ear skin and foot necrosis



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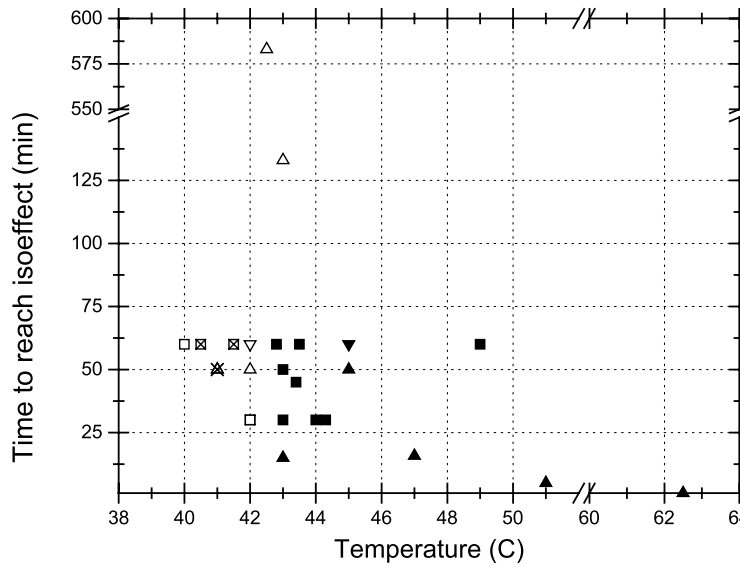

(*b*)

Figure 4. (*a*) Time–temperature relationships for thermal damage to spinal cord across three species. The fill of each symbol refers to the endpoint for damage, as follows: ☙ = no detectable damage, ☒ = subclinical histologic damage, and ■ = severe histologic damage and/or clinical symptoms or death. The shape of the symbol refers to the species used for each study: upward triangle = rat, square = mouse, and inverted triangle = dog. Data obtained from various references[49–52]. (*b*) Time–temperature relationships for thermal damage to brain across three species. The fill of each symbol refers to the endpoint for damage, as follows: ☙ = no detectable damage, ☒ = subclinical histologic damage, and ■ = severe histologic damage and/or clinical symptoms or death. The shape of the symbol refers to the species used for each study: upward triangle = cat, square = dog, and inverted triangle = rabbit. Data obtained from various references[12, 13, 53–57]. Original data are in the Appendix Database.

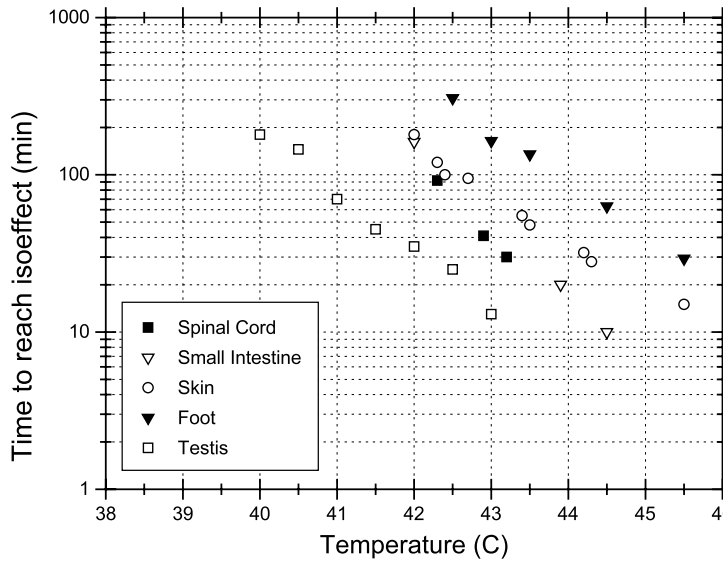

Figure 5. Time–temperature relationships to achieve isoeffective thermal damage in several mouse tissues. Note that the slopes for these isoeffects are parallel, but some tissues appear more sensitive than others (i.e. the thresholds for thermal damage vary from one tissue to the next). There are multiple reasons for this, some of which may not relate to actual differences in tissue sensitivity (see text for details).

data are severe injuries, compared with stem cell survival in the intestine or testis weight (which is reflective of cytotoxicity to stem cells and sperm progenitors). Stem cell survival may recover after heating and it relates to treatment effects in a single cell type and, thus, may not be reflective of the overall thermal sensitivity of a complex tissue. Differences in tissue architecture and kinetics of repair and replacement are different between tissues, so it is also important to control the time of assessment after exposure to be more precise. There has not been consistency in the time of assessement of injuries between authors. It is also known that subtle differences in protein structure can dramatically alter thermal sensitivity to denaturation. For example, the inactivation energy for the protein kanamycin nucleotidyl transferase is $\sim$140 ckal/mole, which is similar to other proteins[14]. When the protein is mutated with single and triple amino acid substitutions, the Arrhenius curve is shifted by 3–9°C without changing the slope of the curve. This means that the inactivation energy remains constant, but the entropy (cal/°C/mole) changes[15]. Thus, there are biological and methodological explanations for why different tissues may have varying thermal sensitivities.

Careful examination of the detailed data in the Appendix Database, however, demonstrate remarkable concordance in the thermal sensitivities of individual tissues across species types. For example, the damage threshold for small bowel mucosa has been reported to be between 20–50 CEM 43°C for mouse and hamster[16–18]. Data are not available for multiple species comparisons for every tissue at this time, but based on what is known about the thermal sensitivity of human cells in tissue culture and the similarity of tissue sensitivities across species, one can conclude that it is very unlikely that human tissues are more thermally sensitive than what has been reported for other species.

