A Bioeffect Produced at Diagnostic Levels

William D. O'Brien, Jr., PhD
Professor
Department of Electrical and Computer Engineering
University of Illinois
1406 West Green Street
Urbana, IL 61801

A Bioeffect Produced at Diagnostic Levels

William D. O'Brien, Jr., Ph. D.
Department of Electrical and Computer Engineering
University of Illinois
1406 West Green Street
Urbana, IL 61801

ABSTRACT

Traditionally, we have thought that there were no ultrasound-induced biological effects in animals at diagnostic ultrasound levels in the clinical frequency range. This has changed with the discovery that diagnostic-level ultrasound can produce lung damage study in mice (Child *et al.*, 1990). These study have been repeated in my laboratory, and confirmed. This was perhaps the first biological effect observed under *in vivo* exposure conditions in a mammalian system similar to those produced by a diagnostic-type ultrasound system. We questioned their applicability in (extrapolation to) humans and thus conducted additional studies with different species (rabbits and pigs). From these studies, we concluded that the mouse is an *inadequate* animal model for the evaluation of whether ultrasound has the potential for producing lung damage in humans. Further, in evaluating the experimentally observed trends of lung damage between mouse, rabbit and pig, it is suggested that there is no experimentally supportable evidence that such damage could occur in humans unless the ultrasonic pressure levels were excessively high.

INTRODUCTION

The Food and Drug Administration regulates diagnostic ultrasound equipment on the basis of the preexisting output levels at the time the Medical Devices Amendments were adopted into law in May, 1976 (FDA, 1985) and not on the basis of any potential risk considerations. In the late 1980s, an activity was initiated to develop a diagnostic ultrasound equipment standard which had, as its basis, biophysical indicators which would provide to equipment operators during a diagnostic procedure a means of assessing the potential risk from either a thermal or a mechanical ultrasound bioeffect. The *Standard for Real-Time Display of Thermal and Mechanical Indices on Diagnostic Ultrasound Equipment* was approved in 1992 and provided manufacturers a standardized procedure to provide on diagnostic ultrasound equipment either a Thermal Index or Mechanical Index (AIUM/NEMA, 1992).

This contribution provides the first *in vivo* bioeffect report which examines whether the Mechanical Index is an appropriate dose-effect quantity. The development of the Mechanical Index was based on theoretical and *in vitro* experimentation by Apfel and Holland (1991) who discovered a simple relationship between acoustic pressure and the onset of cavitation under the assumption that the optimum bubble size is present in tissue. The theory assumed isothermal growth, adiabatic collapse, an incompressible host fluid and neglected gas diffusion into the bubble. These observations were the basis for the adoption of the standard (AIUM/NEMA, 1992) which defined the Mechanical Index, *MI*, as

$$MI = \frac{p_{r.3}}{\sqrt{f}} \qquad (1)$$

where $p_{r.3}$ is the derated (the .3 in the subscript denotes a derating factor of 0.3 dB/cm-MHz) peak rarefactional pressure (in MPa) and f is the ultrasonic frequency (in MHz).

Although the special environment of tissues (and lungs) was not considered in the formulation of *MI*, it has the potential to be a useful predictor of bubble-related or mechanical effects in tissues, an issue which is evaluated by the study reported herein. The study design was based on assessing whether the *MI* had the indicated frequency dependency (see Equation 1) and whether it was an equivalent or better predictor of a mechanical bioeffect than $I_{SPPA.3}$, one of the quantities regulated by FDA (1985).

INTERACTION MECHANISMS

Some of the interactions of ultrasound with tissues are known. These include heating, acoustic cavitation, and some non-thermal, non-cavitational effects (O'Brien, 1992). Of these interactions, the thermal mechanism will be briefly considered. The cavitation-like phenomenon is the principle focus of this contribution. Other mechanisms will not be considered herein.

