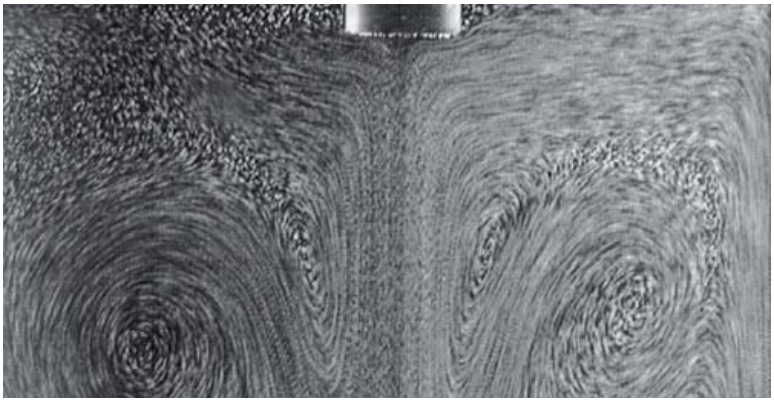
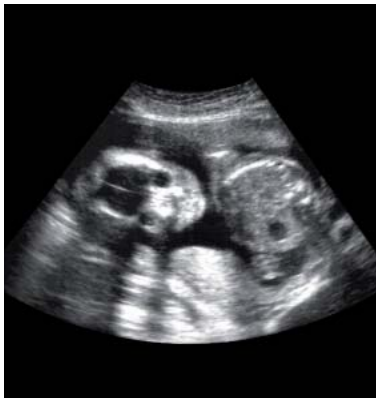
Health Protection Agency

Health Effects of Exposure to Ultrasound and Infrasound

Report of the independent Advisory Group on Non-ionising Radiation





Cover illustrations

TOP ROW

High intensity focused ultrasound (HIFU) unit (courtesy the HIFU Unit, Churchill Hospital, Oxford)

Ultrasound applicator being used for treatment of tendonitis of the elbow (© iStockphoto.com/David Peeters 2006)

MIDDLE ROW

Visualisation of acoustic streaming using corn-starch particles (Nowicki et al, 1998, Eur J Ultrasound, 7, 73–81)

Fetal scan at 20 weeks (courtesy Matthew Pardo)

BOTTOM ROW

Wind turbines (Health Protection Agency 2008)

Lightning over a city at night (© iStockphoto.com/Rick Rhay 2008)

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Foreword

The Health Protection Agency (HPA) has a statutory responsibility for advising UK government departments on health effects and standards of protection for exposure to ionising and non-ionising radiations. This responsibility came to the HPA in April 2005 when it incorporated the National Radiological Protection Board (NRPB).

In 1990, to provide support for the development of advice on non-ionising radiations, the Director of the NRPB set up the Advisory Group on Non-ionising Radiation with terms of reference:

> **'to review work on the biological effects of non-ionising radiation relevant to human health and to advise on research priorities'**

The Advisory Group was reconstituted in 1999 as an independent body and now reports to the subcommittee of the Board of the HPA that deals with radiation, chemical and environmental hazards. Its current membership is given on page ix of this report. For details of its work programme, see the website www.hpa.org.uk. Details of publications by the Advisory Group are given in an appendix.

The Advisory Group has to date issued a number of reports concerned with exposure to ultraviolet radiation and electromagnetic fields. This is the first time it has reviewed ultrasound (frequencies greater than 20 kHz) and infrasound (frequencies less than 20 Hz) relevant to any possible health effects. In this report the Advisory Group considers the available scientific evidence from studies with humans, animals and cells relating to exposure to ultrasound and infrasound.

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We would like to acknowledge the assistance of Professor Tim Leighton, Institute of Sound and Vibration Research, University of Southampton, during the preparation of this report.

Health Effects of Exposure to Ultrasound and Infrasound

Report of the independent Advisory Group on Non-ionising Radiation

Chairman: Professor A J Swerdlow

This report from the independent Advisory Group on Non-ionising Radiation reflects understanding and evaluation of the current scientific evidence as presented and referenced in this document.

Executive Summary

Ultrasound and infrasound are mechanical waves at the extremes of the acoustic wavelength spectrum, approximately above and below, respectively, the human hearing thresholds. Ultrasound is defined as acoustic waves at frequencies greater than 20 kHz and infrasound as acoustic waves at frequencies below 20 Hz. Ultrasound and infrasound can interact with biological tissues by mechanical and thermal processes.

Ultrasound has been widely used in medical practice for at least 50 years. Diagnostic examinations include obstetric, abdominal, pelvic and cardiac imaging; therapeutic uses include promotion of bone and soft tissue regeneration, destruction of kidney stones and tumour ablation. Industrial applications include sonochemistry, emulsification, welding, cleaning and non-destructive testing; and ultrasound has been used in consumer products such as range finders, movement detectors and pest repellents. Natural sources of ultrasound include bats, dolphins and other species that use it for echolocation. Human exposures to ultrasound have not been well quantified except for those from medical devices.

Infrasound is created by various natural sources, such as earthquakes, thunderstorms, wind and waves. Comparable oscillatory changes in air pressure may be experienced when running. Artificial sources of infrasound include explosions, compressors, low speed fans, wind turbines, trains and building sway.

At high levels of exposure, ultrasound is capable of causing permanent damage to biological tissues, including teratogenic effects, through heating, acoustic cavitation and radiation force. At lower levels, such as those used for diagnostic purposes, ultrasound does not generally cause heating beyond the normal physiological range, nor does it cause cavitation in the absence of pre-existing gas bubbles. In one study, prenatal exposure to diagnostic levels of ultrasound has been reported to produce changes in neuronal migration in the developing brains of mice; the functional significance of these changes is unknown, however, and the study has not been replicated. Low intensity ultrasound has also been shown to increase the rate of tissue repair following injury, especially associated with bone fracture.

Animal studies of infrasound have reported biological effects, mainly after exposures at levels above 100 dB, while at levels above 140 dB hearing loss or damage to the ear can occur. At lower levels of exposure there is a sparse literature and no confirmed biological effects. Few animal studies have investigated the consequences of long-term exposure to infrasound and no adverse effects have been established.

Studies of the effects of ultrasound in humans have largely concerned *in utero* exposures to diagnostic ultrasound. The available evidence does not suggest effects on several outcomes, which include perinatal mortality and childhood malignancies, but some observational studies have found increased prevalence of non-right-handedness in males with prenatal ultrasound exposure. The results on handedness might reflect confounding rather than causation, however, and analyses comparing individuals randomly assigned to receive, or not receive, ultrasound in pregnancy have only shown weak evidence of an effect.

There is no consistent evidence of any physiological or behavioural effect of acute exposure to infrasound in humans. There is, however, little good quality research and interpretation is complicated because low frequency noise often includes audible as well as infrasonic frequencies. At high levels of infrasound, aural pain and eardrum rupture can occur. There have been few studies on longer-term effects of infrasound in humans, and no ill-effects have been established.

In summary, high levels of ultrasound and infrasound exposure have well-recognised acute adverse effects; for medical uses of ultrasound, measures and guidelines are in place to avoid these. At lower levels of exposure, notably for diagnostic ultrasound, there is no established evidence of specific hazards, but there are too few research data to draw firm conclusions about their absence, especially in the long term.

In the light of the widespread use of ultrasound in medical practice and its increasing use for 'souvenir' fetal imaging commercially, and given the unconfirmed indications from the biological and epidemiological literature of possible neurological effects of *in utero* ultrasound exposures, there is a need for further research on whether there are any long-term adverse effects of diagnostic ultrasound exposure.

1 Introduction

In September 2004, prior to its incorporation in the Health Protection Agency (HPA), the Board of the National Radiological Protection Board (NRPB) agreed that the independent Advisory Group on Non-ionising Radiation (AGNIR) should be asked to undertake a review of ultrasound (frequencies greater than 20 kHz) and infrasound (frequencies less than 20 Hz) relevant to any possible health effects. A subgroup of the AGNIR was set up in 2005 under the chairmanship of Professor Denis Noble and vice-chairmanship of Professor Francis Duck following the meeting of a scoping group in December 2004 that considered the possible content and shape of a report. In addition, the NRPB Board proposed that the NRPB should host a workshop on ultrasound and infrasound safety. The workshop, which was organised by the Radiation Protection Division of the HPA (the successor organisation of the NRPB), was co-sponsored by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) and the Department of Health and was held at Chilton from 24 to 26 October 2005.

The workshop brought together national and international experts from different disciplines, including medicine, epidemiology, medical physics, biophysics, biology and engineering, to provide reviews of what is known about interaction mechanisms and possible adverse health effects and provide overviews of technological advances both in medicine and in industry. The proceedings of the workshop were published in January 2007 (*Progress in Biophysics and Molecular Biology: Effects of Ultrasound and Infrasound Relevant to Human Health,* **93**(1–3), January/February 2007).

It is clear that ultrasound has become an essential tool in medicine, capable of providing high quality imaging data and a potentially important non-invasive surgical tool. The importance of ultrasound in medicine is no better exemplified than by its widespread use for examining the unborn child. Here, in a clinical setting, where its use is under the control of trained medical staff, there are clear benefits to the fetus and to the mother.

Over the years ultrasound examination has developed into a routine procedure that has found widespread acceptability, indeed enthusiasm, for its use among parents-to-be. Such is this enthusiasm, that it has spread commercially into the 'high street', providing photographs and videos for non-medical purposes, so-called 'souvenir' scanning.

The use of high intensity ultrasound for medical therapeutic purposes is rapidly developing as demonstrated by many ongoing clinical trials. Such applications can be broadly divided into two categories – those that exploit the biological effects arising from the direct interaction of ultrasound with tissue to produce the required effect, and those that use ultrasound to drive specially designed applicators. The two main interaction mechanisms that are of interest are heating and acoustic cavitation. Ultrasonic biological effects are used to give therapeutic benefit in a number of different clinical applications including acceleration of bone repair, treatment of soft tissue injuries, haemostasis and cancer treatment. A novel area of investigation is its use in improving drug and gene delivery. In

addition, ultrasonically powered tools are used in dentistry and surgery. The safety implications for therapeutic and surgical applications, in which the aim is to deliver high intensity ultrasound to a target tissue and achieve beneficial modification of that tissue, are clearly very different from those for diagnostic ultrasound. For these high power devices, the concerns, apart from operator safety, are mainly for accurate targeting of the ultrasound in the desired target treatment volume, while sparing other tissues. Research studies, including clinical trials, are addressing these issues.

Ultrasound, often of high intensity, is used extensively for non-medical applications including many in industry – for example, materials testing, fault location, cleaning, drilling and welding. One of the most active fields in this area is in the use of sonochemistry for industrial applications. Here, processes and techniques are being developed that find application in environmental protection, materials processing and food technology.

Infrasound is widespread in modern society, being generated by cars, trains and many machines and appliances. It is also produced by various natural phenomena, such as earthquakes and volcanic eruptions. Health effects associated with exposure to infrasound are less well understood than for ultrasound. It is important to establish if exposure below hearing thresholds at these low frequencies can cause adverse effects.

In this report the basic principles involved in the production, propagation and physical interaction of ultrasound and infrasound with matter are examined in Chapter 2. The characteristics of sources of ultrasound and infrasound, with particular emphasis on medical and industrial applications, are then explored in Chapter 3.

The evidence for biological and adverse health effects from animal and cellular laboratory studies is examined in Chapter 4 and from human volunteer, clinical and epidemiological studies in Chapter 5.

Conclusions are set out in Chapter 6 and recommendations for action and further research in Chapter 7.

An appendix provides an outline of current regulations and recommendations covering human exposure to ultrasound and infrasound. A glossary of technical, scientific and medical terms used in the report is included.

2 Basic Principles

Infrasound, sound and ultrasound refer to the three frequency bands in an overall spectrum of acoustic waves (Figure 2.1). The division arises from the observed sensitivity thresholds of the human ear and details are given about these thresholds later in this chapter. Acoustic waves of about 20 Hz may be heard as a low rumble and for frequencies below this the term infrasound is used. Acoustic waves at a frequency of 20 kHz can be heard by some as a very high pitched whistle. The term ultrasound is used for all frequencies higher than this. Sound waves lie between these two bands and can be detected by the human ear.

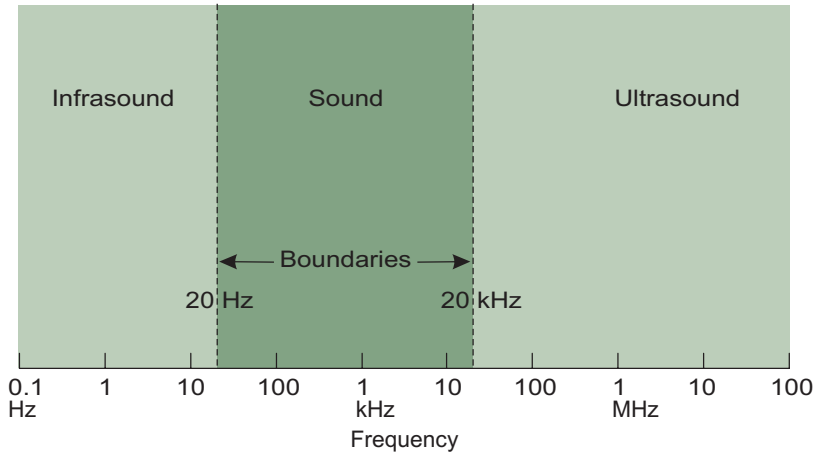


FIGURE 2.1 Frequency bands for acoustic waves

For many medical applications of ultrasound, frequencies above 1 MHz are used, about one hundred times more rapid than the oscillations detected by the ear, and about one million times the infrasonic frequency range. In the following sections, some emphasis is given to the propagation characteristics in the frequency range between 1 and 20 MHz. At such frequencies, practical use is made of these waves in clinical medicine for diagnostic, therapeutic and destructive purposes, and therefore their propagation characteristics are of particular interest and have been more fully studied. In spite of this partial emphasis in the low megahertz range of frequencies, many of the basic principles of acoustics apply equally, appropriately scaled, to other acoustic frequencies, including those of the lower end of the ultrasonic spectrum, and of infrasound. From a knowledge of the wave velocities and of the degree to which tissues absorb, scatter and reflect acoustic waves, it is possible to predict the manner by which acoustic waves propagate within and interact with the body.

2.1 Acoustic Wave Propagation

Acoustic waves, in contrast to electromagnetic radiation, require a physical medium to support their propagation. The particles of the medium oscillate about their equilibrium position, and the pattern of changing particle displacement with time may be used to depict wave propagation (Figure 2.2). For waves in fluids the direction of this particle displacement is the same as the direction in which the wave propagates, giving rise to a longitudinal wave. But this is not always true and, in general, the displacement has both magnitude and direction (ie it is a vector quantity). Elastic solids can also support shear waves. In an isotropic medium, particle displacement in a shear wave is perpendicular to the propagation direction, so waves in solids can be transverse as well as longitudinal. Hard tissues such as bone can support shear waves, but for soft tissues, which tend to have a very small shear modulus, only longitudinal waves are of significance. The discussion in this chapter will concentrate on the specific case of waves in a fluid unless otherwise indicated.

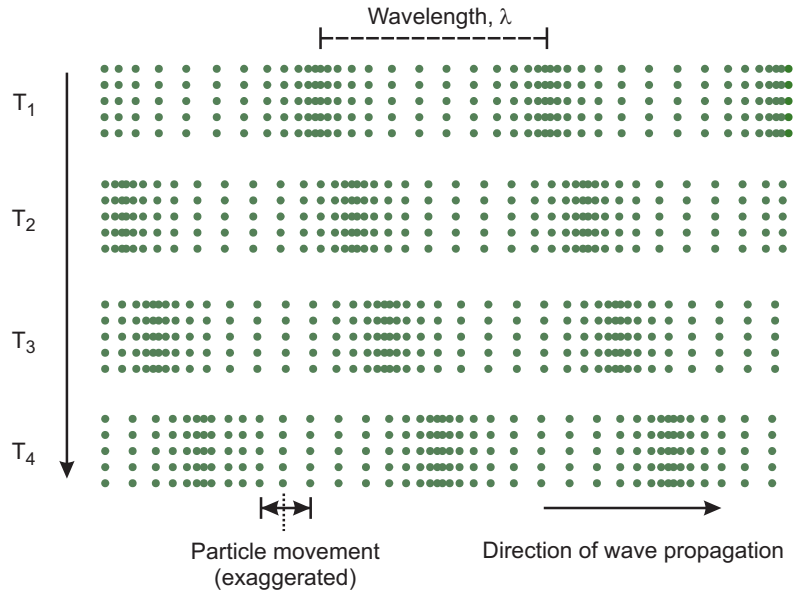


FIGURE 2.2 Progression (from T_1 to T_4) of a longitudinal compressional wave moving forward by about half its wavelength, λ. The time delay between each wave and the one below it is about $\lambda/6c_0$, where c_0 is the speed of the wave. The dots represent the particles, which do not progress with the wave, but oscillate about an undisturbed position

As a result of the varying displacement, the particles of the medium move with a time-varying velocity, u. It should be noted that the particle velocity should not be confused with the speed of sound c_0 with which the acoustic wave travels. As the particles move forward they become closer to those ahead, so increasing both the local density and the local pressure in the medium. Following their maximum forward displacement, the particles return toward and beyond their equilibrium location, resulting in a slight

density reduction, and a reduction in local pressure. The difference between the ambient pressure (atmospheric pressure) and the local pressure as the wave passes is called the acoustic pressure. This may be a compression (pressure above ambient) or a rarefaction (pressure below ambient). For the specific case of a harmonic plane wave the acoustic pressure, p, is related to the particle velocity, u, and the ambient density, ρ_0, by

$$p = \rho_0 c_0 u \tag{1}$$

The SI unit for pressure is the pascal (Pa) and is defined as one newton per square metre. The constant of proportionality, $p/u = \rho_0 c_0$, is the characteristic acoustic impedance, Z. Strictly, Z is a complex quantity – that is, it has both real and imaginary components. Whilst this is of importance for the complete analysis of wave propagation through lossy media, for many practical situations – for example, when considering reflections from plane interfaces – it is not necessary to introduce this complexity.

Particle displacements are very small, being measured in micrometres (μm). The corresponding particle velocities have magnitudes up to the order of metres per second. Whilst the displacements are microscopic, and the particle velocities are small relative to the speed of the wave, the acoustic pressures can become very large compared with the ambient pressure, sometimes exceeding it in magnitude. In diagnostic ultrasound scanners these acoustic pressures can reach more than 2 MPa at the transducer face, or about 20 atmospheres. This is true for positive acoustic pressures (compressions) and also for negative acoustic pressures (rarefactions). Fluids and tissues can withstand large negative pressures (tensions), of magnitude greater than one atmosphere, because of their strength resulting from cohesive molecular forces. This strength, and the brevity of the tension (applied only during the negative half-cycle of the wave), means that, commonly, the fluid can withstand the applied stress. There is, however, a limit to how long the tension can be supported; for longer times, associated with frequencies at the lower end of the ultrasonic frequency spectrum, and at higher acoustic pressures, it is possible for the fluid to cavitate and small gas bubbles can form. This phenomenon is called acoustic cavitation and is one of the physical effects described below. The importance of acoustic pressure as an exposure quantity is when biological effects from mechanical causes are discussed. In particular, the 'peak rarefaction pressure' is strongly related to cavitation events.

The distance between one compression (or rarefaction) and its immediate neighbour defines the wavelength, λ (Figure 2.2). At any particular frequency, f, the wavelength in any medium can be calculated from a knowledge of the wave velocity c_0 (see below), using the expression $\lambda = c_0/f$. At 1 MHz the wavelength in soft tissues is typically between 1.5 and 1.6 mm, whereas at the same frequency the wavelength in bone is between 3 and 4 mm, because the wave travels about twice as fast in bone as in soft tissue. At 20 kHz, the frequency threshold for ultrasonic waves, the wavelength in water and soft tissue is about 7.5 cm, so the breadth of an adult human abdomen is about three or four wavelengths at this frequency. The wavelength of airborne ultrasound at the same frequency is only about 1.6 cm because sound travels much more slowly in air (331 m s^{-1}) than in water (1480 m s^{-1}). At 20 Hz, the upper frequency threshold for infrasound, the wavelength is very much longer, about 16 m in air, and is therefore much greater than any human dimension. This means that the human body experiences the oscillatory pressure and density variations of the infrasonic wave largely in phase, and the body itself becomes a scattering centre of a wave.

When two equal amplitude plane-travelling waves propagate through the same space in opposite directions, a stationary wave is formed. More generally, standing waves can be formed whenever waves interfere with one another, particularly in the vicinity of a strongly reflecting acoustic interface. Although such an arrangement can be generated in the laboratory, it is very rare for conditions giving rise to extended standing waves to occur in an ultrasonic field within the body. Whilst standing waves are associated with continuous-wave, single frequency fields, it is possible for a pulsed beam to create, transiently, interference close to an interface. This can lead, in principle, to local acoustic pressures reaching twice the associated free-field value.

2.1.1 Beam structure

The formal theoretical description of acoustic waves typically starts with that of an infinite monochromatic plane wave propagating in an infinite, lossless medium (see, for example, Kinsler et al, 2000). Since this chapter is intended to develop aspects of relevance to the later discussions of hazard, risk and health, this theoretical formulation will not be duplicated here, and the reader is referred to well-established texts on the subject. It is of more practical interest to describe some aspects of spatial structure of ultrasonic fields, to allow an understanding to emerge as to where tissues may be exposed strongly and where they may be spared.

All acoustic waves originate with a source of finite size, and the geometry of the source and the acoustic wavelength together control the beam structure that is created. This fact cannot be overemphasised. Inadequately specified source and beam conditions can make it impossible to reproduce correctly studies on biological and health effects. Furthermore, users of ultrasound scanners take it for granted that they have wide control over the beam shapes and structures available to them. The important factors in controlling the beam are the shape of the source, its dimensions with respect to the wavelength of the ultrasound being propagated, whether it is pulsed or continuous, and the variation in amplitude and phase over the source. Symmetry is of considerable importance in the structure of acoustic fields. The analysis of beams with strong symmetry – uniform circular sources of continuous sound at a single frequency (monochromatic), for example – has been well developed in the literature (Humphrey and Duck, 1998; Kinsler et al, 2000). However, many common practical sources of ultrasound are not so highly symmetrical, and the fields created differ, sometimes quite markedly, from simple predictions. Examples of this may be seen in the fields used for pulse-echo imaging for which the transducers are rectangular and not circular: the pulses carry a band of frequencies and are not monochromatic, and the beams are focused, often astigmatically. The following overview summarises material that is available more fully elsewhere (see, for example, Humphrey and Duck, 1998, and Szabo, 2004).

The simplest sound field is that generated by a point source. A good approximation is that of the sound field generated by a pulsating microscopic spherical bubble, where the bubble is very much smaller than the wavelength of the sound being generated. Also, any practical infrasound source is a good approximation to a point source, because of the very long wavelengths associated with infrasound. As noted above, the shortest infrasonic wavelength in air is about 16 m, and for much of the infrasonic spectrum the wavelength exceeds 100 m. The spherically diverging wave from such a source is fully isotropic, so its magnitude and phase do not vary with direction. However, even approximately

isotropic ultrasonic fields are quite unusual. The source to generate such a field must be very small compared with the wavelength. A practical example approximating to an isotropic source is that of an ultrasonic horn.

If the radius of a source is comparable with the wavelength, λ, a strongly diverging beam is created, which typically consists of a main lobe and side lobes. The angle, θ, bounding the main lobe (a direction at which the acoustic pressure is theoretically zero) is given by $\sin^{-1}(0.61\lambda/a)$, where a is the source radius: so, for example, the beam divergence of the main lobe of a 2 mm diameter, 1 MHz source will lie between ±66°. As the source increases further in size with respect to the wavelength, the divergence reduces and the term 'ultrasonic beam' can be used to describe this field structure. The region of divergence and side lobes is referred to as the far field, in which the acoustic pressure reduces with the reciprocal of the distance from the source. Many underwater sonar applications operate in this region.

An important alteration in the beam structure close to the source accompanies the formation of a directional far field. An extended near field is formed. This region, illustrated in Figure 2.3, is highly complex. Along the beam axis of symmetry, the acoustic pressure amplitude varies between zero and twice the acoustic pressure at the source. The nulls and peaks are narrowly spaced close to the source, and became more widely spaced as the distance from the source increases. The on-axis minimum and maximum in the near field furthest from the source occur approximately at distances of $a^2/2\lambda$ and a^2/λ, where a is the source radius. The formal transition between near and far fields lies at the Rayleigh distance, $\pi a^2/\lambda$.

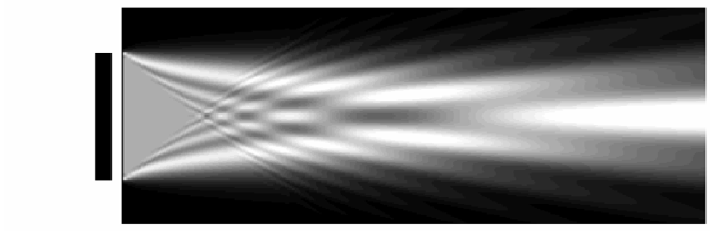


FIGURE 2.3 Near-field variation in acoustic pressure in water calculated for a transaxial plane of symmetry. The source was a plane square of side 20 mm, vibrating at 2.25 MHz. The source is on the left with the propagation direction across the page extending to 200 mm from the source. In the region close to the source, the variations in acoustic pressure are too densely spaced to be shown

In practice, fields produced by physiotherapy ultrasound transducers are amongst those most nearly approximating to this theoretical description, for which the symmetry assumptions of a plane piston (ie uniform) monochromatic circular source are largely valid. So for a typical 1 MHz physiotherapy transducer of 25 mm diameter, the last axial maximum lies theoretically at a distance 10.4 cm and in the far field the beam divergence is 4.2°. Thus whenever ultrasound therapy is applied directly to the skin, most of the exposure lies in this near field. These estimates of beam divergence and near-field patterns have assumed propagation in water. Sound travels much more slowly in air, so the wavelength at any given frequency is proportionally longer, by a factor of about 4.5. For the example given above, the last

axial maximum would be reached at only 2.3 cm and the far field would then diverge more strongly, by about 20°. (This is intended for illustration only: 1 MHz ultrasound is strongly attenuated by air.)

For many applications of ultrasound it has been found valuable to concentrate the acoustic energy density by focusing, either to improve lateral resolution in imaging or to gain greater intensity for destructive purposes. This is achieved by altering the phase of the vibration over the source area, in practice by shaping the source (for example, into a concave bowl), or by inserting a lens (typically plano-convex with a speed of sound lower than that of the medium) or by using appropriate electronic control to an array of ultrasonic source elements. However it is achieved, narrowing of the beam by focusing can only occur within the near field of an equivalent plane source. Focusing is considered to be weak if the amplitude focusing gain is less than 2π, and such regimens are typical of those used for medical imaging. This gain is equivalent to an increase in intensity of up to 40 from the source to the focal zone. High focal gains are used in medicine for applications such as extracorporeal lithotripsy and HIFU (high intensity focused ultrasound). At low focal gains, the complex near-field structure is retained only up as far as the focal zone. For high focal gains, the field complexity characteristic of the near field, with its on-axis minima and maxima, reappears beyond the focus.

The electrical excitation of a source of ultrasound may be regularly switched on and off in practice, generating bursts of ultrasound many cycles long. When this is done using on-times longer than about 10–20 cycles of the ultrasonic frequency, the effect on the field structure is only to switch the whole beam on and off. This happens in pulsed mode in physiotherapy, for which typical pulse lengths of 2 ms are used. The beam structure itself remains largely unchanged during the on-time. The main outcome is that the total acoustic power and time-averaged intensities are reduced proportionally with the mark-to-space ratio.

Pulsed fields with pulse periods of the order of microseconds are different. The diffraction patterns, such as those shown in Figure 2.3, caused by wave interference in continuous-wave fields are much weaker and, for some conditions, are absent. Imaging systems use such beams in order to achieve high spatial resolution. For such beams, the structure is considerably different from that which would have occurred using monochromatic excitation. The acoustic disturbance extends over only one or two wavelengths. The pulse becomes mostly end and no middle, and its ends dominate the beam structure.

Pulsed beams are best described by means of the impulse response function. This function gives the velocity potential as a function of position arising from step movement in the axial direction of any potential source of sound. The time differential of this function is used to predict the impulse response of acoustic pressure, and a time convolution of this function with an arbitrary driving function enables the acoustic pressure field throughout space to be predicted. This approach forms the computational basis to field prediction.

Pulsed fields of this type may best be thought of as a combination of two wave components. The first is that which is caused by the face of the source, and is the main wave, propagating according to the shape and area of that source. For example, if the source is flat and circular, the main wave consists of a disturbance which is circular in section, and whose thickness depends on the pulse length. If the source is shaped as a concave spherical cap, the main wave converges towards the centre of curvature, decreasing in width and increasing in amplitude as it travels. The main wave is accompanied by a second wave,

which propagates from the edge of the source, and so is called the edge wave. The simplest edge wave to imagine is one from a circular source, from which it diverges as a hemi-toroid. It is in anti-phase with the main wave. Interaction between these two waves creates the final beam structure. This interaction depends on the relationship between the pulse period and the time taken for each wave component to travel from its source to the field point. In the far field, and on the pulse axis, wave components arrive more or less together, and so interact in a very similar way to a continuous wave. However, very close to the transducer, there are positions where the main wave pulse has passed before the arrival of the edge wave, and so no interfering interaction can take place. Taking a broader view, the main difference is that the pulsed field is spatially much smoother than the continuous-wave field. In the near field, the differences between maxima and minima are much reduced; near-field nulls in acoustic pressure are absent and there is a region close to the transducer in which the pulse components are entirely separated in time, so no diffraction pattern is created. In the far field, side lobes are reduced in amplitude and the angular nulls that separate side lobes in a continuous-wave, monochromatic beam become minima or largely disappear.

One other important consideration for pulsed fields is their frequency spectrum. In practice, very short acoustic pulses with 100% bandwidths are not uncommon in ultrasound exposure in imaging systems. One method of predicting a specific pulsed field structure uses the weighted superposition of beams from a spectrum of frequencies. When considering the interaction of broadband pulses of this type with the medium supporting their propagation, it is essential to consider any frequency-dependent properties of that medium. These dependencies are discussed below. It is sufficient to say at this point that pulses propagating through tissue lose preferentially their higher frequency components (in contrast to X-radiation, for example, for which, for much of the X-ray spectrum, energy from photons associated with low frequency, spectral components is deposited preferentially in tissue). This is true both for absorption and for scattering, although the respective frequency dependencies are not the same. For pure water and, to a first approximation, all soft tissues, the speed of sound is non-dispersive. This is not true for gassy water, bone or air-filled lungs (Duck, 1990).

2.2 Output, Exposure and Dose

Quantitative reports on acoustic biological effects, to be of value, must always be accompanied by statements about the acoustic conditions used. These statements should be sufficient to quantify fully the acoustic field conditions used for exposure, to quantify the energy transfer into the target and perhaps to quantify biophysical outcomes such as heating or cavitation. Reports of biological experiments have commonly reflected contemporary understanding of these issues and as a result some have suffered from incomplete, inappropriate or even misleading data. What follows gives a structured description of the terminology, parameters and quantities to be used in such statements.

The words output, exposure and dose have each been used, sometimes rather loosely and interchangeably, as terms to describe the acoustic conditions associated with biological effects studies. In what follows, the terms ‘acoustic output’, ‘acoustic exposure’ and ‘acoustic dose’ will each be used with a narrower and more specific meaning.

2.2.1 Acoustic output

The primary measure of acoustic output is total acoustic power, W, emitted from the transducer surface into water. Power is measured in watts. Equivalently, W quantifies the rate at which acoustic energy is propagated from the transducer surface, in joules per second (J s^{-1}). The only other quantity used to measure acoustic output is the spatial average intensity, I_{SA}, measured in watts per unit area (typically W cm^{-2}). I_{SA} is calculated by averaging the total acoustic power over the source area, A:

$$I_{SA} = W / A \tag{2}$$

The source area is sometimes referred to more formally as the effective radiating area, ERA. These two measures, W and I_{SA}, are the quantities used to specify the acoustic output of all ultrasound physiotherapy devices, for which I_{SA} is known as the effective intensity. In addition, acoustic power is the key quantity on which the calculation of thermal index is based (see Section 2.2.3.2). Acoustic power may be measured in water by means of radiation force, if the acoustic wave is propagating in a beam. Calorimetry may be used otherwise. If no convenient means for the measurement of acoustic power exists, it may be possible to estimate this quantity from a measurement of the electrical power delivered to the transducer, and knowledge of its electro-acoustic efficiency. This seems to be the most promising approach to the measurement of acoustic power in air, either at low ultrasonic frequencies or for infrasound.

2.2.2 Acoustic exposure

2.2.2.1 Acoustic pressure

Any of the physical quantities which vary in an acoustic wave are, in principle, candidates for measuring acoustic exposure. These include particle displacement and its temporal derivatives, acoustic pressure and the associated density variations, energy density and intensity. Of these, acoustic pressure has been selected as the primary quantity for acoustic exposure measurement. It is readily measured and simple media are appropriate for standardised measurement conditions. Infrasound and low frequency ultrasound propagating in air may be measured using an appropriately calibrated microphone. Ultrasonic waves propagating in water are measured using a calibrated hydrophone. Furthermore, using acceptable approximations, local intensity can be calculated from the measured acoustic pressure.

'Free-field' conditions are used for standard measurements of acoustic pressure. These conditions approximate to those under which the acoustic field consists only of a travelling wave, propagating into an infinite medium without boundaries. In practice, the conditions are established either by using an anechoic measurement chamber or by using short pulses with time-gated signal capture. Such conditions prevent interference between the wave and any reflections from interfaces, and the resultant possible creation of stationary waves superimposed on the travelling wave.

The acoustic pressure wave varies above and below the ambient pressure, typically with sinusoidal modulation. The amplitude of the wave is given either as the root-mean-square (rms) acoustic pressure, p_{rms}, or the peak acoustic pressure, p_0. Three units have been used for reporting acoustic pressure. Usually the SI unit, pascal (Pa), is used. Alternatively, the acoustic pressure for airborne acoustic waves is

often given in decibels (dB) referenced to 20 μPa, the threshold for human hearing at 1 kHz. For assessment of hearing, over the audible range that largely is not of concern here, a frequency-weighted measure, dB(A), is used, which takes account of the variation in sensitivity of the human ear to different audio frequencies. For underwater sound, the acoustic pressure is measured in dB referenced to 1 μPa.

There are some conditions, most notably for ultrasonic pulses generated by medical diagnostic systems, for which a sinusoidal pressure variation cannot be assumed. Asymmetry between positive and negative half-cycles may be inherent in the pulse waveform itself and also may be caused by non-linear propagation. For these conditions, the peak rarefaction, p_r, and peak compression, p_c, (sometimes called peak negative pressure and peak positive pressure, respectively) are separately reported. In practice, only the peak rarefaction is commonly reported.

One other exposure quantity associated with acoustic pressure should be noted, namely the mechanical index, MI. It is given by

$$\mathrm{MI} = p_r \,/\, f^{0.5} \tag{3}$$

in which f is the centre frequency of the ultrasonic wave. It is thus a frequency-weighted acoustic pressure value. In general usage, MI is normalised by the factor $(1\ \mathrm{MHz})^{0.5}/(1\ \mathrm{MPa})$, allowing the value to be given as a dimensionless quantity.

In one particular form, MI is one of two indices developed to advise users of the acoustic conditions during medical ultrasound scanning, where it indicates the likelihood of cavitation (see Section 2.2.5.2).

2.2.2.2 Acoustic intensity

It is possible to calculate intensity from the acoustic pressure waveform, for those positions in the field where it is reasonable to assume that acoustic pressure and particle velocity are in phase. If so, the instantaneous intensity

$$i = p^2 \,/\, \rho_0 c_0 \tag{4}$$

where ρ_0 is the density and c_0 is the speed of sound. This approximation is valid in the far field and within the focal zone of a focused transducer. It is invalid very close to the transducer and in some regions towards the edges of the beam. This approach allows the calculation of several intensity exposure measures, including some which are used to underpin the regulatory mechanism controlling the sale of medical ultrasound equipment in the USA (FDA, 2008).

The first two intensity measures are related to the two measures of acoustic pressure noted above, namely peak acoustic pressure, p_0, and root-mean-square acoustic pressure, p_{rms}. Temporal peak intensity is calculated by setting p equal to p_0 in equation 4. This measure is now obsolete, having been fully superseded by peak acoustic pressure. Temporal average intensity, I_{TA}, is calculated by setting p equal to p_{rms} in equation 4. The equation for temporal average intensity for a continuous-wave, single frequency condition is thus

$$I_{TA} = \frac{{p_{rms}}^2}{\rho_0 c_0} = \frac{{p_0}^2}{2\rho_0 c_0} \tag{5}$$

For pulsed conditions of exposure, acoustic intensity is calculated from the integral over time of the instantaneous intensity *i*, a quantity termed the pulse intensity integral, PII, averaged over an appropriate time period. If the time period is one pulse repetition period, the measure is of temporal average intensity. If the averaging period is the pulse duration, the exposure quantity is the pulse average intensity, I_{PA}. The symbol I_m was used in the past to indicate another obsolete intensity measure, associated with averaging over a single greatest half-cycle in a pulse waveform.

2.2.2.3 Estimated *in situ* exposures

Specific to ultrasonic medical diagnostic applications, 'estimated *in situ*' exposure quantities have been introduced, in order to predict conditions within the human body. These are termed 'derated' and also 'attenuated' quantities (IEC, 2006). *In situ* exposure quantities form part of the regulatory requirements for sale of medical ultrasound equipment in the USA. In order to calculate these *in situ* exposure quantities, propagation through a very simple homogeneous tissue model is assumed, with an attenuation coefficient of 0.3 dB cm^{-1} MHz^{-1}. Attenuated/derated quantities are derived from the associated free-field exposure quantities using linear assumptions for wave propagation. A similar model may also be used to estimate *in situ* acoustic power from the measured acoustic output power.

2.2.2.4 Localisation of acoustic exposure

There is a key difference between all these exposure quantities and the output quantities discussed above. All the measures of acoustic exposure, whether associated with the acoustic pressure wave or with the intensity derived from it, and whether free-field or *in situ*, are associated with the particular location at which that quantity is measured. The location may be, for example, the position at which a cell culture is placed during an experiment, or the location at which the exposure quantity reaches its maximum value in water, or a specified distance along a specified direction from the source. This means that prediction of acoustic exposure anywhere else in the acoustic field is generally not possible, and can only be made possible by a full knowledge of the geometry of the field, and the coordinates of the location at which the exposure is specified.

Sometimes the location can be inferred from the terminology used. For regulatory requirements in the USA, the estimated *in situ* spatial peak, temporal average intensity, $I_{SPTA,0.3}$, is measured where this exposure quantity reaches its maximum value. This generalisation is not always true, however. Again for regulatory purposes, the MI is calculated using the estimated *in situ* value of peak rarefaction, but the location is that where the pulse intensity integral is greatest. However, in neither case does the location of measurement form an integral part of the statement of exposure. Only the value of the exposure quantity is given and the coordinates of its position of measurement are generally omitted.

A complete description of acoustic exposure would include the acoustic frequency (and frequency spectrum for pulsed exposure), a full spatial distribution of the amplitude and phase of the acoustic pressure field, the pulsing regimen and the scanning regimen.

2.2.3 Dosimetric and associated quantities

2.2.3.1 Acoustic dose and acoustic dose rate

In this section, new definitions of acoustic dose and acoustic dose rate are set out and formulae for their prediction under simplified conditions of exposure are presented (Duck, 2009). They are intended to be applied for exposure to ultrasound and not infrasound.

Acoustic dose is defined as the energy deposited per unit mass of a medium supporting an acoustic wave. The unit of acoustic dose is joule per kilogram (J kg^{-1}). The acoustic dose rate is defined as the energy deposited per second per unit mass of a medium supporting an acoustic wave. The unit of acoustic dose rate is watt per kilogram (W kg^{-1}).

The word 'dose' is thus used, as it is for ionising radiation, to mean a measure of the energy absorbed per unit mass of material. Parallel concepts have been slow to develop in acoustics in part because of the absence of a unique physical mechanism by which energy transfers from the acoustic wave into tissue (Duck, 1987). For example, energy may be transferred by viscous absorption of either progressive or shear waves, or by mechanical means involving either transfer of acoustic energy to an oscillating bubble or transfer of acoustic energy to kinetic and/or potential energy via radiation pressure. There is at present no complete analysis of energy deposition from an acoustic wave to a medium that takes account of all possible conditions and loss mechanisms.

Accepting the complexity of a complete analysis, the following presents a simplified approach that has been used widely to enable approximate predictions of heating from ultrasound (Nyborg, 1981; Cavicchi and O'Brien, 1984). Plane-wave conditions and a uniform medium are assumed. Under these conditions, the rate at which acoustic energy is deposited per unit volume of a material by the absorption of ultrasound is given by $2\alpha_a I$. Hence the rate of energy absorption per unit mass, Q_m, is given by

$$Q_m = \frac{2\alpha_a I}{\rho_0} \tag{6}$$

where I is the intensity, ρ_0 is the density and $2\alpha_a$ is the intensity absorption coefficient of the medium (in nepers per metre). Here α_a is the amplitude absorption coefficient.

The total energy deposited per unit mass of a medium, Φ, during a time t is therefore

$$\Phi = \frac{2\alpha_a I t}{\rho_0} \tag{7}$$

More generally, where a local element of tissue is exposed to a time-varying ultrasound intensity,

$$\Phi = \frac{2\alpha_a}{\rho_0} \int_t i \, dt \tag{8}$$

where i is the instantaneous intensity.

The average rate at which energy is absorbed per unit mass during a time period from t_1 to t_2 is given by

$$Q_m = \frac{2\alpha_a \int_{t_1}^{t_2} i \, dt}{\rho_0 (t_2 - t_1)} \quad (9)$$

By analogy with the definitions of dose and dose rate used for electromagnetic and ionising radiations, equations 8 and 9 give formal expressions for acoustic dose, Φ, and acoustic dose rate, Q_m. Under conditions for which either or both α_a and ρ_0 vary with time (for example, as a result of tissue heating) these quantities may be included within the time integral. The quantity α_a is also dependent on frequency. If the acoustic wave is not at a single frequency – for example, because it is pulsed or because of non-linear distortion – appropriate compensation must be included – for example, by setting α_a to a value appropriate to the pulse centre frequency or by summing over each frequency band.

Examples will serve to relate equations 8 and 9 to thermal and mechanical outcomes of exposure to ultrasound. The initial rate of increase in temperature caused by the absorption of ultrasound, dT/dt, is Q_m/C_v, where C_v is the specific heat capacity of the medium. Equation 8 allows the calculation of the total energy per unit mass, Φ, deposited, for example, during a brief, high amplitude ultrasonic pulse. Concerning direct mechanical effects, the force per unit mass (that is, the acceleration) associated with radiation pressure is Q_m/c_0. This force may be calculated either during the pulse, using I_{PA} to calculate Q_m, or over an extended time from the time-averaged intensity. The streaming velocity in a parallel beam of radius r is $(Q_m r^2/\upsilon c_0)G$, where υ is the kinematic viscosity and G is a geometric factor which takes account of the relative size and shape of the beam and container. The instantaneous intensity i is formally a vector, whose direction is the same as that of the radiation force and streaming. It may be noted that, in general, acoustic dose rate probably has greater relevance than does acoustic dose.

It is more difficult to directly relate these dosimetric quantities to energy transfer due to cavitation. This is perhaps of less concern than it may appear. It is now recognised that the acoustic behaviour of gaseous inclusions within the body probably differs sufficiently from their behaviour in a free fluid to require new approaches to their analysis. In some situations, clouds of bubbles serve to increase the local wave absorption and in principle this could be accommodated by setting $2\alpha_a$ to an appropriate value in the dosimetric formulae. Observed damage to the lung surface cannot be explained through a cavitation mechanism, and a dosimetric approach to the associated radiation force may be profitable. The particular situation when free bubbles are released from gas-body contrast agents will probably not be easily approached directly using the dosimetric considerations outlined here.

The spatial distribution of acoustic dose rate, Q_m, may be mapped simply from the local product of the *in situ* intensity (using any standardised or more realistic model) and the absorption coefficient, taking account of the spatial variability of both quantities. Temporal variation of the dosimetric quantities will arise primarily through the time progression of intensity, although time-dependent variations in absorption coefficient could in principle be included.

2.2.3.2 Thermal index

Thermal index is not a dosimetric quantity, but neither is it an exposure quantity. It is included in this section because of its aim to quantify one outcome of the absorption of acoustic energy by tissue. It does so through calculation of the increase in steady-state temperature arising from acoustic exposure within specified tissue models, which is discussed further below. The calculations arise from the definition of the thermal index, TI:

$$\mathrm{TI} = W / W_{\mathrm{deg}} \tag{10}$$

where W_{deg} is the acoustic power required to cause a maximum temperature rise of 1°C and W is the acoustic power associated with the TI value, under otherwise identical acoustic field conditions (that is, pulsing regimen, beam shape and scanning mode). The TI is one of two safety indices displayed on medical ultrasound scanners to inform users of safety issues associated with their clinical use. There are three simplified tissue models, one for homogeneous soft tissue, one for which bone is heated in the focal region and one for bone adjacent to the ultrasound source. In each case, scanned beams and non-scanned beams are considered separately. If these models were exact replicas of real situations, the TI would quantify the maximum temperature increase in degrees Celsius in tissue caused by ultrasound absorption alone. In practice, the estimates are reasonable approximations to the numerical value of soft tissue temperature rise, for unscanned conditions. However, thermal conditions close to the transducer are dominated by transducer self-heating. Therefore the predictive value of the TI can only be a limiting statement of an estimated temperature rise which is never exceeded for tissues lying sufficiently deep to be unaffected by transducer heating.

2.2.3.3 Thermal dose

Despite its name, thermal dose is a time. The idea of thermal dose has been developed from a thermodynamic or 'Arrhenius-type' analysis of a broad range of biothermal responses. For most biological systems and responses, including those for cells, tissues and whole organs, the risk of a thermal effect increases linearly with time, and exponentially with temperature rise. In general,

$$t_1 / t_2 = R^{T_2 - T_1} \tag{11}$$

where t_1 and t_2 are the minimum times required to cause a specific thermally generated biological effect at temperatures T_1 and T_2, respectively. The response of most biological systems alters at about 43°C, and this change in behaviour is characterised by the value of parameter R. At temperatures above 43°C, $R \approx 2$, so each increase in temperature by 1°C reduces by about two the time to cause a specific effect. Below 43°C, R is slightly higher. For simplicity of analysis, the values of R are usually taken as $R = 2$ for $T > 43$°C and $R = 4$ for $T \leq 43$°C. The thermal dose associated with a specific biological endpoint is the minimum time taken to reach this endpoint, at a specific temperature, and it is common to give this equivalent minimum thermal isoeffect dose at 43°C (emt_{43}). Equation 11 may then be used to calculate times associated with other temperatures.

The basic concept may be extended to include a calculation of the thermal dose associated with conditions where temperature varies over time (Dewey, 1994):

$$\mathrm{emt}_{43} = \int_{t=0}^{t=final} R^{(T_t - 43)}\,\mathrm{d}t \tag{12}$$

Where there is a need to compare thermal responses between different animal species, it is necessary to consider the core temperatures. An alternative symbol $t_{\Delta t,\mathrm{core}}$ has been introduced, to be species specific (Miller et al, 2007). Thus, for a guinea pig for which the core temperature is 39.5°C, $\mathrm{emt}_{43} \equiv t_{3.5,\mathrm{core}}$.

2.2.3.4 Summary of exposure and dose

The careful definition and use of quantities describing acoustic output, acoustic exposure and acoustic dose rate form an essential part of the evaluation of experimental studies of acoustic biological response. Acoustic output, in terms of total acoustic power and spatial average intensity, measures the energy leaving the acoustic transducer. Acoustic exposure, in terms of acoustic pressure and intensity parameters, measures the acoustic field at a specified point. This may be given as a measured free-field exposure or an estimated *in situ* exposure. Acoustic dose rate measures the rate at which energy is absorbed by unit mass of tissue. Each measure is important for the complete specification of the acoustic conditions relating to biological effects. The quantities are summarised in Table 2.1. All quantities apply to ultrasonic fields, but only acoustic exposure quantities may meaningfully be applied to infrasound.