3. Thermal isoeffective dose

The recognition that the rate of cell killing is related to time and temperature has led to several different methods for normalizing time-at-temperature data to a common unit that would allow for comparison of different heating regimes. For clinical applications of hyperthermia, this is particularly important, since temperatures during heating are typically non-uniform and temporally unstable. Spatial variation in temperature is more of a problem typically than temporal variation. For a typical treatment during thermal steady state, temperatures can vary from 37–43°C within the same tumour. Temperatures can even be higher, particularly if there is a focus of necrosis in a tumour[1–4]. The largest temporal variation occurs typically during heat up and cool down. Depending on the tumour location and the efficiency of the heating device it can take from 5–20 min to reach an acceptable thermal steady state. Then, if there are adjustments to applied power or changes in perfusion during heating, this can lead to further temporal variation. Sampling times for temperature measurement vary, depending upon whether the thermometers are fixed in one position or if they are mapped in indwelling catheters. A typical frequency might be one measurement per minute. Thus, a simple thermal prescription defining a desired temperature for a defined period of time is difficult if not impossible to achieve. In a classic paper, Sapareto and Dewey[19] proposed a simple method for converting one time–temperature combination to another. This method is termed 'thermal isoeffective dose'. Typically, the time–temperature data are converted to an equivalent number of minutes at 43°C. There was no particular reason for choosing 43°C as the index temperature, aside from the fact that it is near the break point for CHO and several other cell lines. The equation for doing this conversion is shown here:

$$\text{CEM43°C} = tR^{(43-T)} \tag{2}$$

where CEM $43°C$ = cumulative number of equivalent minutes at 43°C, t = time interval (min), T = average temperature during time interval t. R is the number of minutes needed to compensate for a 1° temperature change either above or below the breakpoint. When there is temporal variation in the temperature of a specific tissue, the time at each temperature must be determined and the CEM 43°C summed over contiguous intervals where temperature is relatively constant. The resultant CEM 43°C value represents the entire history of the exposure.

3.1. *Relevance of the* R-*value for establishing thermal sensitivity of tissues*

There is uncertainty about the slope of the Arrhenius plot below the breakpoint. The method of Sapareto and Dewey[19] assumes that it is 0.25, but there are others who reported that it could be as low as 0.125[20], indicating that the time to achieve an isoeffect at a defined temperature is increased by a factor of 8, as opposed to 4, for every degree drop below the breakpoint. Most rodent data, however, suggest that the R-value below the breakpoint is between 0.25–0.17[21]. For regulatory purposes, precise knowledge of the slope of the Arrhenius plot below the breakpoint would be advantageous, since it would allow for a more detailed description of the thermal limits to achieve a specific tissue endpoint or to avoid tissue damage. With occupational exposure or accidents it is more likely that exposures will be in the region below the breakpoint as opposed to above it. The data in figure 2 show similar R values for rodent and human cells (R above breakpoint = 0.43 vs 0.45 and R below

breakpoint = 0.23 vs 0.25 for human vs rodent cells, respectively). The breakpoint is slightly higher for human (43.5°C) than rodent cells (43°C). The thermal data for skin necrosis in humans, as derived from the work of Moritz and Henriques[5] imply that the *R*-values (figure 3) for human and rodent tissues are significantly different from the *in vitro* estimates. The *R* values for human skin above and below the breakpoint are 0.72 and 0.13, respectively. The rodent data are similar to the *in vitro* results, however, with $R = 0.45$ above the breakpoint and 0.25 below it.

As was discussed above, there is uncertainty about the accuracy of the human skin data. This leads to a lack of precision of the threshold temperature for damage, the breakpoint temperature and the slopes of the Arrhenius plot above and below the breakpoint. In rodent studies, by comparison, the breakpoint derived from *in vivo* and *in vitro* data has been consistently between 42.5–43°C.

3.2. *Implications of using CEM 43°C for development of regulatory guidelines*

A primary advantage of using the CEM 43°C dosimetric unit is that it is not necessary to determine thresholds for damage of a particular tissue at every possible time–temperature combination. If it is well defined at even one time–temperature combination, the results can be extrapolated to define an isoeffect line that will establish the boundaries of what is considered safe exposure times for that tissue at any temperature. However, there may be extrapolation inaccuracies using the CEM 43°C system at very low or very high temperatures (i.e. below 39°C or above 57°C). However, the slope has been found to be constant in *in vitro* studies over the range from 43.5–57°C[22]. Additionally, one has to make the assumption that the *R*-values for the CEM 43°C formulation are constant across a range of tissues. As has been shown above, this is a reasonable assumption.

An example of how Thermal Isoeffective Dose could be used is provided in figure 6. The object of the simulation is to predict the times required to achieve muscle fibrosis over the temperature range from 37–50°C. For comparison three sets of assumptions about the slopes of the Arrhenius plot above and below the breakpoint and the location of the breakpoint are compared. The assumptions consider the parameters derived from (1) figure 1 for rodent cells (which are typical for most rodent cell lines and tissues), (2) the human cell lines shown in figure 1 and (3) the *in vivo* human skin data. A summary of the parameters used for these simulations are shown in table 2. Based on the published work of Meshorer *et al.*[23], the threshold for significant damage to pig muscle, leading to fibrosis at 1 month post treatment, is >43°C for 30 min. From these data, damage threshold curves were derived for the isoeffect of muscle necrosis covering the temperature range from 37–50°C. Using equation (2), the number of minutes of exposure were calculated at set temperatures to reach the same isoeffect. The differences in the slopes of the Arrhenius plots affect the resultant isoeffect curves significantly, particularly at the extremes of the temperature range. The analysis shows that over most of the simulated temperature range, that data derived from tissue culture experiments yield similar predicted heating times when based on parameters derived from either rodent or human cell lines. The apparent increase in thermal sensitivity using human *in vitro* parameters between 43–45°C is the result of small differences in the slopes of the Arrhenius plot and the temperature of the breakpoint for the two species over that temperature range. If the *in vivo* human skin data are used, however, then the prediction for the predicted threshold for muscle damage is much higher than either of the other two predictions. The uncertainties about the human skin data, as explained above, cast