When considering exposimetry issues related to the interaction of ultrasound with tissues, the intensity of the beam is important because it has been most commonly reported and because FDA (1985) regulates diagnostic ultrasound equipment based on intensity (although just recently, the Mechanical Index has become a regulatory quantity (FDA, 1994)). However, is should be noted that intensity is not a dosimetric quantity and is thus flawed as a predictor of heating and cavitation in tissue. The recently approved (AIUM/NEMA, 1992) and FDA adopted (FDA, 1993) Output Display Standard goes a long way in providing to the clinical user real-time information as to the potential for tissue heating in addition to mechanical effects during a diagnostic ultrasound examination. The specific display requirements of this standard was selected to include those quantities whose magnitudes are known or believed to be related to actual damage or to risk of damage to biological tissues as a result of ultrasonic exposure. The basis for this rationale lies in an understanding of the mechanisms by which it is known that ultrasound can affect living systems. Such knowledge comes from fundamental laboratory studies. These mechanisms can be classified and discussed in terms of whether heat is or is not believed to be the principal cause for the biological effect.

Thermal

Whenever high-frequency ultrasonic energy is propagated into an attenuating material such as soft tissues, the amplitude or height of the wave will decrease as it traverses deeper structures. This attenuation results in an overall loss in the power of the wave which is due to either *absorption* or *scattering*. Absorption is a mechanism which represents that portion of the waves energy that is lost by its conversion into heat; scattering can be thought of as that portion which changes direction, some of which is reflected as echoes that produce the images seen on the screen of the scanner. Since the medium interrogated is capable of absorbing energy with the resultant production of heat, a temperature rise may occur as long as the rate at which heat is produced is greater than the rate at which the heat is removed (O'Brien, 1992). The increase in temperature produced by ultrasound can be calculated using mathematical modeling techniques and has been estimated for a variety of exposure conditions *in vivo* (Tarantal and O'Brien, 1994).

Cavitation

Cavitation can be discussed under two general categories, namely 'transient' cavitation and 'stable' cavitation (Flynn, 1964), both of which involve the occurrence of gaseous bubble formation. Transient cavitation refers to a relatively violent activity (*i.e.* bubble collapse) in which "hot spots" of high temperature and/or pressure occur in very short (μsec) bursts. These bursts may be accompanied by localized shock waves and/or by the generation of highly reactive chemical species such as hydroxyl radicals. In contrast, a much less violent form is stable cavitation, which is associated with the vibration of these gaseous bubbles. The nature of this form of cavitation consists of a micron-size gaseous body, which because of the presence of a ultrasound field, may oscillate or pulsate. When such oscillations are established, the liquid-like medium immediately adjacent to the gas bubble flows or streams (termed microstreaming) (Nyborg, 1965). Microstreaming has been shown to produce stresses sufficient to disrupt cell membranes.

Although a known phenomenon in regards to ultrasound, cavitation has been difficult to document in mammalian systems. The presence of small gaseous nuclei (bubbles) is clearly plausible as evidenced by the problems divers may encounter with decompression sickness. Many studies have been performed with *Drosophila melanogaster* due to the natural presence of air in these organisms (Carstensen *et al.*, 1990). Although little work has been done regarding cavitation and the mammalian fetus, studies performed with lithotripters help to shed some light on the potential existence of cavitation nuclei. The peak pressures generated by lithotripters (>10 MPa) greatly exceed those for diagnostic ultrasound scanners (<2 MPa), and it is not anticipated that currently designated peak pressure levels for diagnostic ultrasound systems will be surpassed.

The balance of this contribution focuses on a specific mechanical-like effect which has been shown to produce lung hemorrhage in mammalian species. Terms like *mechanical-like*, *bubble-like* and *cavitation-like* are used because the exact mechanism has not yet been identified although the mechanism is believed to require gaseous bodies in the tissue.