2.2.4 Acoustic properties of body tissues

Ultrasonic wave propagation into and through tissues is controlled by the acoustic properties of those tissues. A number of reviews of these properties are available in the literature (Duck, 1990; ICRU, 1998). The following sections briefly review the more important properties (see Table 2.2). The context for this section is the ultrasonic frequency range, except where noted.

2.2.4.1 Wave propagation speed

The speed at which an acoustic wave propagates is controlled by the mechanical properties of the supporting medium. For liquids and soft tissues, the speed of the wave, c_0, depends on the compressibility and the undisturbed density. Solids support both compressional waves and shear waves, whose speeds depend on the elastic modulus of the solid. However, simple equations are difficult to apply directly to biological solids, including bone. This is because the mechanical properties of hard tissues depend on direction and, consequently, so do their acoustic properties.

Values for the wave speed of ultrasound through selected tissues are summarised in Table 2.2. This table should be regarded as giving reasonable estimates of the speed with which ultrasound propagates in the range from 1 to 10 MHz, at body temperature, in normal adult human tissues. These data should not be considered as definitive and may only be judged to be best estimates of representative values. In particular, it must be remembered that real tissues from a single organ, say liver, demonstrate a range of properties that may depend on age, sex, disease state, perfusion and even dietary habits.

TABLE 2.1 Various quantities for acoustic output, acoustic exposure and acoustic dose*

Quantity	Unit	Symbol	Formula	Dependent quantities
Acoustic output				
Total acoustic power	W	W		
Spatial average intensity	W m^{-2}	I_{SA}	$W/$ERA	ERA = effective radiating area
Acoustic exposure				
Root-mean-square acoustic pressure	Pa Air: dB referenced to 20 μPa Water: dB referenced to 1 μPa	p_{rms}		
Peak rarefaction	Pa	p_r		
Mechanical index		MI	$p_r / f^{0.5}$	f = acoustic frequency
Time average intensity	W m^{-2}	I_{TA}	$p_{rms}^2 / \rho_0 c_0$	ρ_0 = density c_0 = speed of sound
Pulse intensity integral	W m^{-2} s	PII	$(1/\rho_0 c_0) \int_{pulse} p_2 \, dt$	
Pulse average intensity	W m^{-2}	I_{PA}	PII/τ	τ = pulse duration
Dosimetric and associated quantities				
Acoustic dose	J kg^{-1}	Φ	$(2\alpha_a/\rho_0) \int_t i \, dt$	$2\alpha_a$ = intensity absorption coefficient i = instantaneous intensity
Acoustic dose rate	W kg^{-1}	Q_m	$2\alpha_a I/\rho_0$	
Thermal index		TI	W/W_{deg}	W_{deg} = power to raise tissue 1°C
Equivalent minimum thermal isoeffect dose	s	emt_{43}	$\int_{t=0}^{t=final} R^{(T_t-43)} \, dt$	T_t = temperature (°C) at time t R = 2 for T > 43°C R = 4 for $T \leq$ 43°C

* Output quantities are measured at the acoustic source. Exposure quantities are commonly measured on the acoustic axis at a specified distance z from the source. When this distance maximises the value of the exposure quantity, it is identified as the spatial peak and the suffix 'SP' is commonly added. Thus I_{SPPA} indicates the spatial peak value of the pulse average intensity. Spatial average intensity, I_{SA}, may also be used as an exposure quantity, where $I_{SA} = W/A$ and A is the beam cross-sectional area at a specified distance. Estimated *in situ* acoustic exposures $p_{0.3}$ and $I_{0.3}$ may be calculated using $p_{0.3} = p\, 10^{(0.3fz/20)}$ and $I_{0.3} = I 10^{(0.3fz/10)}$, where 0.3 refers to an attenuation coefficient of 0.3 dB cm^{-1} MHz^{-1}. (It should be noted that in some publications, the suffix 'd' has been used to indicate estimated *in situ* quantities.) The formulae for acoustic dose and acoustic dose rate strictly apply only under plane-wave homogeneous conditions.

TABLE 2.2 Representative values for some acoustic properties of tissues at body temperature* [values taken from Duck (1990), ICRU (1998) and Verma et al (2005)]

Property	Cortical bone	Non-fatty tissue	Fat	Blood	Amniotic fluid
Propagation speed (m s^{-1})	3635	1575	1465	1584	1534
Characteristic acoustic impedance (10^6 kg m^{-2} s^{-1})	6.98	1.66	1.44	1.68	1.54
Attenuation coefficient at 1 MHz (dB cm^{-1})	20	0.6	1.0	0.15	0.005
Attenuation coefficient frequency dependence	n/a	1.2	1.0	1.2	1.6
Non-linearity coefficient, B/A	n/a	7.0	10.0	6.1	n/a

* These are representative values only, and there are very wide variations of tissue properties for bone and soft tissues; blood and amniotic fluid are better characterised.

An increase in either water or fat content leads to a decrease in wave speed. Both fatty breast and fatty liver tissue have a lower wave speed than comparable normal tissue. Fetal tissues also have slightly lower speed than comparable adult tissue, but this is because of their higher water content. The presence of collagen, particularly in tendon, skin and arterial wall, gives rise to slightly higher velocities than in other soft tissues.

2.2.4.2 Specific acoustic impedance and interface reflections

Oscillations of particle velocity, u, and pressure, p, in a plane progressive wave propagating in a lossless medium are in phase: that is, the particles move fastest when the acoustic pressure has its greatest magnitude. The magnitudes of p and u are proportional, and the constant of proportionality p/u is called the specific acoustic impedance, Z. A straightforward analysis (Leighton, 2007) shows that the acoustic impedance is equal to the product of the density and speed of sound, $\rho_0 c_0$. Knowledge of the acoustic impedance of a particular tissue is not, by itself, of great importance. The significance of this quantity is demonstrated only when considering the reflection and transmission of an acoustic wave as it passes across a boundary between two materials, or when small-scale variations in Z result in scattering. Acoustic impedance differs little between different soft tissues, and between soft tissues and water. The greatest differences occur at the interface between soft tissue and bone, which reflects about one-half of the incident intensity, and the interface between soft tissue and gas, which reflects almost all the incident wave. Given that the speed of sound is not dispersive with frequency for gas-free soft tissues, acoustic impedance also does not vary with frequency so, similarly, the proportions of reflected and transmitted sound do not have a frequency dependence.

In practice, the very large change in acoustic impedance between air and tissue is of fundamental importance when considering the health effects of exposure to infrasound and ultrasound. It means that, to a very good first approximation, airborne infrasound and ultrasound do not directly enter the body

and are totally reflected by the skin. When used for medical purposes, particular care is taken to exclude all air from between the ultrasound transducer and the skin, by the use of a fluid acoustic coupling material, such as oil or water-based gel. When laboratory experiments involving ultrasound are carried out, ultrasound reaching any air boundary of the apparatus will be entirely reflected back into the experimental volume.

2.2.4.3 Attenuation, absorption and scatter

Earlier (see Section 2.1.1), it was noted that the wave amplitude, or intensity, in a beam of ultrasound varies with distance. In the far field the acoustic pressure reduces as the reciprocal of distance from the source, and in a near field the acoustic pressure becomes greater in the convergent and smaller in the divergent regions of the beam. Superimposed upon these spatial variations in the beam are reductions in amplitude arising from loss associated with the medium itself. This loss, or attenuation, gives rise to potentially hazardous energy deposition in body tissues. In acoustics, attenuation is usually represented either as a reduction in acoustic pressure amplitude or as a reduction in intensity, although it may apply to any of the other parameters describing the wave. Expressed as a loss in acoustic pressure, the attenuation of a plane sound wave at a single frequency by a homogeneous medium is described by the expression

$$p_z = p_0 \, e^{-\alpha_a z} \tag{13}$$

where the initial peak acoustic pressure, p_0, has decreased to p_z after a travelling a distance z. The amplitude absorption coefficient, α_a, is commonly expressed in nepers per centimetre (Np cm^{-1}).

If the medium is inhomogeneous, the wave amplitude may also be reduced as a result of acoustic scattering. In this case equation 13 can be rewritten as

$$p_z = p_0 e^{-\alpha z} \tag{14}$$

where $\alpha = \alpha_a + \alpha_s$, in which α is the total amplitude attenuation coefficient and α_s is the amplitude scatter coefficient. For frequencies up to about 1 MHz it is usually assumed that attenuation by soft tissues due to scattering is very small compared with that due to absorption, so that $\alpha = \alpha_a$. For frequencies above about 10 MHz this assumption becomes increasingly invalid.

Equivalent statements may be made for attenuation of intensity, for which the intensity I_z of a monochromatic plane wave at any depth z, is related to the intensity at $z = 0$, I, by the expression

$$I_z = I e^{-\mu z} \tag{15}$$

where μ is the intensity attenuation coefficient, related to the amplitude attenuation coefficient by $\mu = 2\alpha$.

It is often convenient to give attenuation using decibels (dB) and attenuation coefficient in dB cm^{-1}. This notation uses a base 10 logarithmic scale to express ratios of power or intensity. The loss in decibels is $-10 \log_{10}(I_z/I)$ or, equivalently, $-20 \log_{10}(p_z/p_0)$. Thus, in contrast to the use of nepers, the attenuation coefficient takes the same numerical value in dB cm^{-1} whether considering intensity, μ, or amplitude, α. The factor to convert α from dB cm^{-1} to neper cm^{-1} is 8.68.

The absorption coefficients of materials vary widely depending on the nature of the physical processes giving rise to the loss of energy. These processes include viscous losses, thermal conduction and various forms of molecular relaxation processes (Kinsler et al, 2000). As a result, the attenuation coefficient varies with ultrasonic frequency, typically increasing as frequency increases, and therefore limiting the distance over which ultrasound usefully propagates. The highest frequency used by bats for echolocation in air is about 130 kHz, a limit set in part by higher attenuation in air as frequency increases. In pure water the absorption coefficient varies as the square of frequency. By contrast, soft tissues have attenuation coefficients that increase approximately linearly with frequency. It is common therefore to assume a linear dependence for all tissues, and to give values of the attenuation coefficient for tissue as decibels per centimetre per megahertz (dB cm^{-1} MHz^{-1}). Representative values for some tissues are included in Table 2.2, which gives both the attenuation coefficient at 1 MHz and its frequency dependence. As a rule of thumb, the average attenuation coefficient in soft tissue at any frequency is often taken as 0.5 dB cm^{-1} MHz^{-1}. Safety standards use a lower average figure, 0.3 dB cm^{-1} MHz^{-1}, to avoid overestimating the attenuation caused by a combination of soft tissue and fluid. At frequencies below 1 MHz the attenuation coefficient of water may be considered as negligible compared with that of tissue. However, at higher frequencies, the square-law dependence of attenuation in water means that losses through water become comparable to those through equivalent path-lengths in tissue.

Scattering of sound from tissue arises from small-scale variations in density or bulk compressibility, and hence in sound velocity. For some tissues scattering is anisotropic. This is particularly evident in tendon and is also true for striated muscle. In the low megahertz range there is strong coherent (ie in phase) forward scatter with generally weak scattering in all other directions. Only the very low level backscattered component contributes to pulse-echo imaging, and this constitutes a vanishingly small fraction of the incident energy. The integrated backscattered energy from soft tissue may be as low as 50 dB below the incident energy, which implies that essentially all of the energy entering the body is deposited in the tissue.

Attenuation in bone is much greater than in soft tissue. Attenuation coefficients in the range 10–20 dB cm^{-1} have been reported at 1 MHz for cortical and skull bone. Attenuation in trabecular bone is highly variable, probably due to the contribution from scatter.

Attenuation characteristics have a number of important consequences. First, acoustic beams measured in water differ from those in tissue by an amount dependent on the tissue, frequency and propagation distance. In practice, calibration measurements are often made in water and then reduced to allow for the extra attenuation in tissue, using an attenuation coefficient of 0.3 dB cm^{-1} MHz^{-1}. This process assumes linear propagation, an invalid assumption as is shown below. Second, it is the energy absorption that gives rise to all biological effects mechanisms. Finally, as was mentioned above, the pressure waveform of a broadband pulse changes shape during transmission, because of differential attenuation over the pulse frequency spectrum.

There is little information on transmission of low frequency noise into the body, and apparently none for infrasound. There has been work on sound transmission into the bodies of dead sheep over the range 50–20,000 Hz, aimed mainly at determining criteria for protection of pregnant women who work in high noise levels (Peters et al, 1993). The body cavity of the sheep was cleaned and filled with water, leaving

the organs in place. Internal sound level detection was with a hydrophone, which had been previously calibrated in air, whilst external sound levels were measured with a microphone. Excitation was by a loudspeaker 1 m from the body of the sheep, which was suspended in standing position from a frame. When the different acoustic impedances of air and water were taken into account the insertion loss of acoustic energy from external air to internal body was over 30 dB.

As was noted above (Section 2.1.1), all the wavelengths for the infrasonic spectrum of frequencies are considerably greater than the height of an adult human. This means that, to a very good first approximation, the infrasonic wave couples with the body as a scattering centre, the acoustic pressure external to the body causing cyclic, in-phase, variations in pressure applied to the whole of the body surface simultaneously.

2.2.4.4 Non-linear propagation effects

For all practical purposes, the following section applies only for ultrasound.

Thus far, the discussion has assumed that the acoustic wave is governed by linear laws of acoustic propagation. This may be a very poor approximation to what actually happens when ultrasonic pulses travel through tissue (Duck, 2002; Starritt et al, 1986). So-called 'finite-amplitude' effects occur, the terminology coming from the need to describe waves other than those with vanishingly small amplitudes. An initially sinusoidal pressure wave of finite amplitude does not retain its sinusoidal waveform as it propagates (Baker, 1998; Duck and Starritt, 1984; Hamilton and Blackstock, 1998). This results in a distortion of the wave, in which the compressions catch up the preceding rarefactions, ultimately forming a pressure discontinuity (Figure 2.4). This phenomenon is referred to as an 'acoustic shock' or a 'shocked wave'. The word is used here to describe something quite different from that when used in the context either of an electrical phenomenon or of a physiological state.

The amount of non-linear distortion increases with several factors: the frequency and amplitude of the wave, the non-linear coefficient of the medium and the distance travelled by the wave. A comparison between predicted pulse pressure waveforms after transmission through water and attenuation media of different attenuation coefficients is shown in Figure 2.5. This shows the distortion in wave shape, which has been caused by several centimetres travel, with its accompanying acoustic shock separating the highest amplitude rarefaction and compression. This example also shows that the amount of non-linear distortion decreases with increased attenuation. The competing effects of the non-linear distortion and absorption are quantified through the Goldberg constant, which is the ratio between the absorption length and the discontinuity length (Duck, 2002).

As a result of the distortion caused by the non-linear propagation of the wave, its frequency content is altered and energy is transferred from the fundamental frequency into harmonics (overtones). Theoretically a sawtooth wave is ultimately generated, in which the harmonics reach amplitudes that reduce inversely with harmonic number; the second harmonic is one-half of the amplitude of the fundamental component, the third harmonic one-third amplitude, and so on. The propagation of such shocked acoustic waves is associated with additional energy absorption, which enhances, sometimes significantly, the propagation losses and deposition of energy. At every place in an acoustic field, acoustic saturation places an upper limit on the acoustic pressure that can ever be reached there, no matter how

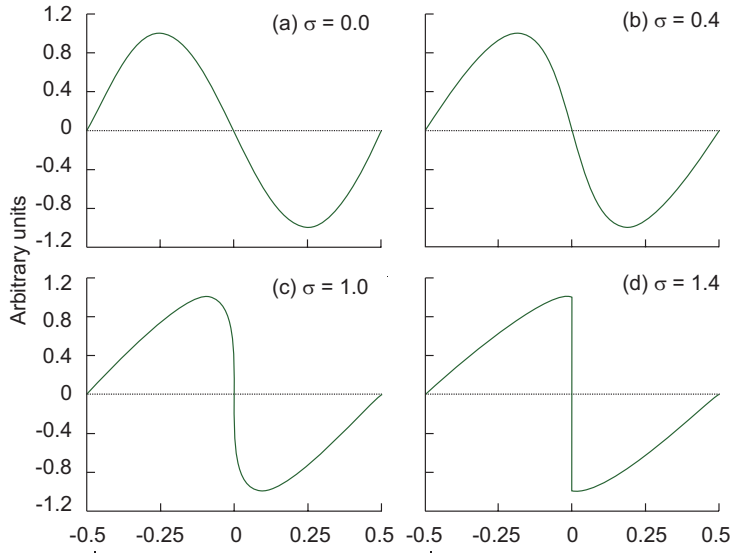


FIGURE 2.4 Non-linear propagation of a plane acoustic wave (propagating to the right) for different values of the shock parameter sigma, σ (equivalent to different propagation distances), showing progressive distortion of the waveform. An acoustic shock front has just formed when sigma equals one

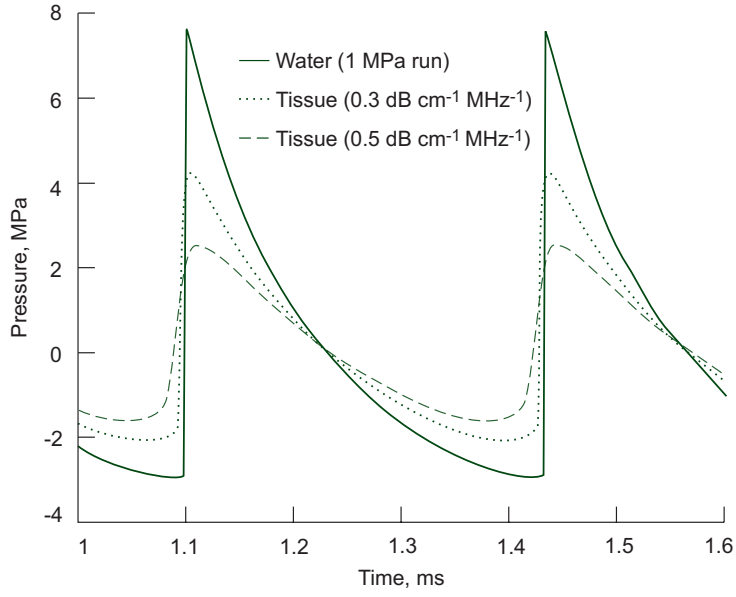


FIGURE 2.5 Model predictions of waveform distortion generated by non-linear propagation through water, and homogeneous tissues with attenuation coefficients of 0.3 and 0.5 dB cm^{-1} MHz^{-1}. The waveforms are plotted at a range of 52 mm from a rectangular, 3.0 MHz transducer, with dimensions of 10 mm by 15 mm. The transducer is focused at 70 mm and the average pressure amplitude at the face of the transducer is 1.0 MPa. The initial waveform is sinusoidal

high the acoustic pressure is at the source. This is called the saturation pressure. In practice, the generation of acoustic shocks is common when ultrasonic pulses generated by medical imaging systems propagate through water. It is predicted that severe waveform distortion and perhaps full acoustic shock generation may also occur within the fluid spaces *in vivo*, because of their low attenuation coefficient. Examples include propagation within urine in the bladder or in the amniotic fluid within a pregnant uterus. Propagation through soft tissue inhibits the formation of high levels of harmonics because of greater absorption losses at higher frequencies (Figure 2.5).

Non-linear propagation is of importance in discussions of biological effects for two main reasons. The first arises because these effects are particularly evident when pulses from diagnostic ultrasound scanners are sent through water, rather than through tissue. All estimates of acoustic exposure within the body are based on such measurements and it is normal to assume a simple linear model to estimate *in situ* exposure. This simple process does not account for the increased loss of energy associated with the propagation of acoustic shocks in water and the resulting exposure estimates can be low (Cahill and Humphrey, 2000). It has been predicted that acoustic saturation can limit the effectiveness of the present FDA limits for the control of ultrasound exposure (see Appendix A), particularly for longer focal depths and higher frequencies (Duck, 1999). The second concern associated with non-linear effects arises because they can enhance the deposition of energy in tissue (Verma et al, 1993). As a result, heating can be greater than may be predicted from linear considerations alone. For example, a three-fold increase in temperature rise has been demonstrated in a tissue-equivalent gel caused by the absorption of harmonic components in a shocked acoustic beam (Bacon and Carstensen, 1990). Similarly, radiation pressure effects, including acoustic streaming, can be enhanced by the increase in effective absorption coefficient associated with acoustic shock propagation.

Analytical methods are limited in scope for the theoretical prediction of acoustic non-linear propagation. Instead, validated numerical methods for propagation through homogeneous media, including layered media, are used. These can take account of non-linearity, absorption and diffraction, and are commonly based on the so-called KZK (Khokhlov-Zabolotskaya-Kuznetsov) equation (Hamilton and Blackstock, 1998). Prediction of non-linear propagation through scattering media remains challenging.

2.2.5 Mechanisms for ultrasonic bulk effects on tissues

The preceding sections have presented in outline the main processes that occur during the propagation of an ultrasonic wave through tissue. As a result of a variety of loss mechanisms, energy is deposited in the tissue. The response of the tissue will depend in part on the mechanism for this deposition and in part on the acoustic properties of the beam. It is conventional to consider two broad categories: thermal effects and mechanical effects. Broadly, mechanical effects can best be predicted from knowledge of individual pulse amplitudes, whilst thermal effects can best be predicted from knowledge of energy flow over an extended time period. In addition, the response *in vivo* is modified considerably by the presence of bone, gas and fluid spaces. For energy to be deposited in the bulk of tissue by ultrasound it is necessary for the acoustic wave to be coupled into the human body. The coupling is profoundly different depending on whether the wave is, or is not, propagating in air. For airborne ultrasound, to a very good

first approximation, no acoustic energy is coupled from air into the skin: all the energy is reflected. Furthermore, at frequencies above about 300 kHz, propagation through air is limited to less than a millimetre by absorption. Coupling to the bulk tissue of the patient or operator can therefore only come about if there is a mechanical liquid or solid coupling between an ultrasound source and the tissue. Coupling through liquids and gels is commonly used to transfer energy efficiently for medical or industrial uses. The operator is normally decoupled from the source by intervening air and may be exposed only by misuse (for example, by placing the hand in an ultrasonic cleaning bath). Operators of therapeutic and diagnostic transducers hold the transducer during use and this might in principle be considered as a route by which leakage of ultrasonic vibration might enter the hand. This is not so, largely because coupling from the piezoelectric element to the transducer housing is minimised by acoustic absorbers and air-spaces. Ultrasonic vibration is limited just to the emitting area of the transducer placed on the skin, an area never in contact with the operator during clinical use.

2.2.5.1 Heating

As noted above, the initial rate of increase in temperature caused by the absorption of ultrasound, $\mathrm{d}T/\mathrm{d}t$, is Q_m/C_v, where C_v is the specific heat capacity of the medium and Q_m is the acoustic dose rate, $2\alpha_a I/\rho_0$. For a beam of finite size, subsequent heating depends on the width of the beam. Broader beams give a higher temperature for a given peak intensity than do narrow, perhaps more highly focused, beams. The steady-state temperature also depends on the thermal conductivity of the tissue and on the effects of blood perfusion. An 'effective thermal conductivity' is commonly introduced into calculations to include convective heat loss due to blood flow. Blood perfusion plays an important controlling role on the actual steady-state temperature reached *in vivo*. Analytical methods for the prediction of ultrasound in homogeneous media have been developed based on the Pennes bio-heat equation (Pennes, 1948; Nyborg, 1988). These have been applied to simplified standard tissue models as the basis for the thermal indices which are used as indicators of safety on diagnostic ultrasound scanners. Modifications to the heat loss term due to blood convection may be required when modelling fetal temperature rise. Examples of predictions of axial temperature rise are given in Figure 2.6. More recently, finite element models have been developed which offer improved realism in modelling temperature rise in more complex geometries (Doody et al, 2000). Methods for thermal modelling from ultrasound exposure have been reviewed by the International Commission on Radiation Units and Measurements (ICRU, 1998) and the National Council on Radiation Protection and Measurements (NCRP, 1992).

Particular sites with higher absorption in the body may experience greater heating than their surroundings. The surfaces of calcified bone absorb energy strongly, although transmission into the bone, and hence its heating, may be reduced for angles of incidence other than those near normal. Fetal bones absorb energy more strongly than the surrounding fetal soft tissue, and the difference becomes greater as the fetal bones calcify. Adjacent soft tissues can experience secondary heating from thermal conduction into the tissue from the bone. The temperature increases caused in tissue by the use of current diagnostic ultrasound equipment are unlikely to extend outside a normal physiological range (including that experienced during vigorous exercise). Hazardous temperature rises in tissue can be induced by physiotherapy ultrasound equipment and by misuse of some non-medical devices, such as ultrasonic cleaning baths.

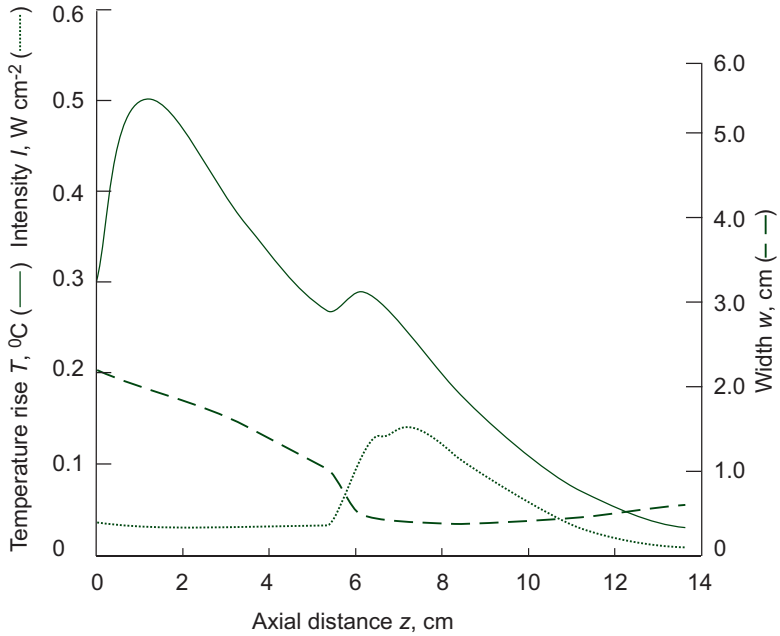


FIGURE 2.6 Predicted temperature rise in tissue for a focused ultrasonic field along the acoustic axis, calculated using a heated disk model. Calculations are for a 3 MHz transducer, 20 mm in diameter with a 100 mm focal length, and source power of 100 mW. The absorption coefficient in tissue is 1.3 dB cm^{-1}. Also shown are the intensity and effective beam width (after Thomenius, 1990)

It is important to remain aware that usually the heating caused by ultrasound is highly localised and limited in extent to the region within, and immediately adjacent to, the ultrasonic beam, whether it is stationary or scanned. The condition which may be experienced in equivalent radiofrequency heating, where the temperature is raised throughout large volumes of tissue or perhaps the whole body, is very rare. This can give rise to difficulties in interpretation of, and extrapolation from, some biological effects experiments, for which a thermal mechanism is hypothesised. For example, it is quite possible to expose, and so heat, the whole of the uterus of a mouse embryo with ultrasound, but such a circumstance can only arise at a very early stage during a human pregnancy.

2.2.5.2 Mechanical effects: acoustic cavitation

Ultrasound can produce direct physical effects in liquids by a second effect known as acoustic cavitation. This term is used to refer to a range of complex phenomena that involve the creation, oscillation, growth and collapse of bubbles within a medium (see, for example, Leighton, 1994 and 1998). Cavitation behaviour can be classified broadly into two categories: non-inertial cavitation and inertial cavitation. The exact behaviour will depend on the frequency, pressure amplitude, bubble radius and environment.

When an existing bubble is exposed to an ultrasonic field, the acoustic pressure acts as a driving force that causes the bubble size and shape to change. The bubble behaves as an oscillator with a stiffness and

inertia. The stiffness is provided by the gas within the bubble; when the gas is compressed it provides a force that resists the compression. The inertia is mainly provided by the liquid surrounding the bubble that moves with the bubble wall. As a result, the bubble has a natural resonant frequency f_r. For the case of a spherical air bubble of radius R_0 in water (assumed to be incompressible and inviscid), a simple calculation based on the linear oscillations gives

$$f_r R_0 \approx 3\,\text{Hz m} \qquad (R_0 \geq 10\ \mu\text{m}) \tag{16}$$

This is an approximate result which neglects surface tension, making the formula less accurate for smaller bubbles.

As a consequence, the bubble will experience a resonant behaviour when driven by an acoustic field at a frequency near to the natural resonance frequency. The amplitude of oscillation will depend on the driving pressure and the driving frequency in relation to the resonance frequency of the bubble. When the pulsation amplitude increases, for bubbles near to resonance, the non-linear character of the oscillation becomes more pronounced.

Bubbles undergoing oscillation can display a range of other behaviours. One possibility is rectified diffusion; this is the process by which the bubble equilibrium radius grows with time. The bubble surface area is larger on the expansion phase than on the compression phase. As a result, more gas diffuses into the bubble when the pressure inside is low than diffuses out when the pressure is high. The processes described so far can all occur during non-inertial cavitation.

Inertial cavitation is an alternative behaviour. It is characterised first by bubble expansion to many times the original bubble size during a rarefaction half-cycle. This is then followed by a rapid collapse phase driven by the inertia of the spherically converging liquid. During this collapse phase the gas temperature may increase transiently to well over 1000°C as it is compressed adiabatically to a fraction of its original volume. These processes can potentially create free radicals. The collapse can also give rise to emission of light, a process known as 'sonoluminescence'. After the collapse the bubble may fragment or may repeat the growth/collapse cycle a number of times.

For a particular spherical bubble nucleus in a given liquid the occurrence of inertial cavitation depends on the acoustic pressure amplitude, the acoustic frequency and bubble radius. For small bubbles the surface tension forces prevent the initial growth so the bubbles do not grow enough. In contrast, large nuclei can grow initially, but do not collapse sufficiently to generate high temperatures. For a given frequency and nucleus radius there is, therefore, a threshold pressure required for inertial cavitation. In any bubble cloud, non-inertial and inertial behaviour may occur simultaneously within the same acoustic field.

Apfel and Holland (1991) investigated theoretically the acoustic pressure needed to cause inertial cavitation, p_{opt}. They considered the case of single-cycle exposure of a fluid containing a wide range of bubble sizes and evaluated the frequency dependence of the threshold pressure for water and blood. They showed that

$$p_{opt}{}^{a} \mid f = b \tag{17}$$

and, for p_{opt} in MPa and f in MHz, found a best fit for the parameter a of 2.10 for water and 1.67 for blood, and for b of 0.06 for water and 0.13 for blood. This work has been used as the basis for the definition of the mechanical index, MI (equation 3), taking $a \approx 2$. The MI can be considered to be an indicator of the likelihood of inertial cavitation, and is now displayed on most medical ultrasound scanners as a safety index.

It should be noted that bubbles provide a mechanism for the conversion of the longitudinal displacements of a compressional wave into shear displacements around the bubble. If the bubbles are in the vicinity of an elastic medium then this may produce shear in the medium and corresponding shear loss mechanism to further enhance the heating effect. Holt and Roy (2001), for example, noted a threshold effect for the heating of agar-based tissue-mimicking phantoms; above a threshold pressure for cavitation there was a considerable enhancement in the temperature rise produced.

The inhomogeneous periodic field around a pulsating bubble can generate a small steady flow of fluid by a process known as microstreaming (Nyborg, 1998). The variation of this flow with distance from the bubble creates extremely high shear stresses near the bubble surface, which have been associated with cell membrane destruction and temporary alteration in permeability (sonoporesis).

Inertial cavitation is a potentially important effect producing high pressures and temperatures within the gas of the collapsing bubble, as well as free radicals. The collapse can also give rise to a radiated acoustic shock wave into the fluid. Additional effects can be observed if the collapse is not perfectly symmetrical as, for example, occurs if the bubble is in the vicinity of an interface. In this case a liquid jet can form that traverses the bubble and impacts on the surface perpendicularly at considerable velocity. It should be noted that these processes illustrate the way in which bubbles provide an effective mechanism for concentrating the energy in the relatively weak ultrasonic field into the very small region of the bubble and its environs.

This discussion of cavitation behaviour has assumed that there are existing bubbles of suitable size, or bubble nuclei that can grow into suitable size bubbles, present within the fluid. Although this is often true for liquids that are saturated with gas, suitable cavitation nuclei may not always be present. Conversely, some diagnostic procedures may rely upon the introduction of contrast agents that are encapsulated bubbles. It is very doubtful if true cavitation of either form occurs at diagnostic levels within soft tissues or fluids in the body, in the absence of gas-filled ultrasound contrast agents. However, there are two conditions when the presence of gas results in mechanical trauma to adjacent soft tissue of a form that has been interpreted as a cavitation-like process: at the surface of the lung and in the intestine. Conversely, acoustic cavitation must be considered as a possible mechanism for all *in vitro* experiments involving cells in liquid suspension.

2.2.5.3 Mechanical effects: radiation pressure

In addition to the direct effects of heating and cavitation, there are a number of secondary physical effects that can also be generated by an ultrasonic field. These result from the non-linear nature of acoustic equations that describe the wave behaviour. As these are secondary effects they tend to increase in proportion to intensity and are generally relatively small in magnitude. They do, however,

have the potential to produce forces and motions at much lower frequencies than those of the incident ultrasonic waves, as explained below.

For a continuous wave, a steady small pressure is exerted on surfaces or media interfaces in the direction of propagation of the wave. This is termed radiation pressure. It is quite distinct from the acoustic pressure that alters as the ultrasonic frequency and is strictly a tensor quantity. Radiation pressure results from the essential non-linearity of the acoustic equations. The Langevin radiation pressure is defined as the difference between the mean pressure on a reflecting or absorbing wall and the pressure behind the wall. For a plane wave incident normally on to a perfect absorber the radiation pressure, P_{rad}, is equal to the time-averaged energy density, $\langle \varepsilon \rangle$, of the wave at the surface so

$$P_{\text{rad}} = \langle \varepsilon \rangle = \frac{1}{2} \frac{{p_0}^2}{\rho_0 {c_0}^2} = \frac{I}{c_0} \tag{18}$$

For the case of an object that extends outside the dimensions of an acoustic beam the total radiation force, F_{rad}, on the target is equal to the power, W, in the acoustic beam divided by the speed of sound:

$$F_{\text{rad}} = \frac{W}{c_0} \tag{19}$$

This expression forms the basis of a standard method for the measurement of acoustic power. For the case of a continuous-wave signal the radiation pressure will be a steady constant pressure. If, however, the acoustic signal is pulsed or modulated then the radiation pressure will vary periodically at the pulsing or modulating frequency. This provides a mechanism of generating a force at frequencies well removed from the normal ultrasonic frequencies, and potentially in the audio range. The resulting motion of structures can in turn be used to image tissue properties; this is the basis of a number of techniques currently under development (Fatemi and Greenleaf, 2000).

For non-absorbing interfaces and small particles the direction and amplitude of the radiation force will depend on the elastic properties of the materials involved. For a small particle, radiation pressure results in a force on the particle that can result in particle movement. In a standing wave this can, in turn, lead to particles collecting at nodes/antinodes, depending on the properties of the particles and fluid; of particular note is the observation that under some circumstances the particles and bubbles may be separated to collect at different locations in a standing wave and so prevent interaction.

Radiation pressure in a fluid gives rise to a further acoustic phenomenon known as acoustic streaming. In linear acoustics it is assumed the particles of the medium vibrate about their equilibrium position with no net flow. However, the attenuation of an ultrasonic beam with distance can be considered to give rise to a 'radiation pressure gradient' in the fluid. As a result of the gradient, each element of fluid experiences a net body force that gives rise to a net flow of fluid. A review of the theoretical understanding of streaming has been given by Nyborg (1998).

For a plane wave, the force per unit volume resulting from radiation pressure, F_v, is given by

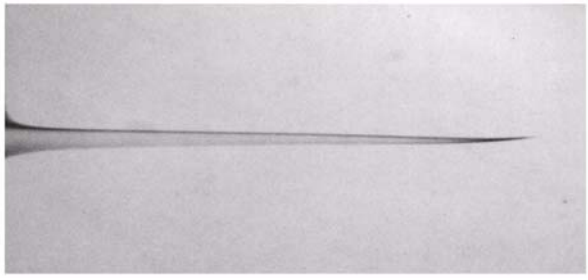
$$F_v \approx \frac{2\alpha I}{c_0} \tag{20}$$

For an unfocused, circularly symmetrical beam of sound, or in the focal region of a weakly focused beam of intensity, I, and beam radius, r, the maximum axial streaming velocity, V, is approximately

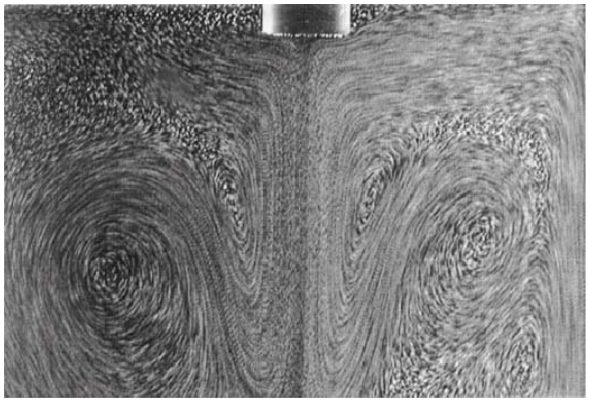
$$V \approx \frac{2\alpha I}{\rho_0 c_0 \nu} r^2 G \qquad (21)$$

where α is the amplitude attenuation coefficient associated with all processes of acoustic loss, υ is the kinematic viscosity of the fluid and G is a constant that depends on the geometry of the beam and vessel containing the fluid. Equation 21 shows that the streaming velocity depends on both the beam intensity and the total attenuation.

Figure 2.7 gives examples of streaming in fluids. Figure 2.7(a) shows a visualisation of streaming using thymol blue indicator in water (Starritt, 1991), caused by a pulsed beam similar to that used for medical scanning. The second example, Figure 2.7(b), is of the field of a 32 MHz Doppler flowmeter, with the flow being visualised by the use of a corn-starch suspension in distilled water. In this case the recirculation flow can be seen.



(a)



(b)

FIGURE 2.7 (a) Streaming visualisation in water using thymol blue indicator. In this case the transducer was a commercial single-element B-scan transducer (Starritt et al, 1991). (b) Visualisation of acoustic streaming using corn-starch particles in the field of a 32 MHz Doppler flowmeter (after Nowicki et al, 1998)

Under conditions of strong acoustic non-linearity, streaming is enhanced in water (Starritt et al, 1989) and radiation pressure is enhanced in soft tissue (Starritt et al, 1991). Streaming can be set up in relatively small fluid volumes; this has been observed *in vivo* in small cysts by Nightingale et al (1995). Used in radiation force elastography, radiation pressure can cause displacements of tissues and gels of the order of tens of micrometres (Nightingale et al, 2001).

2.3 Biological Responses to Acoustic Mechanisms

2.3.1 Direct physiological effects

2.3.1.1 Aural perception

The assessment of direct physiological effects of airborne acoustic waves, including both infrasound and ultrasound, has mainly considered the response of the ear to acoustic waves at frequencies outside the normal hearing range. Normal hearing thresholds are discussed in Section 2.5 (see Table 2.3). At higher sound pressure levels, pain and eventual rupture of the ear-drum occur. The question arises of whether there is a hierarchy of receptors of which the ear is the most sensitive, except at the lower frequencies, when other receptors may come into prominence.

2.3.1.2 Cutaneous perception

Several vibration and contact detectors, which give the sense of touch, reside in the skin, covering different frequency ranges. These include the Pacinian corpuscles and Merkel cells. The Pacinian corpuscles are the most sensitive, with a threshold displacement of about 1 μm in the region of 200 Hz. Their sensitivity into lower frequencies reduces at approximately 50 dB per decade from the maximum sensitivity. A threshold displacement of 1 μm at 200 Hz is similar to the particle displacement in air of a 200 Hz sound wave of 88 dB (0.5 Pa) pressure. Since the particle displacement in a sound wave of constant pressure doubles as the frequency is halved (20 dB per decade), inaudible sound waves will not excite Pacinian corpuscles, whose sensitivity into low frequencies reduces more rapidly than this. In addition, the vibration of the skin is not the same as that of the sound wave, due to mismatch at the air–skin interface.

In the case of Merkel cells a particle displacement in air of 0.2 mm at 15 Hz (threshold of Merkel cells) corresponds to a pressure in the sound wave of nearly 8 Pa, or 112 dB, which is well above the 15 Hz hearing threshold of about 88 dB. At 5 Hz, the pressure in the wave for the same particle displacement is about 10 dB lower, or 102 dB. This is similar to the hearing threshold at 5 Hz, which is about 105 dB, but mismatch between the air and the skin means that the displacement of the skin will be lower than that of the air. In fact, at higher frequencies, the skin is acoustically very reflective, with a measured absorption coefficient of about 0.03 between 1 and 6 kHz (Katz, 2000).

Dalecki et al (1995) showed that pulsed ultrasound may be felt by the skin. Ultrasound with intensity in the range 10–100 W cm^{-2} was coupled to the skin through a water bath. The beams were either pulsed or modulated (turned on and off regularly at a frequency between 50 and 100 Hz). Thresholds for pulsed ultrasound were about ten times greater than those for a modulated beam, and increased substantially

for pulses shorter than 1 ms. A minimum threshold for sensation for the modulated beam was associated with a radiation force of about 0.4 mN.

2.3.1.3 Vestibular system

Another potential mechanism for the perception of infrasound may be direct effects on the vestibular system which, by detecting movement of the head, controls the human balance system. The canals of the vestibular system are part of the inner ear but, in normal state, are a closed system containing fluid which moves when the head changes position. This movement activates hair cells within the canals, transmitting information to the brain. It has been suggested that infrasound upsets the balance system, leading to disorientation, but for this to occur there must be a mechanism for energy to transfer from the external sound into the vestibular canals. Possible paths are directly through the head or through the ear.

Measurement and perception of noise-induced vibrations of the body between 20 and 50 Hz have included measurements on the forehead, which can be taken as representing the vibration of the head. (Takahashi et al, 2002a,b). It was shown that, for example, for an excitation of 110 dB at 50 Hz, the vibration acceleration level at the forehead was nearly 100 dB referenced to 10^{-6} m s^{-2}, which is 0.1 m s^{-2}. Converting this to displacement at 50 Hz gives 10^{-6} m. Considering lower frequencies, at 20 Hz the acceleration level of the forehead is about 74 dB, or an acceleration of 5 10^{-3} m s^{-2}.

Excitation of the head by external sound waves has been reviewed by von Békésy (1960) and by Håkansson et al (1994). There is variation between subjects, but the lowest skull resonance frequency is in the 900 to 1200 Hz range, which is outside the infrasound and low frequency noise region. von Bekesy (1960) determined the threshold of forehead vibrations for hearing by bone conduction between 100 and 5000 Hz. The threshold at 100 Hz is a vibratory displacement of about 10^{-8} m.

Overall, it is unlikely that external low frequency noise at levels below the threshold of audibility will have an effect on the vestibular system, which is a less sensitive detector of sound than by bone or air conduction. High levels of low frequency noise and infrasound do produce vestibular excitation, but the sound levels required are greatly in excess of hearing threshold levels (Parker, 1976).

2.3.2 Heating

Core body temperature is normally about 37°C ± 0.8°C, but this may vary naturally by about 0.5°C during a 24 hour period. During hard exercise it may approach 40°C. Thus humans are able to accommodate small increases in temperature, without obvious harm. Whilst much about these natural responses to heat remains to be discovered, general principles are evident. Pain associated with heating arises because the skin is very sensitive to temperature change, being able to detect changes of considerably less than 1°C. This ability is not shared by deeper organs of the body. At a cellular level, for modest temperature rises, cells develop thermo-tolerance, which correlates with the appearance of intracellular chaperone or heat-shock proteins, typically 10–15 minutes after heating commences. There is an associated decrease in normal cell protein synthesis and cell turnover may be attenuated. An increase in apoptosis (programmed cell death) may occur. In spite of differences in heat sensitivity

between different tissues and cell types, equation 11 represents well the relationship between times and temperatures to cause an equivalent thermal effect. Broadly, for temperatures below 43°C, thermo-tolerance mechanisms serve to protect the cells from the effects of heating. These mechanisms increasingly fail above that temperature, cell death arising from the denaturation of the most thermally sensitive proteins. Above 43°C, the time to cause chronic damage halves for every 1°C increase in temperature. The thermal sensitivity for chronic damage is quite variable between tissues (Dewey, 1994). At 43°C, the time for tissue to be damaged has been reported to be 240 minutes for muscle and fat, and as low as 30 minutes for brain and kidney. Comparable threshold values for thermal damage to cortical bone, in which the highest ultrasonically generated temperatures are to be expected, are not available, but there is no reason to believe this tissue to be unusually sensitive to heat. Bone marrow may exhibit thermal sensitivity, but is usually screened from exposure by cortical layers. Heat is also a known teratogen in animals and a strongly suspected teratogen in humans. There is a large body of evidence demonstrating that heat induces developmental abnormalities in animals (see Chapter 4). The sensitivity to thermal insult varies during gestation. There is a peak in sensitivity at the stage of neural tube closure and neurogenesis. At this stage, a sustained temperature elevation of about 2°C above maternal body temperature results in developmental defects such as micrencephaly, microphthalmia and retarded brain development in a wide range of animals. These defects are also caused following heating to higher temperatures for shorter periods.

2.3.3 Cavitation and gas-body effects

Acoustic cavitation may cause cellular responses as a result of mechanical, chemical or thermal means.

The inhomogeneous periodic field around a pulsating bubble can generate a small steady flow of fluid by a process known as microstreaming (Nyborg, 1998). The variation of this flow with distance from the bubble creates extremely high shear stresses near the bubble surface. It is now accepted that these stresses are responsible for the observed temporary alteration in permeability (sonoporesis) and cell membrane destruction resulting from exposure of cells in suspension in the presence of microbubbles. In addition, mixing from microstreaming is the most likely explanation for enhanced drug transport through the skin mediated by ultrasound. A bubble within a capillary bloodstream will stress that capillary when forced to expand and contract in an ultrasonic field. These stresses may be sufficient to rupture the capillary, allowing extravasation of the contents. Bubble collapse during inertial cavitation can also give rise to a radiated acoustic shock wave into the surrounding medium. Inertial cavitation carries an additional hazard for cells, free radical production. These highly reactive chemical species can be created in the gas contents of an adiabatically collapsing bubble, because of the extremely high temperatures and pressures created by the rapid bubble collapse. They may then be released to the surrounding medium once the bubble fragments. However, in contrast to the association for ionising radiation between free radical production and cell damage, free radicals produced by inertial cavitation lie outside, rather than within, the cell. Whilst such free radicals may in principle migrate through the membrane and damage intracellular components, both the presence of natural free radical scavengers and the very short lifetimes mitigate against this. Were an intracellular bubble to undergo inertial cavitation, cell death would be immediate. Finally, the presence of clouds of bubbles increases the absorption coefficient of a

tissue or liquid, resulting in greater local heating than would have arisen in their absence. It should be noted that these processes illustrate the way in which bubbles provide an effective mechanism for concentrating the energy in the relatively weak ultrasonic field into the very small region of the bubble and its environs.

The above discussion of cavitation behaviour assumes that there are existing bubbles of suitable size, or bubble nuclei that can grow into suitable size bubbles, present within the fluid. Although this is often true for liquids that are saturated with gas, suitable cavitation nuclei may not always be present in tissues. Conversely, some diagnostic procedures may rely upon the introduction of contrast agents that are encapsulated bubbles. However, there are two conditions when the presence of gas results in mechanical trauma to adjacent soft tissue of a form that has been interpreted as a cavitation-like process: at the surface of the lung and in the intestine. At present there is uncertainty about the exact processes causing this trauma.

2.3.4 Radiation force and acoustic streaming

Fluid flow induced by ultrasound in major fluid spaces *in vivo* would appear to have very little importance when assessing heath effects. It is difficult to see why minor fluid movement induced within static fluid spaces, such as urine and amniotic fluid, could be hazardous. In physiologically moving fluids, such as blood, streaming will minimally modify the normal physiological flow patterns. The term 'phonophoresis' has been used to describe a process by which sound waves may induce or alter molecular transport across a membrane. *In vitro* studies suggest that membrane transport may be altered due to local disturbance of concentration gradients, caused by acoustic streaming. No evidence has been reported, however, of phonophoresis *in vivo*. Enhanced cutaneous drug transport depends on acoustic cavitation and there is minimal evidence of such transport in its absence. Induced fluid movement at a cellular level, both within the cell and through the extracellular space, is an unlikely possibility, given the structural dimensions and material viscosities involved. Acoustic streaming may need to be considered when interpreting results of *in vitro* experiments using cells in suspension, for example.