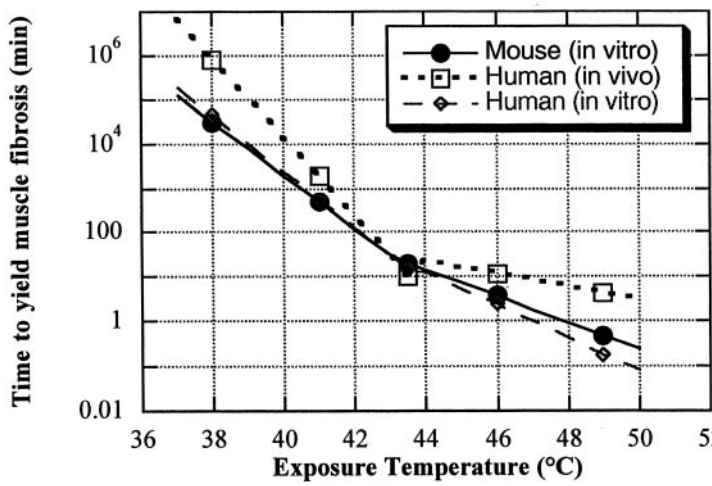

Figure 6. Predicted times to reach muscle necrosis over the temperature range from 37–50°C, based on data by Meshorer *et al.*[23] indicating that 30 min at 43°C is sufficient to cause damage to muscle . The conversion to number of minutes at other temperatures was done using equation (2). Input variables were as described in table 3. The analysis suggests that input variables provided from human *in vivo* studies would predict greater thermal resistance than if parameters from rodent studies or human cells from tissue culture are used. Note, there is considerable uncertainty in the data derived from the human skin data, as described in considerable detail in the text. These data are included to point out the importance of obtaining accurate thermometry.

Table 2. *R*-values and breakpoints used for calculations of time to reach isoeffect, as shown in figure 5.

		R-value	
Species	Breakpoint	< Breakpoint	> Breakpoint
Mouse	43.0°C	0.25	0.5
Man (*in vitro*)	43.5°C	0.233	0.428
Man (*in vivo*)	47.0°C	0.13	0.72

great doubt about the voracity of that prediction. The prediction is left in the simulation set to serve as an example of how important it is to obtain accurate thermal data when doing studies to assess thresholds for thermal damage.

There are other cautions to be aware of in interpreting these simulations. The data in figure 6 have been plotted to cover a large temperature range, but the data from which these predictions were derived do not span that range. Most murine studies have been conducted in the range from 41.5–46°C, while the only human data available, which are based on *skin* tolerance and not *muscle*, span the range from 44–70°C[5]. The slopes of the Arrhenius plots for skin and muscle are likely parallel, as has been shown for several species and tissues (see discussion above and Field and Morris[21]). So the main concerns with respect to interpretation of such predictions are: (1) uncertainty about where the breakpoint is for human tissue, (2) whether or not the threshold for thermal damage in animal studies is similar to humans (if the studies were conducted identically). Given that there is a paucity of human data and uncertainty about the accuracy of the data that exist for human skin, the most

conservative approach is to use the isoeffect dose parameters derived from rodent tissues, which yield a conservative estimate of thresholds for thermal damage to human tissues. This was done to set boundaries for thermal damage to tissues, as described below.

There may be a temperature boundary for most tissues, below which no clinically detectable injury occurs within the practical time-limits to test them. To illustrate how important this is, the time predicted to cause thermal damage to muscle, assuming a temperature of 37°C, and using the *R* factors for human skin from Moritz and Henriques[5], as shown in table 2, is 6.2×10^6 min, or ~12 years. Obviously, one would not expect to see damage to muscle, even if it were at 37°C continuously, and this prediction is consistent with that expectation. Because thermal isoeffect dosimetry is an exponential relationship with temperature, increasing temperature leads to a rapid drop in heating time. At 41°C, the predicted heating time is down to 1800 min, or 1.23 days and, at 42°C, the predicted time of heating to approach the threshold for damage in muscle has dropped to 230 min. Using the rodent data for input, the predicted times to reach damage are much shorter. For example, at 41°C, the threshold would be 0.3 days as opposed to 1.23 days using the human data.

3.3. *Implications for development of regulatory guidelines*

The *R* value and breakpoint assessments derived from large animal and human thermal dose-to-effect studies are likely to yield a more accurate estimate of damage thresholds for man than similar rodent studies. However, the lack of human and/or large animal studies with accurate thermometry creates a significant gap in the knowledge/information necessary to derive thorough and comprehensive extrapolation curves, which would be accurate over a broad range of temperatures and thermal doses. Based on the close association of thermal dose-to-effect for some large animal–human endpoints, it is clear more large animal studies are essential to this end. Such studies are not feasible in humans for ethical reasons, but might be feasible in pigs. It should be recognized that even data obtained from large animals still does not substitute for human data, so a significant margin of safety should be placed on such data to establish boundaries of exposure that are most likely to be safe for human exposure.

It is important to note that thermal isoeffect 'dose' is not a physical quantity such as energy absorbed, as would be used for quantitating radiation dose, for example. Nor is it directly analogous to pharmacokinetic data that are commonly used to measure drug 'dose'. In both of these cases, the dose is based on a physical measurement of the quantity of agent administered. Thermal isoeffect dose is based empirically on the tolerance of specific cell components, the inherent cell tolerance and the *in situ* conditions encompassing the cells/tissue being heated. Some authors have been justifiably critical of this approach, because it does not represent a physical dosimetric quantity and more importantly the value of it is entirely dependent upon the cell or tissue being studied[24]. This means that a thermal threshold for damage assessed from one tissue cannot be extrapolated to another, as was discussed above.