LUNG DAMAGE STUDIES

In regards to *in vivo* studies which have addressed the presence of cavitation-like (a mechanical-like effect) phenomenon, it has been shown that ultrasonically-induced bubble activity can result in lung damage in adult mice (Child *et al.*, 1990). These observations correlate well with the frequency-dependent, *in vitro* cavitation experiments of Apfel and Holland (1991). In an effort to confirm whether the lung sensitivity observed in adult mice was related to the presence of air, Hartman *et al.* (1990) exposed *in utero* mouse fetuses to high peak ultrasonic pressures (20 MPa) on the 18th day of gestation. Results indicated no significant effects on fetal tissues exposed *in situ* (including the lung); peak pressure levels were roughly 10 times the output required for damage in adults in prior studies (Child *et al.*, 1990). As anticipated, marked intestinal and lung hemorrhages were noted in the dams of these fetuses at the higher exposures. These observations led the authors to suggest that (Hartman *et al.*, 1990) "...lung gets its sensitivity [to damage] by virtue of the presence of air bodies which are potential sites for cavitation-related activity." These studies support the hypothesis that cavitation- or bubble-like activity may not be a significant concern in relation to the fetal lung, although the potential for cavitation nuclei in other regions of the fetus are unknown. This is clearly an area of research that will require more extensive study in appropriate animal models in order to improve our awareness regarding the presence of these nuclei and the circumstances under which they may cause damage.

In my laboratory, we have confirmed the findings that diagnostic ultrasound levels can produce lung damage in mice (O'Brien and Zachary, 1994c, Zachary and O'Brien, 1994). Further, we have evaluated this biological effect with three species (mouse, rabbit and pig) using diagnostic-like exposure conditions at 3 and 6 MHz (O'Brien and Zachary, 1994c, Zachary and O'Brien, 1994) to assess whether the *MI* (Mechanical Index) is an equivalent or better indicator of non-thermal bioeffect risk than $I_{SPPA.3}$ (derated spatial peak, pulse average intensity). This study denoted Study 1 (see Table 1) evaluated the frequency dependence (at 3 and 6 MHz) of the degree of lung damage at exposure levels which were in excess of FDA-allowed levels in order to produce an effect. The principle focus of the study was with rabbits. Mice served as positive controls (we knew that the mouse lung would be damaged at the levels being used) and the pigs served as a model much closer to the lung morphology of humans. The pig exposure levels were at the highest possible levels achievable by the diagnostic ultrasound device being used (the regulatory maximum FDA levels were overridden by an engineer from the diagnostic ultrasound company so that we could operate at excessively-high levels known to produce lung damage in mice and rabbits).

Further, we evaluated ultrasonically induced lung damage at a much lower frequency (30 kHz) with the same three species (O'Brien and Zachary, 1994a, 1994b, Zachary and O'Brien, 1994) in order to examine ultrasonic frequency dependency on lung damage. These studies are denoted Studies 2, 3 and 4 (see Table 2). The three species were also evaluated to assess species dependency on lung damage with the view of extrapolating whether this biological effect posed any human risk.

The lung damage scoring criteria were the same for all studies in order to compare directly the observations between mice, rabbits, and pigs. For all animals evaluated, areas of hemorrhage were recorded in gray scale on diagrams representing dorsal-ventral and ventral-dorsal views of all lung lobes (mouse: left, post caval, right cranial, right middle, right caudal; rabbit: combined left apical and middle, left caudal, post caval, right cranial, right middle, right caudal; pig: combined left cranial and middle, left caudal, post caval, combined right cranial and middle, right caudal). The assignment of the numerical score to each lung (see Table 3) was based on clinical variables (survival, respiratory patterns, hemothorax, etc.) and macroscopic assessment of lung for hemorrhage. Lung hemorrhage was evaluated qualitatively on the basis of color, location, and distribution (*i.e.*, there was more intraparenchymal lung hemorrhage with a higher numerical score). Lungs with

intraparenchymal hemorrhage were dark red-brown to black and this color change was apparent throughout affected lung lobes. A score of 0 was assigned to lungs that had absolutely no hemorrhage; lungs with any or questionable (equivocal) foci of intraparenchymal hemorrhage no matter how small were assigned a score of 0.5 for consistency of scoring, animals with minimal intraparenchymal hemorrhage were assigned a score of 1, and so forth.

Table 1: Study 1. Summary of diagnostic-like, pulsed-wave exposure conditions of the derated (at 0.3 dB/cm-MHz) spatial peak, pulse average intensity ($I_{SPPA.3}$), the derated peak rarefactional pressure ($p_{r.3}$) and the Mechanical Index (MI) and a summary of the mice, rabbits and pigs exposed for 5 minutes at each of the exposure conditions.

