

**●Original Contribution****COMPARISON OF MOUSE AND RABBIT LUNG DAMAGE EXPOSURE TO 30 kHz ULTRASOUND**WILLIAM D. O'BRIEN, JR.[†] and JAMES F. ZACHARY[‡][†]Department of Electrical and Computer Engineering and [‡]Department of Veterinary Pathobiology, University of Illinois, Urbana, IL 61801, USA*(Received 15 June 1993; in final form 11 October 1993)*

Abstract—Twenty-four mice and sixteen rabbits were evaluated at one exposure duration (10 min) and at three exposure acoustic pressure levels (0, 100 and 145 kPa) at an ultrasonic frequency of 30 kHz, continuous wave for the purpose of testing whether there was a species difference in the degree of sensitivity to ultrasound-induced lung damage. This study was undertaken because it was hypothesized that 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. The rabbit was selected for comparison to the mouse because the rabbit exhibited sufficient physiological and morphological differences from those of the mouse to test this hypothesis. Using exactly the same exposure conditions and lung assessment criteria, it appeared that the mouse lung was more sensitive to ultrasound-induced damage than that of the rabbit by a factor of between 2.8 and 3.6. Lung lesions in mice and rabbits were similar in character, but were much more severe and extensive in mice. Lesions in both species consisted of intraalveolar hemorrhage that appeared as dark red to red-black areas that were visible on the pleural surfaces and that extend within the lung parenchyma.

Key Words: Mouse, Rabbit, Lung, Ultrasound, Species comparison, Hemorrhage, Damage, Bioeffect, Lesion.

INTRODUCTION

The purpose of this study 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. In a previous study, ultrasonic pressure threshold levels for producing lung damage were determined from exposure to 30 kHz ultrasound. Based on this previous study, the frequency dependency of the ultrasonic pressure threshold appeared to be consistent with that of the Mechanical Index (AIUM/NEMA 1992). Upon completion of the previous study (O'Brien and Zachary 1994), the protocol for the current study was developed.

The rabbit was selected because its lung properties were sufficiently different from those of mice (see Table 8 in O'Brien and Zachary 1994). For example, the rabbit lung exhibited a mean alveoli diameter about 2.5 times greater than that of the mouse, whereas, for humans, it is about 5 times greater (Crosfill and Widdicombe 1961; Tenney and Remmers 1963; Weibel, 1971). The study design did not determine threshold levels; its purpose was to test the species difference hypothesis.

MATERIALS AND METHODS

Six to seven-week old C3H male mice were obtained from Harlen Sprague-Dawley Laboratories (Indianapolis, IN), and sonications were performed within one week of the time of each shipment of mice. Five to 5½ month old (8–9 lb) New Zealand White male rabbits were obtained from Myrtle's Rabbitry, Inc. (Thompson Station, TN), and sonications were performed within one week from the time when each shipment of rabbits was received.

Mice were weighed and then anesthetized with a

Address correspondence to: William D. O'Brien, Jr., Bioacoustics Research Laboratory, Department of Electrical and Computer Engineering, University of Illinois, 1406 W. Green St., Urbana, IL 61801, USA.

combination of ketamine hydrochloride (Ketamine®) (125 mg/kg) and xylazine (Rompun®) (25 mg/kg) administered intraperitoneally. The skin surrounding the rib cage, sternum and vertebral column was clipped with an electric shaver and the hair removed with a depilatory agent (Nair®) to minimize the likelihood of entrapment of air at the skin-water interface. The anesthetized mice were then restrained in a spread-eagle position in a specially fabricated apparatus that permitted the placement of the mice vertically in the waterbath (distilled, degassed water). The ventral surface of the animal was directed towards the ultrasound source. A pointer attached to the ultrasound source was used to position uniformly the mice in the calibrated sound field; the pointer was removed during animal sonication. The individual preparing the mice for sonication (anesthetizing, depilating and placing in exposure structure) was blinded to the exact exposure condition.