Extreme forces are capable of damaging tissue. The ultimate tensile strength of the kidney, one of the weakest, is reported as 50 kPa (Yamada, 1970). Cell-to-cell adhesive forces are of the order of a few kilopascals. Haemolysis of erythrocytes occurs at a shear stress of about 300 Pa. These forces lie generally above the maximum from pulse absorption and therefore mechanical damage is very improbable from diagnostic ultrasound. One condition, radiation pressure on a perfect reflector, brings the estimated highest stress to a value that may exceed the strength of a weak tissue. This gives credence to the argument that lung capillary bleeding results from radiation pressure on alveolar structures. At intensities above those used for diagnosis, soft-tissue damage at the surface of fetal femur (Dalecki et al, 1999) and rat bone (Bigelow et al, 2007) has been reported, an effect attributed to the effects of radiation stress on weak tissue.

Cells also sense and respond to external forces, by mechanisms that are multifaceted and diverse, and yet to be understood (Huang et al, 2003). They do so in order to protect themselves from shear, probably the most threatening force, and to adapt their function to altered mechanical environments.

Integrins are the most likely candidate molecular agents to initiate mechano-sensing, linking extracellular forces to the cytoskeleton, and can initiate a cellular response both by gene expression and by biochemical change. The application of a broadly homogeneous force may be amplified as a result of local cell–matrix or cell–cell adhesions, with amplification factors of 100 being suggested. The effect of haemodynamic shear on the vascular endothelium is the mechanical effect subject to the most detailed study (Davies, 1995). The critical level of fluid shear stress for a variety of biological responses is about 1 Pa. Force thresholds associated with other experiments suggest a response threshold of about 1 nN. It may be expected, therefore, that cells can detect, and may respond to, radiation forces when exposed to ultrasound under a wide variety of situations.

2.3.5 Piezoelectricity and pyroelectricity

Bone and some collagen-rich tissues exhibit piezoelectricity when dehydrated (Fukuda,1968). This is the property exhibited by some anisotropic electrical insulators by which electric charge may be induced from the application of external stress. Conversely, strain is induced in such materials when placed in an electric field. (The majority of ultrasonic transducers in current use exploit the piezoelectricity of crystals, ceramics or polymers for the creation and detection of ultrasonic waves.) The known piezoelectric property of dry collagenous tissues has been suggested as a mechanism by which exposure to acoustic waves might be transduced to an electrical source leading to other possible biological effects. Hydrated tissue has a low electrical impedance, however, so, *in vivo*, bone does not behave as a conventional piezoelectric material. Under such conditions, an electrical streaming potential has been proposed as an explanation of the mechano-electrical potentials observed *in vivo* (Anderson and Eriksson, 1968). Bone and other collagenous tissues have also been shown to possess pyroelectric properties, which could cause secondary effects from acoustically generated temperature rise.

2.4 Metrology

2.4.1 Measurements for ultrasound

Standard measurements of ultrasonic fields are carried out in clean, pure water. The primary quantity for the measurement of acoustic output is acoustic power and the primary quantity for the measurement of acoustic exposure is acoustic pressure. A useful description of these key properties of the acoustic field and the rationale behind their relevance to biological effects may be found in Nyborg and Wu (1993). A recent review of metrology for ultrasonic applications has been given by Zeqiri (2007) and the following presents a précis of this paper.

2.4.1.1 Measurement of acoustic power

The acoustic power generated by an ultrasonic transducer is defined as the acoustic energy emitted per second, measured in watts (W). The parameter may be regarded as a global quantity describing a property of the whole acoustic field, and it does not provide any information regarding the way acoustic pressure or intensity is distributed within the beam. Acoustic power is nevertheless a key property

required to describe acoustic output and plays a pivotal role in assessing health effects, due to its influence on the temperature rise resulting from absorption of ultrasound within tissue. The measurement of acoustic power is generally simple to apply as a relatively low cost means of determining acoustic output.

The standard method for determining total acoustic power is by the measurement of acoustic radiation force on a target surface placed in the beam (equation 10, Beissner, 1993). The target must be much greater than the lateral dimensions of the acoustic field. For propagation through water on to a fully absorbing target perpendicular to the beam, the sensitivity of the technique is 69 mg-force W^{-1} (around 0.67 μN W^{-1}) (Davidson, 1991). In practice, the force also depends on the nature of the target and its shape and orientation. Examples of reflecting and absorbing targets are shown in Figure 2.8. It is usual to calibrate a power balance using a beam of known power, rather than by predicting its response from its design. Some variation occurs in converging and diverging beams but, for many acoustic fields, the method is sufficiently accurate (Beissner, 1993). Exceptions are for very divergent (Hekkenberg et al, 2001) or highly focused acoustic fields (IEC, 1992a), where deviation from the idealised plane-wave behaviour occurs and can significantly impact on power measurement uncertainty.

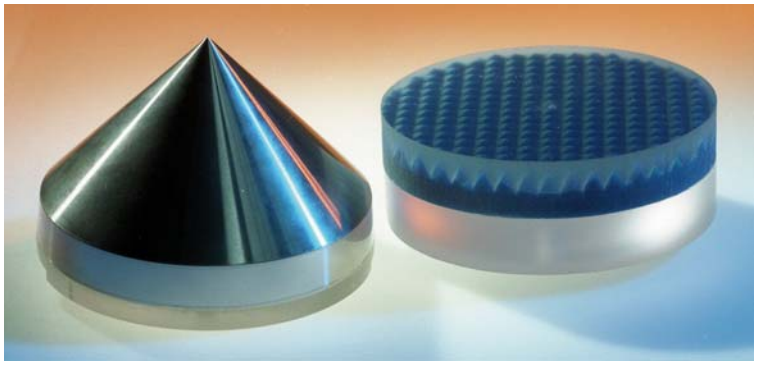


FIGURE 2.8 Totally reflecting (left) and absorbing (right) targets for the radiation force balances used at the National Physical Laboratory, Teddington, to determine total acoustic power. The diameter of both targets is 10 cm (© Queen's Printer and Controller of HMSO, 1998)

Although there are a number of variations in design of power measurement systems, the radiation force is typically determined by measuring the change in weight of an initially buoyant target. Exploitation of the radiation force method has become the internationally accepted method for characterising acoustic power (IEC, 1992a). As radiation force balances are relatively low in cost and easy to use, they often represent the first step in establishing a measurement infrastructure within a laboratory, hospital or manufacturing environment.

2.4.1.2 Measurement of acoustic pressure

The ideal measurement device for the measurement of the acoustic pressure waveform should be capable of determining the acoustic pressure at a point within the acoustic field and should also have a sufficient frequency response to enable the true acoustic waveform to be reproduced. The latter requirement means that the bandwidth of the device, or the way its response varies with frequency, must be sufficiently broad and independent of frequency.

The single most important recent development in ultrasound metrology has been that of ultrasonic membrane hydrophones (Harris et al, 2000). Such hydrophones, manufactured from thin membranes of the piezoelectric polymer, polyvinylidene fluoride (pvdf), have come to be regarded as the gold standard for measurement. Their use for the determination of the spatial and temporal characteristics of ultrasonic fields is now firmly established within national and international standards (IEC, 1992b; Preston, 2000). A thin sheet of pvdf is stretched over a supporting ring. The electrode configuration deployed during manufacture is such that the only piezoelectrically sensitive part of the hydrophone (commonly known as the poled region) is at the centre of the membrane. It is this active region, commonly of sub-millimetre diameter, which defines the spatial resolution of the hydrophone. The frequency response of the hydrophone is governed by the thickness resonance of the film of pvdf used. A single sheet of the piezoelectric polymer of thickness 25 μm has a resonance at roughly 50 MHz. Hydrophones have been manufactured from single sheets (coplanar) or double sheets (bilaminar) (Preston et al, 1983).

Several current commercially available designs of hydrophones, both membrane and non-membrane, are shown in Figure 2.9. From the metrological point of view, the strengths of the membrane hydrophone design are: a sensitivity which is stable (Robinson et al, 2000) and adequate to enable the relevant acoustic pressures to be determined; a broad and flat bandwidth; sufficient spatial resolution to adequately resolve the acoustic field; a non-perturbing construction; and a directional response (angular response) which can be modelled and is predictable. Hydrophones are commonly used in combination with a pre-amplifier to buffer the generated electrical waveform. Nowadays, for many devices, the amplifier tends to be integral to the hydrophone, ie situated as close to the membrane as possible to minimise electrical loading effects. In addition, the gain of this amplifier can be matched to the frequency response of the hydrophone over a limited frequency range in order to counteract any frequency-dependent increase in the sensitivity of the membrane (Robinson, 1991).

Other hydrophone designs may, in some situations, be more appropriate. A description of the properties of different hydrophones is to be found elsewhere (Robinson, 1991; Lewin, 1993). Needle hydrophones have complex frequency responses, arising from internal reflections (Fay et al, 1994) which require compensation if they are to be used to characterise broadband acoustic fields. However, they can be useful for relative measurements of narrowband transducers – for example, determining the spatial distribution of pressure in a physiotherapy field where they are used in determining the effective radiating area (ERA) of transducers (IEC, 1996). As smaller active elements can be achieved with the needle-type hydrophone design, devices made using pvdf active elements may be useful in applications where higher spatial resolution is required or in situations where it may disturb the field less (for continuous-wave applications), or where it is difficult to gain close access to the transducer with a membrane hydrophone due to geometrical constraints.

FIGURE 2.9 Examples of commercially available ultrasonic hydrophones, including membrane and probe (bottom right) designs. The overall diameters of the membrane hydrophones are about 15 cm

A calibrated hydrophone can also be used to determine the spatial distribution of acoustic pressure and intensity with the acoustic field. The procedure involves scanning the hydrophone in a water bath through the field and acquiring the electrical waveform generated by the hydrophone–amplifier combination at various spatial positions (Preston, 1991). Moving a single-element hydrophone through an acoustic field is clearly time-consuming, and systems based on one dimension (Preston 1988) and two dimensions (Hurrell and Duck, 2000) have been produced. There may be significant uncertainties associated with these measurements. These arise from hydrophone calibration, spatial averaging (Radulescu et al, 2002), the frequency response of the hydrophone (Smith, 1986) and the use of a single figure for the hydrophone sensitivity.

2.4.1.3 Measurement of thermal and cavitation responses

From the earlier sections, it is clear that measurement techniques currently applied to characterise key acoustic output and exposure parameters that may be of relevance to biological effects are carried out in water. The use of water makes the estimation of *in situ* exposure likely to be generated at a site of interest within the patient very difficult, primarily due to the acoustic properties of the water medium, which are very different from those of tissue (Duck, 1999). It was recognised that a more direct and therefore appropriate means of assessing the likely heating of tissue caused by ultrasound exposure would be through the application of thermal test objects (TTOs) (Shaw et al, 1999). These consist of a thin-film thermocouple sandwiched between two slices of tissue-mimicking material. The structure of the TTO is such that it attempts to replicate the acoustic path between the transducer and the site of interest within the patient. The time-evolution of the heating can be followed using the

thermocouple output. These devices have been used to underpin important work carried out for the Department of Health on assessing the heating generated by diagnostic ultrasound equipment (Shaw et al, 1998).

For the assessment of the presence of cavitation, a variety of chemical and physical methods are available (Leighton, 1994). Some depend on the creation and detection of free radicals, some on sonoluminescence and some detect acoustic emission from cavitating bubbles. Each has specific strengths and weaknesses, and results from measurements must be interpreted from a basic understanding of the acoustic cavitation processes under investigation.

2.4.2 Measurements for infrasound

The measurement of acoustic pressure at infrasonic frequencies commonly uses similar devices to those appropriate to sound measurement in the audible range. The main differences in the technology are associated with the need to remove ambient pressure noise and the need to extend the bandwidth of the associated electronic circuitry to very low frequencies. The best infrasound observatories can record infrasonic waves at frequencies as low as 10^{-3} Hz, associated with waves as long as 300 km, although typical survey microphones limit the frequency range to 1 Hz. Sensitivities of 10^{-5} Pa may be achieved in the best measurements, whilst survey meters have noise floors around 5 10^{-3} Pa (Whitaker and Mutschlechner, 1997).

Condenser microphone A condenser microphone designed for environmental noise measurements can be easily redesigned to operate at infrasonic frequencies and sound level meters which operate in the frequency range 1–100 Hz in octave bands are available commercially. G-weighting may be applied if required (see Appendix A). It is essential to control noise associated with the wind and its management forms the major limitation on sensitivity of environmental infrasound surveys. Wind protection is achieved typically by the use of a low cost spherical foam protective microphone cover.

Optical fibre infrasound sensor In order to extend the frequency and sensitivity range, optical sensors have been developed. In these devices, two optical fibres are wrapped around a compliant tube and act as two arms of an optical interferometer (Zumberge et al, 2003). As atmospheric pressure changes deform the sealed tube, optical interference varies between the two beams and the signal may be calibrated in acoustic pressure.

Microbarometer By using differential pressure measurements and semiconductor or optical fibre pressure sensors, atmospheric monitoring stations can now operate to 0.02 Hz using commercially available equipment. Environmental noise is reduced in such monitoring stations by the use of radial arrays of linked open-ended pipes, with each array then linked to a common measurement cell through further communication pipes. The whole arrangement is typically about 20 m in overall diameter and several such devices may be used, spaced by between 100 and 1000 m depending on the application.

2.5 Hearing Thresholds and Definitions of Infrasound and Ultrasound

This section gives, in outline, the evidence about human hearing thresholds on which the infrasound-to-sound and sound-to-ultrasound frequency boundaries are based.

2.5.1 Low frequency hearing threshold

There is some confusion over the meaning of the term 'infrasound'. A popular interpretation is that it is sound of such low frequency that it is below the lower frequency limit of hearing, generally taken to be around 20 Hz. The International Electrotechnical Commission (IEC, 1994) and the British Standards Institution (BSI, 1995) define infrasound as 'acoustic oscillations whose frequency is below the low frequency limit of audible sound (about 16 Hz)'. However, sound at frequencies below 16 Hz is clearly audible if the level is sufficient. The hearing threshold has been measured reliably down to 4 Hz for listening in an acoustic chamber (Watanabe and Møller, 1990) and down to 1.5 Hz for earphone listening (Yeowart et al, 1967). These thresholds have been combined with those from ISO 226 (ISO, 2003) in Table 2.3.

TABLE 2.3 Low frequency hearing threshold levels

Freq (Hz)	4	8	10	12.5	16	20	25	31.5	40	50	63	80	100	125	160	200
Level (dB)	107	100	97	92	88	79	69	60	51	44	38	32	27	22	18	14

There is continuity of perception throughout the frequency range and no evidence for splitting into 'infrasound' and 'not infrasound' at around 16–20 Hz. However, there is a reduction in slope of the hearing threshold below about 15 Hz, from approximately 20 dB per octave above 15 Hz to 12 dB per octave below 15 Hz (Yeowart et al, 1967). There is also a change in perception of tonality, occurring around 18 Hz. In addition, the range from 10 to 100 Hz may be considered as low frequency noise, with a possible extension by an octave at each end, but there are no clear divisions between infrasound and low frequency noise (Leventhall, 2007).

The overall hearing threshold is defined as the median of the measured threshold values for otologically normal young adults. Consequently, by definition, 50% of subjects have an individual threshold that is higher than this overall hearing threshold and 50% have a lower individual threshold. The standard deviation of the measured threshold values is typically about 6 dB (Robinson and Dadson, 1956; Watanabe and Møller, 1990) so that approximately 68% of the population is within ±6 dB of the threshold.

Hearing sensitivity reduces with age, particularly at the higher frequencies. Age-related hearing loss has been formally established in an international standard, ISO 7029 (ISO, 2000). The lowest frequency considered in ISO 7029 is 125 Hz, which is in the low frequency, rather than infrasonic, range, but it is shown, for example, that at 125 Hz, 25% of 60 year old men may have a more sensitive hearing threshold than the median 18 year old. As also demonstrated in ISO 7029, hearing loss reduces as the frequency reduces, so it is likely that a similar condition extends into frequencies lower than 125 Hz.

Low frequency hearing thresholds in the range 10–200 Hz, for young and older groups, are shown in Table 2.4. Thresholds for otologically unselected 50–60 year old adults are compared with those for normal young adults (excluding those with any hearing impairment). In addition to the 50% (median) level, a lower threshold below which the most sensitive 10% (most sensitive decile) of the population fall is also given. Comparing medians for 50–60 year olds with those of young people, Table 2.4 shows that the older people are 7 dB less sensitive at the median level than the younger ones, but only 3 dB less sensitive at the most sensitive decile.

TABLE 2.4 Low frequency hearing levels of old and young people (from van den Berg and Passchier-Vermeer, 1999)

Frequency (Hz)	Otologically unselected population 50–60 years old		Otologically selected young adults	
	Median (dB)	Most sensitive decile (dB)	Median (dB)	Most sensitive decile (dB)
10	103	92	96	89
12.5	99	88	92	85
16	95	84	88	81
20	85	74	78	71
25	75	64	66	59
31.5	66	55	59	52
40	58	46	51	43
50	51	39	44	36
63	45	33	38	30
80	39	27	32	24
100	34	22	27	19
125	29	18	22	15
160	25	14	18	11
200	22	10	15	7

There are variations in hearing ability at all frequencies, but these differences reduce into the infrasound region so that, from Table 2.4, there is only a small difference of 3 dB between the 10% most sensitive young and older people. The well-known large differences in hearing sensitivity between old and young people occur at high frequencies, in the kilohertz range, not at low frequencies.

2.5.2 High frequency hearing threshold

The upper frequency limit to human hearing has been used to define the boundary between audible sound and ultrasound. As with the lower frequency hearing threshold, that at the upper limit for hearing is quite ill-defined. The range of normal sensitivity increases towards the upper frequency limit. For example, in a study of hearing in the frequency range 15–20 kHz of a group of 30 normal medical students, Laukli and Mair (1985) recorded a range, between the worst and best, of between 25 and 40 dB, even amongst this selected young group with no known hearing defect. Variation of threshold up to 80 dB has been reported by others. If the sound intensity is sufficiently high, frequencies beyond 20 kHz may be heard (Osterhammel, 1979; Osterhammel and Osterhammel, 1985; Sakamoto et al, 1998). However, taking the full range of response, the average threshold frequency is marginally below 20 kHz, as reported in a large study by Takeda et al (1992). Hearing threshold is related to age. Lawton (2001) has taken account of many studies of the age-related loss of hearing sensitivity and has estimated the median threshold of hearing – this is summarised in Table 2.5.

As a result of these observations, the definition of ultrasound is now accepted as being mechanical waves above 20 kHz.

TABLE 2.5 Age dependence of the middle-value upper frequency limit of human hearing (from Lawton, 2001)

Age band (years)	20–29	30–39	40–49	50–59	60–69	70–79
Hearing limit (kHz)	17.9	16.7	15.7	14.8	13.8	12.8

2.6 Summary

Infrasound and ultrasound are mechanical waves, propagated through oscillatory movements of the medium, and associated with local oscillatory variations in density and pressure. For the purposes of this report, infrasound refers to acoustic waves at all frequencies below 20 Hz, and ultrasound refers to acoustic waves at all frequencies exceeding 20 kHz.

In a homogeneous medium, infrasonic fields typically diverge spherically from the source. In practice, however, they exhibit local variations in acoustic pressure due to acoustic scatter from any solid or liquid objects in the field. Ultrasonic beams can be structurally complex, even in homogeneous media. Ultrasonic field structure depends on symmetry: circular, monochromatic sources give rise to

the greatest spatial variations, especially on axis, whilst pulsed, non-circular sources generate less spatial variation.

Ultrasound propagation through tissue is largely controlled by the speed of sound and attenuation, and by microscopic and macroscopic spatial variations of these properties. Bone, soft tissue and gaseous inclusions have very different acoustic properties from one another. Very little acoustic energy is transmitted across an interface between air and tissue and, to a first approximation, ultrasound above about 300 kHz does not propagate through air. Therefore, for many applications, the operator of ultrasound equipment is effectively screened from exposure. Ultrasonic wave propagation through fluids and tissue can be strongly modified by non-linear propagation effects, especially at frequencies above 1 MHz.

Infrasonic wavelengths exceed the dimensions of the human body by more than an order of magnitude, and so the human body acts as an acoustic scattering centre in an infrasonic field.

The absorption of ultrasound by tissue causes heating. The temperature increases caused in tissue by the normal use of current diagnostic ultrasound equipment are very unlikely to extend outside the normal physiological range. Hazardous temperature rises in tissue may be reached under very unusual conditions, specifically the exposure of bone in the focal zone of a pulsed Doppler beam at maximum power with a stationary beam position. High temperatures can also be induced with physiotherapy ultrasound equipment and by misuse of some non-medical devices, such as ultrasonic cleaning baths. Radiation pressure causes fluid movement in the direction of propagation and can displace tissues slightly. The associated forces lie within those experienced under normal physiological conditions. Bubbles may be created in liquids, and caused to vibrate, by an ultrasonic field. This process is called acoustic cavitation and can create strong shear forces and local fluid motion close to the bubble. Under some conditions inertial cavitation takes place, in which the bubble collapses abruptly creating extremely high, very localised temperatures and pressures, light emission and free radicals. However, the likelihood of cavitation events *in vivo* under diagnostic exposure conditions is vanishingly small. Numerical models have been developed to predict acoustic field propagation and effects, including non-linearity, heating, cavitation and displacement.

Radiation force balances, hydrophones and microphones are used for the measurement of acoustic output and acoustic exposure. Improvements in hydrophone performance and power balance design are still required. Methods for estimating *in situ* exposure and temperature rise from these measurements are still simplistic. Definitions and formulae for acoustic dose and acoustic dose rate are given, that could provide a more robust basis for the development of acoustic dosimetry.

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3 Sources and Exposures

In Chapter 2 sound was described as a form of mechanical energy, transferred through a medium due to the vibrational interaction of the molecules or atoms, which at the microscopic level constitute the material. Here, the characteristics of sources of ultrasound and infrasound are discussed in turn, with particular emphasis on medical and industrial applications.

3.1 Ultrasound

Ultrasound is difficult to propagate through air for any significant distance (Leighton, 2007) and therefore the number of airborne applications of ultrasound is small. The most important applications of ultrasound involve propagation in condensed media: solids, liquids – in particular, water – and biological tissue or tissue-like media. The number of applications of ultrasound is extensive and is increasing. The particular attributes of ultrasound that make it attractive are as follow.

a The technology is reasonably low cost compared with other technologies. Although there are various methods of generating ultrasound, the most commonly applied utilises the piezoelectric transduction effect, where a piezoelectric transducer is used to convert electrical energy into mechanical or sound energy. This process is reversible, such that the same transducer can be used to detect low level acoustic echoes generated in the propagation path of the beam.

b Although transmitted ultrasound can be strongly attenuated by certain types of material, such as air – present either as a single interface or bubbles – and bone, it can be applied to optically opaque media.

c Through the design of the transducer, the generated ultrasonic beam can be steered and focused, such that the location of acoustic echoes originating from within the material can be ascribed to fairly small volumes of the medium, endowing the technique with spatial resolution. This attribute forms the basis of diagnostic ultrasound imaging which represents the major application of ultrasound. Driving the transducer with very short pulses of duration less than a microsecond, additionally provides time (or depth) resolution.

d The transducer may also be driven electrically using long tone bursts, or even continuously, allowing alternative techniques to be applied.

e Through an appropriate choice of acoustic powers, and time-averaged acoustic intensities, exposure conditions can be generated within a medium that actually *modify* the propagation medium, potentially reversibly or irreversibly. This phenomenon has led to a number of industrial applications, but also medical therapeutic and surgical applications. The primary mechanisms for effecting physical and potential chemical changes in a medium are heating and the occurrence of violent acoustic cavitation (Humphrey, 2007).

3.2 Natural Sources of Ultrasound

There are a number of natural sources of ultrasound. Most bats can produce frequencies up to 100 kHz for echolocation. Similarly, dolphins, shrews and a number of whales generate ultrasound to assist in hunting prey.

3.3 Artificial Sources of Ultrasound

Areas of application of artificial ultrasound that might lead to the exposure of people can be divided into three distinct areas: medical, industrial and domestic applications.

3.3.1 Medical applications

There is an extensive and growing number of medical applications of ultrasound. These applications may be divided into diagnostic applications and therapeutic and surgical applications.

Ultrasound accounts for about 20% of diagnostic imaging procedures, and, in total, about 7 million ultrasound examinations are undertaken annually within NHS trusts in England (DH, 2008). There are a number of reasons for the increasing use of ultrasound. These include the relatively low cost of the equipment and the fact that the technique is generally regarded as a benign procedure, with an exemplary safety record.

Within therapeutic applications, the applied ultrasonic field is of sufficient level or strength to generate physical, chemical or biological changes in the propagation medium, such as tissue. Here, the term 'strength' refers to a parameter or parameters that relate to exposure such as applied ultrasonic power, the spatial peak, temporal average intensity or the acoustic pressure (Zeqiri, 2007).

3.3.1.1 Diagnostic applications

The physics and technology of medical diagnostic ultrasound systems have been the subject of reviews by Wells (2006) and Whittingham (2007). These reviews considered key operational characteristics of the transducers applied and, in particular, how the need for improved spatial resolution has driven demands for improved transducer design to achieve more efficient matching and damping of transducers in a way that maximises their operational bandwidth. They described the various transducers and operational modes that were being applied in mainstream applications, but also those emerging as key modalities, providing the clinician with improved diagnostic capabilities.

The numbers of examinations carried out in NHS trusts in England have been reported annually to the Department of Health for over a decade (DH, 2008). Over 7.1 million examinations were reported during the year April 2007 to March 2008. Of these, 2.4 million, about one-third of the total, were identified as obstetric and gynaecological (O/G) examinations. There has been steady year-on-year growth during the past decade (Figure 3.1).

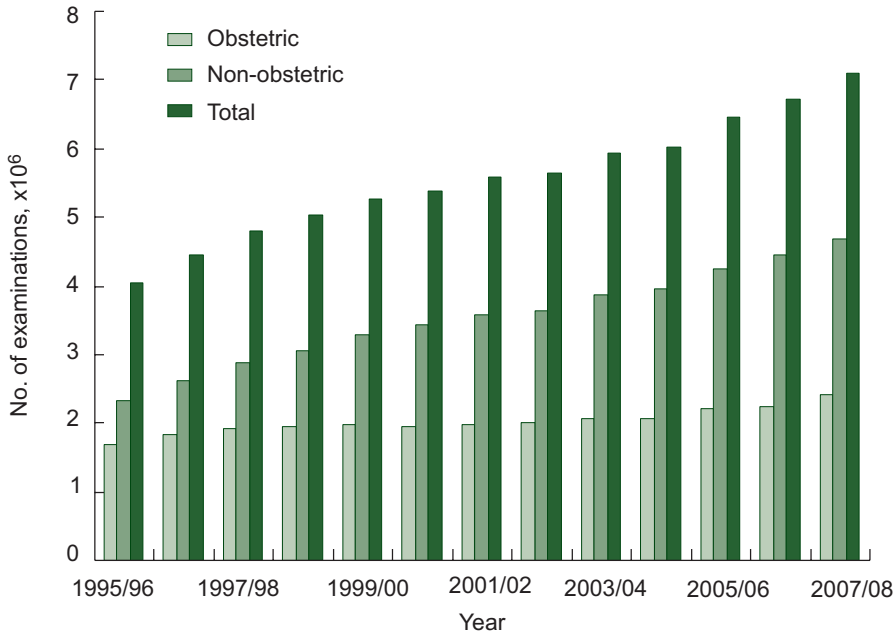

FIGURE 3.1 Number of ultrasound imaging examinations per year reported to the Department of Health, for NHS trusts in England (DH, 2008)

There has been about 43% growth in O/G examinations since 1995, but the overall growth has been dominated by non-O/G examinations which have doubled over the same period. These are primarily cardiovascular and abdominal examinations, but also include neonatal, muscularskeletal, ophthalmological and breast examinations. The DH reporting is part of the annual data collection for medical imaging, and all remaining modalities, such as CT, MRI and X-ray fluoroscopy, are primarily the responsibility of single medical imaging departments. This is not so for ultrasound examinations, which are now used by a wide range of other hospital departments, and also in primary care, for which detailed records are not so readily available. Therefore, it is likely that the number of ultrasound examinations is being under-reported.

Diagnostic examinations can be carried out using various operational modes of the transducers and these are summarised in Table 3.1 (Whittingham, 2007).

Diagnostic applications of ultrasound may be subdivided into two categories of technology: pulse-echo or imaging techniques and Doppler techniques.

Pulse-echo techniques used for ultrasound imaging utilise a transducer that converts an electrical signal (a short pulse) into a short pulse of ultrasound, typically less than a microsecond in duration, which is launched into the body. A simplified schematic of the basic technique is presented in Figure 3.2. The transducer, commonly made from a piezoelectric material (Whittingham, 2007), is held by the clinician with the applicator in contact with the patient. Good coupling is promoted through the use of a

TABLE 3.1 Description of various modes of diagnostic examinations (Whittingham, 2007)

Mode type	Description	Application
A	A stationary beam forms a 'scan line' along its beam axis. Echoes are displayed as a graph of amplitude versus time of arrival, corresponding to depth	Not available on all machines but it is useful for accurate indications of depths. Used for measurement of eye dimensions prior to corneal thinning or lens replacement and characterisation of solid masses in the globe or orbit
M	An extension of A-mode where echo amplitude is represented by grey scale. Echoes from static interfaces arrive coincidentally and trace out a straight line. Those from moving interfaces trace out a graph of range (depth) versus time	Mainly used to assess the dynamic behaviour of heart valve leaflets and heart chamber walls. An application in obstetrics is to provide proof of embryonic or fetal life by recording the cyclic heart movements
B	Name given to cross-sectional imaging and is achieved by scanning the ultrasonic transmit/receive beam across a plane so that many scan lines are interrogated in sequence	B-mode real-time scanning is a major medical imaging modality, with applications in soft tissues which do not lie behind bone or gas

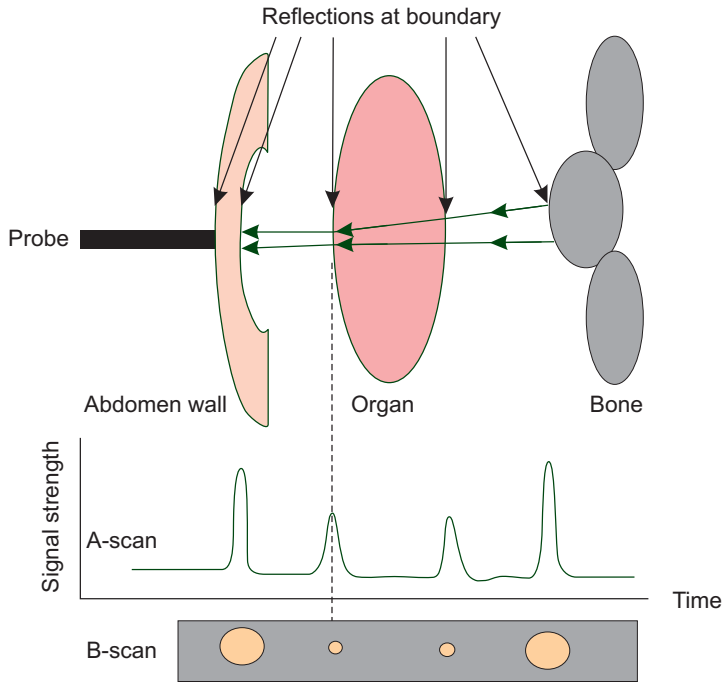

FIGURE 3.2 Simplified diagram of pulsed echo

water-based gel applied to the transducer face. The same transducer, operating in what is called receive mode, detects small echoes arising from the pulse as it propagates into the tissues. These echoes can arise from bone and gas, where the differences in the acoustic properties from the soft tissue are great (Leighton, 2007), but, more typically, will arise from subtle differences in tissue structure. By scanning the beam through the interrogated region either electronically or mechanically, a two-dimensional map can be built up. A key issue relating to deriving diagnostically relevant information is the choice of transducer and, in particular, its frequency. Although better resolution is achieved using higher ultrasonic frequencies (above 5 MHz) due to the shorter wavelengths, the attenuation of ultrasound increases approximately linearly with frequency (Bamber, 1986; Duck, 1990). Consequently, for sites deep within the body, scanning is carried out using low megahertz frequencies.

Doppler techniques enable diagnostic measurements to be made on moving objects within the body. The technique is based on measuring the Doppler shift generated when an ultrasonic wave is reflected by a moving target, resulting in an effective frequency shift between the transmitter and receiver transducers. The frequency of the received ultrasonic signal is compared with that of the transmitted signal and the difference is used to derive the Doppler signal, equal to the Doppler frequency shift of the received signal with an amplitude proportional to that of the received Doppler-shifted signal (Hoskins et al, 2003). In practice, there are numerous moving targets, including both tissue interfaces and millions of blood cells, each with their own speeds and directions. For increased accuracy of velocity measurement, the starting frequency of the ultrasound must be well defined; therefore the equipment utilises long tone bursts of ultrasound (typically 10–15 cycles in length), which could significantly increase applied time-averaged intensities. However, the amplitude is usually decreased, compared with a B-mode emission, to minimise this. When the Doppler spectrum is selected, the transducer stops scanning in B-mode format and all the scan lines are directed along the chosen vector, which now receives a much higher intensity than in B-mode. It is common practice to run the Doppler emissions at a lower frequency than the B-mode emissions. For example, a 3 MHz imaging system will provide tone bursts at 2 MHz and a 5 MHz system may provide tone bursts at 3 MHz. This is because the signals from blood flow are very small; high frequency will give good axial resolution in the image but will need to be lowered in Doppler ultrasound to obtain good 'penetration' for blood flow.

A range of transducers used for diagnostic applications is shown in Figure 3.3.

3.3.1.2 Diagnostic techniques: clinical applications

General medical ultrasound is usually performed by medical staff (radiologists) or technicians (sonographers), both of whom are specially trained and qualified in ultrasound use. In addition, medical staff in several specialty areas perform ultrasound examinations in their area of interest, eg cardiologists, obstetricians, urologists and ophthalmologists. All these practitioners are trained in the use, interpretation and limitations of ultrasound along with the appropriate anatomy and pathology. Although medical ultrasound is performed as a stand-alone examination, it usually forms part of an integrated diagnostic or therapeutic process involving other imaging techniques, relevant clinical information and diagnostic investigations.

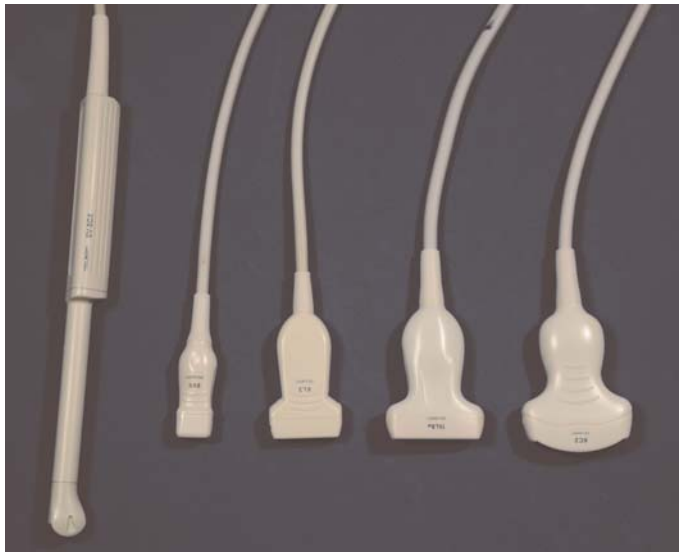

FIGURE 3.3 Diagnostic ultrasound probes

Obstetrics

Obstetric examinations using B-mode ultrasound are used to detect pregnancy, to verify multiple fetuses, to determine the age of the fetus and to determine the position of the placenta. The primary objective of this section is to describe the major clinical applications of obstetric ultrasound, which have found extensive use in the management of pregnancy, from close to inception, through to birth and indeed after birth.

A brief overview of the key uses of obstetric ultrasound may be found in Woodcock (1979), and a summary of the key points is given below.

a Pregnancy can be detected as early as five weeks after the last period and ultrasound plays a key role in detecting the presence or absence of fetal life.

b Fetal heart movements can be detected as early as the seventh week and from the thirteenth week, the fetal skull can be identified.

c Fetal maturity is assessed using ultrasound, through the measurement of the bi-parietal diameter (BPD) and the crown-rump length. This is carried out through electronic calliper measurements of the appropriate distances 'on-screen' and the known relation between the BPD and gestational age.

d Ultrasound can be used to determine the position of the placenta prior to birth. This is important in providing confidence that it is not positioned over the birth canal and for implementing the amniocentesis procedure.

e A number of types of fetal abnormality can be detected, including hydrocephaly, spina bifida and Downs, through the determination of transnuchal transparency.

With the advent of increased capability of modern equipment and the availability of greater image processing power, considerable progress has been made in the quality of the generated images, with the results providing new information on fetal movement within the womb.

Diagnostic continuous-wave (CW) Doppler techniques are also used on the fetus, primarily to study blood flow and for fetal heart monitoring. These involve transmitting continuous waves into the patient from one transducer while receiving the back-scattered and reflected waves with another. Both transmit and receive transducers are typically housed in a single probe. In fetal monitoring applications, a broad region for the acoustic beam is required to ensure the fetal heart remains exposed whatever the position of the fetus.

For this application, the probe is commonly strapped to the mother's abdomen. Although exposures are low in terms of acoustic pressure or power, the fetal monitor can be in place for a number of hours throughout labour, leading up to birth.

Ophthalmology

Some of the first clinical applications of ultrasound were in ophthalmology. This was due to the superficial nature of the organ, its small size and the fact that ultrasound can be readily applied in the absence of the complicating factors of overlying attenuating tissue. Further, the cystic nature of the organ, especially the near acoustically transparent vitreous humour, means that ultrasound can be applied to exploit the acoustic contrast between the fluid in the eye and the solid ocular structures. The low attenuation of the fluid constituents of the eye means that elevated acoustic frequencies can be applied, in excess of 50 MHz, allowing high spatial resolution dimensional measurements to be undertaken. Both A- and B-scan formats provide the primary basis of ophthalmic diagnostic medicine, with imaging being useful in detecting a wide range of pathologies, such as retinal and choroidal detachment, vitreous haemorrhage and tumours of the orbit and choroids, as well as a range of inflammatory conditions. Figure 3.4 shows an example image of the anterior segment of the eye.

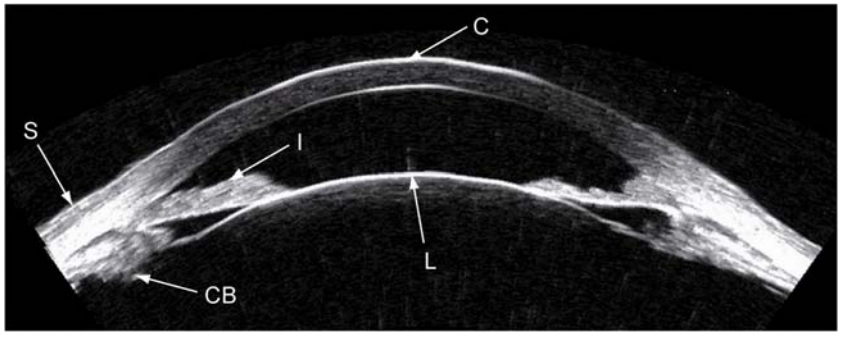

FIGURE 3.4 High resolution ultrasound image of the anterior segment of the eye obtained with arc-scan geometry. Visualised structures include the cornea (C), sclera (S), iris (I), anterior lens surface (L) and ciliary body (CB). (Image reproduced courtesy of Dr R H Silverman, Department of Ophthalmology, Weill Medical College of Cornell University, New York)

Superficial structures

Ultrasound using high frequency (7–20 MHz) probes is widely used to examine the thyroid, breast and testis for diagnosis of focal lesions including tumours and cysts and to guide interventions, particularly percutaneous needle biopsy.

Musculoskeletal system and skin

Joint and soft tissue examinations are also routinely undertaken to identify tumours, collections, foreign bodies and haematomas, and to diagnose tendon and muscle damage. The technique is widely used in sports medicine and is also used to guide joint injection and aspiration for therapy and needle biopsy of lesions for diagnosis.

Cardiology

Echocardiography is an indispensable diagnostic technique in modern cardiology providing essential information on overall cardiac pump and valve function, focal wall lesions, congenital heart lesions and pericardial disease. In 10–15% of patients interposed lung prevents examination of the heart via the skin surface but examination using a transoesophageal probe can overcome this limitation. M-mode studies are routinely employed to provide high temporal resolution functional information on valve function and Doppler studies provide functional flow information relating to valve stenoses and septal wall defects. Contrast examinations are also used in cardiology to detect left to right shunt abnormalities. During conventional invasive cardiac catheterisations, where catheters are introduced into coronary arteries, an intravascular ultrasound probe may also be introduced to detect abnormalities of the arterial wall with high spatial resolution.

Head and neck

Although the adult skull severely attenuates the transmission of sound, the developing neonatal and infant skull has two access windows (fontanelles) that allow views of the neonatal brain using ultrasound. This technique is routinely used by neonatal intensive care units to evaluate and monitor brain and ventricular lesions in premature infants. In adult intensive care units transcranial Doppler measurements are widely used for monitoring cerebral arterial flow in brain injured patients.

Thorax

Normally aerated lung prevents ultrasound examination of the lung but abnormal lung which becomes solid or fluid filled can be visualised and ultrasound is widely used to detect pleural effusions (fluid in the lining of the lungs), and guide diagnostic and therapeutic aspiration of these, and to guide biopsy of superficial tumours (eg mesothelioma) of the lung lining (pleura).

Upper abdomen

Ultrasound is usually the first technique used for imaging the liver, spleen and kidneys, all of which are usually well demonstrated. Ultrasound examination aids diagnosis of both fluid-filled lesions (cysts) and solid lesions (benign and malignant tumours). Such examinations are particularly useful for the detection of gallstones in the gallbladder and blockages of the renal tract. Ultrasound is also used to detect pancreatic lesions, although overlying bowel gas may block the view of this organ in some patients. Ultrasound guided biopsy, drainage and aspiration are also routinely practiced in most hospitals to avoid patients having to undergo surgical procedures for both diagnosis and therapy.

Pelvis

Ultrasound examinations are widely used to evaluate abnormalities of the uterus, ovaries and prostate. Transabdominal ultrasound is used to assess the pelvic organs through the 'window' of a fluid-filled bladder as gas in the large and small bowel blocks the sound beam and prevents visualisation of the structures behind the gas. This may be overcome by using endoluminal or intracavitary probes such as endovaginal and endorectal probes. These are also used to guide aspiration and biopsy procedures.

Endoluminal/intracavitary probes

As noted above these are used in several body areas to overcome the inability of ultrasound to transmit through gas-filled regions. They typically reduce the distance between the transducer face and the observed organ and therefore can increase both the temporal average intensities and the direct heating effects from the transducer. The use of endovaginal examinations during early pregnancy is one example.

3.3.1.3 New imaging technologies

Over the past 10 to 15 years, there have been a number of technological developments whose primary purpose has been to improve the quality of images, thereby increasing diagnostic accuracy.

Contrast agents

Ultrasound contrast agents (UCA) are tiny spherical shells (of diameter 4–10 μm) of proteins, sugars or surfactants filled with inert gas. After injection into a vein, the microspheres are carried along with the blood, increasing its echogenicity. When imaged using special contrast-specific modes (Whittingham, 2007), they are proving very valuable in detecting and characterising tumours, clarifying the boundaries of heart chambers and measuring perfusion, and for a variety of other applications.

Two different approaches are used to increase the contrast between microbubbles and tissue during a diagnostic treatment, distinguished by the lifetime of a UCA within blood. In the first method, they are short lived, their collapse being precipitated by pulses of high amplitude acoustic pressure pulses. The differences in generated acoustic echoes before and after microbubble destruction are detected. The low amplitude approach seeks to prevent microbubble destruction, so that real-time extended examination of the microbubble-perfused region is possible. The detection techniques used for this regime make use of the very-non-linear nature of scattering by microbubbles, even at low pulse amplitudes.

Elastographic techniques

This class of technique mirrors the process of physician palpation, where solid tumours or inclusions are essentially sensed through the feel of the way the stiffness or elasticity of the potential inclusion varies from that of the surrounding tissue. The objective of elastography or elastic imaging is to map properties of the tissue in terms of quantities such as Young's modulus, Poisson's ratio and viscosity (Wells, 2006). Disease modifies these properties, with lesions generally appearing harder than normal tissue. An elastographic image is formed by taking a series of images which investigate the response of the spatial distribution of tissue displacements to an externally applied force. The change in tissue stiffness with time can be imaged and this may be related to the viscoelastic properties of the tissue.

3.3.1.4 Therapeutic and surgical applications

Through the use of appropriate exposure parameters, ultrasonic energy can be used to generate changes in the physiological function of tissue. The exposure parameters determine whether these modifications are temporary (and reversible) or permanent. These are therapeutic or surgical applications, and reviews on the methods may be found in ter Haar (1986) and, more recently, ter Haar (2007). Broadly, applications may be divided into two areas:

a low acoustic intensity (energy) applications, where the aim is to stimulate tissue through a heating related or a non-thermal biological effect, resulting in an acceleration of tissue repair,

b higher intensity applications where the aim is to destroy tissues in a highly selective, controlled manner.

There are also surgical techniques that utilise a form of metallic tool which is driven into oscillation using an ultrasonic transducer. This causes a vibration amplitude of the tool tip in the range 15–30 μm, applied frequencies being typically in the range 10–30 kHz. These surgical tools may be used effectively as knives or scalpels, requiring less force than ordinary knifes to operate, possibly because friction between the two surfaces is reduced if one of them is moving. Benefits are a reduction in bleeding and a cutting tip that does not stick to the tissue. Such devices find applications in tissue aspiration and resection and phaco-emulsification (removal of cataracts), and can have an advantage over alternative modalities in being self-cauterising.

Physiotherapy

Physiotherapy ultrasound is widely used throughout the UK for the treatment of soft tissue injuries, the acceleration of wound healing and the softening of scar tissue. Physiotherapy can also be used for bone injuries and circulatory disorders. It is estimated that there are over 10,000 units in operation within the UK. They typically operate at frequencies in the range 1–3 MHz, with applicators being flat and possessing a physical diameter generally no greater than 25 mm. Small, higher frequency transducers or treatment heads are used to expose small volumes of tissue. In use, the applicator is coupled to the tissue using gel and moved over the treatment area to generate uniform exposure. The applied intensity is selected on the machine, and the upper limit of 3 W cm^{-2} for the effective intensity is prescribed by the relevant international standards (see Appendix A), although the transducer field is typically strongly non-uniform with spatial 'hot-spots' occurring particularly close to the transducer face. It should be noted that these non-uniformities in the acoustic field, coupled with the high time-averaged intensities generated, can lead to patient burns and this has led to the issuing of a recent safety notice in Scotland (Scottish Healthcare Supplies, 2006). A typical applicator being used for the treatment of tennis elbow is shown in Figure 3.5.

The primary mechanism of any therapeutic benefit was thought to be thermal in nature, although, as the basic understanding of bio-acoustic interaction mechanisms improves, treatment regimens are being altered in an attempt to make use of any beneficial non-thermal mechanisms that may exist (by use of lower intensities and of pulsed beams). There is a dearth of scientifically designed controlled clinical trials and, consequently, the ultrasound treatment regimen used is usually empirically determined, often to each hospital department's particular 'recipe'. Until more rigorous scientific studies are available, the

FIGURE 3.5 Ultrasound applicator being used for the treatment of tendonitis of the elbow (tennis elbow) (© iStockphoto.com/David Peeters 2006)

mechanism responsible for any therapeutic benefit will be the subject of speculation and it will not be possible to optimise treatments using an understanding of interaction mechanisms. Water bath immersion can also be used for awkward geometries or transmission of the ultrasound through coupling water bags.

Dental applications

Dental scalers (or, more accurately, descalers) driven by ultrasound are extensively used within dental practices for the treatment of periodontal disease, a disease of the teeth, gums and bone surrounding the teeth, which is the prime cause of adult tooth-loss. It is caused by the build-up of plaque and tartar, which increases the population of harmful bacteria. Dental ultrasound scalers typically operate at low frequencies, 20–30 kHz, and comprise a tool, or scaler tip, which is driven into vibration by the electrical drive unit.