Frequency (MHz)	$I_{SPPA.3}$ (W/cm^2)	$p_{r.3}$ (MPa)	MI	Mice	Rabbits	Pigs
Sham	0	0	0		4	
3	200	2.3	1.3		5	
3	300	2.6	1.5		2	
3	420	3.3	1.9		9	
3	480	3.3	1.9			1
3	510	3.3	1.9			1
3	530	3.3	1.9			1
6	200	2.0	0.8		5	
6	510	2.9	1.2	2	10	
6	1060	4.7	1.9		9	
6	1310	5.4	2.2	1	3	2
6	1480	5.6	2.3			1
TOTALS:				3	47	6

Table 2: Studies 2, 3 and 4. Summary of continuous-wave exposure conditions of the peak acoustic pressure (p_o) at an ultrasonic frequency of 30 kHz for three lung damage studies.

	Mouse (Study 2)			Mouse-rabbit (Study 3)		Mouse-rabbit-pig (Study 4)		
p_o (kPa)	Mice 5 min	Mice 10 min	Mice 20 min	Mice 10 min	Rabbits 10 min	Mice 10 min	Rabbits 10 min	Pigs 10 min
0	15	15	15	4	2		15	16
65	15	15	15					
80	15	15	15					
87	15	15	15					
100	15	15	15	10	7			
145	15	15	15	10	7	18	15	16
290							16	16
340							15	
460							14	17
490								9
TOTALS:	90	90	90	24	16	18	75	74

Table 3: Quantitative numerical criteria for scoring lung damage.

0 - normal lung, normal vital signs.
0.5 - equivocal hemorrhage, normal vital signs.
1 - minimal hemorrhage usually involving 1 to 4 foci measuring approximately < 5 mm in diameter, normal vital signs.
2 - mild hemorrhage that was greater in extent and severity than a score of 1.0, normal vital signs.
3 - moderate hemorrhage that was greater in extent and severity than a score of 2.0, normal vital signs.
4 - marked hemorrhage that was greater in extent and severity than a score of 3.0, abnormal vital signs.
5 - severe hemorrhage that was greater in extent and severity than a score of 4.0, abnormal vital signs, death.

Study 1 summary

Of the 43 rabbits exposed (4 were sham exposed), the highest individual lung damage score was 1 (see Table 3). While this represents a degree of hemorrhage, it is quite minimal.

Figures 1-3 graphically show the rabbit's mean lung damage score values as functions of $I_{SPPA.3}$, $p_{r.3}$ and MI, respectively. In Figure 1, the result at $I_{SPPA.3}$ of 200 W/cm^2 is the combined results from the 3 MHz (MI = 1.3) and 6 MHz (MI = 0.8). Likewise, in Figure 3, the result at MI of 1.9 is the combined results from the 3 MHz ($I_{SPPA.3}$ = 420 W/cm^2) and 6 MHz ($I_{SPPA.3}$ = 1060 W/cm^2).

Note that as all three exposure quantities increase, the mean lung damage score, in general, increases. This suggests that all three would be reasonable indicators of mechanical damage. However, the Mechanical Index is the best of these three exposure quantities since the mean lung damage monotonically increases as the value of the Mechanical Index increases. This is not true for either $I_{SPPA.3}$ or $p_{r.3}$ since both show a slight dip at 510 W/cm^2 and 2.9 MPa, respectively.

Therefore, the Mechanical Index is a better indicator of lung damage, as assess with the rabbit study, than either $I_{SPPA.3}$ or $p_{r.3}$, suggesting that the frequency dependency of Equation 1 is appropriate.

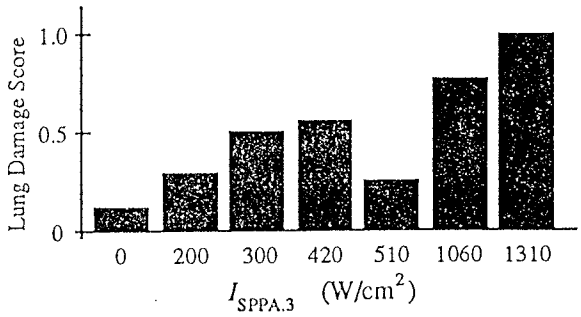

Figure 1: Study 1's rabbit mean lung damage as a function of the derated spatial peak, pulse average intensity.