For specific tissues, at temperatures up to 50°C, the CEM 43°C isoeffect dose method works well to predict defined types of damage from a range of defined time–temperature combinations. This is best illustrated by examining the tables in the Appendix Database. The tables show data for several studies in which the times at

a range of temperatures to reach a 50% probability for an isoeffect have been determined. A typical example is the work of Morris and Field[25], who examined times to reach a 50% probability of losing 10 or more vertebral bodies in baby rat tail at 12 different temperature steps between 41.8–46°C. When these data were converted to CEM 43°C, there was remarkable consistency, averaging 83 ± 14 CEM 43°C to achieve the same level of damage. Put in real terms, this means that 41.8°C for 500 min caused the same amount of damage as 43°C for 85 min or 46°C for 12 min. If these were the only data of this type, one could perhaps dismiss it as being coincidence. However, this type of analysis has been done for a range of tissues, including bowel, skin, ear necrosis, foot loss and testis weight. In addition, a wide range of tumours have been studied[21]. The CEM 43°C for a defined level of damage yields a consistent value over a broad range of temperature–time combinations.

The human skin data from Moritz and Henriques[5] also yield a consistent prediction of CEM43°C to achieve thermal burns, at least over the lower range of temperature–time combinations (Appendix Database). At higher temperatures, the value of the CEM 43°C threshold for burns increases, which may be due to non-linearities in the relationship between surface and deeper layer skin temperatures or uncertainties in what the actual surface temperature was, as was discussed above. The CEM 43°C thermal isoeffective dose concept has been successfully tested in several clinical trials as a predictor of tumour response to the combination of radiation and hyperthermia. Collectively, these data provide some assurance that the parameter has biological validity for determining thresholds for thermal damage to tissues in humans[26–29].

4. Thresholds for thermal damage in tissue

Some studies have carefully documented levels of tissue damage over a wide range of temperatures and times of exposure. From these data it is possible to determine thresholds for thermal damage. In figure 7, the data for onset of burns vs highest time–temperature combinations at which burns were not seen in human skin are plotted[5]. According to most of the current literature, at any defined temperature, the difference in time to achieve thermal injury and avoid it is very small (this appears especially true for acute effects, whereas the differences are less well defined for chronic effects). These data are typical of most of the rodent data seen in the Appendix Database and suggest that the CEM 43°C effect relationships for damage are very steep. As a further demonstration of the steepness of the dose effect curves, the fraction of mice developing ear skin necrosis following water bath heating at 43.5°C is shown in figure 8 (data taken from Law *et al.*[20]). Although clinically apparent thermal injury is avoided for treatment times up to 40 min, heating for 60 min leads to 100% incidence of injury .

4.1. *Implications for development of regulatory guidelines*

For regulatory purposes, the steepness of the dose effect relationship should be an advantage. As shown in figure 3(*a*), there is a large difference between the threshold for thermally induced pain in the skin and damage. Guidelines have generally been set to avoid thermal damage by defining a maximum allowable skin surface temperature that is below the pain threshold. Therefore, very long exposures at such temperatures are unlikely to result in injury.

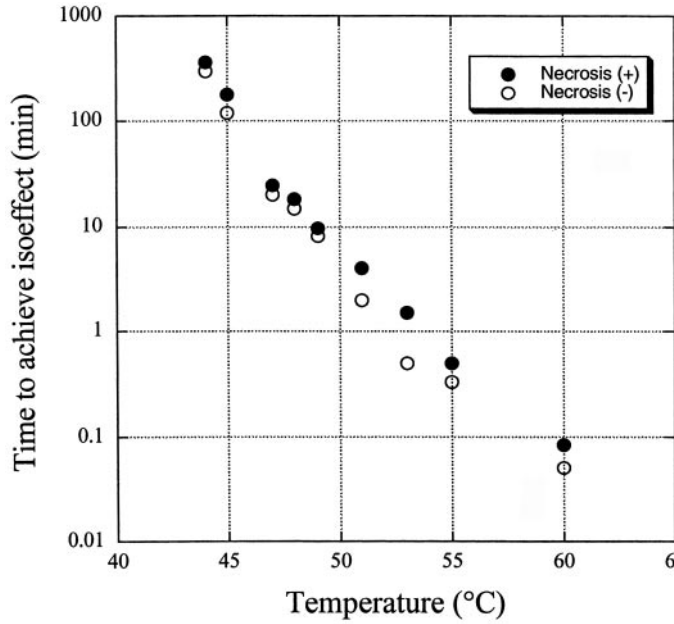

Figure 7. Thresholds for thermal damage. The open circles indicate the highest time–temperature combination studied for which there was no full thickness burn. The solid circles indicate the lowest time–temperature combinations that caused full thickness burns. Data obtained from Moritz and Henriques[5]. This figure demonstrates the very small temperature–time transition between survival and necrosis of tissues subjected to heating.

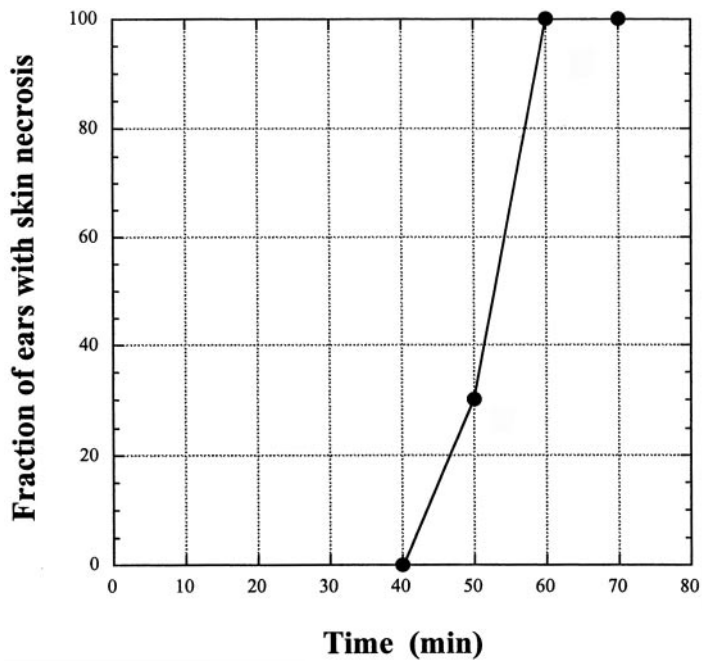

Figure 8. Relationship between time of heating at 43.5°C and incidence of ear skin necrosis. Note the steepness of this dose-effect curve. Assuming 60 min of heating is necessary to achieve 100% incidence of ear skin necrosis, a 30% reduction in heating time will completely avoid the injury. Data replotted from Law[7].