The ultrasound-generating component part of the device – for example, the piezoelectric crystal – is set back in the hand-piece of the tool, and a waveguide generates vibration at the tip. This is applied to the relevant area of the tooth and the tip cleans through what may be described as a mechanical chipping action. An irrigant (water) is applied through the scaler tool tip, ensuring that heating of the tooth, through 'friction burns', is minimised. Apart from heating, other potential damage mechanisms include platelet damage caused by cavitation, auditory damage to patients and the potential release of bacteria in fine aerosols.

Lithotriptors

The application of lithotripsy has become widespread as a clinical treatment for urinary calculosis, or kidney stones. There are a number of methods that fall within this category, but the one which will be

briefly described here is extracorporeal shock wave lithotripsy (ESWL) which is applied to the non-invasive disintegration of kidney stones using short pulses of ultrasound of high acoustic pressure. Introduced in the mid-1980s, this technique has revolutionised the treatment of kidney stones. A non-invasive treatment of this disease has clear advantages over alternative surgical procedures which carry with them the risk of infection.

Although there are various ways in which high amplitude acoustic pulses can be generated – for example, using piezoelectric or electromagnetic ESWL devices – the aim is the same: to generate exceedingly high acoustic pressures at the site of the kidney stone. This is commonly carried out using a focusing arrangement. The therapy head is coupled to the patient through a water cushion which forms an acoustic window. The shock-wave pulse is targeted using either an X-ray or ultrasound imaging system. A treatment will involve applying typically one or two shocks per second, such that about 8000 are delivered in total over two or three sessions. Stone destruction is thought to arise from the resultant shearing forces and the occurrence of cavitation. An imaging system is used to monitor the progress, which continues until the stones are small enough to be passed by urination. Not all stones can be treated in this way, as it depends on their mechanical susceptibility. Side-effects do occur, and these include capillary damage arising from cavitation.

High intensity focused ultrasound (HIFU)

The physical properties of high frequency (megahertz) generated ultrasound in tissue mean that it can be brought to a tight focus at a distance from its source. This can be used to therapeutic benefit when a focused source is driven at high power. High temperatures can be generated solely within the focal region, with surrounding areas remaining largely unheated. It has been shown that, at 1.7 MHz, when focal peak intensities of around 1500 W cm^{-2} are held for one or two seconds, temperatures in excess of 56°C are achieved in liver tissue *ex vivo*. This leads to instantaneous cell death and coagulative necrosis, with a margin of six to ten cells between live and dead cells at the edge of the focal zone (ter Haar, 2007). The ablated volume reflects the focal region of the beam, and at 1.5 MHz is typically an ellipsoid of length around 2 cm and diameter around 2 mm.

It was in the 1990s that HIFU began to be used, largely because of the advent of sophisticated imaging techniques for targeting and monitoring. The interest was largely fuelled initially by the search for non-invasive treatments of benign prostatic hyperplasia (BPH). A number of implementations of HIFU are currently the subject of investigation including transrectal HIFU and extracorporeal HIFU. A detailed description of the current status of HIFU may be found elsewhere (ter Haar, 2007). Within the UK, treatment of prostate cancer through HIFU gained approval from the National Institute for Health and Clinical Excellence (NICE, 2005). However, a subsequent report (NICE, 2008) stated that HIFU is "not recommended for men with localised prostate cancer other than in the context of controlled clinical trials comparing their use with established interventions".

HIFU is still in its infancy and the most appropriate clinical applications will gradually emerge. Its clear advantages are its non-invasive nature, the ability to achieve high spatial specificity, the potential for re-treatment or use of other therapeutic modalities after its use, and its low number of side-effects. A HIFU unit is shown in Figure 3.6.

FIGURE 3.6 HIFU unit in a hospital (photograph reproduced courtesy of the HIFU Unit, Churchill Hospital, Oxford)

Developments and emerging modalities

A more comprehensive description of the current position on the emerging modalities may be found elsewhere (ter Haar, 2007). This covers potentially important areas such as the use of ultrasound in:

a stimulation of bone fracture healing,

b enhancement of drug up-take,

c sonodynamic therapy and sonoporation (the opening of cellular membranes to allow the uptake of molecules),

d sonothrombolysis (dissolution of blood clots).

In many of these areas, the precise mechanism through which the ultrasound operates is a matter of conjecture, and there is a need for carefully controlled studies to develop and improve understanding and optimise treatments.

3.3.1.5 Typical medical exposures

Examples of typical acoustic exposures for a range of medical ultrasound diagnostic and therapeutic applications are presented in Table 3.2 and are based on the values given by Szabo (2004). These show that the systems vary over a very wide range in terms of the acoustic pressures and intensities involved. For example, B-mode and pulsed Doppler diagnostic systems can produce very similar peak negative pressures, but the longer pulses of Doppler systems can result in substantially higher time-averaged intensities. Physiotherapy, surprisingly, operates within the range of acoustic pressures and intensities used for diagnostic Doppler ultrasound. This has been discussed by Starritt and Duck (1992) who concluded that the main differences between these modes lay in the beam dimensions used and total acoustic power. Lithotripsy systems use the highest peak negative pressures, up to 15 MPa, and focused beams for ultrasound surgery (HIFU) use the highest intensities, up to 10 kW cm^{-2}.

TABLE 3.2 General characteristics of ultrasound used for different medical diagnostic and therapeutic applications (based on data from Szabo, 2004)

Application	Frequency	Spatial peak, temporal average intensity (I_{SPTA})	Peak or focal rarefactional pressure
B-mode*	1–15 MHz	0.3–990 mW cm^{-2}	0.45–5.5 MPa
M-mode only*	3–10 MHz	300 mW cm^{-2}	>4 MPa
Colour Doppler imaging	3–7.5 MHz	1 W cm^{-2}	2.5 MPa
Physiotherapy	1 MHz	1 W cm^{-2}	<0.5 MPa
Lithotripsy	0.5–10 MHz	Very low	>20 MPa
Soft tissue lithotripsy†	0.25 MHz	Very low	5–30 MPa
HIFU†	0.5–5 MHz	1000–10,000 W cm^{-2}	10 MPa
Bone growth stimulation	1.5 MHz	30 mW cm^{-2}	50 kPa
Drug delivery†	Up to 2 MHz	Various	0.2–8.0 MPa

* See Table 3.1 for a description of these modes.

† Currently experimental techniques: limited information or wide range of characteristics under investigation. For the therapeutic modalities indicated, acoustic parameters may be a strong function of treatment duration or other factors (from Henderson et al, 1995, and Shaw and Hodnett, 2007).

3.3.1.6 Non-medical exposures to ultrasound

The ultrasound equipment used for obstetric examination has been exploited for non-medical imaging. The basic clinical applications of diagnostic examinations have been described above. Three-dimensional (3D) scanning refers to the acquisition and display of echo information from a volume of tissue, rather

than from a two-dimensional (2D) slice. When the scanning rate is sufficiently high to allow a real-time display of 3D information, it is called 4D scanning, the fourth dimension being time. There are concerns that, driven by the improved quality of the images, and set alongside a description of these images as the first photographs or videos of a baby, this technology could lead to a large-scale use of unregulated obstetric imaging where exposure will be taken out of the hands of the skilled clinician, and the exposure of the developing fetus is primarily carried out for the purposes of entertainment with no clinical benefit. There are currently no regulations covering this activity.

A number of bodies have stated disapproval of non-medical obstetric imaging. The position of the American Institute of Ultrasound in Medicine (AIUM) and the US Food and Drug Administration is clear: that the use of ultrasound for fetal 'keepsake' videos and portraits which provide no clinical benefit to the patient is inappropriate and contradicts the responsible practice of medicine (AIUM, 2005; FDA, 2004).

Doppler fetal monitors are available to the public. Although the output power is low, there is no control over the duration of exposure.

There is also a range of ultrasonic beauty products that are said to improve aspects, such as the firming of skin and the improvement of muscle tone, as well as promoting weight loss.

It is likely that, in future, more ultrasound-related products for the personal care market will emerge. One example is a device for earwax removal using ultrasound, where a probe is introduced into the ear and the ultrasound is used to break up the earwax into small particles which are then removed by suction.

Sonic or powered toothbrushes operate at frequencies outside the ultrasonic range, being generally below 100 Hz. Ultrasonic toothbrushes operate at about 1.6 MHz; it is claimed that the ultrasound breaks up plaque, and furthermore prevents bacteria from adhering to the enamel surfaces of teeth.

Information on the levels of personal exposure from these devices is not available.

3.3.2 Industrial applications

Applications within the industrial area can be divided into lower power and high power applications, where the former category is used for diagnostic applications (non-destructive testing or NDT) and the latter is used in the processing of a wide variety of materials. NDT techniques may be either passive (acoustic emission), listening for acoustic signatures generated from safety-critical events such as cracks, or active, where an acoustic pulse is launched into a structure to monitor changes in its integrity, or the thickness or performance of structures.

For industrial applications, human exposure is occupational in nature, and worker exposure to any generated ultrasound may be readily mitigated through the use of ear defenders, the application of suitable sound-absorbing materials and basic instructions for the operator not to expose their hands, etc, to the ultrasound, which is almost always applied to liquid or liquid-like media.

3.3.2.1 Ultrasonic cleaning

Ultrasonic cleaners are typically used at frequencies between 20 and 40 kHz for the cleaning of a wide variety of objects: jewellery, lenses and other optical parts, watches, dental instruments, surgical instruments and surgical parts. Such devices operate primarily through acoustic cavitation occurring within the commonly water-based fluid-filled vessel. Cavitation collapse occurring close to the surface to be cleaned generates small microscopic jets which clean the surface. Cavitation is accompanied by noise arising from the cavitating bubble generating subharmonics of the applied acoustic frequency, in the range 10–20 kHz, which falls within the audible frequency range.

3.3.2.2 High power industrial processing

Applications of high power ultrasound have recently been reviewed by Mason (2007), and only a brief overview of this area will be presented here.

Water remediation

Ultrasound is being investigated as a means of purifying water. Ultrasound is able to inactivate bacteria, making them more susceptible to biocides, and/or deagglomerate bacterial clusters or flocs. The way this works depends on the applied acoustic power and frequency through a number of physical, mechanical and chemical effects that primarily arise from acoustic cavitation, resulting in the removal of biological and chemical contamination (Mason, 2007).

Air cleaning

The inhalation of airborne particles is a public health concern. Such fine particles originate in the emissions associated with carbon-fired power plants, cement factories, the chemical industry and diesel-powered vehicles which have increasingly become the focus of stricter government regulations. Current filters and electrostatic precipitators have problems in coping with the smallest particles. There is consequently a need for a process by which these particles can be agglomerated to larger particles before being submitted to conventional separation technologies.

It has been shown that airborne acoustic energy in the ultrasonic frequency range can be used to precipitate suspended particles (aerosol or smoke) (Mason, 2007). In order to obtain the efficient generation and transmission of acoustic energy into gases it is necessary to use ultrasonic generators with specifications that include good impedance matching with the gas, large amplitude of vibration, high directional or focused radiation and high power capacity.

Land remediation

Ultrasound has been used as a technology to promote the treatment of contaminated soil wastes. The contaminated soil is mixed with water and exposed to a source of ultrasound. Complex pollutant molecules are destroyed by high temperatures (approximately 4000°C) and pressures (100 MPa).

Treatment of sewage sludge

In biological wastewater treatment, large quantities of biomass (sludge) are generated. Treatment of such material requires several processes that include digestion, settling and dewatering. The overall treatment procedure is referred to as stabilisation since the sludge material will continue to degrade with

the evolution of noxious gases under ambient conditions and must be stopped (stabilised) before the material can be used or disposed of. High power ultrasound breaks down cell walls and releases cellular material. This makes the dissolved organic compounds more readily available in an anaerobic digestion process. The application of ultrasound enables any filtration system to operate more efficiently and for much longer periods without maintenance.

Crystallisation

Ultrasound has proved to be useful in crystallisation processes since it can initiate seeding and control subsequent crystal growth in a saturated or supercooled medium (McCausland and Cains, 2003). This is thought to be due to cavitation bubbles themselves acting as nuclei for crystal growth and to the disruption of seeds/nuclei already present within the medium, thus increasing the number of nuclei present in the medium. Through the correct choice of sonication conditions it is possible to produce crystals of a uniform and designated size, a capability that is of great importance in pharmaceutical preparations.

3.3.3 Domestic applications

The effects of ultrasound, such as acoustic cavitation, have also found application in the domestic market. Examples of systems include an ultrasonic dish washer or vegetable washer machine, and systems cleaning items such as golf clubs.

There are also devices which would appear essentially to be high power ultrasonic horns used in high power sonochemical processing, that use ultrasonic waves in combination with a cleaning fluid to remove tough stain deposits on fabrics.

All of the above devices make use of ultrasound generated in an aqueous medium or coupled to a fabric – for example, using a coupling medium. It is a usual precaution, therefore, not to put hands or fingers in the water contained within these devices, and not to touch the moving surface.

Using a probable combination of acoustic cavitation and radiation force, a mist generator or fountain can be used to add a 'cool' mist to water features. It can also be used to humidify the atmosphere, as well as administer essential oils for aromatherapy. The mist is produced by a high frequency ceramic membrane that vibrates a column of water above the device.

There are a small number of applications of airborne ultrasound. For example, ultrasonically-aided positioning can be used to assist the parking of cars and for distance measurement. A robotic vacuum cleaner uses ultrasound to navigate. The transducer operates at 60 kHz. Some products use airborne ultrasound to scare away animals – for example, cat or rodent scarers – which the manufacturers claim generates a piercing ultrasonic noise that is inaudible to humans. The position with regard to safety-related standards for noise output for airborne ultrasound has recently been described by Leighton (2007).

Ultrasound pressure levels as a function of frequency for a range of medical and industrial applications are shown in Figure 3.7.

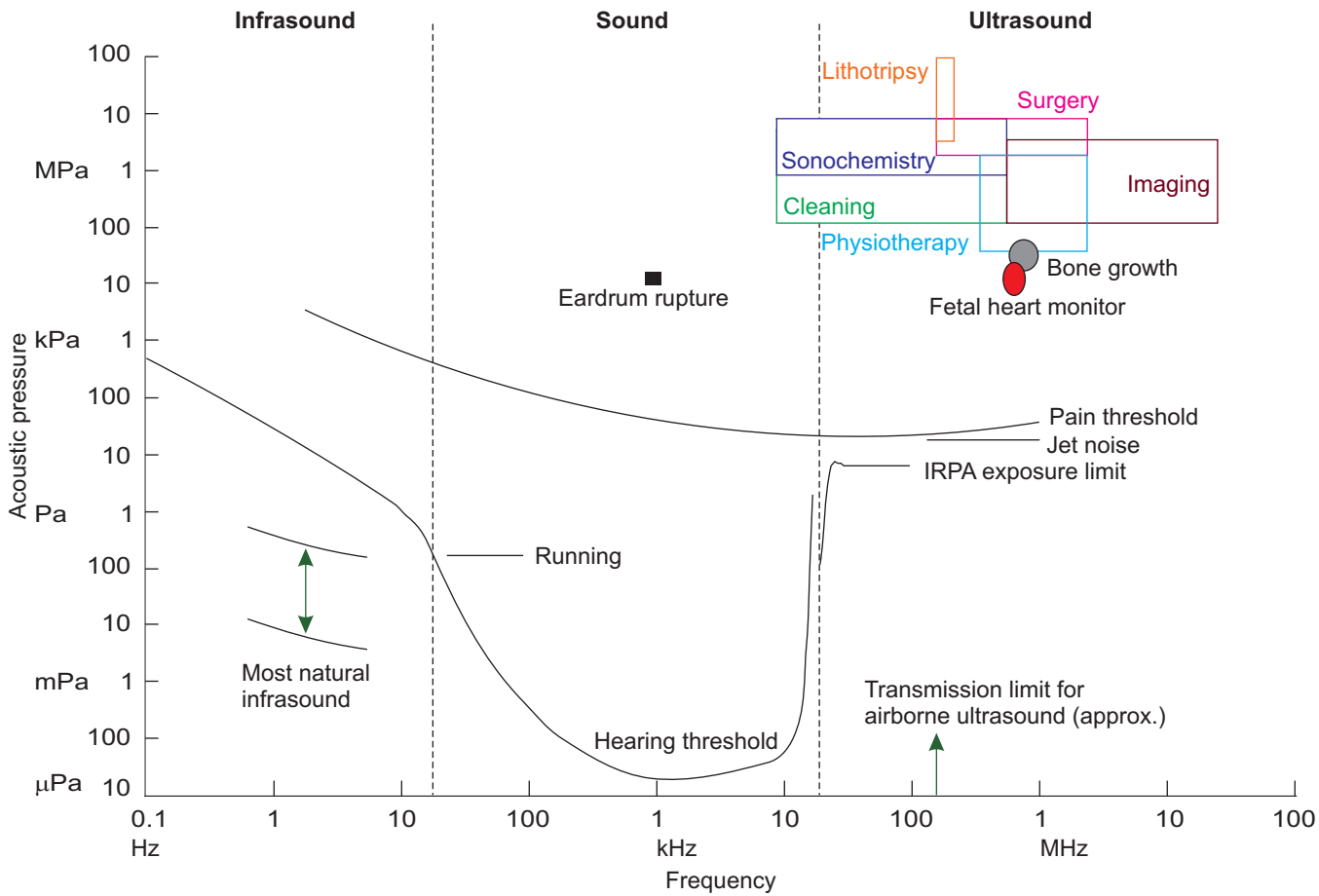

FIGURE 3.7 Pressures and applications as a function of frequency

3.4 Infrasound

Infrasound may be generated through natural geological and weather processes or as the result of man-made activities described below. Infrasound is created whenever a surface moves periodically with a motion that includes frequency components below 20 Hz. Such sources may be natural or artificial, and in both cases a wide range of known or putative sources have been reported. Table 3.3 lists the most important of these.

TABLE 3.3 Summary of some artificial acoustic sources that may give rise to airborne waves in the infrasonic range below 20 Hz

Source	Description
Military	Nuclear tests
	Artillery and other bombs
	Rocket launch
Industrial	Industrial machinery
	Compressors
	Ventilation systems and air conditioning
	Mining explosions
Environmental	Aircraft
	Wind turbines
	Building sway
	Rail traffic and tunnels
	Washing machines

3.5 Natural Sources of Infrasound

A range of natural environmental sources contribute to the complex infrasonic background. Measurements of background infrasound have shown that its acoustic pressure increases with decreasing frequency and most natural sources generate infrasound predominantly in the far infrasound, below 1 Hz. Low frequency alterations in ambient pressure occur during violent storms and tornadoes, in particular associated with wind gusts and their interaction with the land or sea surface. Even under calmer conditions, winds and natural changes in atmospheric pressure may be associated with frequencies as low as 10^{-3} Hz. Atmospheric electrical activity, both thunderstorms and aurora, and the passage of meteor showers may also cause infrasound. High amplitude standing waves sometimes form on the surface of oceans, and these create so-called microbaroms in the atmosphere. Higher frequency infrasound has been predicted from mixing of cold and warm air near the Earth's surface: levels of

around 60 dB were predicted in the 2–20 Hz range at 100 km from the source (Hardin and Pope, 1989). Finally, a number of seismic events may cause the propagation of infrasonic waves into the atmosphere, including earthquakes, landslides and avalanches.

Figure 3.7 compares the frequency ranges and levels of natural infrasound with the hearing threshold (see also Bedard and Georges, 2000, and Hedlin and Romanowicz, 2006). It can be seen that natural infrasound is generally lower in frequency than 1 Hz and at levels below the hearing threshold. The part labelled 'running' towards the left of Figure 3.7 shows the frequency and level of infrasound that is experienced during running due to changes in the height of the head. Similarly, a child on a swing experiences pressure changes equivalent to infrasound at a level of around 110 dB and frequency of 0.5 Hz, depending on the suspended length and change in height during the swing.

3.6 Artificial Sources of Infrasound

The Nuclear Test Ban Treaty has created a need to monitor nuclear explosions and the associated infrasound forms one of the criteria for such monitoring. Similarly, other explosive devices (both civil and military) cause infrasound, as do other ordnance and rocket launches. In the industrial sector, low frequency vibration of some industrial machinery may cause infrasound, especially in association with air compressors and ventilation systems. For example, a reciprocating compressor may have a frequency of 10 Hz and produce harmonics at 20, 30, 40 Hz, etc. Levels of the lower harmonics could be 90–100 dB near to the intake of the compressor. A large, slowly rotating fan produces a peak at its blade passing frequency, which is likely to be in the low audio frequency range, rather than the infrasound range, although badly adjusted fans, running inefficiently, could result in fluctuating levels in the 10–20 Hz range. Infrasound may be created in the environment by aircraft, and by rail traffic, especially when trains travel at high speed through tunnels. These sources are generally accompanied by audible sound. Wind turbines emit acoustic waves in the infrasonic frequency range. The movement of tall buildings during windy conditions may be an important outdoor urban source of infrasound.

3.7 Summary

The major application of ultrasound lies in medicine where, over a period of 50 years, diagnostic imaging has developed into a key modality for the identification and management of disease. Additionally, almost all developing fetuses within the UK are the subject of at least one obstetric examination. It has become well established that ultrasound can have an effect on tissue, primarily through its heating effect arising from absorption by tissue. Indeed, the destructive capability of the applied ultrasonic field is now being harnessed through the emergence of a range of therapeutic techniques in which, at times, the actual mechanism of interaction is not known.

Within industry, the potential of an ultrasonic field to disrupt or modify a material, primarily through acoustic cavitation, has found extensive and growing applications in cleaning, sonochemistry and processing of water.

Many of the sources of infrasound are natural, resulting from geological or meteorological conditions. However, there are also a number of artificial sources such as industrial machinery, ventilation systems and air conditioning, aircraft and rail traffic.

There is very little information on occupational or non-occupational human exposure to ultrasound or infrasound, except for medical exposures of patients.

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4 Biological Studies

There is a considerable body of research concerned with the interaction of ultrasound with biological systems, and there are also many studies investigating the consequences of exposure to infrasound. This chapter reviews the evidence from experimental studies with cell cultures, tissue preparations and whole animals. Effects have been studied in a wide variety of models and exposure conditions, with emphasis on investigating potential biological changes associated with clinical uses of ultrasound, particularly in obstetrics and gynaecology.

In addition, behavioural and neurophysiological data indicate that sound waves at ultrasound and infrasound frequencies are produced and used by various animal species for gathering information about their environment. For example, ultrasound is used for communication, navigation and foraging by most bats, many cetaceans and some rodents (Sales and Pye, 1974) and possibly by amphibians (Feng et al, 2006). There is also some evidence that infrasound may be employed for similar functions by elephants (Larom, 2002; Garstang, 2004), pigeons (Schermuly and Klinke, 1990; Hagstrum, 2000) and some species of fish (Sand and Karlsen, 2000; Karlsen et al, 2004). However, such natural behaviours are outside the scope of this review.

4.1 Ultrasound

Studies on the biological effects of ultrasound have been carried out in a variety of *in vitro* and *in vivo* model systems. However, caution must be exercised when extrapolating the results found in one exposure setting to what will happen in another. The mechanisms of interaction of ultrasound in the liquid environment pertaining *in vitro*, where cultures of cells exist either as a suspension in a nutrient medium or as a monolayer attached to a specially coated surface covered by medium, may be very different from those *in vivo* with more solid structures, such as soft tissue and bone.

In the liquid environment, acoustic cavitation and streaming are likely to be the dominant physical mechanisms for producing biological effects by ultrasound, low acoustic absorption coefficients rendering substantial heating unlikely. There may also be substantial differences between effects found in cell suspensions and monolayers. Cells in suspension are free to move throughout the ultrasonic field, being carried by acoustically induced streaming and thermal currents. The acoustic field experienced by a single cell will therefore vary in time and space. Cells grown in monolayer, however, are unable to move, and the ultrasound parameters to which they are exposed depend on their position in the acoustic beam, temporally varying only if the source is moved.

For experiments in which intact tissues are exposed *in vivo*, thermal mechanisms are likely to be of greater importance than for *in vitro* systems, due to the higher absorption of ultrasonic energy in

tissue. The probability of occurrence of cavitation is usually less in intact tissues than in a liquid, but depends on the tissue state, gas content and temperature.

In addition to the different primary mechanisms for damage between *in vitro* and *in vivo* systems, it is difficult to extrapolate exposure conditions from one to the other. Providing due attention has been paid to the design of the sample holder (avoiding, for example, situations in which the holder itself intercepts the ultrasonic beam or a liquid–air interface provides a near-perfect reflecting surface which sets up standing waves), acoustic pressure levels at any point within an *in vitro* sample will be reasonably well approximated by those measured in water under the same conditions. The same is not, however, true for *in vivo* exposures for which an *in situ* value must be calculated, taking into account the attenuating properties of tissues lying in the beam path, especially interfaces at which reflection may occur. In reality, the acoustic properties of tissues are often imprecisely known, rendering this calculation inaccurate.

Medical ultrasound has never drawn distinctions between exposure and dose, and this can make interpretation of the literature difficult. It is usual in studies of biological effects to report acoustic exposure: it is very uncommon to report energy deposition per unit mass, ie dose. Sometimes the word dose has been used incorrectly, interchangeably for exposure. Different biological consequences may result from different modes of ultrasonic energy delivery. For example, two exposures that use the same total acoustic energy over an identical time span, where one is delivered in continuous mode and the other in short pulses at low repetition rate, may result in very different effects in tissue. The first is more likely to induce thermal effects, while the second may stimulate cavitation activity and its associated characteristic cell damage. In making the transition from exposure to dose in an ultrasonic field, it is necessary to know the *in situ* exposure and the acoustic properties of the target tissue. These parameters are often not well characterised, and large gaps in knowledge exist for normal and malignant human tissues and for the temperature dependence of such parameters.

There are thus some potentially very important differences between therapeutic and diagnostic exposures in the mechanisms for producing biological change, and this must be taken into account when interpreting the literature. Broadly,

a diagnostic ultrasound uses short (1–10 μs duration, pulse repetition frequency around 20 kHz) pulses of high pressure amplitude 0.4–5.5 MPa and intensities (spatial peak, temporal average, I_{SPTA}) less than 1 W cm^{-2},

b therapy ultrasound uses tone burst (over 1 ms duration) or continuous-wave exposures of high pressure amplitude 0.3–10 MPa and intensities (I_{SPTA}) over 1 W cm^{-2}.

The exception to these therapeutic exposure levels is for bone fracture healing, for which the pressure amplitude may be around 50 kPa and the intensity around 30 mW cm^{-2}. Thermal effects are unlikely to be important in soft tissues for the short exposures and relatively low temporal average intensities involved in diagnostic ultrasound. However, high rarefactional pressure amplitudes generated by lithotripters may promote acoustic cavitation in aqueous media, such as that used for cell culture work, even for short pulses. For the tone burst and continuous-wave exposures used in therapy, heating is more probable since the temporal average intensities are higher.

In considering the implications of observed biological effects it is also important to make allowances for the maturity of the individual being exposed. Damage, whether subtle or catastrophic, to a few cells of the developing embryo is generally likely to have far more biological significance than that to a small volume of adult cells. The stage of development of the fetus is also an important factor to consider in the assessment of the potential biological impact of diagnostic ultrasound. For example, the early embryo is likely to have acoustic properties similar to those of water, and thus bulk heating effects are unlikely to be significant, whereas for a third trimester fetus in which bone mineralisation has occurred, some heating at bone surfaces is a real possibility.

4.1.1 Cellular studies

While the interpretation of ultrasound exposures of cells in culture can be problematical as described above, such studies of isolated cells in culture can provide useful information at a fundamental level about changes produced under closely defined exposure conditions. Under optimal conditions, the cells should be contained in sample holders that perturb the ultrasonic field as little as possible.

It is not possible to cite here all the individual publications that describe ultrasonically induced changes in cells. These studies have been reviewed by the NCRP (2002), Feril and Kondo (2004a), Miller (2007) and ter Haar (2007). Instead, representative examples of most importance for concerns about potential adverse effects are given, all of which are drawn from work with mammalian cells.

There are a number of endpoints that have been used to study the effect of external agents on cells. It is convenient here to distinguish between 'gross' effects, such as lysis, effects on cell division capability and damage to cellular ultrastructure, and more subtle effects, such as chromosomal changes, functional changes and altered growth patterns.

4.1.1.1 Cell lysis

The evidence that ultrasound exposure of cells in suspension can lead to cell lysis is extensive and unequivocal. Cavitation has been shown to be a major mechanism in producing complete cellular disruption of this sort, and a number of studies have been published on this subject [see, for example, Kaufman et al (1977), Morton et al (1982), Hallow et al (2006) and Lai et al (2007)]. It is not clear, however, that ultrasound can produce lysis in the absence of cavitation effects. Several authors, (including, for example, Elwart et al, 1988) have demonstrated that the amount of lysis obtained depends on the concentration of cells in suspension, higher cell concentrations exhibiting proportionally less lysis than low ones. It is postulated that this is because high cell densities interfere with bubble activity in the suspension. Brayman et al (1992) investigated this further and suggested that this 'cell density effect' was, in part, due to respiratory consumption of dissolved oxygen and concomitant release of CO_2 into the suspension medium, thus reducing the probability of lysis-inducing effects (Carstensen et al, 1993). Brayman et al (1996) also studied existing data and concluded that, while the proportion of cells that are lysed decreases with increasing cell density, the total number of cells lysed actually increases. These authors hypothesise that cell size is an important determinant of the extent of cell lysis because of its effect on cell–cell and cell–bubble spacings in suspensions of a given concentration. Cell

size is also important in the formation of cell aggregates around pulsating bubbles (Nyborg and Miller, 1982; Brayman and Miller, 1993). Lysis, where it occurs, appears to be an immediate consequence of ultrasound exposure, rather than a delayed effect, and may affect cells in mitosis more than those in other stages of the cell cycle (Clarke and Hill, 1969).

4.1.1.2 Cell division capability

The clonogenic assay is a common measure of biological effect in conventional radiobiology. This assesses the ability of a cell to divide and produce viable daughter cells following a specific insult.

In general, cells that survive ultrasound exposure when intact go on to produce progeny in the same way as their untreated counterparts (Bleaney et al, 1972; Morton et al, 1982). The exception to this appears to be cells that are exposed to ultrasound while being maintained at an elevated temperature (Li et al, 1977; ter Haar et al, 1980; Feril and Kondo, 2004b). It has been found that there is a loss of mitosis in the heated, irradiated cells over and above that of cells subjected to heat alone. The mechanism for this effect is not understood, but is thought to be non-thermal and non-cavitational in origin (Morton et al, 1983).

4.1.1.3 Ultrastructural changes

Changes to the cell membrane following ultrasound exposure are usually manifest as changes in permeability to ions. Examples of this are the sublethal alteration in the thymocyte plasma membrane that leads to a decrease in potassium content following exposure to 1 W cm^{-2} *in vitro* at 1.8 MHz (Chapman, 1974), and the reversible increase in calcium ion uptake in fibroblasts demonstrated by Mortimer and Dyson (1988) (1 MHz, 0.5–1.0 W cm^{-2}, I_{SPPA}). The interaction of ultrasound with the cell membrane has been the subject of particular scrutiny for those interested in ultrasound-mediated drug delivery and sonoporation (ter Haar, 2007).

Electron microscopy of cells following ultrasound treatment at therapy intensities has revealed damage to a variety of subcellular organelles, primarily to mitochondria. When intact tissues have been studied using this technique, damage to lysosomes has been seen, with consequent release of lysosomal enzymes. It is not clear whether lysosomal damage is a direct or indirect result of ultrasound exposure (Dvorak and Hrazdira, 1966; Hrazdira, 1970; Taylor and Pond, 1972).

Damage to the cell membrane of the luminal aspect of endothelial cells of blood vessels irradiated in standing wave fields has also been reported both in chick embryos and in mouse uterine vessels (Dyson et al, 1974; ter Haar et al, 1979). Because vascular endothelial cells mediate important effects on tissue growth and metabolism, damage may have long-term consequences.

Where cavitation is implicated in causing damage, not only has membrane and mitochondrial damage been seen, but dilated rough endoplasmic reticulum and some irregular lesions have been observed (Harvey et al, 1975).

In general, it seems that the cell nucleus is relatively unaffected by ultrasound exposure, the only type of lesion that has been seen being slit-like vacuoles at the nuclear membrane (ter Haar et al, 1979). Watmough et al (1977) have suggested that cavitation microbubbles may be produced within cells and

that nuclear, mitochondrial and granular endoplasmic reticulum membranes could act as nucleation sites. These organelles would be specifically affected, and damage might manifest itself as lesions next to the membrane. There is, however, no direct evidence for this hypothesis.

4.1.1.4 Chromosomal and cytogenetic effects

Ultrasound of sufficiently high intensity may lead to degradation of DNA in solution. It appears that cavitation is a prerequisite for this and that the damage is due to hydrodynamic shear stresses, free radical formation or excessive heating (Thacker, 1973; Miller and Thomas, 1995a, 1996). Such conditions are unlikely to pertain for diagnostic ultrasound exposures.

Considerable effort has been exerted in looking for ultrasonically induced chromosomal alterations and sister chromatid exchanges (SCEs). The vast majority of evidence is that ultrasound up to quite high intensities (100 W cm^{-2} I_{SPTP}) does not produce chromosomal damage [for comprehensive reviews, see Rott (1981) and EFSUMB (1994)]. There is, however, some indication that there may be some synergistic interaction producing chromosomal aberrations when 3 W cm^{-2} ultrasound (810 kHz) follows X-irradiation to 1 Gy but not when it precedes it (Kunze-Muhl, 1981).

Analysis of SCEs has frequently been used as an assay for the effect of potentially mutagenic agents on mammalian cells, although the implications for the cell or whole organism are poorly understood (Latt and Schreck, 1980; Gebhart, 1981). A report that diagnostic ultrasound may be able to produce SCEs *in vitro* (Liebeskind et al, 1979) stimulated a flurry of publications on this topic with a majority showing negative results even for intensities up to 3.0 W cm^{-2} (3.15 MHz, CW).

Although there has been the occasional report that ultrasound may produce chromosomal damage, none has ever been substantiated outside the laboratories of the original authors, and the majority of the most carefully documented studies on the subject have yielded negative reports (EFSUMB, 1994). Most of these studies have been carried out *in vitro*, where, as detailed earlier, interaction mechanisms need not necessarily be the same as those that will pertain in intact tissues *in vivo*. Conceivably, ultrasound could produce epigenetic changes, eg by modification of histone protein structure, which could have long-term effects on gene expression. However, as yet this possibility does not appear to have been investigated.

4.1.1.5 Functional changes

Ultrasound may stimulate or inhibit cellular function. Most functional changes involve interactions at the level of the cell membrane. For example, it has been reported that exposure to ultrasound (1 MHz, 10 W cm^{-2}, pulsed 20 μs – 10 ms, for over 2.5 minutes) may affect the electrophoretic mobility of cells (Taylor and Newman, 1972). This reflects a change in cell surface charge density, probably due to volume changes (Mummery, 1978). In this study, the absence of effect when the exposure was carried out under 170 kPa above ambient pressure suggested that cavitation was not the mechanism of action. However, these *in vitro* conditions have also been associated with cavitationally induced cell lysis (Joshi et al, 1973: 2 MHz, 10 W cm^{-2}).

Time-lapse photomicrography studies of cellular movements have revealed ultrasonically induced changes that may last for several generations of cells (Liebeskind et al, 1982). The implication of this finding for *in vivo* exposure to ultrasound is far from clear.

4.1.2 Animal studies

Small animal models have provided much valuable information about the potential biological effects of ultrasound. Biological systems studied include bone, the blood and vasculature and the lung, and effects on the embryo and fetus. These models have proved particularly useful in exploring the effects of different exposure parameters and consequences of the use of ultrasound contrast agents (UCAs). Some reservations have been expressed, however, concerning the relevance of findings in common laboratory animals to humans, due mainly to their differences in physical size. For example, the scan plane of an ultrasonic beam used in clinical practice to image a human embryo or fetus could result in a whole-body exposure of both the fetus and mother in some small animals. In addition, there are likely to be differences in the insonation times occurring in diagnostic examinations and those used in animal studies.

4.1.2.1 Bone

The main concern for ultrasound exposure of bone has historically been for the induction of pain due to heating of the highly innervated periosteum. This is most likely to be a problem for physiotherapy and hyperthermia treatments. It is also a potential problem with pulsed Doppler exposures at the maximum available output levels. In a conscious patient with normal pain sensitivity, providing that the exposure is stopped when discomfort is felt, no lasting damage is likely to have been caused. Biologically relevant temperature rises (over 2°C) have been recorded at the skull bone during the exposure of laboratory animals. Barrie Smith et al (2001) have shown that the high ultrasonic intensities (over 40 W cm^{-2}) used in thermal ablation therapies can indeed cause osteocyte damage and thermal necrosis.

It is now clear that very low ultrasound intensity levels (12–100 mW cm^{-2}) can affect bone regeneration, and this is used in the treatment of fracture healing (Claes and Willie, 2007). The stimulation is observed when ultrasound is applied during the soft callus formation phase and not for exposures during the remodelling phase. Effects seen are crucially dependent on the intensity used and are thought to arise predominantly from a non-thermal mechanism. Chang et al (2002) have shown that the temperature rise for intensities of 20–50 mW cm^{-2} was well below 1°C. Duarte et al (1983) also reported negligible temperature rise in rabbit fibula osteotomies following 50 mW cm^{-2} (15 minutes per day) treatment (0.01°C ± 0.005°C). However, small increases in temperature (below 1°C) have been shown to affect some enzymes such as matrix metalloproteinase 1, also known as interstitial collagenase or collagenase 1. This enzyme has been shown to be very sensitive to small variations in temperature (Welgus et al, 1981, 1985). However, the biophysical process by which bone regeneration is stimulated remains unknown.

Several studies have suggested that low intensity pulsed ultrasound may have a direct effect on cell membrane permeability (Dyson and Brookes, 1983; Mortimer and Dyson, 1988; Dinno et al, 1989; Ryaby et al, 1989, 1991; Rawool et al, 2003). These changes may result in an increase in capillary hydrostatic pressure leading to accelerated fracture healing (Rawool et al, 2003). It has been suggested that low ultrasonic pulse amplitudes may induce micromotion, producing mechanical stimulation. Such changes may represent an instance of Wolff's law, which states that a bone will adapt to the loads it is placed under (Wolff, 1891). The low energies used for fracture repair treatment cannot penetrate far into tissue. This may explain the inability of ultrasound to stimulate osteogenesis in intact bone (Elmer and Fleischer,

1974; Spadaro and Albanese, 1998; Wimsatt et al, 2000; Warden et al, 2001) or callus in the remodelling phase (Pilla et al, 1990; Wang et al, 1994; Hantes et al, 2004). Spadaro and Albanese (1998) showed that low levels of ultrasound applied for four weeks had no effect on the longitudinal growth or bone mineral density of the femur of four-week-old growing male rats. Similarly, Warden et al (2001) showed that treatment for twenty minutes per day, six days a week for twelve weeks had no effect on bone mineral content or bone mineral density within the distal femur or proximal tibia of either ovariectomised rats or normal rats following sham ovariectomy. These results suggest that the ultrasound treatment does not affect bone remodelling.

It was first suggested that ultrasound might stimulate osteogenesis 60 years ago (Buchtala, 1950). Maintz (1950) reported that histological and radiographic analysis of rabbit radial fractures showed minimal changes after ultrasound exposure at 0.5 W cm^{-2}, but reduced callus formation was observed at higher intensities (1, 1.5 and 2.5 W cm^{-2}). Early reports from the Mayo Clinic (Bender et al, 1954; Herrick et al, 1956; Ardan et al, 1957) using very high intensities (5–25 W cm^{-2}) showed delayed bone healing, necrosis and dense fibrous tissue formation in dog femora. High intensity ultrasound (0.2–3 W cm^{-2}) has also been shown to increase callus formation and to accelerate healing in fractures of rabbit radii (De Nunno, 1952; Corradi and Cozzolino, 1953) and tibiae (Klug et al, 1986) and in guinea pig ulnae (Murolo and Claudio, 1952), relative to untreated controls. Additionally, Chang et al (2002) have shown a 36% increase in new bone formation and an 80% increase in torsional stiffness of limbs stimulated with high intensity ultrasound (0.5 W cm^{-2}) compared with untreated limbs. These mixed results with high intensity ultrasound led to investigation of pulsed exposures and decreased output levels.

4.1.2.2 Blood and vasculature

The role of platelets in the vasculature is to respond to damage by release of factors that lead to thrombus formation. They are the most fragile components of blood and it has been shown that the shear stresses associated with ultrasound exposures *in vitro* can cause platelet activation. Miller et al (1979) showed that, in the presence of stable bubbles, spatial average intensities as low as 0.8 W cm^{-2} can lead to platelet disruption.

Erythrocytes appear to be more resistant to ultrasound damage than platelets. However, in the presence of inertial cavitation, haemolysis has been observed (Rooney, 1970; Williams et al, 1970; Wong and Watmough, 1980). Adenosine-5'-triphosphate (ATP) may be released at lower intensities in the presence of inertial cavitation (Williams and Miller, 1980).

Under normal conditions, cavitation is unlikely to occur in whole blood *in vivo* since the continual filtration of impurities reduces the likelihood of cavitation nuclei. However, Brayman et al (1996) have shown that cavitation may be induced at sufficiently high pressures (around 17 MPa). Damage to blood components has not been conclusively shown *in vivo* (Williams et al, 1977; Deng et al, 1996; Dalecki et al, 1997a; Poliachik et al, 1999). This may not be surprising since it is probable that only a small volume of cells would be affected, and this would rapidly be diluted by the flow of normal cells into the area. Dalecki et al (1997a) detected a clinically insignificant level of haemolysis (below 4%) when mice were exposed through the chest wall. At 2.35 MHz, 0.46% haemolysis was detected for a pressure amplitude of 10 MPa. The addition of gas-filled contrast agents can reduce this threshold to around 10 MPa

(Brayman et al, 1995; Ivey et al, 1995; Miller and Thomas, 1995a,b), still considerably in excess of the pressures found in commercial diagnostic scanners.

van Bavel (2007) has reviewed the potential effects of ultrasonically induced shear stresses on endothelial cells. The shear stress associated with normal blood flow provides a major stimulus for many endothelial responses. The streaming associated with radiation forces and microstreaming in an ultrasonic field induces shear stresses that are considerably higher than physiologically normal levels. They may therefore be expected to give rise to biological effects. This is especially true for microstreaming, since the associated shear stresses may be increased by several orders of magnitude and occur on membranes that may rupture or whose permeability may thus be altered. It seems possible that biological effects that cannot be explained by either thermal or cavitational mechanisms may be a result of these shear stresses. Miller (2004) has shown that lysis of erythrocytes by local shear stresses depends on cell volume. These data predict lysis at 800 Pa. Ohl and Wolfrum (2003) determined a shear stress threshold of 100–160 Pa for the detachment of cultured HeLa cells using a lithotripter. This seems appropriate also for endothelial cells *in vivo*.

It has been shown that ultrasound may induce haemorrhage close to fetal bone (Dalecki et al, 1999: 10 μs, pulse repetition frequency 100 Hz, 3 minute exposure, 1.2 MHz, peak positive pressure 4 MPa and peak rarefactional pressure 2.5 MPa). These authors attributed the effects seen to the relative motion between partially ossified bones and the surrounding tissues, which may result in damage to the fragile fetal blood vessels. Bigelow et al (2007) have studied this further and have postulated that thermal effects may also be involved. Haemorrhage has also been seen in the lung and intestine of mice at pressure levels greater than those found in diagnostic ultrasound devices (see above), but in other tissues these events are more often associated with ultrasound exposures in the presence of gas-filled microbubble contrast agents.

The microvascular effects of ultrasound were examined in isolated rabbit hearts by Ay et al (2001). A cardiac ultrasound system was operated at 1.8 MHz with 1 Hz triggering of image frames. Examinations at a mechanical index (MI) of 1.6 led to indications of capillary damage and erythrocyte extravasation. Potential injurious effects of an ultrasound contrast agent (UCA) – Optison or Definity – were examined in rat hearts *in vivo* (Chen et al, 2002). Imaging was performed at 1.3 MHz with ECG triggering every four cardiac cycles. Elevation of Troponin T in blood plasma, indicating myocardial damage, was detected after 30 minutes for MIs of 1.2 and 1.6.

Ultrasound contrast agents

The use of ultrasound contrast agents (UCAs) containing suspensions of stabilised bubbles is expected to increase the likelihood of causing biological effects through gas-body activation. Miller (2007) has reviewed the safety implications of UCAs.

In tissues, the lining of blood vessels is subject to interaction with UCAs. Brayman et al (1999) modelled the endothelial layer of blood vessels using fibroblast monolayers *in vitro*. Contrast agents in suspension adjacent to the monolayers increased damage and erosion of cells at 1.0, 2.1 and 3.5 MHz. Thresholds for cell erosion in this *in vitro* system were lower than for cell suspensions.

Miller et al (1997) explored the frequency dependence of haemolysis with Albunex (a first-generation UCA) in whole blood. Optison (a second-generation perfluorocarbon-based UCA) led to more haemolysis than the earlier UCAs, particularly for pulsed-mode exposure (Miller and Gies, 1998a). The enhancement of haemolysis was apparently due to the greater persistence between pulses of the perfluorocarbon-based gas bodies compared to the air-based gas bodies of earlier agents. The UCA gas bodies can serve to nucleate inertial cavitation (Miller and Thomas, 1995a). The amounts of ultrasonically induced haemolysis and of inertial cavitation activity showed a good correlation (Everbach et al, 1997; Miller et al, 2001; Chen et al, 2003a,b). Haemolysis induced by ultrasound has a strong dependence on frequency (Brayman et al, 1997; Miller et al, 2001, 2003). The biological effect decreases with frequency much faster than the inverse square root of frequency, and therefore the mechanical index has poor predictive value for the magnitude of this effect.

Injection of contrast agents may increase the risk of capillary rupture by diagnostic ultrasound. Miller and Quddus (2000) reported that the numbers of petechiae (capillary rupture with erythrocyte extravasation) were increased in the intestine and abdominal muscle of anaesthetised, hairless mice scanned using a 2.5 MHz transducer (610 ns pulses with 3.6 kHz repetition frequency and 61 Hz frame rate) after injection of Optison (5 ml kg^{-1}). The increase in petechiae was significant above 0.64 MPa for muscle (equivalent MI = 0.4) and 1 MPa for the intestine (equivalent MI = 0.63). Introducing delays between injection of contrast agent and scanning reduced the numbers of petechiae by about 33% after 200 s, and by 99% after 300 s.

The rat heart provides a convenient model system for exploring the range of potential biological effects of ultrasound and their dependence on dosage and exposure parameters. It has been used to examine microvascular injury (Li et al, 2003, 2004). A 1.7 MHz diagnostic ultrasound system was used to examine rats in a water bath under free-field conditions and with image frames triggered every fourth heartbeat. Bolus doses of three different UCAs were tested. Microvascular leakage was detected by injecting Evans blue dye before exposure and petechiae were counted on the heart surface. Microvascular effects increased in proportion to UCA concentration at low levels, but tended to level-off for high doses. Considered in terms of the volume dose, the effects were comparable for two of the agents (Optison and Imagent) but the third (Definity) produced more microvascular leakage. However, when expressed in terms of the number of gas bodies contained in the volume doses, there was no apparent difference between the three agents. Differences between the gases and stabilisation of the gas bodies in the three agents therefore appeared to have little impact on their potential to cause biological effects. Some microvascular leakage of Evans blue dye was evident at the lowest exposure tested, but other effects were not detected. Apparent thresholds were 0.4 MPa (equivalent MI = 0.31) for petechiae and 1.0 MPa (equivalent MI = 0.77) for premature ventricular contractions, with effects increasing rapidly for higher peak rarefactional pressure amplitudes. The vasoactive drugs propranolol and isoproterenol had little effect on the microvascular leakage, which suggests that it was primarily a mechanical effect (Miller et al, 2004). Samples taken a few minutes after exposure showed evidence of red blood cell extravasation and injured cardiomyocytes in histology. After 24 hours, microlesions with inflammatory cell infiltration were found scattered over the histological sections. After six weeks the microlesions apparently resolved to small fibrous regions interspersed with normal myocytes. This study showed that the delivery mode for

the contrast agent, as well as the dose and ultrasound exposure parameters, have a substantial influence on cardiomyocytes.