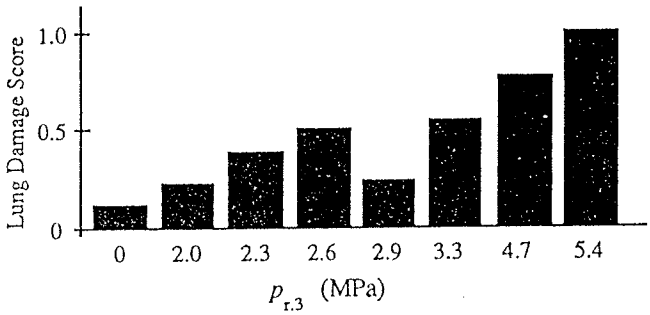

Figure 2: Study 1's rabbit mean lung damage as a function of the derated rarefactional pressure.

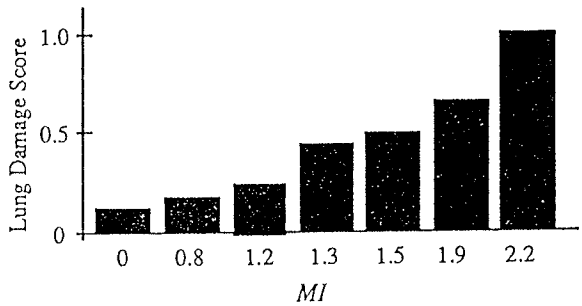

Figure 3: Study 1's rabbit mean lung damage as a function of the Mechanical Index (see Equation 1).

Three mice served as positive controls (see Table 1), all three exposed at 6 MHz. Two were exposed at 510 W/cm^2 and had lung damage scores of 2 and 3. The third was exposed at 1310 W/cm^2 and had a score of 3. These three scores were higher than any of the rabbit scores, suggesting that the mouse was more sensitive to lung damage than rabbits.

The six pig exposures (see Table 1) all yielded a lung damage score of 0. The exposure levels were at the highest possible output setting that the diagnostic ultrasound device could produce, considerable higher than any system used clinically.

Therefore, the degree of sensitivity for producing lung damage, based on Study 1, suggests that mice are the most sensitive, pigs are the least sensitive and rabbits fall somewhere in-between.

Studies 2, 3 & 4 summary

The purpose of Study 2 (O'Brien and Zachary, 1994a; Zachary and O'Brien, 1994), with 270 mice, was to evaluate the acoustic pressure threshold levels at 30 kHz necessary to produce mouse lung damage *in vivo*. Another important purpose was to assess the frequency dependency of the acoustic pressure threshold levels by comparing the 30 kHz threshold findings to those reported in the MHz frequency range by Child *et al.* (1990). The same mouse strain and anesthetic agents were used in both experimental protocols.

Child *et al.* (1990) yielded peak rarefactional acoustic pressure threshold values for individual lung damage between 0.4 and 1.4 MPa as a function of ultrasonic frequency between 1.1 and 3.7 MHz. In terms of the *MI* (see Equation 1), the lung hemorrhage results of Child *et al.* (1990) are shown in Figure 4 (solid columns) and yield *MI* values for individual lung hemorrhage thresholds between 0.4 and 0.7 in this megahertz frequency range.

The *MI* was developed for microsecond-type pulsed ultrasound exposure conditions, However, it is instructive to use the ratio of the exposure acoustic pressure level (p_o in MPa) to the square root of frequency (in MHz) for the 30 kHz results to examine the frequency dependency of the 30 kHz results relative to the megahertz results. Therefore, the $\frac{p_o}{\sqrt{f}}$ quantity is used as the basis of comparison of the frequency dependency.

The $\frac{p_o}{\sqrt{f}}$ quantity for the 30 kHz threshold acoustic pressure level of 65 kPa is 0.38 (O'Brien and Zachary, 1994a). For graphically comparison between these two studies, Figure 4 also includes the $\frac{p_o}{\sqrt{f}}$ quantity of 0.38 at 30 kHz which is in the same range of the Child *et al.* (1990) values, *viz.*, between 0.38 and 0.73.

Therefore, the frequency dependency suggested by the Mechanical Index (see Equation 1) appears to be applicable over the frequency range from 30 kHz to the low MHz frequency range.