Rabbits were weighed and then anesthetized with a combination of ketamine hydrochloride (Ketamine®) (35.0 mg/kg) and xylazine (Rompun®) (5.0 mg/kg) administered subcutaneously. The skin surrounding the rib cage, sternum and vertebral column was clipped with an electric shaver and the hair removed with a depilatory agent (Nair®) to minimize the likelihood of entrapment of air at the skin-water interface. The anesthetized rabbits were then restrained in a spread-eagle position that permitted the placement of the rabbit vertically in the waterbath (distilled, degassed water). The ventral surface of the animals was directed towards the ultrasound source. A pointer attached to the ultrasound source was used to position uniformly the rabbit in the calibrated sound field; the pointer was removed during animal sonication. The individual preparing the rabbits for sonication (anesthetizing, depilating and placing in exposure structure) was blinded to the exact exposure condition.

In the previous study (O'Brien and Zachary 1994), the waterbath (distilled, degassed water) temperature was maintained at 37°C to minimize differences with the Child et al. (1990) study. In this study, the waterbath (distilled, degassed water) temperature was maintained at room temperature ($\approx 22^\circ\text{C}$) to maintain daily experimental protocol schedule, because the water had to be changed much more often than in the previous study.

With an individual mouse or rabbit in position, the mouse or rabbit was subjected to one of three randomly assigned exposure conditions. Following sonication, the animal was removed from the waterbath. Mice were killed by cervical dislocation followed by exanguination and dissection. The rabbits were killed with an overdose of carbon dioxide (CO_2) inhalation, and the lungs were dissected free from the animal.

Mouse lungs were placed in a petri dish that contained sterile saline and examined (blinded to the specific

exposure condition) with a dissecting microscope. Rabbit lungs were washed free of blood and examined visually for hemorrhage. For all animals, areas of congestion and 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: cranial portion of left cranial, caudal portion of left cranial, left caudal, post caval, right cranial, right middle, right caudal). Lung lesions were scored by the numerical criteria listed in Table 1. Lung tissues from all animals were fixed in 10% neutral buffered formalin and stored for future histopathologic evaluation (Zachary and O'Brien 1994).

The differences between individual lung damage score values of 2, 4, 6 and 8 (see Table 1) were determined in part by the relative amount of lung area that had intraparenchymal hemorrhage (i.e., there was more intraparenchymal lung hemorrhage with a higher numerical score), and by the clinical variables listed in Table 1. Intraparenchymal lung hemorrhage was evaluated qualitatively on the basis of color, location and distribution. 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 foci of intraparenchymal hemorrhage no matter how small were assigned a score of 2 for consistency of scoring. Animals with a lung damage score of 10 died and had substantial hemorrhage.

A brief comment should be made about the scoring criteria of this study (Table 1) and the previous study (Table 1 in O'Brien and Zachary 1994). Lung damage score values of 0, 6, 8 and 10 are the same for the two studies. The differences in the two scoring criteria are for lung damage score values of 2 and 4 wherein the previous study used congestion and pleural/subpleural hemorrhage as the biological end-point; this study used intraparenchymal hemorrhage.

The exosimetry procedures are identical to those reported in O'Brien and Zachary (1994) with the exceptions that the previous study was conducted at 37°C and this study was conducted at room temperature ($\approx 22^\circ\text{C}$), and all exposure durations for this study were 10 min. The mice and rabbits were exposed to acoustic pressure (defined (AIUM, 1992) as the total pressure minus the ambient pressure) using one of the two identical, rectangular (16×11 cm) magnetostrictive sources driven by Swen Sonics' Blue Wave amplifier at a frequency of 30 kHz (acoustic wavelength in water is 5 cm) modulated at 120 Hz (100% modulation factor). It was determined that there was no measurable differences in the calibrated Naval Research Laboratory Model F42D/115 hydrophone's response at the two temperatures, that is, 37.6 kPa/V. As in

Table 1. Quantitative numerical criteria† for scoring lung damage following sonication of each animal.