Small animal models are extremely valuable for extensive dose-ranging studies, but reservations are often expressed concerning the relevance of the findings to humans. For example, the ultrasound scan plane covers a substantial fraction of the entire rat heart. The occurrence of microvascular injury in rat mesentery was examined by Kobayashi et al (2002, 2003) using a phased array ultrasound system at 1.8 MHz. Endothelial cell damage was observed in capillaries and venules for all conditions at 0.82 MPa, and also at only 0.14 MPa at 30 Hz frame rate with 1.0 ml kg^{-1} (high dose) of Definity. Wible et al (2002) examined effects of contrast-enhanced diagnostic ultrasound in rat kidneys using Optison and several experimental agents. Diagnostic ultrasound probes with frequencies of 1.8, 4 and 6 MHz (with displayed MI values of 0.4 to 1.6) were placed on the rats. Glomerular capillary haemorrhage was induced from the glomerular tuft into Bowman's capsule and proximal convoluted tubules. Severity, scored by the visual appearance of the kidneys, decreased with increasing ultrasonic frequency, but was significant at 2.0 MPa at 4 MHz (equivalent MI = 1.0). The consequences of this effect were not evaluated, because it was thought to be unlikely to occur in humans. Shigeta et al (2004) reported that diagnostic ultrasound exposure of rat liver at 8 and 12 MHz with a UCA (Levovist) caused platelet aggregation in the liver sinusoids, and endothelial cell damage was seen in samples taken five hours after ultrasound exposure.

Stroick et al (2006) and Härdig et al (2003) have shown that ultrasound exposure in the presence of UCAs did not increase the extent of intracerebral haemorrhage in an experimental animal model. This is an important finding for the use of ultrasound to produce thrombolysis in ischaemic stroke. Vancraeynest et al (2006) studied ultrasound exposure of the rat heart using diagnostic imaging with an experimental UCA. This work confirmed the findings of histologically definable injury in rat hearts (Miller et al, 2005a,b). The microscale injury became so severe, when the parameters were elevated for therapeutic efficacy, that functional impairment of the heart was demonstrated (Vancraeynest et al, 2006).

The phenomenon of sonoporation has been used to induce transfer of DNA into cells that survive the membrane injury. This opens a potential application to gene therapy for diagnostic ultrasound. Studies of these therapeutic applications do not directly address the problem of the risks involved in diagnosis with UCAs and often involve specialised UCAs. Nevertheless, they indicate the substantial nature of the possible biological effects and address concerns about different outcomes between diagnostic and therapeutic applications of medical ultrasound.

4.1.2.3 Adult brain

The presence of the skull renders the brain a difficult organ to image with ultrasound. Despite this, transcranial Doppler is used for monitoring blood flow in the brain, and new techniques are being developed to aid drug delivery across the blood–brain barrier (BBB) using ultrasound and specially designed contrast agents. High intensity focused ultrasound (HIFU) technology is also being modified to allow transmission of a high powered focused beam through the skull to provide localised treatment.

There are no published studies about the safety of ultrasound in the adult brain, and so any conclusions that can be drawn must stem from the therapy literature. This has been reviewed by Meiars and Alonso (2007).

There is convincing evidence that ultrasound and stabilised microbubbles associated with UCAs can be used to open the BBB for targeted delivery of macromolecular agents to the brain. Possible mechanisms for this include transcytosis, passage through endothelial cell cytoplasmic openings, opening of tight junctions and free passage through injured endothelium. Although cavitation was first thought to be primarily responsible for opening the BBB, recent work has demonstrated disruption in the absence of indicators for inertial cavitation. Several studies have addressed the risks of this method for opening the BBB. Although relatively little tissue damage occurs at low acoustic intensities capable of opening the BBB, some BBB injury has been seen in every study in which ultrasound and UCAs have been used in combination.

HIFU has been shown to allow selective and non-destructive disruption of the BBB in rats (Mesiwala et al, 2002). If microbubbles are introduced into the bloodstream prior to focused exposure to ultrasound, the BBB can be opened transiently at the ultrasound focus without acute neuronal damage (Hynynen et al, 2001). Thus, the introduction of cavitation nuclei into the bloodstream can confine the ultrasound effects to the vasculature and reduce the intensity needed to produce BBB opening. This can diminish the risk of tissue damage. In most studies, BBB disruption has been confirmed using contrast-enhanced magnetic resonance imaging (MRI) at targeted locations (Hynynen et al, 2001; Kinoshita et al, 2006; McDannold et al, 2006) or with post-mortem histology (Mesiwala et al, 2002; McDannold et al, 2005).

The issue of the possibility of BBB disruption with ultrasound and UCAs has been addressed. In one study, the effect of peak rarefactional pressure amplitudes up to 3.1 MPa on rabbit brains was evaluated (Hynynen et al, 2005): 10 ms exposures with a frequency of 690 kHz, a repetition frequency of 1 Hz and exposure time of 20 s were used. Using contrast-enhanced MRI to detect localised effects after ultrasound exposure, BBB disruption was demonstrated at pressure amplitudes above 0.4 MPa. The histological findings four hours after exposure indicated that brain tissue necrosis was induced in approximately 70–80% of the sonicated locations at a pressure amplitude level of 2.3 MPa or higher. At lower pressure amplitudes, small areas of erythrocyte extravasation were seen. In another study, ultrasound exposures using a frequency of 1.63 MHz, a pulse length of 100 ms, pulse repetition frequency of 1 Hz and duration of 20 s with pressure amplitudes ranging from 0.7 to 1.0 MPa were performed in the brains of 24 rabbits (McDannold et al, 2005). Contrast-enhanced MRI was used to document BBB disruption. Whole brain was examined histologically using haematoxylin and eosin, vanadium acid fuchsin-toluidine blue (for ischemic neurons) and TUNEL (**t**erminal deoxynucleotidyl transferase d**U**TP **n**ick-**e**nd **l**abelling) (for apoptosis) staining. The study demonstrated that only a few cells in some of the sonicated areas showed evidence of ischemia or apoptosis. No ischemic or apoptotic regions, which would indicate a compromised blood supply, were detected. Importantly, no delayed effects were observed using either MRI or histology up to four weeks after sonication. These results demonstrate that ultrasonically induced BBB disruption is possible without inducing the substantial vascular damage that would result in ischemic or apoptotic death to neurons. However, the fact that red blood cell extravasation into tissue follows ultrasound exposure indicates that BBB injury has occurred and that the method cannot be considered to be completely harmless. This must be carefully taken into account when considering this technique for therapeutic applications for brain disease.

Other studies have addressed the question of whether burst mode ultrasound in the presence of a UCA using parameters similar to those used in diagnostic transcranial Doppler examinations in humans can

cause tissue damage. In one experiment, rabbit brains were exposed to 1.5 MHz, 10 μs bursts repeated at a frequency of 1 kHz at temporal peak acoustic pressure amplitudes ranging from 2 to 12.7 MPa for 20 s duration (Hynynen et al, 2003). Results of contrast-enhanced MRI and histological findings showed that brain tissue damage was induced at a pressure amplitude level of 6.3 MPa. This consisted of vascular wall damage, haemorrhage and, sometimes, necrosis. The authors observed occasional mild vascular damage in about 50% of the sonicated locations at all pressure values tested. However, no signs of ischemia or apoptosis were found. These results provide good evidence that ultrasound exposure levels currently used for blood flow measurements in the brain are below the threshold of BBB opening or brain tissue damage.

As described above, Stroick et al (2006) used transcranial ultrasound applied after experimental induction of intracranial haemorrhages (ICH) to rat brains for 30 minutes during a continuous intravenous infusion of sulfur hexafluoride microbubbles (SonoVue, a UCA) (2 MHz, peak negative pressure of 1051 kPa and spatial peak, temporal peak intensity of 37.3 W cm^{-2} measured at the site of application of ultrasound after attenuation through the skull bone). No significant effects were seen on haemorrhage size, on the extent of brain oedema or on the rate of apoptosis as compared to control rats with ICH but without exposure to ultrasound or microbubbles. These ultrasound exposure parameters are similar to those currently used for thrombolysis of clots in stroke patients.

In another study, ultrasound (2 MHz, peak negative pressure of 1051 kPa and spatial peak, temporal peak intensity of 37.3 W cm^{-2}) and a UCA (SonoVue) were applied in a middle cerebral artery occlusion model in rats to evaluate possible effects upon brain infarct volume, apoptosis, IL-6 and TNF-alpha levels, and on disruption of the BBB (Fatar et al, 2005). Interestingly, the results show that the infarct volume was significantly reduced in the group treated with ultrasound and microbubbles compared with control animals. Moreover, the levels of IL-6 and TNF-alpha concentrations (markers of tissue damage) were not significantly different. No additional BBB disruption was detected using trypan blue uptake. Similarly, there was no increase in apoptotic cell death outside the infarction area in animals treated with ultrasound and microbubbles. These results demonstrate that these treatments do not have an additional harmful effect in ischemic stroke using a middle cerebral artery occlusion model in the rat. The substantial reduction in brain infarction following insonation with ultrasound and microbubbles suggests they may have a novel neuroprotective role in ischemic stroke.

Vykhodtseva et al (2001) investigated the potential of low power focused ultrasound to cause apoptosis in the rabbit brain. Following recovery after surgical removal of a piece of the overlying skull, the brain was exposed to 1.7 MHz ultrasound for 30 s periods using a phase array transducer. MRI was used to estimate the threshold thermal dose and to monitor temperature increases during exposure. Necrosis was evaluated histologically and the TUNEL method was used to detect apoptosis. It was found that exposures close to the threshold for tissue damage resulted in an increase in the incidence of apoptotic cells over 48 hours, but the lesions were dominated by necrosis. These effects were extended in a subsequent study using a UCA (Vykhodtseva et al, 2006). In this study, the brain was exposed to CW or pulsed (500 ms) 1.5 MHz ultrasound for 10 or 20 s at 1.4–8.8 MPa (peak) following injection of Optison. Under these conditions, the lesions were dominated by apoptosis and the number of apoptotic cells was about six times more extensive than that of necrotic cells.

4.1.2.4 Lung

The lung is a delicate, well-vascularised organ containing a large number of air interfaces. As such, it should be considered to be potentially very susceptible to ultrasound damage. While there has been no indication of damage to adult or neonatal human lung, haemorrhage in mouse lung tissue as a result of ultrasound exposure (1.2 MHz, 10 μs pulse, I_{SPTA} 1 mW cm^{-2}, 3 minutes and a peak positive pressure of 0.7 MPa) was first reported by Child et al (1990). This has since been reported by a number of authors and has been observed in rodents, swine and monkeys (Penney et al, 1993; Frizzell et al, 1994, 2003; Tarantal and Canfield, 1994; Zachary and O'Brien, 1995; Baggs et al, 1996; Holland et al, 1996; Dalecki et al, 1997b,c; Kramer et al, 2001; Zachary et al, 2001; O'Brien et al, 2001a,b, 2002, 2003a,b, 2004, 2005, 2006a,b). Damage manifests itself as localised lesions located on the lung surface. Peri-alveolar capillaries are ruptured, thus allowing plasma proteins and erythrocytes to spill out into alveolar spaces. A similar phenomenon has been observed in the gas-containing intestine (see Section 4.1.2.5), but has never been seen in the absence of gas, and so does not present a hazard in, for example, the fetal lung. The biological significance of this observation is not known. It is certainly true that surface haemorrhages can be induced by coughing and that premature babies often have observable lung haemorrhages which are not considered adverse. The mechanism of action by which this effect is produced is not fully understood. One possible explanation is that the gas interface serves as a pressure release boundary which causes a reversal of the incident pressure pulse, leading to high local negative pressures. Church and O'Brien (2007) attempted to establish a safety index to describe this phenomenon. They demonstrated that the MI provided a poor fit to the threshold data from the published literature. They proposed that a better model would be a lung-specific index that relies upon the centre frequency, pulse repetition frequency (prf) and exposure duration.

The introduction of gas-filled ultrasound contrast agents might be expected to alter the threshold for induction of haemorrhage. However, Raeman et al (1997) have demonstrated that the presence of a UCA (Albunex) did not significantly increase the amount of haemorrhage observed in the mouse lung. Animals were exposed to 10 μs pulses of 1.15 MHz ultrasound for five minutes at a peak positive pressure of 2 MPa. The experiments indicated also that the threshold pressure for induction of the effect was not altered by the presence of the UCA.

4.1.2.5 Intestine

Although various sections of the intestine may be exposed during diagnostic imaging, few studies appear to have investigated the effects of ultrasound on the intestine *per se*. Following early observations by Lehmann and Herrick (1953), Dalecki and colleagues have examined the potential of ultrasound to cause vascular damage in the wall of the intestine. Dalecki et al (1995a) exposed C3H mice to pulsed (10 μs, 100 Hz) ultrasound (at 0.7–3.6 MHz) focused at several abdominal sites for five minutes each and observed areas of haemorrhage that depended on the level of exposure, above a threshold of about 1 MPa. Lower frequencies were more effective in producing haemorrhage than higher frequencies. Intestinal temperatures were raised a maximum of 1–2°C during exposure, suggesting the observed effects were primarily induced by gas-body activation. The threshold for ultrasonically induced haemorrhage was explored using a piezoelectric lithotripter, and found to be in the range of 1–3 MPa (Dalecki et al, 1995b). Further, very extensive haemorrhage was observed in the (gas-containing)

intestines of dams compared to an almost total absence of effect in the (gas-free) intestines of their fetuses (Dalecki et al, 1996). In this study, six pregnant mice and their 43 fetuses were exposed on day 18 of gestation to 200 lithotripter pulses at a peak pressure amplitude of 10 MPa delivered at about 1 Hz.

Miller and Gies (1998b) also reported that exposure to ultrasound may induce petechiae and haemorrhage in the intestine. Anaesthetised hairless mice were held in a temperature-controlled water bath and exposed to 0.4 MHz ultrasound for up to 1000 s. Thresholds were inversely related to exposure duration: for the longest continuous exposures at 37°C, the threshold for petechiae was found to be 0.28 MPa (2.6 W cm^{-2}) and that for haemorrhage was 0.65 MPa (14.2 W cm^{-2}). Using pulsed exposures or higher water bath temperatures affected both endpoints, but the threshold for petechiae induction in particular was increased using pulsed exposures, while the numbers of petechiae were enhanced at 42°C. Subsequently, it was reported that injection of a UCA increased numbers of petechiae and haemorrhages for both continuous and pulsed exposures (Miller and Gies, 1998c, 2000).

In a preliminary study, Stanton et al (2001) reported that diagnostic ultrasound could have an effect on the normal progression of epithelial cells in the crypts of the small intestine through the cell cycle. The anterior abdomen of adult CD1 mice was examined at 8 MHz for 15 minutes using B-mode and colour flow modes (TI = 1) and the small intestine was then excised and the distal portion examined histologically. It was found that the number of cells undergoing mitosis was significantly decreased at 4.5 hours after exposure and the number of apoptotic cells was significantly increased.

4.1.2.6 Embryo and fetus

The possibility that ultrasound may adversely affect the development of the embryo and fetus, and result in teratogenic effects, has been studied using a number of animal species and a wide range of exposure conditions. The relevant literature has been reviewed by, for example, Barnett et al (1997), Jensh and Brent (1999), Ziskin and Barnett (2001), Miller et al (2002), NCRP (2002) and Church and Miller (2007). The effects of exposure of the fetus to diagnostic ultrasound have also been examined by Abramowicz et al (2008) and Stratmeyer et al (2008). These reviews have been used to provide a framework against which the results of specific studies are considered in more detail. Deleterious outcomes range from subtle behavioural changes in juveniles and adults, through lowered birth weight and reduced growth, to embryonic and fetal death.

In addition, since exposure to ultrasound during pregnancy may induce localised hyperthermia, information can be derived from studies investigating the teratogenic effects of heat. These studies have been reviewed extensively by Miller and Ziskin (1989), Miller et al (2002) and Edwards et al (2003).

The developing brain and nervous system seem particularly sensitive to the effects of heat, but elevated maternal or fetal temperatures can result in a spectrum of adverse outcomes that affect many developing tissues. It is also possible that pulsed ultrasound could affect the integrity of maternal and developing tissues through non-thermal interactions, and especially by cavitational mechanisms in the presence of ultrasound contrast agents, although the likelihood of occurrence of these interactions for the embryo and fetus is low (Abramowicz, 2005; Sienkiewicz, 2007).

Effects of hyperthermia

It is well accepted that heat can be teratogenic to mammals. Many laboratory studies have investigated the effects of heat on the embryo and fetus in animals and possible pathogenic mechanisms (Miller and Ziskin 1989; Miller at al, 2002; Edwards et al, 2003). Of all the organs and tissues, the developing central nervous system is considered to demonstrate the greatest sensitivity to the effects of heat.

As summarised in Table 4.1, hyperthermia can cause a wide range of consequences, including embryonic death, growth retardation, internal and external abnormalities, developmental deficits and behavioural changes that persist into adulthood. The production of these effects largely depends on the interplay of three main parameters: the degree of elevation of normal core temperature, the duration of this rise in temperature and the particular stage of development (pre-implantation, organogenesis or fetal period) when heating occurred. The importance of the last factor should not be underestimated because the sensitivity of the embryo and fetus to heat varies greatly during development and depends on the particular cellular processes taking place, such as cell proliferation, differentiation or migration (Edwards et al, 2003). As an example, mild exposures during the pre-implantation period can often result in fetal losses, whereas severe exposures are required for these to occur later in gestation during embryonic and fetal development. Thus development can be seen to consist of a progression of various critical periods during which different tissues and organs show an increased sensitivity to heat. The teratogenic effects of heat at different developmental stages have been well described by Edwards et al (2003).

TABLE 4.1 Teratogenic effects of heat at different developmental stages (adapted from Edwards et al, 2003)

Developmental stage	Pre-implantation	Early embryo stage	Mid-embryo/ fetal stage	Fetal/postnatal growth
Biological events	–	Neural tube, eye, face, heart and vertebral formation	Neurogenesis, neural cell migration, development of organs body structures	Glial cell proliferation and myelination
Pathogenic mechanisms	–	Cell death, disruption of gene induction, cell membranes and neural crest migration, over growth of neural tissue	Cell death, disruption of gene induction of organogenesis, neural proliferation and migration, placental damage	Cell death, disruption of gene induction and glial cell proliferation, delayed myelination and vascular disruption
Adverse outcomes	Resorption, death, no defects	Abortion, neural tube defects, heart defects, vertebral defects, microphtalmia, coloboma, blindness	Abortion, stillbirth, microencephaly, behavioural deficits, seizures, abdominal wall defect, clubfoot, Moebius sequence, missing/small toes or teeth, cataract, change in muscle tone	–

The interaction between temperature and time to produce a given teratological or biological effect has led to the concept of the thermal dose (Sapareto and Dewy, 1984; Miller and Ziskin, 1989; Church and Miller, 2007). An analysis of experimental studies that provided empirical information about the lowest temperatures that produced teratological effects suggested that the relation between malformations and thermal dose was linear (see Miller et al, 2002). Thus the risk of a given defect may be increased in a shorter time by a higher temperature. However, there is some uncertainty about the temperature increase necessary to induce these effects: best estimates suggest a threshold range of about 1.5–2.5°C above normal body temperature for short-term exposure lasting up to an hour or so in pregnant animals maintained in laboratory-controlled conditions (Miller et al, 2002). In addition, temperature thresholds vary for each type of defect or malformation, and with strain and species.

Reproductive outcomes and prenatal development

Many studies have investigated the effects of pulsed and continuous-wave ultrasound on reproduction and the prenatal development of the embryo and fetus (Sikov, 1986; Jensh and Brent, 1999). Most of these studies have employed classical teratological techniques and assays to determine endpoints such as the viability of embryos and fetuses, the size and sex ratio of litters, the weight of offspring, and the incidence of internal and external abnormalities and malformations. Other studies have investigated specific effects on selected fetal tissues, such as the brain or testis. Most studies used rodent models.

Some studies have reported significant exposure-related effects in offspring, such as increased malformation rates or changes in weight at term, while other studies have not found any consistent effects in either dams or offspring (Table 4.2). A wide range of endpoints, gestational ages and exposure conditions has been used. This makes direct comparisons between studies sometimes problematic, and differences exist between species: for example, the mouse embryo generally appears more susceptible to insult by ultrasound than the rat. The overall evidence for adverse effects from exposure to ultrasound at diagnostic levels remains inconclusive.

Restraint of dams or the use of anaesthesia may cause adverse outcomes. Vorhees and colleagues used a conditioned immobility procedure to circumvent the need for either restraint or anaesthetic during repeated exposure of rats to either 3 MHz continuous-wave (Vorhees et al, 1991, 1994) or pulsed ultrasound (Fisher et al, 1994, 1996) at up to 30 W cm^{-2} (I_{SPTA}). No significant treatment-related effects were seen on reproductive outcome (including pre-implantation fetal losses), on maternal weight during gestation or lactation, on viability or weight of offspring, or on the incidence of skeletal or visceral malformations. While the methods used avoided some confounders, the possibility that the conditioning procedure itself may have affected the outcomes (perhaps by affecting thermoregulation) was not considered.

An extensive study by Tarantal and colleagues examined the teratological effects of repeated prenatal exposure of cynomolgus macaques to pulsed 7.5 MHz ultrasound (Tarantal and Hendrickx, 1989a,b; Tarantal et al, 1993). Animals were held in a primate chair and exposed at 0.28–12 mW cm^{-2} (I_{SPTA}) using a commercial real-time sector scanner from gestational day 21 to day 150. In addition to a few isolated or transient effects, statistically significant reductions in body weight of offspring were noted that persisted during the first three months of life, and non-significant reductions were noted for the following nine months. Such a lowering of normal body weight would be of great importance if found in humans.

TABLE 4.2 Effects of prenatal exposure to ultrasound on reproductive outcome and embryo and fetal development

Study	Species	Exposure and gestational age	Endpoints and postnatal age at test	Statistically significant outcomes	Comments
Murai et al (1975a)	Albino rat	2.3 MHz 20 mW cm^{-2} for 600 min on day 9	Maternal body weight during pregnancy, length of gestation, resorptions, mortality, weight	No significant effects	Restraint stress produced significant effects. Analysis not by litter, nor adjusted for effects of cross-fostering
Stolzenberg et al (1980)	CFW mouse	2 MHz, CW Spatial average 0.5 W cm^{-2} for 60–180 s or 1 W cm^{-2} for 40 or 60 s on day 8	Litter size, body weight on days 3, 10, 17 and 25; organ weights on day 25	Body weight in males and testicular and seminal vesicle weights increased on day 25 with exposure at 0.5 W cm^{-2} for 100 s. Body weight (both sexes) decreased on day 25 with exposure at 0.5 W cm^{-2} for 180 s; decreased uterine weight with exposure at 0.5 W cm^{-2} for 140 and 180 s, and at 1 W cm^{-2} for 60 s	–
O'Brien (1983)	CF_1 mouse	1 MHz, CW Spatial average 0.5–5.5 W cm^{-2} for 10–300 s on day 8	Fetal weight on day 18	Dose-dependent decrease in weight (5–17.5%)	Results from 272 litters
Kimmel et al (1983, 1989)	ICR mouse	1 MHz, CW or pulsed I_{SATA} 0.05, 0.5 or 1 W cm^{-2} I_{SPPA} 90 W cm^{-2}, for 2 min (CW) or 2 x10 min (pulsed) on day 8	Maternal body weight during pregnancy, numbers of live/dead implants/fetuses, sex ratio, external, visceral and skeletal malformations, weight, on gestational day 17	Intensity-related increase in lethality of dams. No significant effects on offspring	Gestational day 8 considered time of maximal sensitivity. Possible handling or anaesthetic effects on dams

TABLE 4.2 *Continued*

Study	Species	Exposure and gestational age	Endpoints and postnatal age at test	Statistically significant outcomes	Comments
Tarantal and Hendrickx (1989a,b), Tarantal et al (1993)	Cynomolgus macaque	7.5 MHz, pulsed I_{SPTA} 12 mW cm^{-2} (maximum output) 5 times per week on days 21–35 for 10 min, 3 times per week on days 36–60 for 10 min, and once per week on days 61–150 for 20 min	Simian Apgar scores at 1, 5 and 10 mins, physical and morphometric evaluation, gross malformations, weight haematology to 6 months, myeloid lineage maturation in bone marrow	Reduction in weight to 16 weeks; reduction in crown-rump length to 4 weeks ; lower white blood cell counts on gestational day 140 and on postnatal days 3 and 17	Majority of exposures preformed at I_{SPTA} 0.28 mW cm^{-2}. Some inconsistencies between studies
Norton et al (1991)	Sprague-Dawley CD rat	2.5 MHz, pulsed I_{SPTP} 0.78 W cm^{-2} for 30 min to each uterine horn on day 15	Maternal body weight resorptions, litter size, weight and growth	No significant effects	–
Vorhees et al (1991, 1994)	Sprague-Dawley CD rat	3 MHz, CW I_{SPTA} 2, 10, 20 or 30 W cm^{-2} for 10 min on days 4–19 or 20	Number of pregnancies, pre-implantation losses, resorptions, litter size, gestation length, sex ratio, mortality, weight, total, skeletal or visceral malformations	No significant effects when analysed by litter	Animals conditioned to remain immobile. Highest exposure calculated to induce a temperature of 46.3°C in fetus
Carnes et al (1991)	ICR:HD mouse	1 MHz, CW I_{SPTA} 0.5–10 W cm^{-2} for 30–400 s on day 9, 12 or 15	Fetal weight, mortality, sex ratio, external abnormalities, testis weight and histology on day 18	Decrease in body and testis weights, subtle changes in Sertoli and germ cells; dose- and time-related increase in stillborns/fetal resorptions (peak effects day 12)	Effects attributed to non-specific delays in fetal growth and mild hyperthermia

TABLE 4.2 *Continued*

Study	Species	Exposure and gestational age	Endpoints and postnatal age at test	Statistically significant outcomes	Comments
Hande and Devi (1992)	Swiss albino mouse	3.5 MHz ~65 mW for 10 min on day 3.5, 6.5 or 11.5	Prenatal mortality, body weight and size, microphthalmia, sex ratio on day 18	Reduction in fetal weight, day 3.5; increase in number of growth-retarded fetuses, reduction in weight and body length, day 6.5	Maternal core temperature increased to 38.5°C
Hande and Devi (1993)	Swiss albino mouse	3.5 MHz ~65 mW for 10 min on day 3.5, 6.5, 11.5 or 14.5	Litter size at birth, sex ratio at 4 weeks, postnatal mortality, body weight and size to 6 weeks	Decreased birth weight, day 3.5; marginal increase in mortality, day 6.5; transient growth reduction	Maternal vaginal temperature increased by up to ~1.3°C
Fisher et al (1994, 1996)	Sprague-Dawley CD rat	3 MHz, pulsed I_{SPPA} 40 W cm^{-2} I_{SPTA} 2, 20, 30 W cm^{-2} for 10 min on days 4–19 or 20	Number of pregnancies, pre-implantation losses resorptions, litter size, gestation length, sex ratio, mortality, fetal weight, total skeletal or visceral malformations	No significant effects when analysed by litter	Animals conditioned to remain immobile. Actual absorbed intensities 'much lower' than nominal
Devi et al (1995)	Swiss albino mouse	3.5 MHz 65 mW for 10, 20 or 30 min on day 14.5	Postnatal mortality, reflex development	No significant effects	Maternal vaginal temperature increased by up to ~1.3°C
Hande and Devi (1995)	Swiss albino mouse	3.5 MHz 65 mW for 10 min on days 6.5 and 11.5	Prenatal and postnatal mortality, growth at 3 and 6 months	Increased mortality, transient growth retardation	Maternal rectal temperature increased by up to ~1.3°C. Part of larger study with X-rays

TABLE 4.2 *Continued*

Study	Species	Exposure and gestational age	Endpoints and postnatal age at test	Statistically significant outcomes	Comments
Carnes et al (1995)	ICR:HD mouse	1 MHz, CW I_{SPTA} 1–10 W cm^{-2} for 20–200 s on day 9, 12 or 15	Weight, mortality, sex ratio at birth, testis weight and histology, daily sperm production (DSP) on day 50	Decreased litter size, and increased stillbirths and postpartum deaths, significant above 1125–1500 W^2 s cm^{-4}; reduced weight mainly gone by weaning. Decreased testis weight and volume, and DSP. Most consistent changes seen with day 9 exposure	Variability in sham-exposed controls, and only limited evidence of dose response
Harding et al (1996)	Merino sheep	3.5 MHz I_{SPPA} 28 or 58 W cm^{-2} I_{SPTA} 1.3 or 2.45 mW cm^{-2} for 440 min over 40 sessions of 10 or 20 min each, on days 42–140	Analysis of cord and maternal blood, biometry and weight of offspring on day 141 (term)	No effects on maternal weight gain during pregnancy, or on birth weight, organ weights, biometric parameters, bone mineral content or on haematology and blood biochemistry	Two diagnostic scanners used for exposures. Protocol based on that used by Tarantal and Hendrickx (1989a)
Oh et al (2000)	ICR mouse	7.5 MHz 4.2 mW I_{SPTA} 7.9 W cm^{-2} for 10 or 30 min on day 18	Frequency of apoptosis of neurons in external granular layer of the cerebellum using *in situ* end labelling technique 6 h post-exposure	Increase in apoptosis, suggestion of dose dependency, but no statistical analysis	No increase in maternal temperature. Comparable effects seen on intestinal crypt cells of adult mice. Part of a study comparing effectiveness of gamma rays
Ryo et al (2001)	Crj; CD-1, ICR mouse embryo	1.875 MHz, pulsed I_{SPTA} 2.96 W cm^{-2} for 1 or 5 min at two cell stage *in vitro*	Development and uptake of [^{3}H]-2-deoxy-D-glucose cultured blastocysts	Time-dependent effects attributable to procedure, no significant effects of exposure	Temperature maintained at 36–38°C

TABLE 4.2 *Continued*

Study	Species	Exposure and gestational age	Endpoints and postnatal age at test	Statistically significant outcomes	Comments
Gu et al (2002)	ICR mouse	1 MHz, CW I_{SPTA} 1 or 2 W cm^{-2} for 10 min 2 h post-conception on day 0	Post-implantation losses, litter size, sex ratio, weight, external malformations on day 18	No significant effects	Dams restrained but not anaesthetised; peak rectal temperature of ~40°C at 2 W cm^{-2}. Effects seen with 0.5 Gy gamma rays
Brown et al (2004)	CD 1 mouse	40 MHz I_{SPTP} 1.9 W cm^{-2} Doppler for 60 min or 2.6 mW cm^{-2} B-mode for 3 min on day 8.5 or 10.5 MI ~1 in B-mode	Gestation length, visceral and skeletal abnormalities, body and crown-rump length, weight on days 1, 8, 15 and 22, and organ weights on days 36–41	Decreased weight postnatal day 1 and days 36–41, decreased body length postnatal days 8–22 and days 36–41 with day 8.5 Doppler; decreased body weight and length postnatal days 1–22 with day 10.5 Doppler; decreased weight postnatal day 8, and decreased length from postnatal day 8 with day 10.5 B-mode	Frequency well above that used for diagnostic imaging. Dams anaesthetised and restrained, body temperature maintained between 35 and 37°C during exposure. Small numbers of litters/treatment (n = 5)
Jia et al (2005)	Sprague-Dawley rat	3.0 MHz Doppler for 30 min on day 14 MI = 1.6	Apoptosis of myocardial cells on day 15 or postnatal day 10	Increase in apoptosis after 24 h only observed using *in situ* TUNEL and electron microscopy	–
Karagöz et al (2007)	Sprague-Dawley rat	3.75 MHz $I_{SPTA,0.3}$ <720 mW cm^{-2} $I_{SPPA,0.3}$ ≤190 W cm^{-2} B-mode or Doppler for 20 min on day 3 and 7 MI ≤ 1.9	Activities of SOD, CAT and GSHPx, and level of TBARS in brain tissue on day 20	Increase in CAT both modes, increase in GSHPx and TBARS in Doppler mode	Exposures not well described. Results not analysed by litter

Brown et al (2004) investigated the effects of high frequency ultrasound associated with biomicroscopy on fetal development. Pregnant mice were exposed to 40 MHz in either Doppler or B-mode on day 8.5 or 10.5. Exposure did not affect length of gestation or litter size, nor did it increase the incidence of abnormalities in offspring; however, small, but significant, changes in weight and body length were reported.

Carnes et al (1991, 1995) reported that prenatal exposure to ultrasound reduced fetal weight and increased mortality in mice, and resulted in subtle, sometimes significant changes in the size and morphology of the testis in neonates and adults, which became manifest as reductions in testicular function in adults. Despite some internal inconsistencies, these results provide some evidence that short-term *in utero* exposure to ultrasound may be capable of causing lasting changes in male reproductive capacity.

Jia et al (2005) reported that exposure of fetal rats to diagnostic colour Doppler ultrasound for 30 minutes resulted in a short-term increase in apoptosis of myocardial cells.

Karagöz et al (2007) studied the effects of B-mode and Doppler ultrasound on the activities of three antioxidant enzymes and a lipid peroxidation endproduct in the fetal rat brain. Measured changes in superoxide dismutase (SOD) activity were not significant, but significant increases in activity were seen for catalase (CAT) in both modes, and for glutathione peroxidase (GSHPX) and thiobarbituric acid reactive substances (TBARS) in Doppler mode. These responses were taken by the authors as evidence of the benign nature of B-mode ultrasound on the brain and the potential for Doppler mode to cause harmful effects, possibly through an increase in free radicals generated by high temperatures.

Reflex ontogeny and physical development

The appearance of physical landmarks, such as eye opening or pinna detachment, and the acquisition of typical reflex behaviours are considered to be sensitive indicators of the normal sequence of development and maturation of the brain. For each species, these indexes show a well-defined and characteristic appearance and timing, so significant deviations from these norms can indicate the expression of underlying functional changes.

Several studies have investigated the effects of ultrasound on the development of various landmarks and reflexes (Table 4.3). Overall, no consistent pattern of exposure-related effects has been found (Jensh et al, 1994; Vorhees et al, 1994; Fisher et al, 1996), although a few studies have reported transient changes. For example, in a primate study, it was reported that repeated ultrasound examination of the fetus between gestational days 21 and 150 had no consistent effect on a variety of reflexes or on motor development, although muscle tone was transiently increased for the first few days of life (Tarantal and Hendrickx, 1989a; Tarantal et al, 1993).

Rao et al (2006) investigated the effects of diagnostic ultrasound given at different gestational days on postnatal growth and development in the mouse. Ultrasound was more likely to increase mortality and cause reductions in weight and growth when given during the late organogenesis and early fetal periods, corresponding to days 11–16, with greatest sensitivity seen on days 14 and 16 and lesser vulnerability seen in the late fetal period (days 17 and 18). In addition, exposures on all days induced significant and lasting reductions in head size: no other study appears to have investigated this endpoint.

The effect of stress caused by sustained immobilisation cannot easily be discounted in many of these studies. This was revealed in an early study using rats: Murai et al (1975a) reported that prenatal exposure to ultrasound caused a delay in the maturation of several reflexes, although only one (the grasp reflex) was significantly different from that shown by restrained, sham-exposed control animals.

Some studies suggest that prenatal insonation may affect brain function. Short-term and reversible changes in auditory brain stem responses were reported by Siddiqi et al (1988, 1990) following ultrasound exposure of near-term fetal and neonatal lambs. Suneetha and Kumar (1993) reported increased activities of acetylcholine (ACh), acetylcholinesterase (AChE) and gamma amino butyric acid (GABA) in the brains of fetal mice following short-term, repeated exposure during organogenesis.

However, Horder et al (1998a,b) found that brief, prenatal exposure of fetal guinea pigs, sufficient to cause a rise in temperature of around 1.5°C in the hypothalamus, and around 3°C in the parietal cortex, did not significantly alter fetal heart rate as measured by the ECG. Similarly, Duggan et al (1993) reported that exposure to low intensity pulsed ultrasound had no effect on the electrical activity of the brain in fetal sheep.

Juvenile and adult behaviours

Behavioural endpoints and tests of motor function are considered to be among the most sensitive indicators of teratogenic effects (Weiss and Laties, 1975; Geller et al, 1979). Postnatal functional evaluation therefore represents a very sensitive, non-invasive indicator of the potential teratogenic activity of ultrasound. Effects on postnatal behaviour following prenatal exposure to ultrasound have been evaluated in a number of studies using rodents and primates (Table 4.4).

There are conflicting reports of behavioural changes following prenatal exposures, with some studies reporting that fetal insonation can cause changes in adult behaviours while others have not observed significant effects. Many of these differences have been attributed to different methodologies and incomplete descriptions of exposure (Jensh and Brent, 1999; Rao et al, 2006). In particular, stress caused by immobilisation restraint of pregnant animals is known to be able to cause adverse developmental outcomes, although this caveat does not appear to apply to the restraint of anaesthetically induced unconscious animals. However, in the latter there may be confounding cardiovascular effects that affect tissue perfusion.

In an early study, Murai et al (1975b) reported that exposure of rats on gestational day 9 resulted in animals displaying increased emotional responses under stress. Restrained animals were exposed for 300 minutes to continuous-wave ultrasound at 2.3 MHz, with a spatial average, temporal average intensity of 20 mW cm^{-2}. Offspring were cross-fostered, and males tested as adults in an open field on an active avoidance task and a negatively reinforced visual discrimination task. Compared with sham-exposed controls, exposure significantly increased vocalisation during handling, increased the amount of time animals spent in the escape chamber during the avoidance task and reduced the numbers of crossings between chambers; other measured endpoints were not affected. However, the data were analysed by subject, not litter. The study design and methodology have been criticised by Vorhees et al (1994) who also questioned the biological significance of the results. Other early studies have been reviewed by Sikov (1986).

TABLE 4.3 Effects on prenatal exposure to ultrasound on the appearance of physical landmarks and simple reflexes

Study	Species	Exposure and gestational age	Endpoints and postnatal age at test	Statistically significant outcomes	Comments
Murai et al (1975a)	Albino rat	2.3 MHz 20 mW cm^{-2} for 600 min on day 9	Spontaneous movements, pinna detachment, incisor eruption, eye opening, righting reflex, negative geotaxis, cliff aversion, grasp reflex, visual placing, vibrissae placing, air righting	Delay in grasp (compared to sham exposures)	Restraint stress produced significant effects. Analysis not by litter, nor adjusted for effects of cross-fostering
Sikov et al (1977)	Rat	0.93 MHz, CW I_a 0.01–1 W cm^{-2} for 5 min on day 15	Pinna detachment, eye opening, incisor eruption, negative geotaxis, testes decent/vaginal opening, forepaw grasp, surface righting, air righting, posture, reflex suspension, from day 1	Transient delay in grasp reflex, air righting. Reduction in suspension by forefeet	Uterus surgically exteriorised during exposure under anaesthesia. No statistical tests
Siddiqi et al (1988, 1990)	Lambs	34.5 mW, pulsed I_{SPTA} 15.5 W cm^{-2} for 15 min on days 120–125, or 3 MHz, pulsed I_{SPTA} 22.1 W cm^{-2} for 15 min on postnatal days 0.5–6	Evoked auditory brain stem response at 5 and 15 min during, and 30 min post-exposure	Amplitude and latency changes during exposure only	Transient response. Frequency not specified generated by scanner used in clinical practice
Tarantal and Hendrickx (1989b), Tarantal et al (1993)	Cynomolgus macaque	7.5 MHz, pulsed I_{SPTA} 12 mW cm^{-2} (maximum output) 5 times per week on days 21–35 for 10 min, 3 times per week on days 36–60 for 10 min, and once per week on days 61–150 for 20 min	Standardised neurobehavioural test battery on days 1–10, 17 and 24	No significant effects on development of reflexes, muscle tone or motor development	Majority of exposures preformed at I_{SPTA} 0.28 mW cm^{-2}
Norton et al (1991)	Sprague-Dawley CD rat	2.5 MHz, pulsed I_{SPTP} 0.78 W cm^{-2} for 30 min to each uterine horn on day 14	Weight at birth, growth to 28 days negative geotaxis, reflex suspension on days 6–14	Suspension times increased	–

TABLE 4.3 *Continued*

Study	Species	Exposure and gestational age	Endpoints and postnatal age at test	Statistically significant outcomes	Comments
Suneetha and Kumar (1993)	Swiss-Webster mouse	875 kHz, CW 1 W cm^{-2} for 60 or 90 s on days 6–10	ACh, AChE, GABA activities in brain on day 10, 15 or 20	All activities increased compared to cage and sham-exposed controls	Skin surface temperature increased by ~2°C
Jensh et al (1994)	Wistar rat	5 MHz, pulsed 2. 5 mW I_{SPTP} 500 mW cm^{-2} and I_{SPTA} 24 mW cm^{-2} or I_{SPTP} 1500 mW cm^{-2} and I_{SPTA} 58 mW cm^{-2} for 35 min on days 15, 17 and 19	Weight at birth, growth to 77 days, surface righting, negative geotaxis, auditory startle, air righting, visual placing, pinna detachment, eye opening, testes descent/vaginal opening, from day 1	No significant effects at either intensity	No significant increase in maternal temperature during exposure
Vorhees et al (1994)	Sprague-Dawley CD rat	3 MHz, CW I_{SPTA} 2, 10, 20 or 30 W cm^{-2} for 10 min on days 4–20	Pinna detachment, incisor eruption, eye opening, testes decent/vaginal opening, from day 1, olfactory orientation on day 9, 11 or 13, air righting on days 15–18, acoustic startle on days 18–20	Increased orientation towards home cage scent on day 13 at 10 W cm^{-2}	Animals conditioned to remain immobile. Highest exposure calculated to induce a temperature of 46.3°C in fetus
Fisher et al (1996)	Sprague-Dawley CD rat	3 MHz, pulsed I_{SPPA} 40 W cm^{-2} I_{SPTA} 2, 20 or 30 W cm^{-2} for 10 min on days 4–20	Olfactory orientation on day 9, 11 or 13, acoustic startle on day 19	No significant effects	Animals conditioned to remain immobile. Actual absorbed intensities 'much lower' than nominal
Horder et al (1998a,b)	Guinea pigs	3.5 MHz, pulsed 240 mW I_{SPTA} 2.8 W cm^{-2} for 2 min on days 57–66	Temperature profile of fetal brain during *in vivo* exposure, ECG	Mean peak increases of 4.9 to 1.2°C from parietal cortex to midbrain, 1.5°C in hypothalamus, no effect on heart rate	Fetal heads clamped during exposures. Variable effects of perfusion

TABLE 4.4 Effects on juvenile and adult behaviour from prenatal exposure to ultrasound

Study	Species	Exposure and gestational age	Endpoints and postnatal age at test	Statistically significant outcomes	Comments
Tarantal and Hendrickx (1989b), Tarantal et al (1993)	Cynomolgus macaque	7.5 MHz, pulsed I_{SPTA} 12 mW cm^{-2} (maximum output) 5 times per week on days 21–35 for 10 min, 3 times per week on days 36–60 for 10 min, and once per week on days 61–150 for 20 min	Spontaneous motor behaviour days 4–95, object constancy day 20 to criterion, fine motor control day 76 to criterion, hand coordination day 83 to criterion, discrimination reversal learning 7–10 months	Increase in amount of siting or laying down in test cage weeks 1–5, more agitated during object constancy task	Majority of exposures performed at I_{SPTA} 0.28 mW cm^{-2}
Norton et al (1991)	Sprague-Dawley CD rat	2.5 MHz, pulsed I_{SPTP} 0.78 W cm^{-2} for 30 min to each uterine horn on day 14	Activity, exploration gait analysis on day 28	Narrower and longer gait	Water bath exposure
Hande et al (1993)	Swiss albino mouse	3.5 MHz, 65 mW for 10 min on day 11.5 or 14.5	Activity, learning and memory at 3 and 6 months	Decreased preference for activity in dark, deficits in learning passive avoidance task on day 14.5 at 6 months	Abdominal hair not removed during exposure
Vorhees et al (1994)	Sprague-Dawley CD rat	3 MHz, CW I_{SPTA} 2, 10, 20 or 30 W cm^{-2} for 10 min on days 4–20	Activity on day 20 and 60, passive avoidance on days 60 and 74, spatial learning on days 50–54, acoustic and tactile startle on day 75	Increased activity and time spent in corner regions of open field at 30 W cm^{-2}, increased errors in water maze at 30 W cm^{-2}	Animals conditioned to remain immobile. Highest exposure calculated to induce a temperature of 46.3°C in fetus
Hande and Devi (1995)	Swiss albino mouse	3.5 MHz 65 mW for 10 min on days 6.5 and 11.5	Activity, exploration, learning and memory at 3 and 6 months	Decreased activity in open field, deficits in learning passive avoidance task	Maternal rectal temperature increased by up to ~1.3°C Part of larger study with X-rays

TABLE 4.4 *Continued*

Study	Species	Exposure and gestational age	Endpoints and postnatal age at test	Statistically significant outcomes	Comments
Jensh et al (1995)	Wistar rat	5 MHz, pulsed 2. 5 mW I_{SPTP} 500 W cm^{-2} and I_{SPTA} 24 W cm^{-2} or I_{SPTP} 1500 W cm^{-2} and I_{SPTA} 58 W cm^{-2} for 35 min on days 15, 17 and 19	Activity, learning and memory at 90 days	Dose-dependent decrease in activity wheel, in light males only	Maternal temperatures did not rise more than 1°C. Highly controlled water bath exposure and very detailed dosimetry
Devi et al (1995)	Swiss albino mouse	3.5 MHz 65 mW for 10, 20 or 30 min on day 14.5	Activity, exploration, learning and memory at 3 months	Decreased activity in open field, increased exploration of hole board, deficits in conditioned avoidance	Maternal vaginal temperature increased by up to ~1.3°C
Fisher et al (1996)	Sprague-Dawley CD rat	3 MHz, pulsed I_{SPPA} 40 W cm^{-2} I_{SPTA} 2, 20 or 30 W cm^{-2} for 10 min on days 4–20	Olfactory orientation on day 9, 11 or 13, acoustic startle on day 19, activity on day 20 and 60, spatial learning on days 50–55, acoustic and tactile startle on day 75	Tactile startle response increased in males 2 W cm^{-2} on final trial block	Animals conditioned to remain immobile. Actual absorbed intensities 'much lower' than nominal
Suresh et al (1996)	Swiss albino mouse	3.5 MHz 65 mW for 10 min on day 14, 16 or 17	Activity, exploration (age not specified)	Decreased activity in open field, increased exploration of hole board	No significant increases in vaginal temperature
Suresh et al (2002)	Swiss albino mouse	3.5 MHz 65 mW for 10, 20 or 30 min on day 14	Activity, exploration, learning and memory at 4 and 12 months	Decreased activity in open field, increased exploration of hole board, deficits in conditioned avoidance	Maternal vaginal temperature increased by up to ~1.3°C

Tarantal and Hendrickx (1989b) reported that prenatal exposure of cynomolgus macaques to pulsed 7.5 MHz ultrasound resulted in a few short-term changes in behaviour. Animals were repeatedly exposed between gestational days 21 and 150 using a commercial real-time sector scanner and subjected to extensive tests to evaluate their behavioural state and motor skills during their first year. Exposure increased the amount of time animals spent quietly sitting on the floor of an observation cage and exposed animals appeared more agitated while performing one of the cognitive tasks. Otherwise exposure was without notable effect and, in particular, had no significant effect on discrimination reversal learning.

Norton et al (1991) found prenatal exposure to 2.5 MHz ultrasound had no effect on exploratory activity of juvenile rats as measured using a continuous corridor. Subtle changes in gait were observed, but these were similar to those seen in sham-exposed animals.

Using a protocol that was designed to eliminate the effects of restraint or the need for anaesthesia, Vorhees et al (1994) reported that prenatal exposure to continuous-wave ultrasound at 30 W cm^{-2} produced subtle changes in locomotor behaviour of adult rats and altered performance of a spatial learning task in a Cincinnati water maze principally by increasing the number of errors made. No consistent effects were seen on other behaviours or when using lower intensities. The highest intensity was calculated to induce peak fetal temperatures of about 46°C. In a companion study, Fisher et al (1996) reported that *in utero* exposure to pulsed ultrasound had no consistent effect on locomotor activity, startle response or learning in a water maze. Sex-related changes in many parameters were observed to be independent of treatment.

Jensh et al (1995) found prenatal exposures of rats during the late fetal period to 5 MHz pulse-echo ultrasound at 500 or 1500 W cm^{-2} did not affect adult behaviour in an open field, the performance of a water T-maze task, or the acquisition or retention of a conditioned avoidance response. Dose-dependent decreases in wheel running activity were found, but these were limited to males during the light phase.