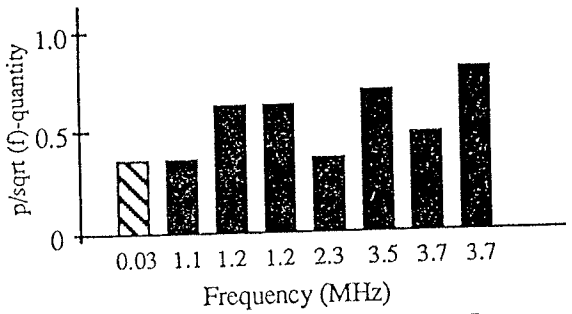

Figure 4: Calculated values of the $p/\sqrt{f}$ quantity as a function of frequency of the mouse lung hemorrhage threshold results reported by Child *et al.* (1990) (solid columns), and the 30 kHz threshold acoustic pressure level result of Study 2 (hatched columns).

The purpose of Study 3 (O'Brien and Zachary, 1994b; Zachary and O'Brien, 1994) was to test the hypothesis that mouse pulmonary ultrasound studies cannot be generalized to other species, that is, the mouse may not be an acceptable or suitable animal model for studies that examine the effects of ultrasound on lung tissue for purposes of extrapolating or estimating the degree of potential damage in other species.

Figures 5 suggests that species difference (mouse *vs* rabbit) is a strong function of lung damage. There is a clear difference between the mouse and rabbit sensitivities to lung damage at each of the exposure pressure levels of 100 and 145 kPa. The rabbit was much less sensitive to damage than the mouse.

The purpose of Study 4 (Zachary and O'Brien, 1994) was to test further, with mice, rabbits and pigs, the hypothesis that there was a species difference in the degree of sensitivity to ultrasound-induced lung damage. Figure 6 clearly demonstrates a strong species dependency of

ultrasonically-induced lung damage with mice being the most sensitive, pigs the least sensitive and rabbits in-between. This observation is consistent with the results from Study 1 (O'Brien and Zachary, 1994c).

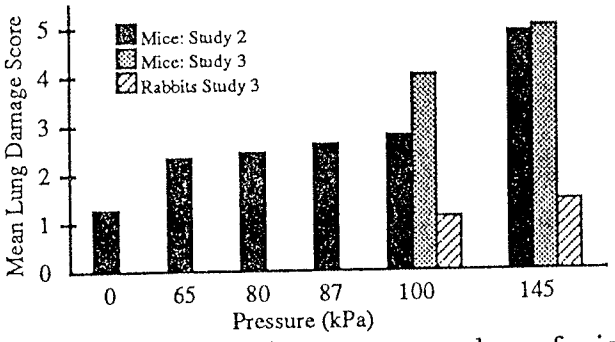

Figure 5: Mean lung damage score values of mice from Study 2 (O'Brien and Zachary, 1994a), and mean lung damage score values of mice and rabbits from Study 3 (O'Brien and Zachary, 1994b), as a function of exposure acoustic pressure level for the 10 minute exposure duration.

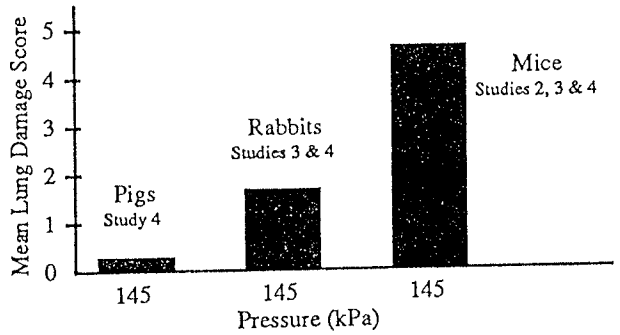

Figure 6: Comparison of the mean lung damage scores when mice, rabbits and pigs from Studies 2, 3 and 4 are combined.

DISCUSSION

Let us evaluate the differences in the lung properties between species (O'Brien and Zachary, 1994b). Pulmonary pleura of human and large animal lungs tends to be thick whereas pulmonary pleura of lungs from smaller animals tends to be thinner. Thick and thin are relative terms that do not precisely describe the pleura thickness since there are no precise data available on the thickness of pleura in specific regions of the lungs from most species (Tyler and Julian, 1992).