Table 3. Summary of tissue sensitivites to hyperthermia, as assessed by thermal isoeffect dose measures.

		Type and degree of change				
		Acute		Chronic		
CEM 43°C	Tissue type	Minor	Significant	Minor	Significant	Species
0–20	BBB		F			Dog
	Bone marrow	F/H				Mouse
	Brain	H		H		Dog/cat
	Conjuctiva	G				Rabbit
	Kidney	H				Mouse
	Retina			G/H		Rabbit
	Spleen	F (enzyme)				Mouse
	Testicle	F	H	F	F	Mouse
21–40	BBB		F			Dog
	Brain	H/G		H/G		Dog
	Cornea	G				Rabbit
	Eyelid	G				Rabbit
	Prostate			H		Dog
	Rectum	H				Pig
	Retina	G				Rabbit
	Rodent appendage	G		G		Mouse/rat
	Skin	F				Mouse
	Small intestine		H			Mouse
41–80	Anterior chamber	G				Rabbit
	Brain		H/G		H/G	Dog
	Choroid			G		Rabbit
	Cilliary body	G				
	Cornea		G			Rabbit
	Fat	H				Pig
	Lens		G		G	Rabbit
	Liver	H				Rabbit
	Muscle	H				Pig
	Peripheral nerve		F/H		F/H	
	Rectum	H				
	Retina		G	G		Rabbit
	Rodent appendage		G	G		Mouse/rat
	Sclera	G				Rabbit
	Skin		G/H		G/H	Mouse
>80	Anterior chamber	G				Rabbit
	Bladder				G	Dog
	Choroid			G		Rabbit
	Cilliary body		G			Rabbit
	Conjunctiva	G				Rabbit
	Cornea		G			Rabbit
	Esophagus	H	H	H	H	Pig
	Eyelid	G				Rabbit
	Fat			G/H	G/H	Pig
	Lens		G		G	Rabbit
	Liver	H				Rabbit
	Muscle			G/H	G/H	Pig
	Peripheral nerve		H/F/G		F/G	
	Prostate	H	G			Dog
	Rectum		H			
	Retina		G			Rabbit
	Rodent appendage		G		G	Mouse/rat
	Sclera	G		G		Rabbit
	Skin		G/H		G	Pig
	Small intestine		H		G/H	Pig/dog

Acute (Tissue evaluated 0–30 days after heat exposure); Chronic (Tissue evaluated >30 days fater heat exposure); BBB = blood brain barrier.

Histolopathology (H); Gross appearance (G); Function (F).

In addition to many references already cited, references 58–105 were used as a basis for development of this table. More complete information on these additional references can be found in the Appendix table (available on request from MWD or PJH).

Most of the data that have been acquired to establish thresholds for thermal damage to tissues have been in the context of using hyperthermia to treat tumours. The data largely relate to local or regional heating of the body and nearly all of the data deal with single thermal exposures. A brief summary of the Appendix Database data is shown in table 3. The data in the Appendix Database have been compiled by examining the literature via electronic databases as well as by cross referencing older citations against reference lists. Each paper has been examined in detail to obtain the following parameters for each entry: endpoint used, post-heating time that assessment was made, target time and temperature of thermal exposure and method of temperature measurement or estimate (in footnote of each table in the Appendix Database). When appropriate data were available, the highest CEM 43°C exposure was indicated for which there was no measureable damage and the lowest CEM 43°C exposure for which damage was observed. In some cases, there is disagreement between different publications with respect to these numbers. This can be seen by examining the individual entries. Unless data was believed to be internally unclear or inconsistent, the lowest thermal dose at which a tissue effect was observed was shown. A handful of available papers were excluded because these minimal sets of data were not available.

There are a number of issues that are important to consider when examining and using the tissue thermal threshold data.

(1) Thresholds are endpoint dependent. In the Appendix Database the endpoints are indicated. The sensitivity of the endpoints varies considerably. For example, histologic assessment of tissues can reveal damage that may be sub-clinical. Alternatively, functional endpoints may miss subtle changes that, if cumulative over repeated thermal exposures, could lead to significant consequences.
(2) Most of the studies listed in the table are for acute one-time exposures. There is virtually no information on whether repeated sub-clinical exposures can cause cumulative damage. One exception to this is an epidemiological study that was done to establish the risk of developing cataracts in steel workers[30]. This study demonstrated that the temperature of the working environment was strongly related to the liklihood of developing cataracts. The risk was highest for those persons working in the regions of the steel mills that had the highest ambient temperature. The temperature of the eye or the lens was not measured under these circumstances, however, so one cannot completely rule out that some other environmental factor that existed in the regions of highest ambient temperatures may have contributed to the development of these lesions.
(3) The amount of damage observed will depend upon the time post exposure that the assessment is made. For example data is shown indicating that the threshold for acute damage to muscle is lower than for chronic damage. Transient acute damage to this organ that does not progress to chronic damage might be relatively unimportant. In other organs, such as the brain, the acute physiological changes resulting from heat exposure could be lethal (brain oedema), even when few cells actually die from direct thermal injury.
(4) The importance of damage is often judged by the criticality of the tissue being evaluated, but this makes the various degrees of heat injury difficult

to weigh and assess since the judgement of severity of injury is subjective. For example, scattered loss of hepatocytes is probably much less important for the overall heath of a patient than scattered loss of neurons. Judgements such as this are included in the summary data, which is why the brain is listed as being one of the two most thermally sensitive tissues. It is assumed that any loss of neurons should be considered significant. It should also be noted that a number of brain-assessment endpoints were designed to identify relatively minor loses in brain cellularity. The relative clinical importance of such changes remain unclear.