In a series of studies, Devi and colleagues have found that exposure of mice to diagnostic levels of ultrasound from early organogenesis to the late fetal period can result in significant changes in adult behaviour, including effects on exploratory activity and deficits in learning conditioned responses (Hande and Devi, 1992, 1995; Hande et al, 1993; Devi et al, 1995; Suresh et al, 1996, 2002). In these studies, the appearance and severity of any effect depended on the day of gestation on which exposure took place, and were most pronounced during the early fetal period (gestational day 14). There were also suggestions that increasing the length of exposure (from 10 to 30 minutes) increased the magnitude of the behavioural change, but a clear dose response was not observed. Exposures resulted in modest increases in temperature, generally to less than 39°C, a rise of no more than 1.5°C. There were some subtle differences in outcomes between studies, but these were attributed to testing at different times of day. However, the studies were performed using a commercial obstetrics scanner (with a maximum acoustic output of I_{SPTP} 1 W cm^{-2} and I_{SATA} 240 mW cm^{-2}) and the exposure power (65 mW) was calculated using calorimetry (Hande and Devi, 1992). Nevertheless, it was concluded that lasting effects on the behaviour of mice are possible from brief diagnostic ultrasound exposure. Jensh and Brent (1999) questioned whether the slight changes in behaviour observed by Devi and colleagues had any biological significance.

Thus, although several studies indicate that many postnatal behaviours are not affected by even repeated prenatal exposure to ultrasound at diagnostic levels, there is some evidence that ultrasound may produce long-lasting functional effects and result in subtle deficits, particularly in cognitive behaviour. Whether these changes are attributable in any part to the effects of maternal restraint or anaesthesia, or to localised heating of the mothers or offspring, is unclear.

Cytoarchitectonics

The development of the cerebral cortex in mammals involves a complex and highly organised schedule of neuronal proliferation and migration. Various environmental agents and genetic factors are known to affect the development of the cerebral cortex (see, for example, Rakic, 2007, and Métin et al, 2008) but, as yet, very few studies have investigated the effects of prenatal exposure to ultrasound on this process.

Whilst shielding the left side of the abdomen, Norton et al (1990, 1991) exposed the right uterine horn of pregnant rats to pulsed 2.5 MHz ultrasound at 0.78 W cm^{-2} (I_{SPTP}) on day 15 of gestation. The fetal cortex was examined after 24 hours. Exposures of 10 or 20 minutes resulted in slight changes in cortical morphology, but the only significant effect was a marked increase in the nuclear area of neuroblasts in the developing cortical plate of fetuses from the shielded side of the uterus. Exposures of 30 minutes, which resulted in peak temperatures of the uterus between 39.7 and 41.1°C, resulted in more pronounced effects after 24 hours, with significant changes observed in fetuses on the exposed side: the area of the nuclei of the subventricular zone was increased; the number of mitoses in the ventricular zone was decreased; and the percentages of fetuses with pyknotic cells in the subventricular and interventricular zones, or macrophages in the ventricle, were increased. However, when offspring were examined on postnatal day 28, exposures of 30 minutes were not associated with changes in the thickness of each of the cortical layers, suggesting that exposure had not caused any long-term morphological changes.

More recently, in a comprehensive study, Rakic and colleagues reported that prenatal exposure to ultrasound may have subtle effects on neuronal migration in embryonic mice (Ang et al, 2006). Following injection on gestational day 16 with bromodeoxyuridine (BrdU) to label cells undergoing mitosis, unanaesthetised, pregnant mice were exposed to pulsed 6.7 MHz ultrasound, with a pulse duration of 0.2 ms and a scanning rate of 11 frames per second. The spatial peak, pulse average intensity (I_{SPPA}) was estimated to be about 1 W cm^{-2} at the position of the fetus (0.48 W cm^{-2} average, 1.31 W cm^{-2} peak).

Beginning 12 hours after BrdU injection, animals were exposed to diagnostic ultrasound for a single session of 5 or 15 minutes, or two sessions of 15 or 30 minutes (separated by an interval of 12 hours). Other animals were exposed for seven sessions of 30 minutes (with a 6 hour interval between each), or for 12 sessions of 35 minutes (separated by 4 hours), both of these schedules beginning 8 hours after injection. Overall, total exposure times ranged between 5 and 420 minutes. Complementary studies suggested that these exposures did not result in any significant change in maternal heart rate or core temperature.

On postnatal day 10, the brains of offspring were removed and cut into sections and the neurons that had incorporated BrdU were immunolabelled to allow their distribution to be determined within the layers of the (somatosensory) cortex. Three brain slices were taken from each offspring and two cortical sections were taken from each slice.

It was found that ultrasound exposure did not result in any differences in brain size or gross cortical cytoarchitecture. However, a highly significant and dose-dependent increase in dispersion of neurons was seen in the animals exposed to ultrasound for 30 minutes or more: the mean dispersion was 5% in the controls (measured as the portion of BrdU positive cells in the deeper cortical layers and adjacent white matter) and the dispersion increased to 9% for animals exposed for 60 minutes, and to 19% in animals exposed for 420 minutes. Specifically, the majority of BrdU positive cells in the controls were found in cortical layers II and III, whereas there were smaller numbers of cells in these layers in the exposed animals, and greater numbers of BrdU positive cells in the lower cortical layers and in the subcortical white matter. In addition, exposure was associated with the formation of ectopic layers of labelled cells close to the ventricle. There was evidence that the heterotopic neurons retained their cell class-specific characteristics.

It was concluded that these differences in dispersion resulted from a changed pattern of neuronal migration, and more neurons in the exposed animals had failed to migrate to their proper positions in the superficial cortical layers and so had remained in the deeper layers of the cortex compared with the controls (see Figure 4.1). The molecular or cellular mechanisms responsible for these changes were not explored.

The pregnant animals were held in position during exposure in this study using a cardboard tube to limit their movements. The effects of prolonged restraint in this system had an effect on migration, because dispersion was significantly increased in the sham-exposed animals restrained for 420 minutes (mean dispersion of 13%) compared with equivalent values for animals held for shorter periods (mean dispersion values between about 3 and 6%). Thus maternal stress was considered to have had an indirect effect on dispersion and to have caused effects comparable in magnitude to those produced by ultrasound. Further evidence of possible maternal stress was provided by the observation that some offspring were either resorbed *in utero* or cannibalised at birth following exposures of 420 minutes and no offspring survived to postnatal day 10 following exposures of 600 minutes.

However, some caveats exist concerning the statistical methods used to analyse these data*. Further commentaries on this study are provided by Caviness and Grant (2006) and Gressens and Hüppi (2007). Of interest, Hinoue et al (2001) heated pregnant mice using a water bath to 43°C for 12.5 minutes on day 13.5 or 14.5 of gestation and reported that the proliferation of neuronal precursor cells in the brains of fetuses was suppressed and that their migration to the cortical plate was reduced.

* The t-test that was used by Ang and colleagues to compare dispersions does not take account of their absolute values or their restricted range, and the use of repeated pair-wise comparisons should usually be avoided, if possible. It would have been preferable to have performed a more sophisticated generalised linear regression (GLR) analysis based on the binomial distribution. This analysis could have been performed using the measure of dispersion reported in the paper, or it might have been more informative if dispersion was defined as the probability that a cell attains the correct position only if it is present in cortical layers II or III. Statistical models could have been fitted to examine the effects of modifying factors such as duration, dose or treatment group. It would also have been possible to have tested if a trend in the probability of attaining a correct position with dose or duration was detectable: the authors mentioned that they considered a trend in dispersion with duration and dose but provided no quantitative results. For either measure of dispersion, a GLR analysis would be more sensitive as the distributional assumptions would be more appropriate. The *post hoc* test used for comparing the sham-exposed and unrestrained controls would also be more reliable if it was susbstituted for tests designed to compare proportions rather than normally distributed means.

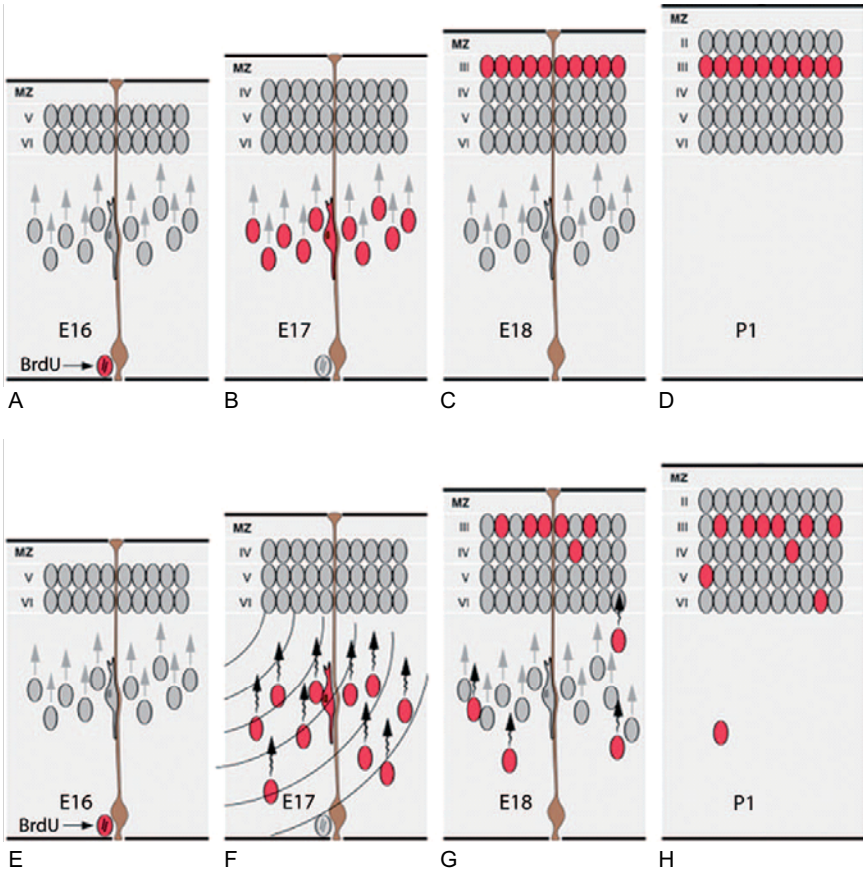

FIGURE 4.1 Progression of neuronal migration to the superficial cortical layers in the normal mouse
A–D: Most cells labelled with BrdU at gestational day 16 (E16) arrive in the cortex by gestational day 18 (E18), and, by postnatal day 1 (P1), those cells become surpassed by subsequently generated neurons. Eventually, these cells will settle predominantly in layers II and III of the cerebrum
E–H: Model of the ultrasound effect – when cells generated at E16 are exposed to ultrasound, they slow down on gestational day 17 (E17) and some remain in the white matter or are stacked in the deeper cortical layers (Reproduced from Ang et al (2006), Prenatal exposure to ultrasonic waves impacts neuronal migration in mice, *PNAS*, 103(34), 12903–10, © 2006 National Academy of Sciences, USA)

Overall, Ang et al (2006) suggest that exposure to diagnostic ultrasound is capable of altering neuronal migration in the developing mouse cortex, with increasingly long exposures above a threshold of about 30 minutes causing greater effects. The observed changes also seem to be an enhancement of processes that otherwise occur naturally, and that are sensitive to manipulation by other factors. It is possible that the heterotopic neurons would not persist into adulthood, being eliminated by apoptotic mechanisms during the further development of the cortex. Hence is not clear whether these changes

have any functional significance or if they are consistent with the alterations in juvenile and adult behaviour that have been reported in some other animal studies.

The results of this study in mice are important because there is the possibility that qualitatively similar effects could occur in humans: the same tissues in different species tend to exhibit the same acoustic properties, suggesting the same interactions will occur. However, direct extrapolation to humans is problematic, as recognised by Ang et al (2006), due in part to the obvious differences that exist between the developing cerebral cortex in rodents and humans in terms of numbers of neurons and the time and complexity of their migratory processes. This limitation applies to all studies using animal models. Further, the interaction mechanism by which the effects were caused was not identified, but was assumed to be non-thermal. Finally, the current concerns that even moderate challenges during prenatal development – for example, in maternal diet, exercise or stress levels – can have long-lasting and delayed effects on the offspring (Gluckman and Hanson, 2007) might extend to obstetric ultrasound. Such effects need not necessarily be accompanied by reduction in fetal growth or obvious teratogenic effects, but nonetheless might alter normal development.

4.1.3 Summary of biological effects of ultrasound

There is a considerable body of published work concerned with the interaction of ultrasound with biological systems. However, few of these studies have been directed at providing basic information on the biological responses that are necessary to provide an understanding of the health effects of diagnostic ultrasound. Particularly in the early work, it was rare that clinical devices were used to provide the required ultrasound exposures. The main thrust has been towards therapeutic applications and thus concentration has been on biological endpoints that are useful for these.

In surveying the relevant literature, it is important to distinguish between ultrasound exposures that are carried out with therapeutic intent and those designed to yield diagnostic information. Clearly, the aim of ultrasound imaging is to obtain the required information from tissue without causing biologically significant cellular effects, while for therapy beneficial changes, reversible or irreversible depending on the treatment goal, are sought. An important consideration in deriving this information from therapy exposures is the difference in ultrasound exposure regimens that are used. Therapeutic ultrasound usually employs continuous-wave or tone burst (10 ms – 10 s and a pulse repetition frequency around 1–20 Hz) exposures, whereas diagnostic ultrasound imaging uses microsecond (μs) pulses at pulse repetition frequencies in the kilohertz (kHz) range, although continuous wave is also used for Doppler-based analysis of blood flow patterns. These different pulsing regimens may alter the balance of importance of thermal and non-thermal mechanisms for exposures of otherwise identical total energy.

In this context, it must be remembered that a biological effect that is viewed as an advantage from a therapeutic standpoint may constitute a hazard under different circumstances, such as in obstetric diagnostic applications. Nonetheless, it is possible to extract some relevant information from these studies and to use this to help establish future directions for further research. However, it is possible that more subtle biological effects that may give rise to adverse events, especially in the developing fetus or embryo, may be missed by such an approach. Modern biology has some powerful technologies to

reveal changes in physiology and biochemistry at the cellular and molecular levels, which will be required to investigate these possibilities; however, the use of these techniques is not yet common in studies with ultrasound.

Despite the emphasis on therapy, studies investigating the biological effects of ultrasound have been carried out in a variety of *in vitro* and *in vivo* models. Studies with cells in culture under closely defined exposure conditions have provided much useful information, as have studies with pregnant animals concerned with effects on the embryo and fetus. Ultrasonically induced hyperthermia, raising temperatures by between 2 and 4°C, can cause damage and necrosis to cells and tissues, and result in teratogenic effects.

Exposure of cells in suspension can lead to lysis, primarily through cavitational mechanisms. Provided that cells remain intact, ultrasound does not affect their reproductive ability, unless the cells are also heated during exposure. Ultrasound may induce long-lasting changes in the permeability of the plasma membrane and may also cause damage to the nuclear, mitochondria and granular endoplasmic reticulum membranes, most likely through cavitation.

Ultrasound of sufficiently high intensity may lead to degradation of DNA in solution and cavitation appears to be a prerequisite for this, the damage being due to hydrodynamic shear stresses, free radical formation or excessive heating. However, ultrasound does not produce consistent cytogenetic effects *in vitro*.

Low ultrasound intensity levels are able to affect bone regeneration and fracture repair (but not bone remodelling), although the biophysical process responsible for these effects remains largely unknown it may involve changes in cell membrane permeability. For this application, pulsed exposures with decreased output levels seem more effective than high intensity ultrasound.

Other studies have investigated the effects of ultrasound on blood and circulatory system. Platelet disruption can occur through shear stresses, and cavitation may disrupt erythrocytes in the presence of an ultrasound contrast agent (UCA). Haemolysis induced by ultrasound has a strong dependence on frequency. Endothelial cells may also be damaged due to shear stresses associated with microstreaming, and possibly through thermal effects and cavitational mechanisms. Long-lasting injury to heart myocytes has been seen in the presence of UCAs at diagnostic levels, with functional impairment of the heart seen at therapeutic levels.

The effects of ultrasound on the integrity of the blood–brain barrier (BBB) have been investigated. There is convincing evidence that ultrasound and the stabilised microbubbles associated with UCAs can be used to open the BBB. Possible candidate mechanisms include transcytosis and opening of tight junctions, although investigations aimed at determining how ultrasound and microbubbles interact at the molecular level of the BBB are still needed. Selective and non-destructive disruption of the BBB may be induced following the introduction of cavitation nuclei (in the form of UCAs) into the blood, although there is no evidence of adverse actions of UCAs at the exposure levels of current ultrasound equipment used for transcranial investigations.

The lung is considered at risk of ultrasound damage. Haemorrhage of the lung resulting from rupture of peri-alveolar capillaries has been seen in a number of animal studies. The mechanism by which this effect

is produced is not fully understood. The presence of a UCA did not alter the amount of haemorrhage observed in the mouse lung.

Few studies have investigated effects on the intestine, but one preliminary study has suggested that diagnostic ultrasound may affect cell turnover and apoptosis. Other studies suggest that ultrasound may cause heating or activate gas in the intestine to induce petechiae and haemorrhage.

Regarding possible effects on the embryo and fetus, most studies have reported that exposure to ultrasound does not produce adverse events in the absence of overt heating; however, a few studies have reported increased malformation rates or transient changes in weight, and there is some evidence that exposure may result in changes in cognitive behaviour of juveniles and adults. The stage of development is an important factor in the assessment of the effect of ultrasound. Of potential importance is the suggestion that prenatal exposure to diagnostic ultrasound may alter neuronal migration in the developing mouse cortex, with longer exposures causing greater effects. Although the functional significance of these results has yet to be fully assessed, they indicate that prenatal ultrasound exposure may induce subtle changes in the structural organisation of the brain that could result in long-lasting or even deleterious outcomes.

4.2 Infrasound

The biological effects of infrasound have not been extensively studied. Most laboratory studies have investigated short-term exposures to high intensities, and there is a general lack of high quality research on the biological effects of exposure to long-term, low intensity infrasound. In addition, many published studies only have an abstract in English, while other studies are only available from proceedings of conferences. Nevertheless it is clear that much of what has been written about the effects of infrasound by the media and in popular books is incorrect and misleading.

Previous reviews of the biological effects of infrasound include those by Haneke et al (2001), Leventhall et al (2003) and Jauchem and Cook (2007). Low frequency acoustic sources typically produce both infrasound and low frequency noise. Therefore, some studies that also used frequencies above 20 Hz have been included here.

4.2.1 Cellular studies

Very few studies appear to have investigated the effects of infrasound *in vitro*. Yount et al (2004) reported that infrasound did not affect the colony forming ability of a human glioma cell line, SF210, either alone or in combination with 2 Gy of X-rays. However, there was a highly significant decrease in the number of colonies when infrasound was applied with 5-fluorouracil, a chemotherapy agent. In these pilot experiments, cell cultures were exposed to 8–14 Hz at 72–79 dB for ten minutes eight times a day for two days using a qigong device that is used in traditional Chinese medicine. Wang et al (2005) reported that the appearance of the surface of L929 cells became smooth following exposure to 16 Hz at 130 dB for two hours each day for three days.

4.2.2 Animal studies

Experimental studies have been mainly performed with rats, mice, guinea pigs and chinchillas. Most of these involved acute or short-term term exposures to infrasound lasting from a few minutes to a few months, and many used high intensities, of about 100 dB or more. Biological endpoints studied include effects on vestibular and auditory function, and effects on morphology and histopathology. The long-term effects of infrasound on animals have not been much addressed. No large-scale studies (comparable to those performed by the National Toxicology Program in the USA, http://ntp.niehs.nih.gov) appear to have investigated whether exposure is associated with the initiation or development of any kind of tumour or leukaemia, and no multigenerational studies have investigated effects on reproduction and development.

4.2.2.1 Hearing and vestibular function

In a series of experimental studies, Harding and colleagues have investigated the various effects of noise on cochlear function in animals. Recently they reported that exposure to infrasound had minimal effects alone but increased the damaging effects associated with intense audible noise (Harding et al, 2007). Chinchillas were exposed to a 30 Hz tone at 100 dB and a 4 kHz octave band noise at 108 dB for 105 minutes. Distortion product otoacoustic emission (DPOAE) level shifts and auditory brain stem response threshold shifts were determined before and after exposure, and losses of inner hair cells and outer hair cells were measured histologically. It was suggested that infrasound increased cochlear damage because the large fluid movements induced by infrasound exposure (as described by Salt and DeMott, 1999) caused more intermixing of cochlear fluids through the noise-damaged reticular lamina. Exposure of infrasound and a moderate noise (86 dB for 24 hours) did not cause these effects, nor did exposure to the intense noise following attenuation caused by tympanic perforation. Taenaka (1989) reported that exposure of guinea pigs to 15 Hz at 135–140 dB for 24–72 hours had no effect on the endocochlear potential or on cochlear microphonics.

Some studies have reported exposure to infrasound may cause effects on the ear. An intensity-dependent increase in overt damage to the cochlear and tympanic membrane was reported by Lim et al (1982) following acute exposure to infrasound. Chinchillas were exposed for 7.5–10 minutes to 1, 10 or 20 Hz infrasound at 150, 160 or 170 dB. In addition, the amount of damage observed histologically decreased with increasing frequency. Hiraide et al (1985) reported a threshold of 133 dB to induce damage to the cochlear in guinea pigs. Animals were exposed for one hour to 1, 10 or 20 Hz at 120–163 dB and the cochlear was examined 14 days later under a scanning electron microscope. Hair cell damage and globus formation of the tectorial membrane of the apical turn of the cochlea were revealed. However, exposure was not associated with damage to any other structures of the ear (Hiraide et al, 1987). Feng et al (2001) reported that exposure of guinea pigs to 8 Hz infrasound at 135 dB for 90 minutes resulted in reduced amplitudes of DPOAE and ultrastructural changes to the inner ear.

4.2.2.2 Morphological effects

The morphological effects of exposure to low frequency noise, including infrasound, on rats have been studied by Castelo Branco and colleagues (reviewed by Alves-Pereira and Castelo Branco, 2007, and Branco et al, 2007). Exposure-related effects, characterised by the abnormal growth of extracellular

elastin and collagen, have been reported in various tissues, including larger blood and lymphatic vessels (Martins dos Santos et al, 2002, 2004) and especially in the respiratory system (De Sousa Pereira et al, 1999; Grande et al, 1999; Castelo Branco et al, 2003a, 2004a,b), where a fusing of the microvilli of the brush cells of the bronchial epithelium has been observed. The latter appears to be transient and reversible, and any changes gradually disappear within seven days following termination of infrasound. Exposure-induced degenerative lesions and functional changes have also been observed in the gastric epithelium (da Fonseca et al, 2006) and the parotid gland (Oliveira et al, 2007).

In addition, effects on the ciliated cells of the tracheal epithelium were observed following *in utero* and postnatal exposure of rats (Oliveira et al, 2002; Castelo Branco et al, 2003b).

Animals in these studies (usually consisting of a small treatment group of between five and twenty rats) were exposed to low frequency noise at frequencies of 0–500 Hz at above 90 dB (A-weighted values are less meaningful here due to the spectral characteristics of the noise) for periods of about one to three months or longer, with exposures lasting eight hours per day, five days per week. However, the highest levels were from frequencies above 100 Hz, and the actual intensity of infrasound in the noise is uncertain. Castelo Branco and colleagues suggest that low frequency noise generates a mechanical signal that particularly affects the cytoskeleton. This results in changes to the cell signalling pathways and so produces the observed morphological and functional changes, resulting in an increase in the structural integrity of exposed tissues. Empirical evidence for these suggestions is lacking.

A few studies appear to provide some general support for these results. For example, Nekhoroshev and Glinchikov (1990, 1992) reported morphological and pathological changes in the ear, cardiovascular system, liver and other organs typically following exposures to 4–16 Hz at up to 140 dB for up to four months: see Haneke et al (2001) and Leventhall et al (2003) for a description and review of these and other relevant studies.

4.2.2.3 Cardiovascular effects

Pei et al (2007) investigated the effects of infrasound exposure of 5 Hz at 130 dB on cardiac ultrastructure and function in rats. Rats were exposed for two hours per day for up to 14 days, and time-dependent increases in systolic and diastolic pressure were reported along with cellular and molecular changes in cardiac cells.

Nishimura (1988) reported that exposure of rats to 16 Hz at 120 dB for 20 minutes resulted in changes in plasma adrenocorticotropic hormone (ACTH) and corticosterone. Exposure also resulted in significant reductions in gastric mucosal blood flow.

4.2.2.4 Neurobehavioural effects

A few studies suggest that acute exposure to high intensity infrasound may affect brain neurochemistry. Guo-You et al (1999) found exposure to 8 or 16 Hz at 120 dB (but not at 90 dB) for two hours resulted in significant increases in glutamate concentrations in rat brain. Spyraki et al (1978) found modest but significant reductions in norepinephrine concentrations in rat brain immediately following exposure for one hour to 7 Hz at 122 dB, or 16 Hz at 124 dB, but not following exposure to 2 Hz at 105 dB. A subsequent experiment (Spyraki et al, 1980) found that concentrations of norepinephrine and

dopamine were significantly reduced following repeated exposure to infrasound: animals were exposed for one hour a day for four consecutive days to 16 Hz at 124 dB. Treatment with diazepam (5 mg per kg ip) increased the effect on dopamine but significantly increased the concentration of norepinephrine.

Exposure to high intensity infrasound has been reported to affect locomotor activity. Petounis et al (1977a) reported that spontaneous activity and exploration were generally reduced in female rats during a two-hour exposure period to 2–16 Hz infrasound at 104–124 dB. Exposure was also associated with a pronounced hypnotic effect. Spyraki et al (1980) reported that activity was unaffected following exposure of male rats to 16 Hz infrasound at 124 dB for one hour a day for four days. Animals were tested 24 hours after the last exposure.

Yamamura and Kishi (1980) investigated the effects of infrasound on Rota-rod performance in rats: this test measures the length of time an animal is able to remain on a rotating cylinder by running. Rats were exposed to 16 Hz infrasound at 75–105 dB for durations of 70–150 minutes. The mean running times of the six best performing animals (determined in ten trials before exposure) were unaffected by exposure to infrasound, but the times of the six poorest performing animals were reduced at 95 dB and above. There was a suggestion that the deficits increased with length of exposure. Exposure at 105 dB was accompanied by an audible noise, but exposure to pink noise (250 Hz – 8 kHz) at this level (72 dB) for 70 minutes did not result in any changes in performance. Pink noise is a random-like signal in which power is proportional to the inverse of frequency.

Lehmann and Busnel (1979) used a forced swimming test to assess the effects of combined effects of alcohol and infrasound (6–50 Hz at 100–106 dB for two hours) on mice. No effects on latency to submersion were seen with either treatment alone. However, when alcohol was administered (by gavage) shortly before exposure to infrasound, swimming times were significantly reduced; this was attributed to an initial increase in activity leading to a more rapid fatigue. Normal mice and deaf mutants showed similar results, suggesting the involvement of non-auditory mechanisms. Audible noise (10 kHz at 60 dB) did not produce this interaction. Previously, Busnel and Lehmann (1978) had found that exposure to infrasound alone could reduce swimming times in both hearing and deaf mice above a threshold of 106 dB at 50 Hz and 112–115 dB for lower frequencies.

In addition, Petounis et al (1977b) reported that exposure to 2 Hz infrasound at 104 dB resulted in significant deficits in the acquisition and short-term retention of a conditioned avoidance response, particularly to an auditory stimulus, with exposed animals showing significantly increased latencies to avoid and escape. In contrast to controls that showed a consistent improvement of an avoidance response, rats exposed to infrasound maintained the same level of performance across four training sessions (Spyraki et al, 1980). Animals were exposed to 16 Hz infrasound at 124 dB for one hour immediately after each session.

Short-term exposure of rhesus monkeys to high intensity infrasound was reported by Sherry et al (2008) to induce significant decrements in the performance of an over-trained, continuous compensatory tracking task. Using a custom-made test system, five adult males were exposed to 10 Hz at 160 dB for 75–509 s whilst held in a primate chair. The chair was randomly rotated in pitch, and the task required the animals to manipulate a joystick to negate these perturbations and maintain their balance. Deviations in pitch above a set threshold produced a mild electric shock. Compared with unexposed baseline values,

infrasound resulted in large excursions in the position of the chair and a concomitant increase in the numbers of shocks delivered, with performance quickly returning to baseline values after cessation of exposure. Neither longer exposures nor repeated exposures appeared to cause greater deficits in performance, although the experimental design was not optimal to test this. No effects were seen on cochlear or hearing function as assessed by tympanometry, DPOAE or auditory brain stem responses either immediately after exposure or 24 hours later. No mechanism could be offered to explain the disruption in behaviour.

Finally, infrasonically induced phonophoresis has been described in the rabbit eye (Filatov, 2001, 2005) following acute, repeated exposure to intense infrasound (173 dB at 4 Hz).

4.2.3 Summary of biological effects of infrasound

The biological effects of infrasound have not been extensively investigated and some possibilities and uncertainties remain to be explored, particularly concerning the effects of long-term, low level exposures. *In vitro* techniques do not appear to offer a particularly suitable approach and too few cellular studies have been performed to reach a meaningful conclusion. Animal studies have reported that exposure to infrasound may cause biological effects, but these changes mostly occurred only after exposures to infrasound at levels generally above 100 dB, while exposures above about 140 dB may result in loss of hearing or damage to the ear. It is possible that infrasound may potentiate the damaging effects of high intensity audible noise. Very few animal studies have investigated the effects of long-term exposure. One group has consistently reported morphological and functional changes in epithelial tissues and endothelial cells following *in utero* or adult exposures of rats to low frequency noise which appear to result in an increased resistance to mechanical stress. These data are very suggestive, but have not been independently replicated, and the relative contributions of infrasound and audible noise to the results remain unclear. Too few studies have investigated potential effects on the brain to allow statements to be made regarding effects on neurophysiology or behaviour. No studies appear to have investigated the carcinogenic or teratogenic potential of infrasound (although there are no obvious reasons to suspect that the risk of cancer could be affected). Overall, the available data are sparse, and comparison between studies is made difficult due to differences in experimental protocols and techniques.

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5 Experimental Studies, Randomised Trials and Observational Studies in Humans

5.1 Ultrasound

Examination of the fetus with imaging ultrasound and of the umbilical cord or uterine circulation with continuous-wave Doppler ultrasound have both been used for many years. A number of clinical trials have been carried out in which pregnant women were randomly assigned either to a protocol that involved one or more ultrasound examinations routinely or to a protocol in which an ultrasound examination was given only if the woman's physician judged it to be necessary. Most of these randomised trials were concerned with evaluating the beneficial effect of ultrasound on the management of the pregnancy, rather than with evaluating any adverse biological effects of the ultrasound either during the pregnancy or later on. They do, nevertheless, provide information on the possible adverse effects of ultrasound exposure on the fetus.

In interpreting the trials it is important to bear in mind that in none of them would an ultrasound examination have been withheld if the woman's physician judged it necessary. Therefore, in analyses of outcome according to randomised group, a proportion of those assigned to the 'no routine ultrasound' group will, in fact, have been exposed to ultrasound, while in analyses of outcome according to exposure, the exposed group will include women who were assigned to 'no ultrasound', but where ultrasound was judged necessary, as well as those who were assigned to the group to receive ultrasound.

In addition to the randomised trials of ultrasound in pregnancy, a number of observational studies not involving any randomisation have been carried out. In these studies the association between ultrasound exposure in pregnancy and one or more outcome measures in the children has been investigated. Some of these studies have been in the form of follow-up (cohort) studies in which an entire group of children, some of whom were exposed to ultrasound *in utero*, have been identified and followed over time. Other observational studies have been in the form of case–control studies in which a group of children who have experienced an adverse event are identified as well as a group who have not experienced the event. The ultrasound history of the children in the two groups has then been obtained and compared.

Ultrasound is also widely used in medicine in contexts other than pregnancy. A number of studies evaluating its effects have been carried out.

5.1.1 Experimental studies

Very few studies have been carried out with volunteers exposed to ultrasound. van der Wouw et al (2000) observed a dose-dependent increase in premature ventricular contractions (PMVs) in healthy male volunteers during imaging with an ultrasound contrast agent (AIP 101) for myocardial perfusion assessment. Average numbers of PMVs increased significantly from 0.03 per minute during control imaging without the contrast agent to 1.06 per minute during imaging with a mechanical index (MI) of

1.5 and end-systolic triggering (at the end of the T wave). No increase in PMVs was observed using an MI of 1.1, or with end-diastolic triggering (at the first deflection of the QRS complex). It was suggested that imaging under these circumstances should be performed using low acoustic pressures and with end-diastolic triggered imaging to avoid any possible adverse consequences.

Fatemi et al (2001, 2005) reported that pulsed ultrasound used during routine examinations may affect fetal behaviour and cause an increase in motor activity. The heads and ears of nine healthy fetuses (at 25–40 weeks) were intermittently exposed for 3 minutes (10 s on, 20 s off) using a clinical ultrasound scanner equipped with a 2 MHz probe. Compared with either no exposure or exposure in continuous-wave Doppler mode, gross fetal movements were significantly increased during exposure to combined pulsed Doppler and B-modes; fetal heart rate was also significantly increased. Fetal movements were assessed using a low power ultrasound monitor (1.5 mW cm^{-2} at 990 kHz). The scanner could be used while measuring movement, but had to be turned off during determination of heart rate.

One study has investigated the integrity of the blood–brain barrier in humans after destruction of two ultrasound microbubble contrast agents (Levovist and Optison) with transcranial colour-coded sonography (Schlachetzki et al, 2002). Gadolinium-enhanced MRI (Gd-MRI) examinations were performed during both early and late phases after insonation. Ultrasound transmission power levels were kept within diagnostic limits and resembled standard settings in brain perfusion studies. Using a triple dose of gadolinium to increase sensitivity, and considering the potential time dependence of blood–brain barrier changes, insonation with neither Levovist nor Optison led to any detectable difference in T1 signal intensities in two defined brain regions using Gd-MRI. Moreover, Schlachetzki et al found no signs of focal signal enhancement or focal brain damage. This study provides further evidence of the absence of adverse effects produced by these contrast agents at the exposure levels of ultrasound equipment currently used for transcranial investigations. The results are reassuring but not totally conclusive in terms of ultrasound safety, since hypothetically more subtle effects of ultrasound and microbubbles on the blood–brain barrier might have been missed by the Gd-MRI assessment.

5.1.2 Randomised trials of ultrasound during pregnancy

In evaluating the evidence* on the safety of ultrasound provided by the randomised trials, the primary outcomes were considered to be perinatal mortality (excluding malformations) and measures of long-term development that might reflect an adverse effect of ultrasound on the fetus. In addition, low

* The evidence from clinical trials presented in this chapter was obtained by searching the Cochrane Library (issue 1/2008) including the Cochrane Database of Systematic Reviews, the Cochrane Database of Abstracts of Reviews of Effects and the Cochrane Database of Technology Assessments for articles where the word 'ultrasound' appeared as a keyword or in the title or abstract. In addition, a search of the Cochrane Central Register of Controlled Trials was carried out of all the trial reports submitted by the Cochrane Pregnancy and Childbirth Group during the period 1996–2007 (ie covering the entire period since all the Cochrane Reviews of ultrasound in pregnancy were last updated).

The odds ratios and confidence intervals presented have been taken from those presented in Cochrane Reviews, where they are available. Where they are not available they have been taken from the original papers. Where only numbers of events were available in the original papers odds ratios have been calculated using binomial regression. Odds ratios have been combined and confidence intervals and tests of significance or heterogeneity calculated using standard statistical methods.

birth weight (below 2.5 kg or, if this is not available, another similar measure) and low Apgar score (≤7 at 5 minutes) have been considered as secondary outcomes, although it is acknowledged that these secondary outcomes may to some extent reflect changes in clinical management as well as safety. Other outcomes considered in the trials are likely to be determined primarily by alterations in clinical management due to information provided by the ultrasound examinations and are not considered here.

5.1.2.1 Trials of ultrasound imaging

A total of twelve trials of routine versus selective ultrasound imaging in pregnancy were identified. One used 'pseudo-randomisation' based on the hospital number, and in two trials women in both groups were exposed, with the results remaining concealed in one group. After exclusion of these three trials, nine trials remained in which perinatal mortality (excluding malformations) was reported. They included a total of 17,901 women randomly assigned to the routine ultrasound group and 17,818 women to the control group (Table 5.1).

When all these trials were considered together, there was no evidence either of heterogeneity between the findings of the different trials (p=0.25) or of an increased risk of perinatal mortality (excluding lethal malformations) in the intervention arm (Table 5.1). This finding did not change when the analysis was limited to the eight trials in which ultrasound before 24 weeks was offered in the intervention arm or when just the four trials in which ultrasound was offered after 24 weeks in the intervention arm were considered.

In addition, data from seven trials (including 9090 in the routine ultrasound group versus 9021 controls) were available for the assessment of low birth weight (below 2.5 kg) in singletons. Overall there was no evidence of an increase in the intervention arm (367 versus 367 events, odds ratio (OR) of 0.99, 95% confidence interval (CI) of 0.85–1.15). However, there was significant heterogeneity between the trials (p<0.001), with two trials having a significantly decreased risk of low birth weight in those randomly assigned to receive ultrasound and one trial having a significantly increased risk in those assigned to receive ultrasound. One further trial reported on birth weight less than the 10th percentile and found a decreased risk in those randomly assigned to receive ultrasound (994 versus 999 participants, 69 versus 104 events, OR 0.67, 95% CI 0.50–0.89, p=0.008).

Data from six trials (including 5353 versus 5350 participants) were available on Apgar score (≤7 at 5 minutes). There was no evidence either of heterogeneity between the findings of the different trials (p=0.14) or of an increased risk in the intervention arm (5353 versus 5350 participants, 86 versus 93 events, OR 0.91, 95% CI 0.67–1.23).

In two Norwegian trials (ie Alesund and Trondheim) a follow-up study designed to look for possible long-term adverse effects of ultrasound on the fetal brain has been carried out on as many as possible of the children from singleton pregnancies at age 8–9 years. The children of mothers who were randomly assigned to receive ultrasound showed no statistically significant differences in growth compared with control children (Salvesen et al, 1993a). The study also tested six hypotheses, stated in advance, to evaluate an effect of fetal ultrasound on the developing brain. No significant differences between those in the ultrasound and control groups were found for five of them: delayed speech development, poor school performance, impaired neurological development, poor vision or poor hearing

TABLE 5.1 Randomised trials of routine versus selective ultrasound imaging in pregnancy: odds ratio for perinatal mortality excluding malformations

Study	Year of exposure	Comparison	Treatment *n/N**	Control *n/N**	Odds ratio (95% CI)
Alesund, Norway (Eik-Nes et al, 1980, 2000)	1979–1980	18 + 32 + some 36 weeks vs selective	6/792	7/761	0.82 (0.28–2.45)
Helsinki (Saari-Kemppainen et al, 1990)	1986–1987	16–20 weeks vs selective	17/4,389	28/4,347	0.61 (0.34–1.09)
Missouri (Ewigman et al, 1990)	1984–1986	10–12 weeks vs selective	2/404	4/420	0.53 (0.11–2.65)
RADIUS, USA (Ewigman et al, 1993)	1987–1991	(18–20) + (31–33) weeks vs selective	42/7,685	27/7,596	1.53 (0.95–2.45)
Sweden (Waldenstrom et al, 1988)	1985–1987	15 weeks vs selective	10/2,413	10/2,432	1.01 (0.42–2.43)
Trondheim, Norway (Bakketeig et al, 1984)	1979–1980	19 + 32 weeks vs selective	5/516	5/507	0.98 (0.28–3.41)
Tygerberg, South Africa (Geerts et al, 1996)	1991–1982	18–24 weeks vs selective	6/467	11/460	0.54 (0.21–1.41)
Adelaide (Crowther, 1999)	1991–1995	<17 weeks vs selective	3/290	3/296	2.00 (0.18–22.25)
Belfast (McKenna et al, 2003)	~2000	30 weeks vs selective	2/999	1/999	0.50 (0.05–5.17)
All trials with ultrasound at <24 weeks			91/16,902	95/16,819	0.94 (0.70–1.25) *p for heterogeneity: 0.19*
All trials with ultrasound at 24+ weeks			55/9,992	40/9,863	1.35 (0.90–2.02) *p for heterogeneity: 0.53*
All trials			93/17,901	96/17,818	0.95 (0.71–1.26) *p for heterogeneity: 0.25*

* *n* = number of perinatal mortalities, *N* = group size.

(Salvesen, 1992a,b, 1993b; Salvesen et al, 1994). For non-right-handedness (ie left-handedness and ambidexterity), however, there was a statistically significant increase in the ultrasound group (861 versus 802 participants, 162 versus 120 events, OR 1.31, 95% CI 1.02–1.70) (Table 5.2 and Salvesen, 1993b).

Long-term follow-up has also been carried out in the children of mothers in the Swedish trial. The study used a questionnaire consisting of 52 questions. Based on 3052 children evaluated at 8–9 years old, no significant differences between the children of mothers allocated to the screening group and the children of mothers allocated to the control group were found for analyses that considered endpoints related to non-right-handedness (253 versus 240, OR 1.04, 95% CI 0.85–1.26) or to growth, vision, hearing and neurological development (Kieler et al, 1997, 1998a,b; Salvesen and Eik-Nes, 1999). Separate results for all endpoints were also presented for boys alone. No significant difference was found for handedness (156 versus 134, OR 1.19, 95% CI 0.92–1.53, based on 1574 boys) or for any of the other endpoints when the data for boys were analysed according to randomised group. In a separate analysis of the Swedish data in which children were classified according to whether or not they had been exposed to ultrasound (rather than according to the group to which their mothers were randomly allocated in the trial), there was no significant increase in non-right-handedness for all children combined (OR 1.09, 95% CI 0.90–1.33, based on 3052 children). However, when the analysis based on exposure received rather than randomised group was repeated considering boys alone, a significant increase was found (odds ratio exposed *versus* unexposed of 1.30, 95% CI 1.01–1.68, based on 1574 boys). A further paper, evaluating the school performance of children of mothers in the Swedish study at age 15–16 years, is currently in preparation (H Kieler, personal communication).

A meta-analysis has been carried out of the Norwegian and Swedish data on handedness (Salvesen and Eik-Nes, 1999). This found no significant increase in non-right-handedness when the data were analysed according to randomised group and data for boys and girls were considered together. However, when data for boys were considered alone, there was a significant excess of non-right-handed children born to the mothers randomly assigned to receive ultrasound (Table 5.2). In both countries, almost all the women in the ultrasound group were actually exposed (97% in Norway and 99% in Sweden). In addition,

TABLE 5.2 Odds ratios (and 95% confidence intervals) for non-right-handedness (ie left-handedness and ambidexterity) in follow-up studies of children included in randomised trials of ultrasound for fetal assessment in early pregnancy (based on Salvesen and Eik-Nes, 1999)

	Norway	Sweden	Both
Analysis according to randomised group			
All children	1.31 (1.02–1.70)	1.04 (0.85–1.26)	1.13 (0.97–1.32)
Boys only	1.40 (1.00–1.96)	1.19 (0.92–1.53)	1.26 (1.03–1.54)
Analysis according to exposure received			
All children	1.37 (1.06–1.77)	1.09 (0.90–1.33)	1.19 (1.02–1.38)
Boys only	1.42 (1.02–1.99)	1.30 (1.01–1.68)	1.34 (1.10–1.65)

a considerable proportion of those randomly assigned to the control group were also exposed (19% in Norway and 35% in Sweden). When the analysis was repeated with the children classified according to whether or not they had actually been exposed to ultrasound, there was a significant excess of non-right-handedness in those exposed when data for both boys and girls were considered together and also when data for boys were considered alone (Table 5.2).

5.1.2.2 Randomised trials of Doppler ultrasound in pregnancy

A total of seventeen trials were identified of routine Doppler ultrasound of the umbilical artery and/or uterine circulation in pregnancy versus selective or no Doppler ultrasound. In five of these, women in both trial arms received the Doppler scan and the results were concealed in the control arm. Exclusion of these left a total of twelve trials, with 8776 women randomly assigned to the routine Doppler group and 8820 women to the control group (Table 5.3).

When all twelve trials were considered together there was no evidence for an increase in perinatal mortality (excluding malformations) in the children of women assigned to receive ultrasound (68 versus 80, OR 0.82, 95% CI 0.59–1.14) and there was no significant heterogeneity between the trials. This finding did not change when the analysis was limited to the eight trials carried out in high risk pregnancies (3150 versus 3223 participants, 23 versus 34 events, p for heterogeneity = 0.26, OR 0.72, 95% CI 0.45–1.15). When the analysis was limited to the four trials carried out in unselected/low risk pregnancies, there was no overall increase in perinatal mortality for the routine Doppler group (5626 versus 5597 participants, 36 versus 38 events, OR 0.94, 95% CI 0.59–1.48). However, for one study there was a significant increase in perinatal mortality (17 versus 7 deaths, OR 2.29, 95% CI 1.02–5.11) and, as a result, there was significant heterogeneity between the four trials (p=0.03).

Only one follow-up study designed to look for possible long-term adverse effects of Doppler ultrasound on the fetal brain has been carried out. This is the Perth low risk study, in which follow-up examinations were carried out at 1, 2, 3, 5 and 8 years of age on the children born in this study from singleton pregnancies without congenital abnormalities (Newnham et al, 2004). Information was available from 1362 children of mothers allocated to the intensively ultrasound-examined group and 1352 children of mothers allocated to the control group. By one year of age and thereafter, physical sizes were similar in the two groups, and there were no significant differences indicating deleterious effects of multiple ultrasound examinations at any age as measured by standard tests of childhood speech, language, behaviour and neurological development. Preliminary results are available from a further follow-up carried out at ten years of age on 1569 children, ie 58% of the eligible singleton cohort (Doherty et al, 2007). There was no effect of multiple ultrasound examinations on hand preference (10.4% versus 12.0%, adjusted OR of 1.17, 95% CI 0.86–1.61). However, in a further analysis that investigated factors associated with handedness, there was a substantial increase in left-handedness in boys born to mothers with proteinuric pre-eclampsia (40% versus 12%, p=0.002, based on 826 boys; adjusted OR of 5.73, 95% CI 2.12–15.44), although the proportion of girls who were left-handed did not differ significantly according to whether or not their mothers had pre-eclampsia (12% versus 10%, p=0.7, based on 743 children). The authors speculated that left-handedness may, in some cases, result from an adverse intrauterine environment.

TABLE 5.3 Randomised trials of continuous-wave Doppler ultrasound in pregnancy: odds ratios for perinatal mortality, excluding malformations

Study	Year of exposure	Comparison	Treatment *n/N**	Control *n/N**	Odds ratio (95% CI)
Unselected/low risk pregnancies					
France (Doppler French Study Group, 1997)	1988–1990	28–34 weeks vs selective	3/1,950	9/1,948	0.37 (0.12–1.14)
Leeds (Mason et al, 1993)	1988–1990	28 + 34 weeks vs selective	4/1,015	5/1,001	0.79 (0.21–2.92)
London (Davies et al, 1992)	1989	19–22 + 32 weeks vs selective	17/1,246	7/1,229	2.29 (1.02–5.11)
Perth, Australia (Newnham et al, 1993)	1989–1991	Ultrasound and Doppler at 18 + 24 + 28 + 34 + 38 weeks vs ultrasound at 18 weeks	12/1,415	17/1,419	0.70 (0.34–1.48)
High risk pregnancies					
Perth, Australia (Newnham et al, 1991)	1989–1991	Doppler during third trimester vs no Doppler	7/275	7/270	0.98 (0.34–2.83)
Leeds (Tyrrell et al, 1990)	Before 1990	Doppler at 28 weeks and modified biophysical examination vs selective Doppler and standard biophysical examination	1/250	2/250	0.51 (0.05–4.94)
Sweden (Almstrom et al, 1992)	1989–1990	Doppler vs cardiotocography	0/214	2/212	0.13 (0.01–2.14)
Chester (Biljan et al, 1992)	~1990	Doppler during third trimester vs no Doppler	1/338	3/336	0.36 (0.05–2.60)
Edinburgh (Johnstone et al, 1993)	1989–1991	Doppler vs no Doppler	8/1,132	10/1,197	0.85 (0.33–2.14)

TABLE 5.3 ***Continued***

Study	Year of exposure	Comparison	Treatment *n/N**	Control *n/N**	Odds ratio (95% CI)
Dublin (Burke et al, 1992)	~1990	Doppler during third trimester vs no Doppler	3/241	2/235	1.46 (0.25–8.49)
Oxford (Hofmeyr et al, 1991)	~1990	Doppler vs selective Doppler	3/438	8/459	0.42 (0.13–1.37)
Australasia (Giles et al, 2003)	1993–1997	Doppler vs no Doppler	7/202	8/264	0.88 (0.31–2.46)
All unselected/low risk pregnancies			36/5,626	38/5,597	0.94 (0.59–1.48) *p for heterogeneity: 0.03*
All high risk pregnancies			23/3,150	34/3,223	0.72 (0.45–1.15) *p for heterogeneity: 0.26*
All pregnancies			68/8,776	80/8,820	0.82 (0.59–1.14) *p for heterogeneity: 0.13*

* *n* = number of perinatal mortalities, *N* = group size.