As a generalization (Tyler and Julian, 1992), thick pleura is supplied by branches of the bronchial artery and thin pleura is supplied by the pulmonary artery. Differences in pressures within these two systems of blood vessels should influence the rates of pleural fluid formation. Also, animals with thick pulmonary pleura tend to have the most extensive network of lymphatics.

The size of the pulmonary acinus is dependent in part on the anatomical makeup of the transitional zone between the last conducting airway (the terminal bronchioles) and the alveolar ducts. In some species, this transition is extremely abrupt, going directly from the terminal bronchiole to the alveolar duct. In other species, airways beyond the terminal bronchiole contain alveolar outpocketings within their walls. These airways, called respiratory bronchioles, may consist of one to three generations before reaching an alveolar duct (Tyler and Julian, 1992; Pinkerton *et al.*, 1992).

In evaluating the experimentally observed trends of lung damage between mouse, rabbit and pig, it is suggested that there is no experimentally supportable evidence that such damage could occur in humans unless the ultrasonic pressure levels were excessively high. Consider these observations between mouse, rabbit, pig and man:

1) *Total lung volume*: rabbit is about 100 times larger, pig is about 5000 times larger and human is about 6500 times larger than mouse.

2) *Alveolar surface area*: rabbit is about 65 times larger and pig and human are about 1500 times larger than mouse.

3) *Mean alveoli diameter*: rabbit is about 2 times larger, pig is about 2.3 times larger and human is about 5 times larger than mouse.

4) *Capillary surface area*: rabbit is about 85 times larger and human is about 2300 times larger than mouse. Capillary surface area for pigs does not appear to be available.

5) *Capillary volume*: rabbit is about 95 times larger and human is about 2800 times larger than mouse. Capillary volume for pigs does not appear to be available.

6) *Lung compliance*: rabbit is about 175 times larger and human is about 4000 times larger than mouse. Lung compliance for pigs does not appear to be available.

7) *Pulmonary pleura size*: both mouse and rabbit are thin and pig and human are thick.

8) *Pulmonary pleura blood supply*: both mouse and rabbit are supplied by the pulmonary artery and both pig and human are supplied by the bronchial artery. Pulmonary artery typically has a higher blood pressure value than the bronchial artery.

9) *Interlobular and segmental connective tissue*: both mouse and rabbit have little, if any, and both pig and human are extensive.

10) *Pulmonary plural lymphatics*: both mouse and rabbit have very few and both pig and human are extensive.

11) *Nonrespiratory bronchiole*: mouse, rabbit, pig and human have several generations.

12) *Respiratory bronchiole*: mouse, rabbit and pig are absent or single short generation and human has several generations.

13) *Terminal respiratory bronchiole*: mouse, rabbit and pig end in alveolar ducts or very short respiratory bronchioles and human ends in respiratory bronchioles.

14) *Ultrasound-induced lung damage*: A significant greater degree of lung damage was induced in mouse and rabbit compared to pig using same scoring criteria (see Table 3).

Although the mechanical properties leading to pulmonary hemorrhage following ultrasound treatment are poorly understood, these properties are important in determining the ability of lung tissue to respond to and recover from pulmonary ultrasound. Cavitation-type activity and/or bubble-related formation adjacent to the visceral pleural surface and/or within the underlying alveoli may be a function of alveolar diameter assuming, of course, that such actions do occur. The genesis of cavitation-like activity and/or bubble-related formation and the resultant alveolar and capillary damage may be related directly to a relationship between acoustic pressure levels and ultrasonic frequency and the size of the air space in which resonance might occur. It is possible that there is an optimum combination (threshold) of alveolar diameter, frequency, and acoustic pressure that results in alveolar damage and hemorrhage.

ACKNOWLEDGMENTS

The author gratefully acknowledge support from NIH grant CA09067, Swen Sonic, Division of Arjo Hospital Equipment, Inc., Morton Grove, IL and Advanced Technologies Laboratories, Bothell, WA.

REFERENCES

AIUM/NEMA (1992). "Standard for Real-Time Display of Thermal and Mechanical Indices on Diagnostic Ultrasound Equipment," AIUM Publications, American Institute of Ultrasound in Medicine, 14750 Sweitzer Lane, Suite 100, Laurel, MD 20852.