0—	alive; no lesions in lungs; normal respirations; no blood in chest cavity. <i>No lung tissue damage, septa and capillaries (blood vessels) are normal.</i>
2—	alive; minimal focal (localized) intraparenchymal hemorrhage in one or two lung lobes; normal respirations; no blood in chest cavity. <i>Lung damage, injury to alveolar septal capillaries (probably trauma (sonication) associated with tearing of septa and capillaries) resulting in bleeding into alveoli.</i>
4—	alive; mild focal (localized) intraparenchymal hemorrhage in one or a few lung lobes; normal respirations; no blood in chest cavity. <i>Lung damage, injury to alveolar septal capillaries (probably trauma (sonication) associated with tearing of septa and capillaries) resulting in bleeding into alveoli.</i>
6—	alive; moderate to marked intraparenchymal hemorrhage in one or more lung lobes; normal respirations; no blood in chest cavity. <i>Lung damage, injury to alveolar septal capillaries (probably trauma (sonication) associated with tearing of septa and capillaries) resulting in bleeding into alveoli.</i>
8—	near death but still alive; intraparenchymal hemorrhage (dark red to black) in one or more lung lobes; hemorrhage associated with formation of gas bubbles of varied sizes mixed with blood; gasping respirations; blood in chest cavity caused by rupture of lung tissue. <i>Lung damage, injury to alveolar septal capillaries (probably trauma (sonication) associated with tearing of septa and capillaries) resulting in bleeding into alveoli (hemorrhage) and chest cavity (tearing of lung pleura with bleeding into the chest cavity).</i>
10—	death; intraparenchymal hemorrhage (dark red to black) in one, a few, or all lung lobes; hemorrhage associated with formation of gas bubbles of varied sizes mixed with blood; gasping respirations during exposure; blood in chest cavity caused by rupture of lung tissue. <i>Lung damage, injury to alveolar septal capillaries (probably trauma (sonication) associated with tearing of septa and capillaries) resulting in bleeding into alveoli (hemorrhage) and chest cavity (tearing of lung pleura with bleeding into the chest cavity). Animals die from hypovolemic shock (inadequate circulating blood volume) and respiratory failure (unable to effectively transfer oxygen from alveoli to red blood cells because of hemorrhage into alveoli).</i>

† The scoring criteria were developed to compare hemorrhage in the mouse lung to that in the rabbit lung. These criteria are not the same as those utilized in O'Brien and Zachary (1993). Also text in *italics* is a speculative interpretation of the potential mechanism from lung hemorrhage and the cause of death.

the previous study (O'Brien and Zachary 1994), spot checks of the calibration were conducted periodically during the study and the results were consistent.

The overall acoustic pressure lateral distribution from Source # 2 (active rectangular source area was 16 cm horizontally × 11 cm vertically) was not very uniform at a range of 5 cm from the source surface. The mouse's thoracic area was about 2 × 2 cm and the rabbit's thoracic area was about 10 × 10 cm. To assure that each animal was positioned at the same field location where the acoustic pressure was known, a removeable metal pointer that

could be placed on the face of the transducer was designed and fabricated. The acoustic pressure calibrations and animal placements were performed relative to the position of the tip of the removeable metal pointer; the tip was at a distance of 5 cm from the face of the source. The lateral area chosen to perform the calibrations, and hence the animal exposures, was a 4 cm² square region about the pointer's tip location for mice, and a 100 cm² square region about the pointer's tip location for rabbits. A two-dimensional lateral beam plot at a range of 5 cm was performed (20 × 20 cm in 0.5 cm steps) and the horizontal and lateral scan relative to the pointer tip location is shown in Fig. 1. Within the mouse's 4 cm² square region, the hydrophone's zero-to-peak acoustic pressure (p_{hop}) ranged from 99 to 118 kPa, that is, p_{hop} varied by 19 kPa with a maximum p_{hop} within this area of 118 kPa. Within the rabbit's 100 cm² square region, the hydrophone's zero-to-peak acoustic pressure (p_{hop}) ranged from 23 to 118 kPa, that is, p_{hop} varied by 95 kPa with a maximum p_{hop} within this area of 118 kPa.

The p_{hop} values reported herein are the maximum p_{hop} values within either the 4 or 100 cm² square region, either 100 or 145 kPa. In addition, the sham exposure conditions are reported at 0 kPa. These ultrasonic acoustic pressure levels were selected because they represent levels at which mice were mostly killed for a 10 min exposure.