(5) There is a range of thresholds of thermal sensitivity for different tissues, but thresholds for damage within a defined tissue type are remarkably similar, when multiple species have been evaluated for the same endpoint. By extrapolation, those organs most thermally sensitive in rodents are likely to be the most thermally sensitive in humans. However, additional data over a wider temperature range in species with tissues more like the human would add considerable confidence to this statement.

(6) The accuracy of the thermal threshold for damage is highly dependent upon the accuracy of the temperature measurement. This is a particular issue for the only data that exist for human skin, as was discussed in detail above.

(7) The Appendix Database tables include thresholds for thermal damage to some organs following whole body hyperthermia. The thresholds for damage when hyperthermia is administered this way are typically higher than what is observed following local hyperthermia, which may be due to effects of thermotolerance development occuring during induction of whole body hyperthermia. Thermotolerance has been shown to develop following whole body hyperthermia exposure. Kapp and Lord[31] found that the LD50 (time of heating at 42.5°C to result in 50% lethality) for rats exposed to a conditioning whole body hyperthermia treatments (41.8°C for 60 min at 30 h prior to the test hyperthermia treatment) was two fold longer than the LD50 for animals that did not recieve the conditioning treatment. However, thresholds for tissue damage from accidental whole body exposure are dependent upon other variables, such as the age and cardiovascular condition of the subject as well as degree of hydration. More detail on whole body exposures to hyperthermia are provided in a separate report in this issue (Donaldson *et al.*).

(8) There are physiological responses that occur in response to heat stress that may or may not be related to thermal damage, *per se*. For example, perfusion in skin of both rodents and humans increases at temperatures near 40°C[10, 32]. The increase in perfusion may not reflect damage; it is a normal physiologic response to increased temperature. Some of these types of responses are listed in the Appendix Database tables, but are not considered to be indicative of toxicity by themselves. Some authors have examined time–temperature relationships for vascular damage, however. Two examples related to perfusion reduction in mouse muscle and rat subcutis are provided in the Appendix Database[33, 34]. The slope of the Arrhenius plot for vascular damage is the same as that seen for other types of damage, as reported above.

(9) The ranking of thermal sensitivities by tissue type does not follow the general guidelines that one would associate with tissue sensitivity to other cytotoxic entities such as radiation or chemotherapeutic drugs. For those entities, the

proliferative status of target (most sensitive) cells necessary for tissue function is a dominant feature in determining sensitivity. For example, tissues most sensitive to chemotherapeutic drugs include testis, bone marrow and gut epithelium. Clearly, this general rule does not seem to follow for hyperthermia, since brain tissue seems to be meaningfully affected by relatively low thermal doses. Most types of brain cells have low or almost no proliferative potential, whereas testis, which has high proliferative potential, is also demonstrated to have high functional and morphologic sensitivity to heat. Thus, there is no clear ranking by tissue type. For the nervous system, for example, peripheral nerves are very heat resistant, the spinal cord is intermediate and the brain falls into the very sensitive category. Similarly, different components of the eye have widely varying thermal sensitivities. Although some tissues of relatively similar makeup show large variations in thermal sensitivity, it is believed that the heat sensitivity differences shown in similar organs (even in different species) would be smaller if all studies had been performed under the same conditions using the same endpoints and parameters. For example, in the studies that have examined thermal sensitivity of gut, some investigators have reported LD50 values, whereas others have reported histologic endpoints. In some cases, histologic endpoints may identify sub-clinical lesions that may not have significant clinical consequences. In other cases, such endpoints may identify lesions that may be of potential importance that would not be seen clinically. The most pertinent example in this latter case is the neuronal apoptosis observed following local heating of the brain that would not be found if the tissue had not been assessed quantitatively using histomorphometry. Additionally, the time at which the damage is assessed can make a large difference in the damage measurement, as was discussed above for muscle damage in the pig. When the assessment is made acutely, the tissue appears more sensitive than when it is assessed 30 days after exposure.

4.2. *Implications for development of regulatory guidelines*

There has been no systematic study to evaluate the sensitivity of different organs to thermal injury, using a standardized set of outcome variables over a defined set of time–temperature combinations. Studies to establish such guidelines would be extremely valuable. Given the relative similarity of the skin data between humans and pigs, such studies may be more translatable if they were done in pigs, as opposed to rodents. It will be important to conduct such studies using careful thermometry so that the temperature of the heated tissue is known precisely.

5. Factors that influence thermal sensitivity of tissues

There are several factors that influence the thermal sensitivity of tissues. Some of these could occur from occupational or accidental exposure to RF fields and, therefore, should be discussed. The factors that will be outlined include thermotolerance, pH and pressure effects and a phenomenon referred to as ‘step down’ heating. The effect of these factors on thermal sensitivity are well characterized and can be described quantitatively based on Arrhenius analysis.

5.1. *Thermotolerance*

Thermotolerance is defined as an acquired resistance to thermal cytotoxicity. It occurs when tissues or cells are exposed to thermal stress. It is regulated by a special class of proteins, known as heat shock proteins. During and for some time following heat stress, the production of most proteins is down-regulated. The exception to this rule are the heat shock proteins, which are upregulated following heat stress. The purpose of these proteins in the context of heat shock is to act as 'chaperones' to either target proteins for degradation or refolding[35].