R. E. Apfel and C. K. Holland (1991). Gauging the likelihood of cavitation from short pulse, low duty cycle diagnostic ultrasound. *Ultrasound in Medicine and Biology*, 17,179-188.

E. L. Carstensen, D. S. Campbell, D. Hoffman *et al.* (1990). Killing of *Drosophila* larvae by the fields of an electrohydroulic lithotripter. *Ultrasound in Medicine and Biology*, 16, 687-698.

S. Z. Child, C. L. Hartman, L. A. Schery and E. L. Carstensen (1990). Lung Damage from Exposure to Pulsed Ultrasound, *Ultrasound in Medicine and Biology*, 16, 817-825.

FDA (1985). 510(k) Guide for Measuring and Reporting Acoustic Output of Diagnostic Ultrasound Medical Devices, December, 1985, Center for Devices and Radiological Health, US Food and Drug Administration, Rockville, MD

FDA. "Revised 510(k) Diagnostic Ultrasound Guidance for 1993." Center for Devices and Radiological Health, US Food and Drug Administration, Rockville, MD February 17, 1993.

FDA. "Use of Mechanical Index in Place of Spatial Peak, Pulse Average Intensity in Determining

Substantial Equivalence." Center for Devices and Radiological Health, US Food and Drug Administration, Rockville, MD April 14, 1994.

H. G. Flynn (1964). Physics of acoustic cavitation in liquids. In Mason WP (ed): Physical Acoustics vol. 1B, p 57, Academic Press, New York, NY.

C. Hartman, S. Z. Child, R. Mayer *et al.* (1990). Lung damage from exposure to the fields of an electrohydraulic lithotripter. *Ultrasound in Medicine and Biology*, 16, 675-683.

W. L. Nyborg (1965). Acoustic streaming. In Mason WP (ed): Physical Acoustics vol. 2B, p 265, Academic Press, New York, NY.

W. D. O'Brien, Jr. (1992). Ultrasound Dosimetry and Interaction Mechanisms. In Non-Ionizing Radiation: Proceedings of the Second International Non-Ionizing Radiation Workshop, M. Wayne Greene, ed., pp. 151-172, Canadian Radiation Protection Association Vancouver, BC.

W. D. O'Brien, Jr. and J. F. Zachary (1994a). Mouse Lung Damage from Exposure to 30 kHz Ultrasound. *Ultrasound in Medicine and Biology*, 20, 287-297.

W. D. O'Brien, Jr. and J. F. Zachary 1994b). Comparison of Mouse and Rabbit Lung Damage Exposure to 30 kHz Ultrasound. *Ultrasound in Medicine and Biology*, 20, 299-307.

W. D. O'Brien, Jr. and J. F. Zachary (1994c). Rabbit Lung Damage Assessment from Exposure to Pulsed Wave Ultrasound. *Ultrasound in Medicine and Biology*, Submitted.

K. E. Pinkerton, P. Gehr and J. D. Crapo (1992). Architecture and Cellular Composition of the Air-Blook Barrier. In R. A. Parent (ed) "Comparative Biology of the Normal Lung", Chapter 11, pp 121-128, CRC Press, Boca Raton, FL.

A. F. Tarantal and W. D. O'Brien, Jr. (1994). Discussion of Ultrasonic Safety Related to Obstetrics. In Ultrasound Applied to Obstetrics and Gynecology, 3rd Edition, Rudy E. Sabbagha, ed., J. B. Lippincott Co., Philadelphia, PA, pp 45-56.

W. S. Tyler and M. D. Julian (1992). Gross and Subgross Anatomy of Lungs, Pleura, Connective Tissue Septa, Distal Airways, and Structural Units. In R. A. Parent (ed) "Comparative Biology of the Normal Lung", Chapter 4, pp 37-47, CRC Press, Boca Raton, FL.

J. F. Zachary and W. D. O'Brien, Jr. (1994). Lung Hemorrhage Induced by Continuous and Pulsed Wave (Diagnostic) Ultrasound in Mice, Rabbits, and Pigs, *Vet Pathology* (in press)