STATISTICAL ANALYSIS

Nonparametric statistical tests are used, because the assumption that the population from which the sample under observation is drawn is normally distributed is not necessary met. This results from the arbitrary scoring criteria (see Table 1), which is a quantitative means to indicate the degree of lung damage. The Kruskal–Wallis Analysis of Variance (ANOVA) test is a nonparametric method corrected for ties used to compare the medians of three or more unpaired groups. The Mann–Whitney test is a nonparametric method used to compare the median of two unpaired groups. For the time of death comparison between mice of this and the previous (O'Brien and Zachary 1994) studies at the exposure pressure level of 145 kPa, the parametric unpaired t-test is used to compare the means of the two groups. Linear regression analyses are used to quantify the best-fit straight line between two variables, and yields the correlation coefficient (r) that describes the amount of linear association and slope's p value, which indicates the slope's significance relative to a zero slope. Statistical significance is at the 0.05 level, and all statistical calculations were performed using InStat® Macintosh Version 2.0 (GraphPad Software, San Diego, CA).

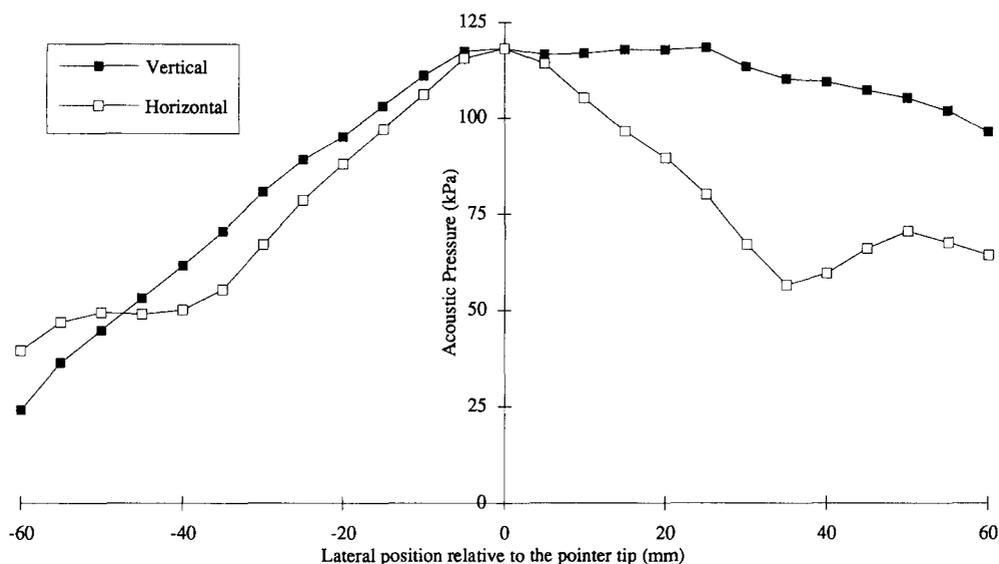


Fig. 1. Vertical and horizontal lateral acoustic pressure field plots relative to the pointer location at a range from the source of 5 cm.

RESULTS AND DISCUSSION

The study consisted of 24 mice and 16 rabbits. The exposure conditions selected for this study were based on the results from the previous study (O'Brien and Zachary 1994). One exposure duration (10 min) and the two highest exposure acoustic pressure levels (p_{hop} : 100 and 145 kPa) from the previous study were selected to limit the number of animals. Both mice and rabbits were included so that (1) a direct comparison between mice from the two studies could be made to evaluate experimental protocol consistency; and (2) a direct comparison between the two species could be made to evaluate the hypothesis that the two species would be affected differently under identical ultrasonic exposure conditions.

Prior to this study, 12 mice and 12 rabbits were exposed and evaluated to establish the experimental protocol procedures and scoring criteria (see Table 1). These preliminary animals were not included in the following analysis.

Figure 2 shows the individual lung damage score values of the 24 mice (4 mice at 0 kPa, 10 mice at 100 kPa, 10 mice at 145 kPa) and 16 rabbits (2 rabbits at 0 kPa, 7 rabbits at 100 kPa, 7 rabbits at 145 kPa) for the 10 min exposure duration. Their mean \pm standard deviation are summarized in Table 2, along with those of the previous 10 min mouse study (O'Brien and Zachary 1994). The greatest lung damage score value of the rabbits evaluated was 4, whereas the greatest lung damage score value of the mice evaluated was 10.