Thermotolerance is a transient phenomenon. The degree of protection afforded to cells from thermal stress is dependent upon the severity of the initial thermal damage and the amount of time elapsed from the time that the initial injury occured. Many papers have been published examining these kinetics. Three examples will be provided here for discussion. Law *et al.*[20] examined the resistance of mouse ear to skin necrosis using fractionated thermal exposures, following an initial thermal injury at 43.5°C for varying lengths of time, ranging from 2–40 min. A second heat treatment at 43.5°C for varying lengths of time was administered at time intervals between 1–160 h after the first treatment. The time to achieve 50% incidence of ear skin necrosis was used as the endpoint. For a single heat treatment, 50 min at 43.5°C was sufficient to achieve this effect. The degree of thermotolerance achieved was dependent upon the length of the initial exposure, but maximized at a value of 2. Thus, when thermotolerance was at its maximum, it took twice as long to achieve 50% incidence of ear skin necrosis than when the treatment was administered without prior treatment. The time required to reach maximum thermotolerance was also dependent upon the initial exposure. The time required to reach maximal thermotolerance increased by 0.7 h for each minute of initial heating (figure 9). The time required for complete decay of thermotolerance was not reported in this study, but based on limited data presented it was over 80 h for the most severe treatment. A

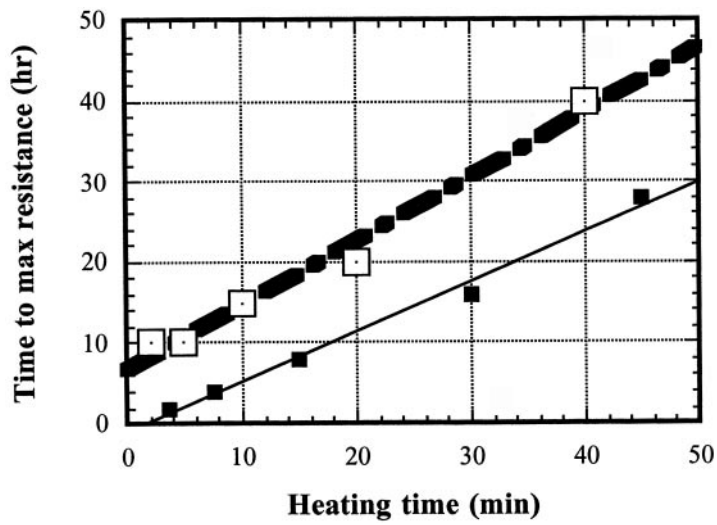

Figure 9. Time for development of a maximum amount of thermotolerance as a function of length of heating time at 43.5°C. Data are plotted for a mouse tumour line (open symbols)[36] and for mouse ear necrosis (closed symbols)[20]. The lines are the best linear fits to the data. The heavy dashed line is the fit to the mouse tumour line data.

similar study was done by Nielson and Overgaard[36], using a mouse tumour model. The temperature of the test heating was also 43.5°C . In this case, the endpoint was tumour growth delay time. The time to reach maximum thermotolerance increased by 0.8 h for each additional minute of heating, similar to the data by Law *et al.*[20] for mouse ear. Better data were available for time to decay of thermotolerance in the Nielson and Overgaard[36] data. The fit for these data were linear and predicted a time to thermotolerance decay to increase by 2 h for each additional minute of heating (figure 10).

Other investigators have studied the effect of thermotolerance by examining the temperatures required to achieve an isoeffect either alone or after a priming heat dose to induce thermotolerance. When such results have been examined on an Arrhenius plot, it has become clear that the effect of thermotolerance is to shift the curve to the right, but in parallel with the original curve. The shift can be between 1–2°C. Thus, when tissues are maximally thermotolerant, the temperature required to achieve an isoeffect increases by ~1–2°C, as compared with a single heat treatment[37, 38]. The one degree shift is consistent with the requirement to double the heating time at a temperature above the breakpoint for rodents, as discussed above.

Thermotolerance is an ubiquitous phenomenon, occuring in nearly all living organisms, ranging upward from algae and bacteria to mammalian cells. It has been demonstrated following fractionated hyperthermia applications to a variety of tumour and normal tissues[35] and in experiments testing fractionated exposures to whole body hyperthermia[31]. Thermotolerance also affects vascular response to thermal injury. Normally, tissue perfusion increases in response to mild thermal injury. However, it has been demonstrated that this response is reduced if the tissues were heated previously. Presumably, this type of physiologic thermotolerance is still based on development of tolerance at the cellular level[39]. For example, it has been shown that gaps between endothelial cells open up upon non-lethal thermal stress. However, these gaps close again after 4–6 h and, if heating is repeated 8 h after the first exposure, the gaps do not open again[40]. These results suggest that cellular thermotolerance can be expressed physiologically.

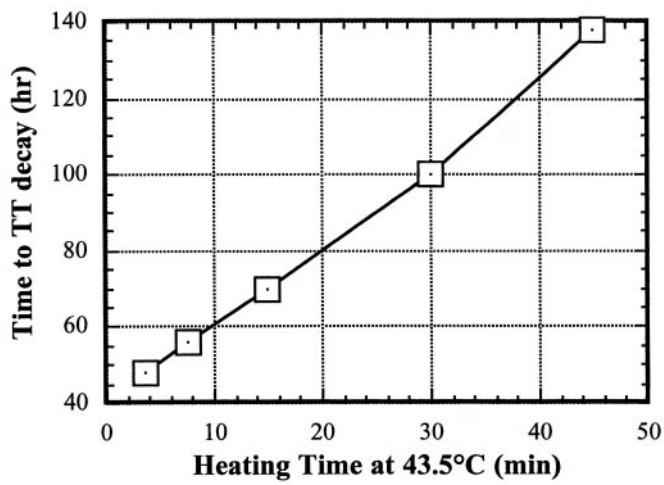

Figure 10. Time for decay of thermotolerance, as a function of length of heating time at 43.5°C. Data abstracted from *in vivo* studies of thermotolerance in a mouse tumour line[36].

There are no data published regarding thermotolerance in humans. Moritz and Henriques[5] performed a limited study using the pig skin model, however. Using a test temperature of 49°C, they found that 9 min of continuous heating was sufficient to induce a full thickness burn. If the treatment was broken into 3 min segments and spread evenly over a 48–72 min time interval, the damage was less severe. When the three treatments were spread over 4, 24 or 48 h, only mild erythema was seen. These results are consistent with the induction of thermotolerance. They did not follow the animals long enough to determine the kinetics of thermotolerance decay.