Data on babies with birth weight below 2.5 kg were not available in any of the twelve trials, but three trials (London, Perth low risk and Perth high risk) reported on babies born with birth weight less than the 10th percentile. For the Perth low risk trial the risk of birth weight below the 10th percentile was greater in the intensive group of the trial (1415 versus 1419 participants, OR 1.35, 95% CI 1.09–1.67, p=0.006), while for the other two trials there was no suggestion of an increase (for the London trial 1246 versus 1229 participants, 125 versus 127 events, OR 0.97, 95% CI 0.77–1.23; and for the Perth high risk trial 275 versus 270 participants, 93 versus 89 events, OR 1.04, 95% CI 0.73–1.48). The heterogeneity between the result for the three trials was not statistically significant (p=0.10). Low birth weight was slightly increased in the babies of mothers randomly assigned to the routine Doppler group, but the increase did not quite reach statistical significance (2938 versus 2918 participants, OR 1.14, 95% CI 0.99–1.32). Data on low Apgar score (≤7 at 5 minutes) were available for nine trials (including 6671 versus 6699 participants). Overall there was no significant increase in the group assigned to receive the Doppler scan and there was no significant heterogeneity between the trials (OR 0.97, 95% CI 0.72–1.31, p for heterogeneity = 0.09).

5.1.3 Observational studies of ultrasound in pregnancy*

5.1.3.1 Cohort (follow-up) studies of children exposed *in utero*

A number of cohort (follow-up) studies have been carried out comparing children exposed to ultrasound in pregnancy with those who were not exposed (Scheidt et al, 1978; Stark et al, 1984; Lyons et al, 1988; Moore et al, 1988; Stålberg et al, 2007). None of these studies found any effects that could be clearly attributed to ultrasound, although one study found an association between dyslexia and ultrasound (Stark et al, 1984).

Following the suggestion from the Norwegian and Swedish trials of a possible link between ultrasound imaging and handedness, a study was carried out in Sweden specifically to examine this hypothesis (Kieler et al, 2001). In Sweden handedness, determined in a standardised manner, was available for large numbers of young men as it was determined as part of eligibility testing for military service. Preferred hand was assessed by handing a replica rifle to the enrolee, who was then asked to take up the alert position. Only enrolees who shot left-handed were registered as such. Those not registered either shot right-handed or were not tested.

Prior to October 1972 ultrasound imaging was not offered in Sweden. The University Hospital in Malmö was the first medical centre in Sweden to use ultrasound examinations as part of standard prenatal care. From October 1972 one ultrasound examination was offered at 28 weeks to pregnant women attending Malmö University Hospital for prenatal care. During 1974–75 the time of this examination was gradually changed to 19 weeks. Then, from October 1976, two examinations were offered, at 19 and 32 weeks.

* The evidence from observational epidemiological studies was obtained by considering the review papers of Salvesen (2007) and Kieler (2007) and the papers mentioned in them. In addition, a MEDLINE search of articles published during the period 2005–2007 (ie since the Salvesen and Kieler reviews were prepared) was carried out.

Information on the hospital of birth is available from 1973, when the Swedish Medical Birth Register was established. Prior to this the only information on place of birth available for military recruits is the administrative district of birth. Data were published on the handedness of boys born in the three time periods: 1969–72, 1973–75 and 1976–78 (Table 5.4). During 1969–72 there was no difference in left-handedness between boys born in Malmö and boys born in the rest of Sweden. During 1973–75 and 1976–78 the odds ratios of left-handedness for boys born in Malmö University Hospital compared with boys born at 48 hospitals in Sweden that did not give ultrasound before 1980 were 1.04 (95% CI 0.91–1.18) and 1.32 (95% CI 1.16–1.51), respectively.

In view of the suggestion of an association between handedness and exposure at 19 weeks in boys born in Malmö University Hospital, the same investigators looked at the records for other hospitals in Sweden that had given ultrasound imaging early in pregnancy. In Linköping University Hospital ultrasound was not given prior to 1978, but from 1 January 1978 a single ultrasound examination was given at 12 weeks

TABLE 5.4 Relative risk of non-right-handedness (ie left-handedness or ambidexterity) among Swedish military conscripts according to date and place of birth (from Kieler et al, 2001, 2002)

Birth cohort	Likely exposure	Comparison*	Odds ratio of non-right-handedness (95% CI)
1969–1972	Ultrasound not offered in Sweden during this period	Malmö† Stockholm Gothenburg Rest of Sweden	0.98 (0.90–1.07) 1.03 (0.95–1.10) 1.04 (0.96–1.13) 1.00 (reference group)
1973–1975	Women attending Malmö University Hospital for prenatal care offered ultrasound from October 1972, 90% were examined, usually at 28 weeks. Time of first examination in Malmö changed gradually to 19 weeks during 1974–75	Malmö University Hospital vs 48 hospitals not giving ultrasound before 1980	1.04 (0.91–1.18)
1976–1978	As above. Then, from October 1976, 2 examinations (at 19 and 32 weeks) performed. From April 1978, first examination at 17 weeks	Malmö University Hospital vs 48 hospitals not giving ultrasound before 1980	1.32 (1.16–1.51)
Jan–July 1978	Ultrasound not given prior to 1978 in Linköping University Hospital	Linköping University Hospital vs 48 hospitals not giving ultrasound before 1980	0.9 (0.6–1.3)
Aug–Dec 1978	From 1 January 1978, examination given at 12 weeks at Linköping University Hospital	Linköping University Hospital vs 48 hospitals not giving ultrasound before 1980	1.4 (1.0–2.1)

* Based on place of birth of infant.
† Hospital of birth not available before establishment of the Swedish Birth Register in 1973.

(Kieler et al, 2002). For boys born during January–July 1978 in Linköping University Hospital there was no suggestion of an increase in the risk of left-handedness compared with the 48 hospitals not giving ultrasound before 1978, but for boys born during August–December 1978 the odds ratio was 1.4 (95% CI 1.0–2.1).

A further study compared intellectual performance in these Swedish men as measured by the battery of tests given to them on military enrolment (Kieler et al, 2005). Those born in Malmö University Hospital had lower intellectual performance scores than those born elsewhere (mean difference −0.16, 95% CI −0.21 to −0.11) and an increased risk of subnormal performance (OR 1.28, CI 1.18 to 1.38). However, men born in Malmö before ultrasound examinations were introduced also had lower scores, and the decrease in test scores after the introduction of ultrasound was small. Moreover there was no difference in intellectual performance within pairs of brothers that could be attributed to a likely difference in the extent of ultrasound exposure.

5.1.3.2 Case–control studies of the effects of ultrasound exposure *in utero*

Seven case–control studies of childhood malignancies and ultrasound during pregnancy have been carried out (see Table 5.5). No significant associations between *in utero* ultrasound exposures and childhood malignancies were found.

In a case–control study of prenatal ultrasonography exposure in children with delayed speech, a network of community physicians affiliated with the Primary Care Research Unit, University of Calgary, Canada, identified 72 children aged 24 to 100 months who had undergone a formal speech-language evaluation and were found by a speech-language pathologist to have delayed speech of unknown cause. For each case subject, two control subjects matched for sex, date of birth, sibling birth order and associated health problems were identified via the same network (Campbell et al, 1993). The children with delayed speech had a higher rate of ultrasound exposure than the control subjects (OR 2.8, 95% CI 1.5–5.3, p=0.001).

5.1.4 Other medical uses of ultrasound

No study* of medical uses of ultrasound other than in pregnancy has provided any indication of harmful effects of ultrasound, although none had been designed to evaluate the safety of ultrasound and several observed that the quality of the available studies was poor. An assessment of the safety and efficacy of internal ultrasound lipoplasty (or endothermolysis) (Almazan and Gallo, 1999) concluded that short-term complications (seroma, dysesthesia, discolouration and skin surface irregularities, as well as skin thermic lesions), though not very frequent, deserve further consideration, mainly due to their potential severity and the fact that they may be avoided with appropriately performed procedures. It further concluded that research is needed to evaluate the safety of the procedure in areas close to vessels and nerves (mainly neck, face, breast, internal thigh, knee and ankle).

* Searches of the Cochrane Library identified a total of 62 Cochrane Reviews and 33 Other Reviews with the word 'ultrasound' in the title or abstract, or as a keyword that concerned medical uses of ultrasound other than in pregnancy.

TABLE 5.5 Case–control studies of ultrasound exposure during pregnancy and childhood malignancies

Study	Year of ultrasound exposure	Year of diagnosis	Cancer type	Cases		Controls		Odds ratio	p-value*
				Number	% exposed	Number	% exposed		
UK (Wilson and Waterhouse, 1984)	<1981	1972–1981	Leukaemia	665	6	665	6	1.00	ns
			Solid tumours	1066	6	1066	6	0.98	ns
UK (Cartwright et al, 1984)	<1983	1980–1983	Leukaemia	149	23	298	22	1.12	ns
			Other tumours	406	27	812	29	0.86	ns
China (Shu et al, 1994)	<1991	1986–1991	Leukaemia	166	36	166	38	0.90	ns
			Other tumours	476	23	476	27	0.80	ns
UK (Sorahan et al, 1995)	<1994	1982–1984	Neoplasms of the reticuloendothelial system	212	25	212	25	1.03	ns
			Solid tumours	308	28	308	29	0.94	ns
Sweden (Naumburg et al, 2000)	<1995	1973–1989	Lymphatic leukaemia	71	41	66	41	1.00	ns
			Myeloid leukaemia	534	37	532	40	0.85	ns
USA (Shu et al, 2002)	<1993	1989–1993	Acute lymphoblastic leukaemia	1789	35	1936	34	0.9	ns
Sweden (Stålberg et al, 2008)	<1999	1975–1999	Childhood brain tumours	503	44	524	46	0.9	ns

* ns = not significant.

A retrospective study* aiming to identify the incidence of adverse events of a second-generation ultrasound contrast agent in clinical practice (Piscalgia et al, 2006) found the rate of reporting of adverse events to be lower than or similar to that reported for other radiological and MRI contrast agents. However, as the authors pointed out, mild or moderate adverse events would probably be underestimated in this study, as would reactions occurring after the patient's discharge.

5.1.5 Discussion

5.1.5.1 Randomised trials of ultrasound in pregnancy

Although the randomised controlled trial is generally taken as the gold standard for medical evidence, interpretation of the trial data on the use of ultrasound in pregnancy is complicated by a number of factors.

Firstly, the majority of studies were designed to evaluate the possible benefits of ultrasound imaging on the management of the pregnancy and not to evaluate the safely of ultrasound. Therefore, in most of the trials, possible adverse effects of ultrasound were not included as primary endpoints. This complicates the interpretation of information on adverse endpoints even when they have been reported. Where a small number of endpoints relating to adverse effects was stated in advance of the trial, then interpretation of the results and their significance levels need to take into account the fact that several comparisons were carried out. Even more so, where the number of adverse endpoints to be examined was not clearly stated in advance, it is possible that many endpoints have been evaluated and the evidence presented only for a highly selected subset, giving greater uncertainty to interpretation.

A second problem, also arising from the fact that most of the trials were designed to evaluate the possible benefits of ultrasound on the management of the pregnancy, is that for most of the trials there has been no long-term follow-up of the children who were exposed *in utero*.

Thirdly, in most of the trials ultrasound examination was available to women randomly assigned not to receive ultrasound if examination was requested by their physician, and in many of the trials a substantial proportion of the mothers who were in the no-ultrasound group did, in fact, receive an examination. This reduces considerably the power of the trial to detect any harmful effects. In addition, the results from several trials have been reported according to the exposure received, as well as according to randomised group. Where these results differ from the results as reported by randomised group, any associations observed in relation to exposure received may not necessarily be causal.

Considering just the evidence from the trials analysed according to randomised group there are a few suggestions of possible adverse effects from fetal exposure to ultrasound, including:

a an increase in non-right-handedness (ie left-handedness and ambidexterity) from ultrasound imaging examinations given at 18/19 and 32 weeks in Norway, following the levels of exposure typical around 1980 (Salvesen et al, 1993b), and a significant increase also in trials for Norway and Sweden combined, but only for males, not for both sexes combined,

* One study identified from a MEDLINE literature search of the period 2005–2007.

b an increase in perinatal mortality from a single continuous-wave Doppler scan at 19–22 weeks following exposure at the levels prevalent in the late 1980s (Davies et al, 1992),

c an increase in the risk of intrauterine growth restriction following multiple Doppler scans (at 18, 24, 28, 34 and 38 weeks) and at the exposures of around 1990 (Newnham et al, 1993).

Each of these findings is based on the results of just one trial (or, for handedness in Norway and Sweden combined, on a subset analysis without strong prior rationale) and, when they are taken together with the results of trials that have reported no significant adverse effects for these endpoints, the overall conclusion must be that there is no strong evidence of any adverse outcome on the exposed fetus from clinical ultrasound.

The evidence from the randomised trials is, however, limited and relates only to ultrasound examinations as they were given around 20–30 years ago. There is good evidence that exposure from ultrasound of the fetal population has increased since the majority of these randomised trials were carried out. There are several reasons for the increase. Firstly, there has been a general trend by all manufacturers to design equipment that uses higher intensities and pulse amplitudes, often approaching the regulatory limiting values (Duck and Martin, 1991; Whittingham, 2000), and these modern machines tend to give higher exposures per examination. Secondly, the increased exposure levels of more modern machines increases their imaging ability, thereby making them more useful in clinical practice and, as a result, more widely used. Thirdly, in the early 1990s, the FDA regulations in the USA altered, allowing ultrasound intensities to be used for obstetric examinations that had been previously reserved only for peripheral vascular examinations. In particular, this allowed pulsed Doppler and Doppler imaging to be used effectively for obstetric examinations for the first time. A final factor is the increasingly widespread use of transvaginal examinations especially during early pregnancy.

5.1.5.2 Observational studies of ultrasound in pregnancy

In contrast to the lack of strong evidence of a causal relationship between fetal ultrasound exposure and handedness from the randomised trials, there is greater evidence of an association between non-right-handedness and *in utero* exposure to ultrasound from observational studies. In particular, the studies of Swedish military recruits found evidence of an association between non-right-handedness (ie left-handedness and ambidexterity) and prenatal ultrasound exposure. The evidence from analyses of randomised trials in which the individuals are classified according to the exposure actually received, rather than the group to which the mother was randomly assigned, is also essentially observational in nature as, in most of the trials, an appreciable proportion of the mothers who were assigned to the control group were referred for selective ultrasound by their physicians.

There are many factors known to increase the risk of left-handedness and several of these, including possible multiple pregnancy (Davis and Annett, 1994), also provide a strong reason for a pregnant woman to receive an ultrasound examination. Pre-eclampsia would be another reason for an examination and it is noteworthy that a strong association was seen between left-handedness and pre-eclampsia in the Perth study. The long-term associations of left-handedness in terms of life-expectancy and disease incidence are still not fully understood, but a recent study of over 13,000 middle-aged Dutch women

followed for 13 years found that left-handedness was associated with higher mortality from several major diseases, including total cancer and stroke (Ramadhani et al, 2007).

The case–control studies of childhood malignancy and ultrasound exposure *in utero* are sufficient to rule out a large association between exposures of the type given in this period and childhood leukaemia or all childhood cancers combined. However, they are not sufficient to rule out a moderate association for individual types of solid cancer. The single study reporting an association between fetal ultrasound exposure and delayed speech development was well carried out and this association merits further study (Campbell et al, 1993).

5.1.5.3 Other medical uses of ultrasound

Although procedures involving ultrasound are widely used throughout medicine, there have been very few studies concerned with the health effects, even of established uses of ultrasound.

5.1.6 Summary

There is no established evidence that medical uses of ultrasound are causing long-term adverse health effects. Nevertheless, there is very little evidence on such effects, especially in circumstances other than obstetric use.

Very few experimental studies have been performed with volunteers. However, consistent with anecdotal reports, one study found that fetuses may perceive and respond to pulsed ultrasound at diagnostic levels by increasing their body movements.

Trials of ultrasound in pregnancy analysed by intention to treat have not found strong evidence of an association between prenatal ultrasound exposure and non-right-handedness. Observational studies of the same question have found some evidence of an association but with limited power and with difficulty in distinguishing whether associations are causal or not. The possibility of an effect therefore has mixed evidence, but is not established.

5.2 Infrasound

The proposition that exposure to infrasound may induce adverse effects on health has a long history, and remains highly controversial (Leventhall, 2007). However, few volunteer or observational studies appear to have investigated these possibilities (see Leventhall et al, 2003). It has been proposed that specific weather conditions, possibly associated with changes in levels of natural infrasound, may affect behaviour and health (Moos, 1963, 1964; Green and Dunn, 1968), although these suggestions have not been substantiated.

In addition, there are reports that chronic exposure to unwanted, low frequency acoustic sources in the environment may also induce non-specific symptoms of stress, including headaches, nausea and loss of sleep (see Leventhall et al, 2003, 2008) but it cannot be excluded that these effects occur due to audible noise.

5.2.1 Experimental studies

Despite some claims that infrasound may produce a variety of unpleasant symptoms, the effects of infrasound on volunteers have not been well studied, and there appears to be little consensus about possible biological responses to low level exposures. The literature has been reviewed, for example, by Westin (1975), Harris et al (1976), Broner (1978) and Landström and Pelmear (1993) and, more recently, by Haneke et al (2001), Leventhall et al (2003) and Jauchem and Cook (2007). The perception of sound at frequencies below 200 Hz has been well reviewed by Møller and Pedersen (2004).

Most volunteer studies have investigated the immediate consequences of intense exposure (above about 100 dB) often for just a few minutes, and these have tended to concentrate on hearing and balance, or the effects on the cardiovascular system.

5.2.1.1 Hearing and vestibular changes

Research into the possible biological effects of infrasound was stimulated by the American space programme, and there were concerns about the safety of astronauts during launch when intense levels of infrasound and low frequency noise were generated. Mohr et al (1965) reported that short-term exposure to infrasound at 150 dB was unpleasant, but within voluntary tolerance limits. There was no change in subjective hearing sensitivity and no temporary threshold shifts (TTS) in hearing were detected two minutes after exposure. Subjects were five United States Air Force officers who were well used to exposure to intense noise. They were exposed, usually whilst wearing ear protectors, to broadband, narrowband and pure tone low frequency noise for up to two minutes. Moderate chest wall vibration and changes in respiratory rhythm were observed, but no substantial effects were seen on visual acuity, spatial orientation, dexterity or speech intelligibility (although no data were presented to justify these results). Middle ear pain was reported by two out of three subjects during exposures without ear protectors.

Exposure to infrasound has been found to induce modest TTS in some subjects. Jerger et al (1966) reported that repeated exposures to 2–12 Hz at 119–144 dB for three minutes induced TTS of 10–22 dB at 3–8 kHz, with full recovery after a few hours, in 11 out of 19 subjects tested. Using a piston-phone coupled to the ear via an earmuff, Nixon (1973) observed TTS that persisted for about 30 minutes in about one-third of volunteers exposed to an 18 Hz signal at 135 dB. Subjects were exposed for six periods of five minutes with a one to two minute rest period between each. Similar responses have been reported using higher frequencies (Mills et al, 1983). Overall, these studies suggest that infrasound may induce a temporary shift in hearing threshold in some subjects, but recovery is usually rapid and complete.

Hensel et al (2007) investigated the impact of infrasound (6 Hz at 130 dB) on cochlea function by measuring distortion product otoacoustic emissions (DPOAEs). Infrasound induced changes in sound processing by the cochlea, but did not cause damage to the outer hair cells (as assessed by the absence of temporary DPOAE level shifts). Subjects described perceiving the infrasound as a sensation at the eardrum and not as a tonal stimulus. No symptoms were reported following exposure.

Takigawa et al (1988) suggested exposure to infrasound could result in very subtle changes in vestibular function. Exposure to 16 Hz at 95 dB(C) for 5 minutes resulted in an inhibition of the body sway normally produced by closing the eyes. This effect was not seen with 5 Hz or using wide octave band noise. Taenaka (1989) found that exposure to 10–15 Hz at 130–135 dB for 30 minutes did not result in changes in auditory or vestibular function.

5.2.1.2 Cardiovascular changes

Several studies have investigated the possible effects of infrasound on the cardiovascular system: results are somewhat mixed. A small, but significant, elevation in diastolic blood pressure was reported by Borredon and Nathie (1973) in males exposed to 7.5 Hz infrasound at 130 dB for 50 minutes. Danielsson and Landström (1985) reported that acute exposure to infrasound (6, 12 or 16 Hz at 95, 110 or 125 dB) for 20 minutes caused minor effects: exposure tended to increase diastolic blood pressure and to very slightly decrease systolic blood pressure and pulse rate. However, exposure to 16 Hz at 125 dB for 60 minutes resulted in significant changes and mean diastolic blood pressure increased by about 5 mm Hg, and mean systolic blood pressure decreased by about 3 mm Hg. Strandberg et al (1986) found exposure to infrasound for one hour at 16 Hz and 125 dB increased diastolic blood pressure, decreased systolic blood pressure and increased pulse rate.

Martiník and Opltová (1986) compared the physiological effects of exposure to several sound bands. It was found that exposure of males to white noise (4–10,000 Hz at 30–110 dB), pure tones (125–500 Hz at 110–110 dB) or infrasound (8–12.5 Hz at 80–100 dB) for 180 minutes resulted in transient and non-uniform increases in heart rate and blood pressure, with infrasound producing the greatest variation in heart rate. It was suggested that the initial increase in heart rate depended on the intensity of the sound, and that the speed at which the heart rate recovered depended on the frequency used. Performing a mental task during exposure was reported to mask the effects of the sound, and individual variations in responsiveness were attributed to differences in personality characteristics among the subjects. However, no statistical analysis of the data appears to have been performed, and the limited amount of data presented suggest that some of the reported responses may not have been statistically significant.

No consistent changes in blood pressure, pulse rate, ECG and other physiological variables were reported by Okamoto et al (1986) in volunteers exposed to 10 or 15 Hz at around 105–130 dB via loudspeaker or headphones, although there was some evidence that body sway increased, particularly with exposures at 15 Hz. Nystagmus was not elicited by exposure. Hearing thresholds were raised following whole-body exposures in subjects with ear plugs.

In contrast, a variety of physiological and subjective effects were attributed to infrasound in an early study by Karpova et al (1970). Subjects were exposed to 5 or 10 Hz at 100 or 135 dB for 15 minutes. Reported changes to the cardiovascular system included increased heart rate and reductions in the strength of cardiac contraction. In addition, behavioural and neurophysiological changes were also observed, and exposure to infrasound was reported to induce fatigue, apathy and depression.

5.2.1.3 Neurobehavioural changes

Some studies have investigated effects of infrasound on brain function and behaviour, but very few effects have been reported. Harris and Johnson (1978) investigated the effects of infrasound and audible noise on the performance of two cognitive tasks by men and women. No statistically significant effects were observed on a serial search task during exposure for 15 minutes to a 7 Hz tone at 125 dB either alone or in combination with a low frequency background noise at 110 dB, or on a complex counting task for 30 minutes. No effects were seen on the latter task during exposure for 15 minutes to 7 Hz at 125, 132 or 143 dB with a background noise at 110 dB. In addition, there were no reports of increased dizziness or disorientation during exposure.

Kyriakides and Leventhall (1977) explored the effects of infrasound, audible noise and alcohol intake on the simultaneous performance of a visuomotor pursuit task and a serial reaction time task. Whereas both noise and alcohol produced observable effects, exposure to a band of infrasound from 2–15 Hz at 115 dB for 40 minutes had no detrimental effect on the performance of either task. Exposure also produced no significant shifts in hearing threshold.

Møller (1984) found that exposure for three hours to infrasound at levels just below the hearing threshold (1–30 Hz at 100 dB) did not produce any consistent impairments in the performance of a battery of nine cognitive tasks that measured reasoning, short-term memory and reaction time. Exposure produced no significant cardiovascular changes or shifts in hearing threshold, nor did it increase the incidence of symptoms. However, a feeling of annoyance and pressure on the ear were reported with exposures at 20 dB above the hearing threshold when the errors made on a cue utilisation task were also increased. Previously, Slarve and Johnson (1975) reported that exposure to 1–20 Hz for eight minutes at up to 144 dB resulted in pressure effects on the ear and in vibration of the chest and abdomen.

A few studies have investigated the cognitive and behavioural effects of low frequency noise. Landström et al (1991) investigated the effects of short-term exposure to noise on a hidden pattern recognition task, where subjects had to identify five different patterns within fifteen different figures: no further details were given of this task. Twelve volunteers were exposed to either broadband ventilation noise at 40 dB(A), tonal noise (which consisted of the ventilation noise plus a tone at 100 Hz) at 40 dB(A), or pink noise (which consisted of the tonal noise plus a pink masking noise of 50–200 Hz) at 41 dB(A). No significant differences in response were observed between broadband noise and tonal noise, or between tonal noise and pink noise, although performance was slightly improved during exposure to pink noise.

Persson Waye et al (1997) studied the cognitive and subjective effects of low frequency ventilation noise associated with working in an office environment. Fourteen volunteers were exposed for 60 minutes to either a mid-frequency broadband noise at 41 dB(A) or to the same noise to which low frequency sounds (31.5–125 Hz) had been added. Effects were quantified using a figure rotation test, a test of short-term memory and a verbal reasoning test; questionnaires were used to assess symptoms, mood, annoyance and perceived interference with performance. Despite some suggestions that low frequency noise increased response times and increased reports of pressure on the eardrum compared with mid-frequency noise, there were no significant effects on any of the objective measures. However, the subjective ratings of interference were higher and subjects felt more annoyed with low frequency noise. In an extension of this study, Persson Waye et al (2001) explored the effects of exposure for 130 minutes

to these sounds on 32 volunteers who reported themselves as having a high or low sensitivity to noise in general and to low frequency noise in particular. Two of the previous tests were used with the addition of a simple reaction time task and a proof-reading task. Questionnaires were again used to assess mood, symptoms and annoyance, and to rate changes in work capacity. Compared with the values obtained with mid-frequency noise, there was a smaller decrease in response time in the verbal reasoning task with low frequency noise, and an impairment in the performance of the proof-reading task. In addition, increased levels of annoyance and decreased work capacity were reported. These effects were more pronounced for subjects reporting high sensitivity to low frequency noise.

5.2.1.4 Effects on wakefulness and sleep

There have been anecdotal reports that exposure to infrasound may cause drowsiness. This possibility has been examined in a series of laboratory investigations by Landström and colleagues (see Landström, 1987). In these studies, healthy male volunteers were seated within a bespoke, low frequency pressure chamber, and changes in wakefulness were monitored principally by analysis of the EEG. Evidence of reduced wakefulness was indicated by an increase in theta activity and a decrease in alpha activity with the eyes closed, or by an increase in alpha activity with the eyes open.

In the first study, Landström et al (1982) exposed subjects to infrasound at 125 dB(Lin) for periods of 20 minutes at frequencies of 6, 12 and 16 Hz or intermittent 16 Hz (23 bursts of sound in ten seconds with a random time interval in between). It was found that intermittent 16 Hz induced a mean increase in theta activity. However, there was a large variability in the size of individual responses, and about half of the subjects showed no change or a decrease in theta activity. In addition, intermittent 16 Hz reduced heart rate (by one to two beats per minute, bpm) and reduced systolic and diastolic blood pressure. Similar trends were observed for the other frequencies, although not all of these changes reached statistical significance. No significant changes in wakefulness were found by studies using deaf volunteers exposed to infrasound (Landström et al, 1983).

Subsequently, Landström and Byström (1984) exposed subjects for 15 minutes to 6 Hz at 95 or 115 dB and 16 Hz at 95 or 115 dB. Exposure at the higher level (which was 10 dB above the hearing threshold for each frequency) was reported to result in a significant increase in mean theta activity, but individual responses were very variable. No effects were observed with exposures below the hearing threshold. It was concluded that infrasound had to be audible to result in a decrease in wakefulness.

Landström et al (1985) investigated the effects of infrasound at EEG frequencies on wakefulness in six volunteers. Subjects were exposed for 15 minutes to infrasound with a spectrum equal to that of their own EEG recorded online, with the dominant frequency held at 10 dB over the hearing threshold. Changes in EEG were variable and none was significant, although there was a suggestion that mean theta activity had increased with the eyes closed, and thus exposure had reduced levels of arousal.

Finally, Landström et al (1991) investigated the effects of exposure to broadband ventilation noise, tonal noise (the ventilation noise plus a tone at 100 Hz) or pink noise (the tonal noise plus a pink masking noise of 50–200 Hz), at 40–41 dB(A). Wakefulness and annoyance were also assessed by the use of visual analogue scales, and the only statistically significant change was an increase in self-reported wakefulness with the pink noise compared with that reported with the tonal noise.

Overall, these studies indicate that short-term exposure to infrasound under laboratory conditions can decrease wakefulness. The mechanism appears to be based on auditory perception, as the changes only occur with exposures above the hearing threshold.

The effect of infrasound on sleep has also received some attention, although most work has involved low frequency noise (see the review by Persson Waye, 2004). In an early study, Fecci et al (1970) observed that exposure to very low level infrasound at 50–65 dB increased sleep. No other physiological effects were reported. Okada and Inaba (1990) studied the effects of night-time exposure to infrasound and low frequency noise on sleep. This was assessed by using the EEG; the ECG and the electrical activity of the jaw and eye muscles were also measured. Compared with the results from a no-exposure night, no statistically significant effects were observed on sleep pattern. Volunteers were exposed to 10, 20, 30 and 63 Hz for 30 seconds every 20 minutes at between 50 and 105 dB for three or four successive nights in a specially equipped laboratory.

5.2.1.5 Annoyance

Annoyance to a low frequency sound is considered to result from the complex interaction of a number of variables, including the attitudes and expectations of the people hearing the noise, and not just from the properties of the noise itself. Thus assessments that attempt to quantify annoyance by only considering acoustic factors are of limited use, and it is important to consider the extent to which a noise may lead to changes in behaviour. Many laboratory studies have investigated the effects of infrasound and low frequency noise in causing annoyance, including those with self-reported enhanced sensitivity to noise. These studies have been reviewed by Leventhall et al (2003). Overall, it was concluded that there is a large variability between subjects in noises that cause annoyance, and that the use of the dB(A) scale underestimates perceived annoyance for frequencies below about 200 Hz. Studies also suggest that annoyance of tones increases with sound pressure level more rapidly with decreasing frequency, while the increases for bands of noise with sound pressure level are more gradual. The importance of fluctuations in the intensity of noise as a contributor to annoyance was also noted, indicating that noises that are perceived to pulse may be far more annoying than predicted by their average sound levels. Coping strategies for low frequency noise have been described by Leventhall et al (2008).

5.2.1.6 Body vibration

High levels of low frequency noise induce body vibrations (Smith, 2002; Leventhall et al, 2003; Leventhall, 2007). The most prominent body response is a chest resonant vibration in the region of 50–80 Hz, which occurs at high sound levels. The low frequency perception thresholds of volunteers with normal hearing and those who were profoundly deaf have been investigated by Yamada et al (1983). It was found that the profoundly deaf subjects perceived noise through their bodies at levels in excess of normal thresholds. The threshold of sensation of the deaf subjects was 40–50 dB above the hearing threshold of those with normal hearing up to a frequency of 63 Hz, and greater at higher frequencies – for example, about 100 dB greater at 1 kHz, at which level perception was by the subjects' residual hearing. Deaf subjects experienced chest vibration in the same frequency range as subjects with normal hearing.

5.2.1.7 Aural pain

Pain may arise in the ear as a consequence of exposure to high sound levels. This is not a hearing sensation, but results from mechanical displacement of the middle ear system beyond its comfortable limits. People with both hearing ability and hearing loss, but with normal middle ear function, exhibit aural pain at a similar stimulus level, which is about 165 dB at 2 Hz, reducing to 145 dB at 20 Hz (Figure 3.7). Static pressure produces pain at 175–180 dB, whilst eardrum rupture occurs at 185–190 dB (von Gierke and Nixon, 1976). A pressure of 50 kPa, which is about half atmospheric pressure, is equivalent to 188 dB. Eardrum rupture is most likely to occur from unprotected exposure to blast waves.

A sensation of pressure in the ear may occur at levels of 125–130 dB, which is about 60 dB lower than eardrum rupture, and in the audible range of infrasound and low frequency noise. There is no firm evidence that inaudible levels of noise generally produce ear pressure sensations, although there are wide individual differences in response.

5.2.2 Observational studies

No epidemiological study appears to have been published into the effects of infrasound on any aspect of health or well-being.

5.2.3 Case studies

One group of researchers has adopted the title of vibroacoustic disease (VAD) to define a claimed whole-body, multi-system pathology which the researchers have attributed to occupational or chronic exposure to large pressure amplitude and low frequency noise (Castelo Branco, 1999; Gomes et al, 1999; Pimenta et al, 1999; Castelo Branco and Alves-Pereira, 2004; Ferreira et al, 2006a,b; Alves-Pereira and Castelo Branco, 2007; Branco et al, 2007). The concept of VAD was developed by Castelo Branco from an observation that a technician displayed disorientation while working around military aircraft with engines operating. As with animals (see Chapter 4), VAD in humans has been claimed to be characterised by an abnormal proliferation of collagen and elastin in the absence of an inflammatory response. The clinical picture claimed involves respiratory disorders, neurological disturbances and cardiovascular problems. Castelo Branco (1999) suggested that the clinical progression is insidious, and lesions are found in many systems throughout the body.

The symptoms presented by those with long-term exposure to high levels of low frequency noise and infrasound from aircraft have not been generally accepted as resulting only from the noise in this complex environment (Leventhall et al, 2003). Further, sound (and vibration) levels near ground running aircraft are very high and cannot be compared to the low levels of infrasound that are produced by environmental sources.

In addition, it is important to distinguish VAD from the range of conditions known as hand–arm vibration syndrome (HAVS), which include vibration white finger and carpal tunnel syndrome. HAVS is a well-recognised source of much pain and discomfort, and is caused by the repeated and frequent use of powerful, hand-held vibrating tools.

There have been a few other case studies involving exposure to infrasound at work, but none is particularly informative. Using a questionnaire, Kawano et al (1991) found that occupational exposure of long-distance lorry drivers to infrasound at about 115 dB(A) did not increase the incidence of adverse symptoms, hearing impairment or hypertension. Huang et al (2003) reported that the use of infrasonic minesweeping equipment aboard a ship resulted in small, but significant changes in mood and performance of members of the crew. Compared with those aboard a similar minesweeper that did not use infrasound, the score of a digit memory test was reduced, vigour-activity was lower and fatigue-inertia was higher. No significant differences were seen initially between the crews. Zinkin et al (2007) examined the hearing sensitivity of 80 aircraft maintenance staff at airports who were exposed to noise containing infrasound at 102–108 dB and higher frequency components at 94–120 dB(A). Hearing thresholds were raised at all measured frequencies, which correlated with duration of exposure to noise.

In an investigation of persistent complaints made by a married couple concerning an audible hum which was attributed to a nearby heating plant, Feldmann and Pitten (2004) found measured levels of infrasound in the couple's home to be below the auditory threshold.

Some of the clinical features attributed to VAD overlap with those claimed for electrical sensitivity and multiple chemical sensitivity. The HPA has reviewed the evidence for the definition, epidemiology and management of electrical sensitivity (Irvine, 2005).

5.2.4 Summary

Volunteer studies investigating the potential effects of infrasound are relatively limited. The quality of many of the early studies is questionable, and they often yield conflicting results. Very few studies appear to have been published in recent years. Overall, there is little evidence to suggest that acute exposure to infrasound at levels commonly experienced in the environment is capable of causing any consistent physiological or behavioural effect, although there is a general paucity of high quality research in this area. Nevertheless, there is some evidence that infrasound at levels above the hearing threshold may cause a decrease in wakefulness. Finally, aural pain and damage may result from very intense exposures, above a threshold of about 140 dB or more depending on frequency.

There is also a lack of useful epidemiological and clinical data. One group has suggested that long-term occupational exposure to large pressure amplitude and low frequency noise may cause a diverse pathology, termed vibroacoustic disease (VAD), that is claimed to involve neurological, respiratory and cardiovascular disturbances. While those working in very high levels of audible noise may suffer some adverse consequences, and the prolonged use of hand-held vibrating tools may cause ill-health, there is no evidence that infrasound at levels normally encountered in the environment will lead to the development of VAD. Further, this disease itself has not gained clinical recognition. The few other case studies are not particularly informative regarding possible risks to health.

Overall, there is a paucity of useful information regarding the potential of infrasound to cause health effects.

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6 Conclusions

This report considers the potential health risks arising from intentional and adventitious exposure to ultrasound and infrasound. Like audible sound, infrasound and ultrasound are mechanical waves, propagated through oscillatory movements of the medium, associated with local oscillatory variations in density and pressure. Ultrasound is defined here as acoustic waves at all frequencies exceeding 20 kHz, and infrasound as acoustic waves at all frequencies below 20 Hz.

Ultrasound has been used in medical practice for at least 50 years worldwide. Specific uses include diagnostic ultrasound examinations during pregnancy, soft tissue imaging and ultrasound therapy. It is also used to good effect in a large number of industrial applications, including sonochemistry, emulsification, welding, cleaning and non-destructive testing. Ultrasound has also found applications in a variety of consumer products, as range finders, movement detectors and pest repellents.

Infrasound is widespread in modern society, being generated by cars, trains and aircraft, and by industrial machinery, pumps, compressors and low speed fans. Under these circumstances, infrasound is usually accompanied by the generation of audible, low frequency noise. Natural sources of infrasound include thunderstorms and fluctuations in atmospheric pressure, wind and waves, and volcanoes; running and swimming also generate changes in air pressure at infrasonic frequencies.

6.1 Physics, Sources and Dosimetry

In a homogeneous medium, infrasonic fields typically spherically diverge from the source. In practice, however, they exhibit local variations in acoustic pressure due to acoustic scatter from any solid or liquid objects in the field. Ultrasonic beams can be structurally complex, even in homogeneous media. Ultrasonic field structure depends on symmetry: circular monochromatic sources give rise to the greatest spatial variations, especially on axis, whilst pulsed non-circular sources generate less spatial variation.

Ultrasound propagation through tissue is largely controlled by the speed of sound and attenuation, and by microscopic and macroscopic spatial variations of these properties. Bone, soft tissue and gaseous inclusions have very different acoustic properties from one another. Very little acoustic energy is transmitted across an interface between air and tissue and, to a first approximation, ultrasound above about 300 kHz does not propagate through air. Therefore, for many applications, the operator of ultrasound equipment is effectively screened from exposure. Ultrasonic wave propagation through fluids and tissue can be strongly modified by non-linear propagation effects, especially at frequencies above 1 MHz.

Infrasonic wavelengths substantially exceed the dimensions of the human body, and so the human body acts as an acoustic scattering centre in an infrasonic field.

The absorption of ultrasound by tissue causes heating. The temperature increases caused in tissue by the normal use of current diagnostic ultrasound equipment are very unlikely to extend outside the normal physiological range. Hazardous temperature rises in tissue may be reached under very unusual diagnostic conditions, specifically the exposure of bone in the focal zone of a pulsed Doppler beam at maximum power with a stationary beam position. High temperatures can also be induced with physiotherapy ultrasound equipment, and by misuse of some non-medical devices, such as ultrasonic cleaning baths. Radiation pressure causes fluid movement in the direction of propagation, and can displace tissues slightly. The associated forces lie within those experienced under normal physiological conditions. Bubbles may be created in liquids, and caused to vibrate, by an ultrasonic field. This process is called acoustic cavitation, and can create strong shear forces and local fluid motion close to the bubble. Under some conditions, inertial cavitation takes place, in which the bubble collapses abruptly creating extremely high, very localised temperatures and pressures, light emission and free radicals. However, the likelihood of cavitation events *in vivo* under diagnostic exposure conditions is vanishingly small. Numerical models have been developed to predict acoustic field propagation and effects, including non-linearity, heating, cavitation and displacement.

Radiation force balances, hydrophones and microphones are used for the measurement of acoustic output and acoustic exposure. Improved hydrophone technology and a commercial portable ultrasonic power balance both remain to be developed. Methods for estimating *in situ* exposure and temperature rise from these measurements are still simplistic. While the acoustic outputs of medical sources of ultrasound have been much investigated and well described, there is an absence of information regarding the levels of exposures that may occur from non-medical sources of ultrasound.

There is also very little information on occupational or non-occupational human exposure to infrasound.

6.2 Biology

Ultrasound at high levels of exposure is capable of causing overt biological changes to living tissues, and these may be achieved through heating, cavitation and acoustic radiation force. For example, ultrasound can cause the destruction of kidney stones and induce localised necrosis to soft tissues, and prenatal exposure at levels well above diagnostic levels can result in teratogenic effects in animals. All these responses and the relevant mechanisms of interaction are listed in Table 6.1. Interestingly, the mechanisms through which ultrasound may facilitate bone and soft tissue regeneration, and also cause an increase in apoptosis, have not yet been positively identified. Biological responses at lower levels of exposure are more controversial, but a few possibilities (see Table 6.2) enjoy some experimental support. For example, ultrasound at diagnostic levels has been reported to affect the normal progression of epithelial cells in the small intestine of adult animals through the cell cycle, and prenatal exposure has been shown to induce changes in neuronal movement in the developing brain of mice, leading to subtle morphological changes in the cerebral cortex. It is not known whether these effects have any long-term functional significance.

TABLE 6.1 Established biological effects of exposure to ultrasound

Biological effect or endpoint	Attributed mechanism(s)
Local tissue destruction (HIFU)	Heating, boiling, acoustic cavitation
Cutting or cleaning (soft tissue scalpel, emulsification of cataract, tooth descaler)	Mechanical, acoustic cavitation
Increased cell apoptosis	Not known
Lithotripsy	Mechanical, acoustic cavitation
Sensation and pain	Acoustic radiation force, heating
Teratology	Heating
Bone and soft tissue regeneration	Not known
Increased fetal movement	Acoustic radiation force
Local lung surface tissue damage/bleeding	Gas-body activation
Intestinal petechiae	Heating, gas-body activation
Degradation of DNA (*in vitro*) (and repair mechanism)	Mechanical, acoustic cavitation, heating
Sonoporesis (*in vitro*)	Acoustic cavitation
Lysis (*in vitro*)	Acoustic cavitation
Capillary damage, ventricular extra-systolic contractions, effects on blood–brain barrier (from contrast agents)	Acoustic cavitation
Topical drug delivery	Acoustic cavitation

TABLE 6.2 Possible biological effects of exposure to ultrasound

Biological effect or endpoint	Attributed mechanism(s)
Increased incidence of non-right-handedness in males following prenatal exposure	Not known
Fetal cortical neuron migration	Not known
Behavioural and cognitive deficits following prenatal exposure	Not known
Increased apoptosis of intestinal cells and decreased cell turnover	Not known

Infrasound at high levels of exposure may have adverse biological consequences, causing annoyance or aural pain or resulting in the rupture of the eardrum. These responses are listed in Table 6.3. When machines and vehicles produce infrasound they also tend to produce low frequency noise, and these audible frequencies may be more responsible than infrasound *per se* for the annoyance and resulting stress that may be caused by persistent sources of unwanted noise in the environment. Biological responses at lower levels of exposure have not been established, although several types of effect have been suggested in volunteer and animal studies (Table 6.4). In particular, exposure of animals to infrasound has been associated with changes in cellular structure and tissue morphology. The mechanism responsible for these changes is not known, but it is possible that it may be at least partly attributable to mechanical vibration caused by exposure to infrasound.

TABLE 6.3 Established biological effects of exposure to infrasound

Biological effect or endpoint	Attributed mechanism(s)
Aural pain	Mechanical vibration
Body vibration	Mechanical vibration
Hearing loss	Mechanical vibration
Annoyance*	Acoustic perception

* May include contributions from audible, low frequency noise.

TABLE 6.4 Possible biological effects of exposure to infrasound

Biological effect or endpoint	Attributed mechanism(s)
Cardiovascular changes	Not known
Impairment in cognitive function	Not known, but may include acoustic perception
Decrease in wakefulness	Acoustic perception
Effects on cytoskeleton (VAD)	Not known, but may include mechanical vibration

6.3 Epidemiology and Randomised Clinical Trials

Although there is no firm evidence to show that prenatal exposure to ultrasound causes any adverse health effect, there are insufficient data on long-term outcomes to be confident that there are no material effects, and there is some evidence for possible neurological effects. The need for more investigation is reinforced by the increasing use of ultrasound for commercial 'souvenir' imaging of the developing fetus and increasing intensity and number of medical ultrasound investigations in pregnancy.

The potential effects of such exposures are particularly difficult to assess because the brain undergoes a lengthy and highly complex period of development, in which different areas mature at different times. Even similar insults at different times of gestation would exert different effects on different areas of the brain, and so have the potential to induce different biological outcomes.

To date there have been very few studies concerned with the safety of medical uses of ultrasound other than in pregnancy and the available information on exposures associated with ultrasonic consumer products is too sparse to draw any conclusions regarding health.

For infrasound, aural pain and damage can occur at exposures above about 140 dB, the threshold depending on the frequency. The best-established responses occur following acute exposures at intensities great enough to be heard and may possibly lead to a decrease in wakefulness. The available evidence is inadequate to draw firm conclusions about potential health effects associated with exposure at the levels normally experienced in the environment, especially the effects of long-term exposures. The available data do not suggest that exposure to infrasound below the hearing threshold levels is capable of causing adverse effects.

7 Research Recommendations

7.1 Physics, Sources and Dosimetry

There is a need to further develop exposure and dosimetric quantities for assessment of health effects and safety of ultrasound and infrasound that relate more closely to biological responses.

Further surveys are required to assess occupational exposures to ultrasound and infrasound in the workplace. Assessments of environmental exposure to infrasound should be considered.

Improved methods for the prediction of ultrasonic wave propagation should be developed and validated, to create realistic models including the effects of propagation non-linearity, macroscopic structure and scatter.

Improved methods for the prediction of temperature rise caused by ultrasound should be developed, to include particular models for exposure of safety-critical anatomical sites.

7.2 Biology

Studies should place emphasis on gaining improved understanding of the biological responses to ultrasonically induced temperature changes and mechanical forces. Dose–response thresholds should be studied.

A report of subtle histological effects of ultrasound on the developing brain in animals requires confirmation and further study as a high priority, and this should include investigation of whether there are any behavioural or functional changes.

A deeper investigation is required of the effects of low power ultrasound on tissue regeneration, and particularly on bone healing.

The clinical use of ultrasound contrast agents is established, and known effects include microvascular damage and adverse effects on the heart. Further studies are needed to determine thresholds for these effects.

The general absence of adverse effects that have been observed with low levels of infrasound does not suggest that further studies should be given a high priority, However, the possibility that acute exposure to high intensity infrasound may affect behaviour and lead to decrements in the performance of cognitive tasks could be studied further, with better definition of the thresholds and time course for any effects.

Additional animal studies investigating whether infrasound can potentiate the damaging effects of intense audible noise would be useful.

7.3 Epidemiology and Randomised Clinical Trials

It would be desirable to determine the variation in the number, mode, intensity and duration of ultrasound examinations in different populations during pregnancy.

For further observational studies of non-right-handedness (or of left-handedness) to be helpful in providing evidence regarding the possible effects of prenatal ultrasound exposure, they would need to include better exposure assessment and information on potential confounding variables. It is possible that observational studies of other endpoints might be useful in the future. For example, a future case–control study might be informative about a putative causative association between prenatal ultrasound and a specific health outcome that either had not been evaluated or else was too rare to be either demonstrated or excluded, in the randomised controlled trials.

In view of the extent to which ultrasound examinations are established as part of standard medical care during pregnancy, it seems unlikely that further large-scale trials in which some women are randomly assigned to receive no routine examination can be contemplated. Nevertheless, there is at present variation in the number and timing of examinations in different countries and in the exposure received from different types of machine. If trials are conducted in future for clinical reasons in which women are randomly assigned to receive different numbers of examinations at different stages of pregnancy or to different levels of exposure, then it would be desirable that such trials include a component evaluating adverse effects of ultrasound as an endpoint. The list of adverse effects to be evaluated in such trials needs to be clearly specified in advance and to include those adverse effects that have been suggested by the existing trials. These effects include perinatal mortality (excluding malformations) and restricted intrauterine growth. In addition, long-term follow-up of children in such trials is desirable, including evaluations of growth, development, speech, hearing and vision, non-right-handedness and intellectual performance.