Table 2 and Figs. 2 and 3 suggest that species

difference (mouse versus rabbit) is a strong function of lung damage. At the 0 and 145 kPa exposure pressure levels, the Mann-Whitney test was not possible to analyze the differences between the two groups because at least one of the groups in each exposure pressure level exhibited a zero standard deviation (all of the observations were the same value). However, there is a clear difference between the mouse and rabbit sensitivities to lung damage at each of the exposure pressure levels, save the sham groups. The rabbit was much less sensitive to damage than the mouse.

Likewise, as a function of exposure pressure level for each species, the Kruskal-Wallis ANOVA test was not possible to analyze statistically the data because at least one of the groups for each species exhibited a zero standard deviation (all of the observations were the same value). However, the Mann-Whitney test was applied to the 100 and 145 kPa rabbit groups and indicated a nonsignificant difference. To compare the difference between the 100 and 145 kPa mouse groups, it is noted (see Fig. 2) that 100% of the mice had hemorrhaged lungs (score ≥ 6), although the degree of lung damage was, in general, less in the lower pressure level group.

Linear regression analyses of lung damage score values versus exposure acoustic pressure levels for the two species from this study yielded:

$$\text{score} = 0.069 \text{ pressure} + 0.50$$

$$r = 0.94 \quad p < 0.0001 \quad \text{mice, } n = 24 \quad (1)$$

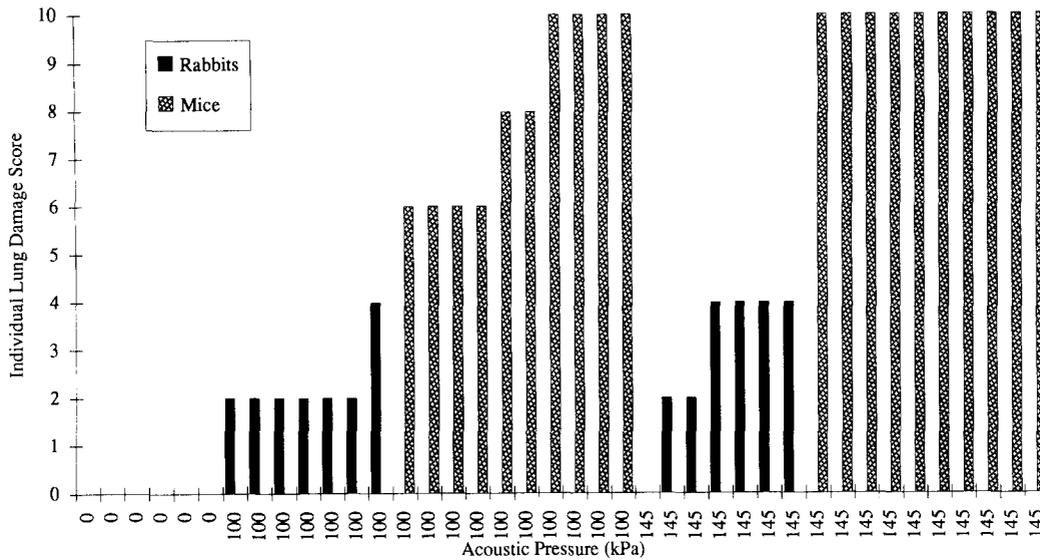


Fig. 2. Individual values of the mouse and rabbit lung damage score (based on Table 1 scoring criteria) for 24 mice (4 mice at 0 kPa, 10 mice at 100 kPa, 10 mice at 145 kPa), and 16 rabbits (2 rabbits at 0 kPa, 7 rabbits at 100 kPa, 7 rabbits at 145 kPa), as a function of exposure acoustic pressure level for the 10 min exposure duration.

score = 0.019 pressure + 0.19

$r = 0.63$ $p < 0.009$ rabbits, $n = 16$. (2)

Both regressions are highly significant, with a much greater slope for mice compared with that for rabbits by a factor of 3.6 (0.069/0.019), demonstrating the difference in degree of sensitivity to lung damage between these two species.

To compare the two mouse studies, it is first necessary to examine the criteria for the lung damage score values of the two studies, viz., Table 1 reported

herein for this study and Table 1 in O'Brien and Zachary (1994) for the previous study. Lung damage score values of 0, 6, 8 and 10 are the same between the two studies. The differences in the two sets of criteria are for lung damage score values of 2 and 4, wherein the scoring criteria used for this study uses intraparenchymal hemorrhage as the biological endpoint, and those for the previous study used congestion and pleural/subpleural hemorrhage.