5.2. *Implications for development of regulatory guidelines*

It is clear that thermotolerance can play an important role in providing protection against thermal injury. However, more information is needed to establish its kinetics of induction and decay under a range of thermal injuries. Based on the rodent data above, it was found that the kinetics of thermotolerance induction and decay are predictable, for an initial thermal exposure above the breakpoint. What happens at other levels of thermal injury? Are the kinetics of induction or decay independent of the severity of the initial exposure? It is known that the degree of thermotolerance induced is related to the amount of damage induced, irregardless of what time–temperature combination is used to reach that level of damage. For example, in the paper by Nielson and Overgaard[36], the same degree of initial thermal damage was caused by 42.5°C (60 min), 43.5°C (30 min) and 44.5°C (15 min). The amount of thermotolerance induced at 16 h after these priming heat doses was also identical. The CEM 43°C for all of these exposures was 42 min. More information needs to be identified to indicate what the kinetics of thermotolerance induction and decay are for lower thermal exposures, particularly involving temperatures below the breakpoint.

5.3. *Vascular occlusion and acidosis*

If the blood supply to a region of the body is partly or completely shut down, the thermal sensitivity of the tissue increases. This difference in tissue sensitivity could occur if the reduction in perfusion leads to a decrease in thermoregulatory capability. However, even in studies where this has been controlled, the tissue sensitivity increases. Using rat baby tail stunting as an endpoint, it has been determined that, above the breakpoint, the primary effect is to shorten the overall time to reach an isoeffective level of damage. The Arrhenius plot slope, however, is parallel to that for heating applied without clamping[25]. Below the breakpoint, there is a dramatic effect. Under control conditions, dropping the temperature by 1° below the breakpoint required an 8-fold increase in heating time. If the tail was clamped for 20 min prior to heating, this ratio dropped from 8 to 1.3. Since the slope above the breakpoint is relatively unaffected, the primary effect of clamping is to eliminate the breakpoint. In addition, clamping of the tail lowered the temperature threshold for thermal damage. A significant percentage of animals lost their tails after several hours of heating at 37°C with clamping whereas this was not seen without clamping. Normal skin temperature in the rat is 34°C, so 37°C does represent an elevation above normal for this tissue. The biological implication of this is that abrupt loss of perfusion blocks the onset of thermotolerance during heating. It is known from *in vitro* studies that acute acidification of cells prior to heating will accomplish the same effect. Thus, the underlying mechanism leading to increased thermal sensitivity as a result of clamping may be due to conversion to anaerobic metabolism and acidosis.

During an RF exposure, thermal burns could occur at lower than predicted temperature if perfusion to a portion of the heated volume is reduced. In clinical applications of hyperthermia, it has been reported that burn injuries over pressure points can develop during whole body hyperthermia procedures using water blankets where the skin temperature does not exceed 43°C[41]. Moritz and Henriques[5] examined this question in one series of experiments using pig skin. They applied extra pressure to the skin at a pressure of 90 mmHg at the time of the burn application. This amount of pressure did not affect the degree of thermal injury achieved, suggesting that a greater amount of pressure would be needed to reduce perfusion enough to increase the likelihood for thermal injury.

5.4. *Step-down heating*

This phenomenon occurs when there is a fluctuation in temperature from being above to below the breakpoint during exposure. If the initial heat stress is at a level above the breakpoint, thermotolerance induction will be inhibited, even if the temperatures drop back below the threshold . Thus, the slope of the Arrhenius plot will be identical above and below the normal breakpoint. The net result will be that the amount of thermal damage resulting from the thermal exposure will be greater than predicted[42].

5.5. *Rate of heating*

The rate that cells or tissues are heated can have a profound effect on the degree of cytotoxicity. Most tissue culture experiments have been done by heating cells in a water bath and the contents reach target temperature within 1–2 min. Even in the clinical setting where hyperthermia is used to treat tumours, target temperatures are reached within 10–20 min. If the heating period occurs over 1 h to reach the same target temperature, there will be less killing[24, 43]. The effects of high vs low heating rates on vascular damage in normal tissues[44]. The threshold for arteriolar stasis was between 45–46°C for heating rates ranging from 0.1–0.7°C/min. At a heating rate of 1.0°C/min, the stasis temperature dropped to 42°C. Venular stasis temperature dropped from 43.4 to 42.1°C when comparing 0.1 to 1.0°C/min heating rates, respectively. The difference in temperature for damage onset occurs because thermotolerance develops to a greater degree during slow vs fast heating rates.

5.6. *Differences between resting temperature and final temperature*

The degree of cytotoxicity that occurs from heating depends upon what the resting temperature is. It is known that the resting temperature of peripheral tissues is lower than core temperature, as would be measured in a major vessel or internal organ[45]. Normal skin temperature in humans is ~34°C. The amount of damage from a thermal exposure would be greater for the temperature differential between 34–42°C than for the differential between 37–42°C[46–48]. Even though this phenomenon has been demonstrated in tissue culture and rodent models, there is no clear evidence to suggest that skin is more thermally sensitive than other tissues because of it.

5.7. *Implications for development of regulatory guidelines*

Scenarios could occur that lead to either perfusion occlusion or step down heating. Under such circumstances, the thermal sensitivity of the affected tissue may be much greater than would occur under normal circumstances. Differences in the rate

of heating could also affect the level of damage from a thermal exposure, as very rapid heating is likely to be more cytotoxic than a slower rate of heating.

Acknowledgements

This work was supported by a grant from the NIH/NCI CA42745. The authors acknowledge that Paul Stauffer was the first person to suggest the use of CEM43°C to indicate cumulative equivalent minutes of heating at 43°C.

An electronic Appendix database accompanies this paper for those readers who are interested. It can be obtained by contacting either of the senior authors (*MWD*, *PJH*).

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