There have been very few studies concerned with the safety of ultrasound other than in pregnancy. Consideration should be given to the desirability and feasibility of such studies including the health effects of new technologies involving ultrasound.

Appendix A
Guidelines and Standards for Protection

Usually, reports from the Advisory Group on Non-ionising Radiation are restricted to scientific evidence and its discussion, and omit reviews of any associated exposure regulations or guidelines. It has been decided to make an exception in this case. For information, this appendix has been added to give, in outline, the current international regulations and recommendations by which exposures of humans to infrasound and ultrasound are limited and controlled to assure protection against inappropriate exposure.

A1 Introduction

Members of the public are commonly exposed to ultrasound as patients for diagnostic, therapeutic and surgical purposes. Individuals may also be exposed to ultrasound in a quasi-medical context, but without a diagnostic outcome. Examples of such exposures include the demonstration of new equipment to a potential customer, during teaching and training, or when an ultrasound examination is carried out for personal reasons, such as to view an image of an unborn child. Ultrasound may be also used for cosmetic applications. For these examples, the conditions involve deliberate exposure, with some end purpose. The protection of those exposed to ultrasound under these conditions arises both from appropriate equipment design, for which the manufacturer is responsible, and from appropriate use, for which the operator is responsible.

Exposure to ultrasound and infrasound may also be associated with occupational conditions – for example, where equipment machinery vibration generates acoustic noise in the frequency bands above 20 kHz or below 20 Hz, respectively. Those working under water may be similarly exposed as a result of their submarine occupation. Members of the public may also be exposed to airborne or water-borne infrasound or ultrasound, under conditions comparable to those experienced during occupational exposure. This second group of conditions of exposure is environmental, and protection control becomes more a matter of regulatory management and monitoring than professional practice.

A2 Protection Standards for Medical Uses of Ultrasound

Protection against inappropriate exposure to ultrasound when used for medical applications is mediated by governmental regulatory bodies, guided by international standards, most notably those published by the International Electrotechnical Commission (IEC). In Europe, the Medical Devices Directive (EC, 1993) places demands on manufacturers regarding the safety of their scanners and provision of information to purchasers and users. In the USA, the Federal Food, Drug and Cosmetic Act (FDA, 2005) controls the sale of ultrasound equipment for medical uses. Manufacturers apply through the US Food and Drug

Administration using a process known as 510(k) (FDA, 2008). Since in each of these national cases reference is made to international standards, these will first be described.

A2.1 IEC standards for medical ultrasound equipment

IEC safety standards for medical electrical equipment are contained in the IEC 60601 series. Part 1 (IEC, 2005) applies to all electro-medical equipment, and specifies general criteria for protection against thermal, electrical and mechanical hazards. This standard has undergone recent substantial revision, including a change in emphasis towards risk assessment. It is yet to be determined what effects these changes may have on the management of hazards and protection.

IEC 60601 Part 1 has nothing to say about exposure to ultrasound. There are, however, two particular standards in the 60601 safety series concerning medical ultrasound. These are Part 2-5 for ultrasound physiotherapy equipment (IEC, 2001a) and Part 2-37 for ultrasound diagnostic and monitoring equipment (IEC, 2001b). No Part 2 safety standards have yet been published for surgical ultrasound equipment such as extracorporeal shock-wave lithotripters or high intensity focused ultrasound devices.

A2.2 IEC 60601-2-5: ultrasound physiotherapy equipment

The IEC standard for physiotherapy equipment includes two limits for the purpose of patient protection. The first limits the temperature of the front face of the transducer to be no more than 41°C when operated under water with an initial temperature of 25°C. The second protection limit applies to the effective ultrasound intensity, which must not exceed 3 W cm^{-2}. Exposure of tissue to this intensity causes temperature rises that can result in tissue damage, particularly at the surface of bone. The relatively high intensity limit is allowed because some physiotherapy applications require brief exposures, during which the transducer is kept in continual motion with respect to the tissue under treatment.

A2.3 IEC 60601-2-37: ultrasound diagnostic and monitoring equipment

The IEC standard for diagnostic and monitoring equipment differs from the standard for therapeutic equipment in that it sets no upper limit on output intensity, nor any other output exposure quantity. Thus IEC standards establish no protective limit on ultrasound exposure from equipment whose purpose is diagnostic imaging or monitoring. Instead, the standard specifies how a user shall be informed about potential hazard, through displayed indices related to exposure and safety. This approach to protection casts a responsibility on the user, requiring for its success an appropriate level of training and competence for all practitioners using ultrasound diagnostic equipment. The philosophy and definitions for index display were developed in the USA in the early 1990s, and were first published in the so-called 'Output Display Standard' (AIUM/NEMA, 1992).

The standard defines two indices related to safety, the thermal index (TI) and the mechanical index (MI), and specifies methods for their determination under a set of particular conditions. The values of the MI and TI appear, typically, on the screen of the scanner, and alter dynamically as the practitioner changes the mode of operation or output power. The conditions for which the indices must be displayed

are specified, allowing index values not to be displayed for low output: although it is common for manufacturers to display index values for all conditions of use. The methods for determination of the safety indices (without specifying when they should be displayed) are now also published in a separate IEC standard (IEC, 2006).

A2.4 Safety indices

Thermal indices

The thermal index (TI) warns the user that the tissue may be warmed, specifically through the absorption of ultrasound energy. The TI calculation assumes that temperature increase is linearly related to acoustic power, and so depends on the ability to measure this primary acoustic quantity (NCRP, 1992). The conditions assume that sufficient time has elapsed for steady-state thermal conditions to apply, mimicking a condition where the ultrasound beam has remained stationary with respect to the tissue for a period of typically several minutes. The formulation has led some to misinterpret the TI as being numerically equal to the actual temperature increase in degrees Celsius in tissue while scanning. Whilst the TI can be taken as an approximate indication of the temperature rise, this may not be assumed for many conditions, and this can cause problems when relating the TI value to known biological responses to heating.

A set of six formulae allows the TI to be calculated for specific conditions, and these are described in detail elsewhere (Abbott, 1999; Duck, 2006). Three simple generic physical models are used. The soft-tissue thermal index (TIS) model assumes a uniform homogeneous medium with an attenuation coefficient of 0.3 dB cm^{-1} MHz^{-1} to predict the ultrasound exposure at depth. The selected attenuation coefficient is rather lower than the average for soft tissue. This allows it to be assumed that the propagation path may include a fluid path, such as amniotic fluid or urine, for which the attenuation coefficient is much lower than that for other soft tissues. The bone-at-focus thermal index (TIB) model includes a layer of strongly absorbing bone-mimic material within the soft-tissue model and assumes that half of the incident power is absorbed in this layer. The cranial bone thermal index (TIC) model omits soft tissue, and considers the absorption of ultrasound in a bone-equivalent layer coupled directly to the transducer.

For each of the three tissue models, two conditions of ultrasound exposure can be considered, giving six conditions in all. These are for scanned and unscanned exposure. Scanned conditions are associated with pulse-echo B-mode images and Doppler images of tissue cross-sections. Unscanned conditions are used for M-mode and pulsed Doppler studies of local tissue movement and blood velocity waveforms. Heating patterns differ between these two conditions, requiring different approaches to the prediction of temperature rise from acoustic power in each case.

The IEC standard specifies how to derive the thermal index value in each of the six circumstances described, using measurements of acoustic quantities at prescribed positions within the beam (IEC, 2006). The methods for calculation use measurements in water of acoustic quantities such as acoustic power, temporal average intensity, acoustic pressure and beam width, and therefore relate directly to other IEC standards specifying measurement procedures.

Mechanical index

The mechanical index (MI) is used to indicate the possibility of mechanical damage to the tissue as a result of acoustically driven bubbles or gas bodies. The formula is based on a mathematical model which describes the behaviour of a cloud of air bubbles of a wide spectrum of diameters in water and blood when exposed to a single cycle of ultrasound (Apfel and Holland, 1991). From this analysis, and making some simplifying assumptions, a standard formulation of MI was defined (AIUM/NEMA, 1992; IEC, 2001b, 2006) (see Chapter 2). The new 'mechanical index' is calculated at a specified location in the beam, so enabling the MI to be used to characterise the beam as a whole, rather than being dependent on position. It is defined as

$$\mathrm{MI} = p_r / f^{0.5}$$

where p_r is the peak rarefaction pressure and f is the acoustic working frequency (centre frequency) of the pulse. The tissue model used for the mechanical index is the same as that used for the soft-tissue thermal index. There is a standard protocol to identify the depth at which the peak rarefaction pressure is measured in water, and the attenuation coefficient is used to correct for the loss in the tissue model, to give an attenuated peak rarefaction pressure. The threshold for the MI for inertial cavitation in water is 0.7.

The MI informs the user about changes in acoustic pressure amplitude. In contrast to the TI, there is only one tissue model for the MI, and this has raised concerns about its limited generality. Furthermore, the cavitation threshold applies to bubble clouds in water. Much higher values are required to initiate bubble generation in tissue *in vivo* (Church, 2002). It has been suggested that tissue damage in the presence of gas inclusions *in vivo* (lung alveoli, intestinal gas and contrast agents) follows the same frequency dependence as that for the MI, but this is not supported strongly by experimental evidence. Therefore it is not generally possible to compare outcomes at different frequencies. Furthermore, different contrast agents behave differently, and MI thresholds established for one cannot be used for another.

A2.5 Transducer surface temperature

IEC 60601-2-37 places limits on the allowed temperature of the surface of the transducer (Table A1). In air this is 50°C, considerably higher than the temperature of 43°C allowed in contact with tissue. Higher increases in temperature are allowed for transducers in contact with the skin than for those used internally, such as transvaginal or intra-rectal probes. These temperatures are not only of theoretical interest, since many scanners can now drive transducers at levels approaching these temperatures (Duck et al, 1989). Under all reported conditions the allowed contact temperature is the dominant limit (Calvert and Duck, 2006).

TABLE A1 Limits on surface temperature and surface temperature rise specified in IEC 60601-2-37 (IEC, 2001b)

	In air	On tissue (external use)	On tissue (internal use)
Maximum temperature	50°C	43°C	43°C
Maximum temperature rise	27°C	10°C	6°C

A2.6 Measurement standards

All the IEC standards to which reference has been made depend on IEC acoustic measurement standards. Of these, four may be selected for particular reference. These are IEC 61102 and IEC 61220 (IEC, 1991, 1993) which deal with the measurement of acoustic beams in the frequency range 0.5–15 MHz using hydrophones in water; IEC 61828 (IEC, 2001c) for definitions and measurement methods for focusing transducers; and IEC 61161 (IEC, 1992) which is concerned with the measurement of acoustic power in the frequency range 0.5–25 MHz.

A2.7 Regulation by the US Food and Drug Administration

Equipment sold in the USA must meet the US Food and Drug Administration 510(k) regulations. Whilst these regulations have no formal status outside the USA they have had, and continue to have, a fundamental effect on the control of acoustic output, and consequent protection of patients, throughout the world. The industry is now dominated by four or five international companies, most with manufacturing facilities in the USA. Since government controls in the USA affect both manufacture and the sale of equipment, the FDA 510(k) process affects a large proportion of equipment in clinical use at present. The process requires manufacturers to supply information on acoustic output and ensure that certain 'derated' acoustic parameters do not exceed allowable levels. Details of the procedure and requirements may be found on the FDA website (FDA, 2008). Most applications use so-called 'Track 3', which places stricter control just on equipment for ophthalmic use. The lower limits applied for eye scanning reflects concerns that the eye may be particularly susceptible to thermal damage as a result of very low blood perfusion.

To comply with Track 3 requirements, manufacturers must display the safety indices according to the IEC 60601-2-37 standard. Manufacturers complying with Track 3 must also meet the upper limits on derated (attenuated) spatial peak, temporal average intensity I_{SPTA}, derated spatial peak, pulse average intensity I_{SPPA}, MI and TI as set out in Table A2. Manufacturers may use conditions giving a TI greater than 6.0 for applications other than ophthalmology, but must justify why this is necessary.

TABLE A2 Threshold exposures allowed by the US Food and Drug Administration under the 510(k) process for marketing clearance for diagnostic ultrasound equipment. All values are for estimated *in situ* quantities, for which the tissue attenuation model has an attenuation coefficient of 0.3 dB cm^{-1} MHz^{-1}, so-called 'derated' values. Values of TI above 6.0 are allowed but require justification

Applications	Spatial peak, temporal average intensity (mW cm^{-2})	Spatial peak, pulse average intensity (W cm^{-2})	Mechanical index	Thermal index
All except ophthalmology	720	190	1.9	(6.0)
Ophthalmology	50	Not specified	0.23	1.0

When considering the FDA controls for the protection of the patient from inappropriate exposure, it is perhaps useful to understand the origin of the particular levels set in Table A2. When the limits were first established, in 1987, it was argued that there was no evidence of harm from the use of diagnostic ultrasound up to the date of the Medical Device Amendments Regulations (1976). Surveys of maximum output were carried out on all commercial systems in use in the USA up to that date. From these surveys the upper limits for intensity were taken from the overall maxima in each of four categories of clinical application. When Track 3 and output display were introduced, the temporal average intensity previously reserved only for peripheral vascular applications was allowed for all non-ophthalmic applications, including obstetrics.

A2.8 Advice to users

The introduction of the output display and its implementation as an international standard has cast a greater responsibility on users to limit exposures and helps to protect patients from inappropriate exposure. Advisory documents have been published explaining the meaning and use of the safety indices (AIUM, 1994; EFSUMB, 1996). The British Medical Ultrasound Society (BMUS) has published guidance that advises on actions to be taken by the user depending on the value of the displayed safety indices (Table A3). The recommended BMUS thresholds for action are based on a rationale that takes account of available scientific evidence, together with a safety factor which recognises the inadequacy of the models used for the calculation of the safety indices (Hoskins et al, 2003). The MI value of 0.3 is based on a previous AIUM statement (AIUM, 1993), and the MI value of 0.7 on the threshold given by Apfel and Holland (1991). The TI value of 0.7 is based on the World Federation for Ultrasound in Medicine and Biology (WFUMB) recommendations on temperature rise (WFUMB, 1998) with a safety factor of two to allow for practical situations for which the TI will underestimate temperature rise. The BMUS guidance also gives recommendations for limiting exposure time at higher TI values. The upper limit of 3.0 for the TI for any embryological or fetal exposure relates to an overall temperature rise of 6°C, giving an estimated *in situ* temperature of 43°C, again applying a safety factor of two. The limit on the TI of 1.0 for eye scanning is the same as that set by the US regulations (FDA, 2008) and given by Herman and Harris (1999).

TABLE A3 Levels of the safety indices for which user action is recommended by the British Medical Ultrasound Society (Hoskins et al, 2003)

Safety index	User action
MI > 0.3	Reduce duration of exposure to neonatal lung or intestine
MI > 0.7	Potential hazard with gas contrast agents
TI > 0.7	Restrict exposure times of embryo or fetus
TI > 1.0	Eye scanning not recommended (not applicable for fetal scanning)
TI > 3.0	Use not recommended for embryo or fetal scanning

Users can also obtain advice from the WFUMB recommendations (WFUMB, 1998). Amongst the WFUMB conclusions and recommendations, two contain specific advice about heating. It is stated that a diagnostic exposure that produces a maximum temperature rise of no more than 1.5°C above normal physiological levels (37°C) may be used clinically without reservation on thermal grounds. For higher temperature elevations, it is advised that "A diagnostic exposure that elevates embryonic and fetal *in situ* temperature above 41°C (4°C above normal temperature) for five minutes or more should be considered potentially hazardous".

Barnett et al (2000) summarised the recommendations of some national and international bodies including those from the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB), the Australasian Society for Ultrasound in Medicine (ASUM) and the American Institute of Ultrasound in Medicine (AIUM). These recommendations are largely qualitative, and are in broad agreement with each other. This review also usefully identifies some perceived gaps in the policy documents from the professional organisations. Of the gaps noted at that time, safety recommendations for the use of echo contrast agents have since been developed by the WFUMB, EFSUMB and AIUM, and the non-clinical use of ultrasound is now under active discussion by the WFUMB and others. Appropriate recommendations covering the use of ultrasound to examine the uncomplicated pregnancy during the first trimester have yet to be fully developed, and possible effects on the human central nervous system have yet to be given serious consideration.

A2.9 European Medical Devices Directive, MDD

One of the main drivers for the development of European standards is the European Medical Devices Directive or MDD (EC, 1993), which has now been adopted into the national legal framework of European member states. Each state appoints a Competent Authority which, for the UK, is the Medicines and Healthcare products Regulatory Agency (MHRA). The Authority in its turn designates Notified Bodies whose function is to make assessments of new products under the MDD procedures and to carry out any necessary conformity assessments described in the annexes of the MDD. Several Notified Bodies may be designated in each member state, but only those with appropriate products within their designated scope may be selected by manufacturers of medical ultrasound devices. Manufacturers can use any of these Notified Bodies within the European Union. A CE mark on a device means that the manufacturer has declared that the device satisfies the requirements essential for it to be fit for its intended purpose. All medical devices (except custom-made and devices intended for clinical investigations), whether used in private or public hospitals and nursing homes, or sold in retail outlets, must carry the CE marking.

Annex IX of the MDD describes three general classes of equipment and a set of rules for establishing the class of any particular type of equipment. Almost all diagnostic ultrasonic devices lie in Class IIa, because they are "active devices intended for diagnosis". Class IIb is specifically intended for monitoring vital physiological parameters, where the nature of the variations is such that they could result in immediate danger to the patient. Some Doppler devices used during surgery may be deemed to fall into this category. Intravascular ultrasound transducers are usually designated as Class III since they are invasive devices. Therapeutic ultrasonic devices including lithotripters are classified as Class IIa, unless they are deemed to carry out their function in a potentially hazardous way, in which case they are Class IIb. Initially

a manufacturer determines the class of a product, and selects an appropriate Notified Body to carry out the conformity assessment procedure. The assessment in all categories requires appropriate audits of the manufacturer's production quality assurance systems. In addition, there is a provision for examination and testing of products or batches, although this is not mandatory, even for Class III products provided the design dossier is submitted. It is probable that most medical ultrasound devices gain a CE mark without practical independent assessment, being based substantially on inspection of required documentation. Manufacturers are also required to maintain a post-market surveillance system and report certain types of incidents to a Competent Authority.

The MDD does not give any guidance specific to ultrasound emissions. The general statements of Clause 11 (on protection against radiation) and of Clause 12 (on equipment with an energy source) may be taken to apply, however. These require that "Devices shall be designed and manufactured in such a way that exposure to patients ... shall be reduced as far as possible compatible with the intended purpose, whilst not restricting the application of appropriate specified levels for therapeutic or diagnostic purposes". Devices may emit hazardous levels of radiation necessary for a specific medical purpose, but it must be possible to control them. Where radiation is "potentially hazardous", devices must be "fitted, where practicable, with visual displays and/or warnings of such emissions". It may be taken that some ultrasonic diagnostic devices fall into this category. Furthermore, "accessible parts of devices ... must not attain potentially dangerous temperatures under normal use". The normal method for a manufacturer to demonstrate that they have complied with these requirements is to follow procedures laid down in international standards.

Prior to the introduction of any novel medical device, the manufacturer may have to carry out a clinical investigation to demonstrate compliance on efficacy and declare any undesirable side-effects. These data are submitted to a Competent Authority for approval.

The Medicines and Healthcare products Regulatory Agency and the European Commission have issued a number of general guidance documents on the application of European directives on medical devices. Further information may be found on the following websites:

www.MHRA.gov.uk

and http://ec.europa.eu/enterprise/medical_devices/meddev

A2.10 Overview of protection against excess exposure during medical use

The standards and recommendations summarised in the previous sections constitute the present framework with the objective of ensuring that those deliberately exposed to ultrasound for medical reasons are protected from inappropriate exposure, whilst not placing inappropriate constraints preventing effective diagnosis or treatment. For diagnostic ultrasound equipment, it is generally understood that the most structured and effective control is still exercised through the agency of the US FDA marketing clearance process.

Apart from the FDA limits, transducer self-heating is the other practical control on ultrasound exposure. Further increases in output power are not possible, using present transducer designs, without the

transducer getting too hot to use. Already, higher contact temperatures are tolerated for ultrasound transducers than for most other electro-medical devices, and this is particularly true for transducers intended for internal use – that is, for rectal, vaginal or oesophageal insertion. Manufacturers are responding to this technological challenge, and a number of means for the thermal control of diagnostic transducers have been patented (Saunders et al, 2004). It may be expected that the safe balance between exposure and surface temperature will remain a central consideration for diagnostic systems in the future.

A2.11 Summary of medical exposure guidelines and standards

Protection from excessive exposures during use of ultrasound for physiotherapy is assured through an IEC upper limit on acoustic intensity of 3 W cm^{-2}. Whilst this intensity would certainly allow hazardous temperature increases, especially in any exposed bone, it is acceptable, because of the need to achieve a therapeutic outcome. For surgical applications such as extracorporeal shock-wave lithotripsy and high intensity focused ultrasound (HIFU), there are no protection standards. Allowed exposures are sufficient to cause the desired destructive effects. Protection of non-target tissue regions is achieved entirely from appropriate placement of the beam. For example, no equipment design standards prevent excessive heating of overlying tissues in HIFU. Lung damage from lithotripters is prevented by appropriate beam positioning.

A3 Protection from Non-medical Exposures of Ultrasound

By comparison with exposure to ultrasound for medical purposes, the protection of humans from exposure to other sources of ultrasound has received very much less attention. It is now over 25 years since the publication of interim guidelines on limits to airborne ultrasound by the (then) International Non-Ionizing Radiation Committee of the International Radiation Protection Association (IRPA, 1984). The recommendations were based on scientific evidence contained within a companion document prepared by the Division of Environmental Health of the World Health Organization (WHO, 1982). Since then, perhaps the only extensive publication has been from the Environmental Health Directorate of Canada (1991), which presented a further review and update of the earlier IRPA publication.

These documents make reference to the range of industrial and domestic devices capable of giving human exposure to airborne ultrasound, including industrial equipment for cleaning, drilling, welding, mixing, extraction, foaming and emulsification (Shoh, 1975) and domestic products including door openers, remote controls, intrusion alarms, pest repellents and guidance devices for the blind. The frequency spectrum considered is limited to the lower ultrasonic range, from 20 to 100 kHz, although some devices such as fuel atomisers may operate at frequencies up to 300 kHz. For all the devices being considered, it is appropriate to manage protection in a different way, depending on whether the ultrasound wave is either airborne or coupled by direct or liquid contact, and these different conditions are considered separately below. Advice concerning the deliberate use of medical diagnostic equipment for non-medical purposes is also addressed.

A3.1 Protection from airborne ultrasound

The protection of personnel from hazardous exposure to airborne ultrasound is similar to that for audible noise. Threshold levels are set for sound pressure level (SPL) appropriate for 1/3 octave bands centred at selected frequencies over the range of interest. The SPL is quantified as a logarithmic ratio, with a practical unit the decibel, dB:

$$\mathrm{SPL} = 20 \log_{10}\left(\frac{p_{\mathrm{rms}}}{p_{\mathrm{rms}}(\mathrm{ref})}\right) \mathrm{dB}$$

where p_{rms} is the root-mean-square acoustic pressure and $p_{\mathrm{rms}}(\mathrm{ref})$ is the reference rms acoustic pressure. The $p_{\mathrm{rms}}(\mathrm{ref})$ is equivalent to approximately the lowest level of audible sound perceived by humans at 1 kHz, which is the frequency at which the human ear is most sensitive to sound. The accepted reference acoustic pressure is 20 μPa rms. Thus, for example, an SPL of 20 dB is equivalent to an acoustic pressure of 200 μPa, 40 dB to 2 mPa, 60 dB to 20 mPa (all rms), and so on. An increase in sound intensity by a factor of two is equivalent to an increase in SPL of 3 dB, whilst a doubling of acoustic pressure increases the SPL by 6 dB.

Limits to exposure have been considered by a number of national and international bodies, and are summarised elsewhere (auf der Maur, 1985; Environmental Health Directorate of Canada, 1991; ACGIH, 2003). Table A4 presents two sets of recommended limits for 1/3 octave bands, centred in the range 20–100 kHz. The IRPA limits assume exposure during a full eight-hour day, and upward modification to the limit is allowed for shorter exposures (see Table A5). Lawton (2001) commented that the American Conference of Governmental Industrial Hygienists (ACGIH), by consistently setting higher levels than all other recommended levels, "has pushed its acceptable exposure limits to the very edge of potentially injurious exposure". All recommendations retain an upward change in limit between the 1/3 octave bands at 20 and 25 kHz, based on an empirical analysis of industrial exposure (Acton, 1983).

For the exposure of the general public the recommended levels are lower. A comparison between the recommended levels for occupational and public exposure is given in Table A6 (IRPA, 1984). The scientific basis for these levels depends substantially on considerations of damage to human hearing (Lawton, 2001). For occupational exposure, exclusions are allowed if workers are provided with ear protectors which reduce the SPL at the ears to those given in Tables A4 and A5, depending on the exposure time. No such exclusion is recommended for public exposure. Where ear protection may be used, the Canadian guidelines recommend that an overall upper limit on total linear SPL should be 137 dB, in order to prevent inappropriate heating to any other part of the body (Environmental Health Directorate of Canada, 1991).

A3.2 Protection from contact exposures

Some industrial and commercial sources of ultrasound can, in principle, allow the ultrasonic wave to couple directly with body tissue. This may occur when the transducer face is touched, when tissue comes into contact with a solid which itself is coupled to an ultrasound source, or when part or all of the body is immersed in a liquid through which ultrasound is propagating. Under all these conditions the acoustic

TABLE A4 Occupational exposure limits for sound pressure level, SPL, for occupational airborne exposure (dB referenced to 20 μPa)

1/3 octave band centre frequency (kHz)	ACGIH (2003)	IRPA (1984)
20	135	75
25	140	110
31.5	145	110
40	145	110
50	145	110
63	–	110
80	–	110
100	–	110

TABLE A5 Adjustment to occupational exposure limits given in Table A4 for exposure durations not exceeding four hours per day (IRPA, 1984)

Total exposure duration per day (hours)	Correction to SPL (dB)
2–4	+3
1–2	+6
Less than 1	+9

TABLE A6 Exposure limits for sound pressure levels, SPL, for occupational and public exposure (dB referenced to 20 μPa) for 1/3 octave bands (IRPA, 1984)

1/3 octave band centre frequency (kHz)	Occupational exposure	Public exposure
20	75	70
25, 31.5, 40, 50, 63, 80 and 100	110	100

wave is coupled with the tissue with considerably greater efficiency than for airborne ultrasound, and energy transfer may approach 100% for some liquid coupling. This provides a potential route for high power ultrasound to penetrate the body and cause damage, which could be severe. Examples of devices for which such inadvertent exposure might occur, if the device was misused, are ultrasonic humidifiers, ultrasonic cleaners and ultrasonic bonding machines. The limits given by the ACGIH (see Table A4) assume that no coupling with water or other substrate exists. When such coupling does exist, these thresholds should be reduced by 30 dB.

When the ultrasound source is coupled directly with the body, the values in the table do not apply. The Canadian guidelines (Environmental Health Directorate of Canada, 1991) contain some useful advice, however. It is stated that contact exposure to high power ultrasound must be avoided at all times. In addition, only qualified personnel should operate the equipment and those involved should have knowledge of possible harmful effects. Appropriate safety warning labels should be used, and a design for an ultrasound warning sign is suggested. The use of gloves lined with flock or fur is suggested if hand immersion is unavoidable when using cleaning baths.

A3.3 Use of medical diagnostic equipment for non-medical purposes

The widespread use of ultrasound examination as a diagnostic aid during pregnancy has led the general public to expect recordings of ultrasound images of the unborn child to form part of the family photograph collection. It is now common for such recordings to be made available, at a cost, when routine ultrasound examinations are carried out during pregnancy. In addition, it is becoming more common for hospitals and private studios to offer ultrasound examination services purely to provide keepsake images, which may include two- and three-dimensional video sequences of fetal images. A number of professional bodies representing the medical use of ultrasound have given advice about this trend. This advice has been based on a number of considerations, including those of ethical, medico-legal, safety and operator training issues. The following summary is derived from information obtained at the time of writing from the websites of the respective professional bodies.

The American Institute of Ultrasound in Medicine (AIUM, 1994) does not oppose ultrasound examinations for souvenir images on any grounds, although it specifically recommends that such examinations should be carried out by qualified medical professionals. Recognising that "the general use of ultrasound for medical diagnosis is considered safe .... Ultrasound bioeffects may result from scanning for a prolonged period or inappropriate use of color *(sic)* or pulsed Doppler ultrasound without medical indication".

The British Medical Ultrasound Society (BMUS), within its safety guidelines, gives other examples of the non-medical use of ultrasound such as repeated examinations for operator training and equipment demonstration, in addition to the keepsake images mentioned above (Hoskins et al, 2003). It recommends limits on the safety indices such that the TI is less than 0.5 and the MI is less than 0.3. BMUS recommends that such examinations should not be carried out during the first trimester, nor should exposure levels or times be extended beyond those needed for clinical purposes.

The European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB, 1996) has taken the most critical view, recommending that "Ultrasound scans should not be performed solely for producing souvenir images or recordings of a fetus or embryo". The rationale for the EFSUMB position is based on the possible existence of subtle biological effects of ultrasound on the developing embryo or fetus, allowing a justification of the use of ultrasound exposure where there is a diagnostic benefit, but not otherwise.

A3.4 Consumer products

Consumer products should comply with the requirements of the General Product Safety Regulations, 2005 (DTI, 2005). The regulations place a general duty on all suppliers of consumer goods to supply products that are safe in normal or reasonably foreseeable use. Safety takes into account factors such as the product's characteristics, instructions and warnings, and the categories of consumers at serious risk when using the product, particularly children. Relevant British or European standards can be taken into account in assessing the safety of a product. There are currently no specific standards covering consumer products generating or emitting infrasound or ultrasound.

A3.5 Overview of protection against excess exposure during non-medical use

Guidelines for occupational and public exposure to airborne ultrasound were published by IRPA over 25 years ago in an 'interim' report. Threshold limit values are given as sound pressure level (SPL) in decibels. The IRPA report remains the main international document which sets protection levels for the public and workers from any hazard associated with airborne ultrasound. Canadian and US guidance documents, restricted in scope to occupational exposures and based broadly on the same scientific evidence, reach broadly the same conclusions. The US levels for occupational exposure are 5–10 dB above those given by IRPA and the Canadian guidelines do not allow for higher levels to be used for shorter times. Criteria for protection levels are largely based on the response of the ear. Where the ear is protected, limits depend on estimated tissue heating. Greater hazard may be associated with directly coupled ultrasound, particularly when parts of the body are immersed in water or other liquid carrying an ultrasound beam. Industrial, commercial and occupational situations where this may occur are not uncommon, including the use of humidifiers, cleaning baths and decorative fountains, and underwater occupations. No established protection standards or recommendations exist for any of these circumstances.

A3.6 Overall summary of exposure guidelines and standards

For most medical uses of ultrasound, the protection of those exposed arises both from appropriate equipment design, for which the manufacturer is responsible, and from appropriate use, for which the operator is responsible. For environmental and occupational exposure to ultrasound, protection control is a matter of regulatory management and monitoring rather than professional practice.

Protection from inappropriate or hazardous exposure to ultrasound for medical purposes is controlled through international standards and national regulations. The International Electrotechnical Commission has established particular manufacturing standards for diagnostic medical ultrasonic equipment, and for therapeutic ultrasound equipment. The diagnostic standard sets no upper limits on ultrasound exposure (although limits to transducer contact temperature are imposed). Instead, safety indices are defined to advise clinical users on thermal and mechanical hazard. In the USA, there are national regulatory limits on diagnostic exposure, based on acoustic exposure from clinical equipment in use over 20 years ago. These limits are mediated through the FDA marketing clearance process. There are no international safety standards for surgical ultrasound equipment. For surgical and therapeutic uses of ultrasound, protection from inappropriate or unsafe exposure of non-target tissue regions is achieved by the appropriate

exposure time and placement of the beam. The European Medical Devices Directive, transposed into national legislation, allows manufacturers to place CE marking on medical ultrasound equipment once a declaration of conformity has been made through an appropriate Notified Body.

Protection recommendations exist for occupational and public exposure to ultrasound. For airborne ultrasound, interim guidelines on limits of human exposure published by IRPA are now over 25 years old. A limit on sound pressure level of 100 dB for the general population is recommended.

No formal recommendations currently exist for the establishment of management infrastructures specifically for protection against inappropriate exposure to infrasound or ultrasound, comparable to those in use for ionising radiation. Such structures might include, for example, systems to ensure that all equipment conforms to current national and international standards, is used according to current professional guidelines, and is subject to appropriate risk assessment. For such management structures to operate effectively, individuals with appropriate knowledge and skills would be required.

A4 Protection from Infrasound

The World Health Organization has recognised that the normal A-weighted assessment measures for environmental noise are deficient for evaluation of disturbance from noises with large low frequency components (Berglund et al, 2000).

The Department for Environment, Food and Rural Affairs (Defra) has recently supported the development of criteria for limiting environmental low frequency noise (Moorhouse et al, 2005). The average limiting levels expressed as equivalent sound level, L_{eq}, are given in Table A7 for night-time fluctuating noise.

TABLE A7 Proposed infrasound and low frequency noise limiting criteria

Freq (Hz)	10	12.5	16	20	25	31.5	40	50	63	80	100	125	160
Level (dBL_{eq})	92	87	83	74	64	56	49	43	42	40	38	36	34

The criterion levels are a few decibels below the median threshold at the lowest frequencies, whilst with increasing frequency the difference decreases and the criterion rises above threshold at 63 Hz. The criterion is for annoyance, not for direct effects on health, and there are relaxations for steady noises and noise that is present only in the day-time.

Other agencies also tend to manage infrasound as a subset of considerations on protection from noise (NOHSC, 2003; Jakobson, 2003). However, the relevant European directive on noise protection (EC, 2003) omits specific reference to protection levels for infrasound. The American Conference of Governmental Industrial Hygienists (ACGIH, 2003) advises that, for frequencies between 1 Hz and 80 Hz, sound pressure level (SPL) should not exceed 145 dB and the overall SPL should not exceed 150 dB, where weighting of

the frequency components, commonly applied for protection against audible noise, is not applied. Japanese guidelines recommend a limit of 92 dBG for infrasound generated by wind turbines.

The dBG frequency weighting was introduced by the International Organization for Standardization (ISO) to be applied in the infrasonic range (ISO, 1995) comparable with the A-weighting scheme used for the audible spectrum of frequencies. G-weighting follows assumed hearing contours with a slope of 12 dB per octave from 20 Hz down to 2 Hz (Figure A1). This slope is intended to give a subjective assessment to noise in the infrasonic range. A G-weighted level of 95–100 dBG is close to the perception level. G-weighted levels below 85–90 dBG are not normally significant for human perception. Alternatively, linear weighting, also known as Z-weighting, is specified, which has a flat frequency response from 10 Hz to 20 kHz. More detail of the noise – in particular, the presence of tones – can be found from a third octave or narrowband analysis.

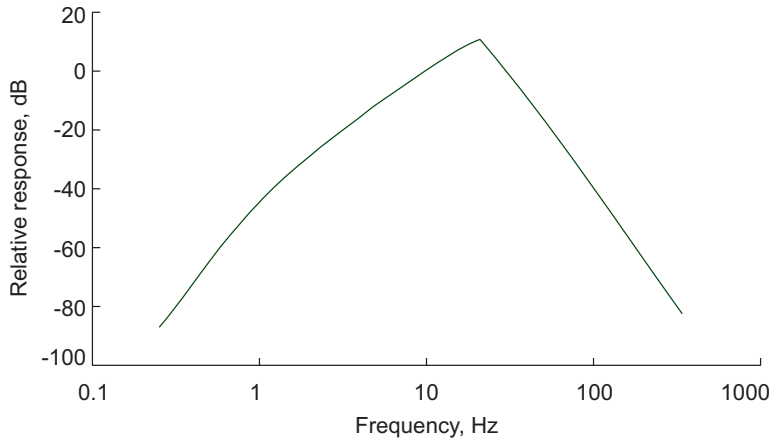

FIGURE A1 G-weighting curve specified by ISO 7196 (ISO, 1995)

One of the IEC standards associated with wind turbines (IEC, 2006b) specifies the appropriate method of measurement for environmental noise, including infrasound, close to such a device. In particular, it specifies the standard distance for measurement shall be the sum of the height of the turbine to its blade axis and twice the length of one turbine blade.

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Appendix B
Publications of the Advisory Group on Non-ionising Radiation

1 Electromagnetic fields and the risk of cancer. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **3**(1), 1–138 (1992).

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3 Health effects related to the use of visual display units. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **5**(2), 1–75 (1994).

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5 Health effects from ultraviolet radiation. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **6**(2), 7–190 (1995).

6 Use of sunbeds and cosmetic tanning. Statement by the Advisory Group on Non-ionising Radiation. *Radiol Prot Bull,* No. 218, 11–15 (1999).

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8 ELF electromagnetic fields and the risk of cancer. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **12**(1), 1–179 (2001).

9 Possible health effects from terrestrial trunked radio (TETRA). Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **12**(2), 1–86 (2001).

10 ELF electromagnetic fields and neurodegenerative disease. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **12**(4), 1–24 (2001).

11 Health effects from ultraviolet radiation. Report of an Advisory Group on Non-ionising Radiation. *Doc NRPB,* **13**(1), 1–276 (2002).

12 Health effects from radiofrequency electromagnetic fields. Report of an independent Advisory Group on Non-ionising Radiation. *Doc NRPB,* **14**(2), 1–177.

13 Particle deposition in the vicinity of power lines and possible effects on health. Report of an independent Advisory Group on Non-ionising Radiation and its Ad Hoc Group on Corona Ions. *Doc NRPB,* **15**(1), 1–55 (2004).

14 Power frequency electromagnetic fields, melatonin and the risk of breast cancer. Report of an independent Advisory Group on Non-ionising Radiation. *Doc HPA,* **RCE-1,** 1–169 (2006).

15 Static magnetic fields. Report of an independent Advisory Group on Non-ionising Radiation. *Doc HPA,* **RCE-6,** 1–143 (2008).

Glossary and Symbols

Glossary

General

Adverse health effect A biological effect which is detrimental to the mental or physical well-being of exposed individuals, either in the short term or in the long term.

Biological effect A measurable change in a biological system in response to an applied stimulus.

Biology

Alveoli Small air-filled sacs in the mammalian lung, the primary site of gaseous exchange between the blood and the air.

Apoptosis Process of programmed cell death.

Blood–brain barrier (BBB) Specialised endothelial cells that regulate the passage of substances between the blood and the tissue of the brain.

Bowman's capsule Small, cup-shaped structure within the kidney.

Capillaries Smallest of the blood vessels.

Cardiomyocytes Muscle cells in the heart.

Cell lysis Death of a cell caused by destruction of the cell membrane.

Ciliated cells Cells with hair-like projections on their exposed surfaces.

Clonogenic assay A microbiology technique for studying the effectiveness of specific agents on the survival and proliferation of cells.

Cochlea Auditory part of the inner ear.

Cortical layers Distinct sheets of neurons forming the cerebral cortex of the brain.

Cytoarchitectonics Study of the composition and arrangement of the cells of the brain and other tissues.

Electrophoretic mobility The observed rate of migration of a component divided by the electric field strength in a given medium.

Embryo The earliest stage of prenatal development. In humans, from the moment of implantation until the end of the 8th week of gestation, after which it is called a fetus.

Endothelial cells Specialised cells lining blood vessels and lymphatics.

Enzymes Biological catalysts, which lower the amount of energy needed for chemical reactions.

Erythrocyte A red blood cell that contains haemoglobin and can carry oxygen.

Extravasation The leakage of substances from a blood vessel into the surrounding tissues.

Fetus (foetus) The stage of prenatal development between the embryo and birth.

Fibroblast A type of cell found in connective tissue with the potential to develop into any one of a range of different cell types.

Gene expression The process whereby the information contained in genes is read and used by cells, usually to make proteins.

Glioma A type of cancer in the brain or spinal cord.

Glomerulus A knot-shaped collection of capillaries within the kidney.

Haemoglobin Protein found in red blood cells that contains iron and transports oxygen.

Haemolysis The process of breaking open red blood cells, release of haemoglobin into the surrounding fluid.

Haemorrhage Escape of blood from blood vessels through accident or disease.

Histology Study of the microscopic anatomy of cells and tissues.

Histone Major structural proteins found associated with chromosomes.

Hyperthermia Overheating of the body.

Infarct A volume of dying tissue caused by the loss of an adequate blood supply.

Ischemia Restriction in the local blood supply.

Macrophage A type of white blood cell that ingests foreign matter and cellular debris.

Mitosis The type of cell division associated with growth and repair through which two daughter cells are produced from one parent cell.

Myocyte A muscle cell.

Necrosis Death of a cell or tissue as a result of injury or disease.

Neonate Newly born.

Organelle A specific component of a cell which has a particular function or role.

Organogenesis The stage of development during which the major organs of an organism are formed.

Osteogenesis The process of forming new bone.

Periosteum Connective tissue which covers the major surface of bones.

Petechiae Sites of minor haemorrhage.

Phonophoresis Use of ultrasound to enhance the delivery of drugs applied to the skin.

Platelets Irregular shaped bodies in the blood which play an important role in blood clotting.

Pyknotic cells Dying cells in which the DNA in the nucleus has condensed into a shrunken mass.

Sister chromatid exchanges Exchange of genetic material between identical copies of a chromosome.

Teratogenic effects Defects or malformations occurring during prenatal development of the fetus.

Thrombolysis Breakdown of blood clots.

TUNEL (or Terminal deoxynucleotidyl transferase dUTP nick-end labelling) A method for detecting DNA fragmentation.

Venule A small vein or blood vessel.

Vestibular system The sensory organs of the inner ear associated with balance.

Viscera The internal organs of the body.

Epidemiology and statistics

Bias A systematic tendency to overestimate or underestimate a parameter of interest because of a deficiency in the design or execution of an epidemiological study.

Case–control study An epidemiological study in which people who have developed a health outcome (cases) are identified, and their earlier exposure to putative causes is compared with that of controls who have not developed the health outcome.

Cohort (cohort study) An epidemiological study in which people who differ in their exposure to putative determinants of a health outcome are followed up and the subsequent occurrence of the health outcome is compared according to exposure. Cohort studies may be conducted prospectively or retrospectively.

Confidence interval (CI) An interval calculated from data when making inferences about an unknown parameter. In hypothetical repetitions of the study, the interval will include the parameter in question on a specified percentage of occasions (eg 95% for a 95% confidence interval).

Confounding A tendency to overestimate or underestimate the strength of a causal association in an epidemiological study because the putative cause that is under investigation is associated with another variable that independently determines the risk of the health outcome. Confounding can lead to a false conclusion about whether or not there is a causal relationship between exposure and disease.

Cross-sectional study An epidemiological study in which the prevalence of one or more health outcomes and/or their determinants is assessed in a population at a point in time or over a relatively short period.

Odds ratio (OR) The ratio of the odds of a health outcome in people exposed to a risk factor to that in people who are unexposed or exposed at a different level. The odds of a health outcome are defined as $p/(1 - p)$, where p is the probability of the outcome.

Proportional mortality ratio (PMR) The ratio (often expressed as a percentage) of the number of deaths in a study group from a specified cause to the number that would have been expected if, for each combination of sex, age and/or other potential confounding variables, the proportion of all deaths that were from that cause was the same as in a specified standard population (often the national population).

Prospective study An epidemiological study in which data on health outcomes are collected as they occur (cf retrospective study).

Relative risk (RR) The ratio of the risk (probability) of a health outcome in people exposed to a risk factor to that in people who are unexposed or exposed at a different level. Relative risks may be estimated with or without adjustment for possible confounding factors, such as age. For rare health outcomes, the relative risk is numerically similar to the odds ratio (see above).

Retrospective study An epidemiological study in which data are collected on health outcomes that occurred before the study began (cf prospective study).

Standardised incidence ratio (SIR) The ratio (often expressed as a percentage) of the number of incident cases of a disease in a study group to the number that would have been expected if, for each combination of sex, age and/or other potential confounding variables, the group had experienced the same incidence as that in a specified standard population (often the national population). An SIR greater than 100 (expressed as a percentage) signifies risk raised in the study group compared with the standard population, and an SIR of less than 100 signifies a reduced risk.

Standardised mortality ratio (SMR) Defined in the same way as an SIR, but with death from a specified cause, rather than incidence of a disease, as the health outcome.

Statistical power The probability that, with a specified degree of statistical confidence, an underlying effect of a given magnitude will be detected in a study. A study with low power might easily fail to detect an important effect, simply by chance.

Statistically significant result A finding in a study that deviates from a stated (or assumed) null hypothesis to an extent that would rarely occur (usually meaning with a probability of less than 5%) simply by chance if the null hypothesis were true.

Physics and dosimetry

Acoustic cavitation A range of complex phenomena that involve the creation, oscillation, growth and collapse of bubbles within a medium.

Acoustic dose Energy absorbed per unit mass of tissue from an acoustic wave.

Acoustic impedance Ratio of the pressure in a plane progressive wave to the particle velocity.

Acoustic pressure The difference between the ambient pressure (approximately atmospheric pressure) and the local pressure as an acoustic wave passes.

Acoustic transducer A device that, for example, converts electrical energy into acoustic energy, or *vice versa*.

Doppler shift Change of frequency of a wave when reflected from a moving target.

Infrasound Acoustic waves below 20 Hz.

***In situ* spatial peak, temporal average intensity** Maximum intensity occurring in an acoustic wave averaged over the pulse repetition period.

Intensity Acoustic power per unit area.

Mechanical index (MI) An indicator of the likelihood of cavitation.

Microstreaming Localised flow of liquid which can produce extremely high shear stresses, particularly associated with cavitation.

Peak acoustic pressure Usually the peak rarefaction or negative pressure of an ultrasonic wave.

Peak rarefactional pressure The amplitude of a negative instantaneous sound pressure in an ultrasonic beam. Rarefaction is the reduction in pressure of the medium during the acoustic cycle.

Pink noise Also termed $1/f$ noise. Each octave carries an equal amount of noise power.

Pulse average intensity Intensity averaged over the duration of a pulse.

Radiation force The force produced when an acoustic wave strikes a reflecting or absorbing surface.

Radiation pressure A local pressure exerted in a medium associated with energy loss from an ultrasonic wave.

Shear Transverse displacement.

Sonoporation A process where ultrasound can be used to temporarily increase the transmission of drugs or other compounds through a cell membrane.

Spatial average intensity Acoustic power in an acoustic wave, divided by the beam area.

Thermal index (TI) An indicator of tissue warming by an ultrasonic wave.

Total acoustic power Overall power emitted by a transducer as ultrasound.

Ultrasound Acoustic waves above 20 kHz.

Symbols

a	Source radius
A	Beam area at source
c_0	Speed of sound
C_v	Specific heat capacity
emt_{43}	Equivalent minimum thermal isoeffect dose
f	Acoustic frequency (also used for pulse centre frequency)
f_r	Resonant frequency
F_{rad}	Radiation force
G	Geometric factor
i	Instantaneous intensity
I_{PA}	Pulse average intensity
I_{SA}	Spatial average intensity
$I_{SPTA,0.3}$	Attenuated (derated) spatial peak, temporal average intensity
I_{TA}	Temporal average intensity

I_z	Intensity at distance z
MI	Mechanical index
PII	Pulse intensity integral
p	Instantaneous acoustic pressure
p_0	Peak acoustic pressure
p_c	Peak compression (peak positive acoustic pressure)
p_{opt}	Acoustic pressure for inertial cavitation
p_r	Peak rarefaction (peak negative acoustic pressure)
p_{rms}	Root-mean-square acoustic pressure
P_{rad}	Radiation pressure
Q_m	Acoustic dose rate (energy absorption rate per unit mass)
r	Beam radius
R	Constant for thermal dose
R_0	Initial bubble radius
t	Time
T	Temperature
TI	Thermal index
u	Particle velocity
V	Streaming velocity
W	Acoustic power
W_{deg}	Acoustic power for a 1°C maximum increase in tissue temperature
z	Distance along beam axis
Z	Acoustic impedance
α	Amplitude attenuation coefficient
α_a	Amplitude absorption coefficient
α_s	Amplitude scatter coefficient
$\langle\varepsilon\rangle$	Energy density
λ	Wavelength
μ	Intensity absorption coefficient (=2α)
ρ_0	Density
Φ	Acoustic dose (energy absorbed per unit mass)
υ	Kinematic viscosity

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