For comparison purposes, the mean lung damage score values for 100 kPa and 145 kPa of the two mouse studies show considerable agreement. At an exposure

Table 2. Mean ± standard deviation of the lung damage score values for this and the previous mouse studies. Scoring criteria for the two studies were slightly different (scores of 0, 6, 8 and 10 are the same, whereas 2 and 4 are different; see Table 1 for this study and Table 1 in O'Brien and Zachary (1993) for the previous study). Entries in parentheses indicate the number of animals.

Exposure zero-to-peak acoustic pressure P_{hop} (kPa)	(Previous study) (Mice) Score for 10 min exposure duration	(This study) (Mice) Score for 10 min exposure duration	(This study) (Rabbits) Score for 10 min exposure duration	Mann-Whitney p value†
0	2.7 ± 1.8 (15)	0 ± 0 (4)	0 ± 0 (2)	‡
65	4.5 ± 1.8 (15)			
80	4.8 ± 2.0 (15)			
87	5.2 ± 1.7 (15)			
100	5.3 ± 1.6 (15)	8.00 ± 1.9 (10)	2.29 ± 0.8 (7)	0.0001
145	9.9 ± 0.5 (15)	10.0 ± 0 (10)	2.86 ± 1.6 (7)	‡

† The Mann-Whitney test was performed between mouse and rabbit groups from this study. The (‡) denotes that it was not possible to analyze statistically because one or more of the group's standard deviation was zero (all observations were the same value).

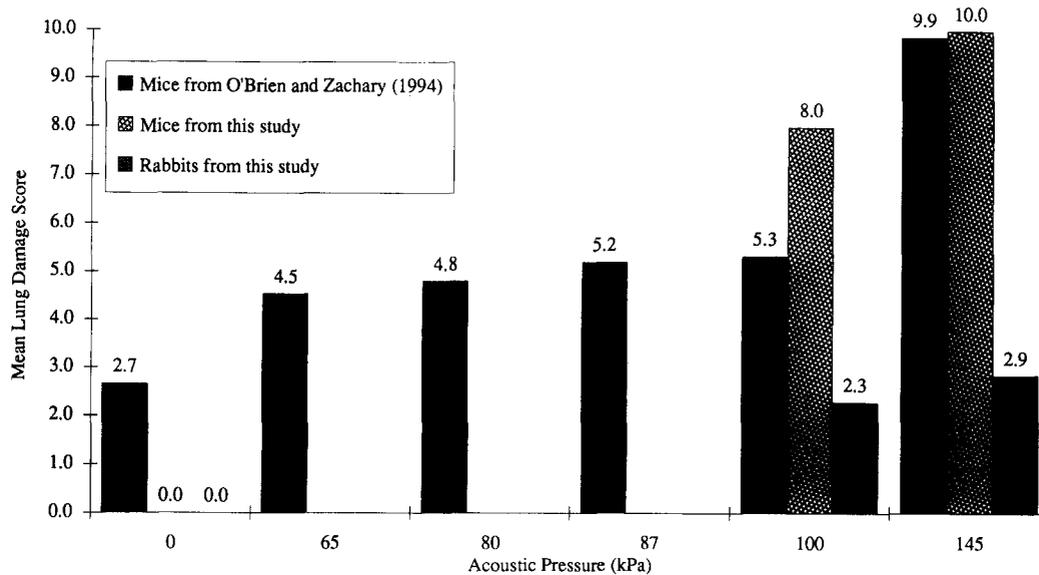


Fig. 3. Mean lung damage score values of mice from previous study (O'Brien and Zachary 1993), and mean lung damage score values of mice and rabbits from this study, as a function of exposure acoustic pressure level for the 10 min exposure duration.

acoustic pressure level of 100 kPa for the 10 min exposures, the mean lung damage score values for the previous study and this study are 5.3 and 8.0, respectively, whereas, at 145 kPa, the values are 9.9 and 10.0, respectively. If anything, the results from this study suggest a slightly greater degree of damage, but the differences could simply be due to biological variability.

To further compare the results of the two mouse studies, the number of mice that died (individual lung damage score value of 10—criterion same for both studies) during the 10 min exposure is listed in Table 3, along with the mean \pm standard deviation time to death. There were no deaths resulting from exposure to the rabbits. All mice were observed continually during the exposure period. Animals that had severe lung hemorrhage and hemothorax developed a gasping respiratory pattern early in the exposure period. The gasping pattern became more exaggerated as the exposure period progressed. Terminally, these animals would take several deep breaths and then stop breathing (defined as the moment of death). Animals with no lung damage, or with moderate lung hemorrhage without hemothorax, would maintain a normal respiratory pattern throughout the exposure period (normal and regular respiratory pattern).

The death rate, particularly for the 145 kPa exposure acoustic pressure level, suggests considerable agreement between the two studies. Fourteen out of 15 mice (93%) died in the previous study and 10 out of 10 mice (100%) died in this study, with their mean

times to death of 309 and 300 s (within 3%), respectively. For the 100 kPa exposure acoustic pressure level, none of the 15 mice died in the previous study, whereas 4 out of 10 died in this study, again suggesting, if anything, a slightly greater degree of damage in this study.

Figure 3 summarizes the mean lung damage score values for the 10 min exposure duration studies of the previous study (90 mice) and this study (24 mice and 16 rabbits). This figure graphically demonstrates that the mean lung damage levels of mice are substantially greater than those of rabbits. A marked difference is noted between the lung damage score values and lung hemorrhage for similar exposure conditions of mice and rabbits.

Table 3. Comparison of this study with the previous study for mice only. Number of mice that died during exposure. Entry in parenthesis indicates the mean \pm standard deviation time to death (in seconds).

Exposure zero-to-peak acoustic pressure p_{hop} (kPa)	(Previous study) Death for 10 min exposure duration	(This study) Death for 10 min exposure duration
0	0	0
65	0	
80	0	
87	0	
100	0	4 (405 \pm 150)
145	14 (309 \pm 92)	10 (300 \pm 73)

Linear regression analyses of lung damage score values versus exposure acoustic pressure levels for the two species from the previous mouse results ($n = 90$), and the mouse ($n = 24$) and rabbit ($n = 16$) results of this study yielded:

$$\text{score} = 0.054 \text{ pressure} + 1.33$$

$$r = 0.80 \quad p < 0.0001 \quad \text{mice, } n = 114 \quad (3)$$

$$\text{score} = 0.019 \text{ pressure} + 0.19$$

$$r = 0.63 \quad p < 0.009 \quad \text{rabbits, } n = 16 \quad (4)$$

Both regressions are highly significant, with a much greater slope for mice compared with that of rabbits by a factor of 2.8 (0.054/0.019), demonstrating the difference in degree of sensitivity to lung damage between these two species. Clearly the mice are much more sensitive to lung damage than rabbits.

It is likely that lung hemorrhage and hemothorax occurs early in the exposure period, and that the clinical observations of gasping and death are sequelae to hypovolemic shock. Macroscopic lesions in lung consisted of dark red to black areas of intraparenchymal hemorrhage that extended from visceral pleural surfaces into lung parenchyma, and that appeared spatially related to the edges of lung lobes. Lesions occurred in specific lung lobes anatomically related to lung that was closest to and in contiguous alignment with the ultrasound transducer (Zachary and O'Brien 1994). Lung hemorrhage could be induced by ultrasound directly from damaged endothelial cells, or indirectly from mechanical tearing (laceration) of blood vessels in association with a cavitation-like or bubble-related phenomena in lung alveoli.

Let us further evaluate the differences in the lung properties between species. Comparative lung morphology between different species is shown in Table 4. 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, because 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 are supplied by branches of the bronchial artery and thin pleura are 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 (see Table 4). 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).

Lung damage and hemorrhage induced by ultrasound are hypothesized to be a direct reflection of structural, functional and physiological differences between lungs of mice and rabbits. These differences include the two important properties of mean alveolar diameter and lung compliance, as discussed previously (O'Brien and Zachary 1994). Consider these observations between mouse and rabbit:

1. *Total lung volume*: Rabbit is about 100 times larger than mouse.
2. *Alveolar surface area*: Rabbit is about 65 times larger than mouse.
3. *Mean alveoli diameter*: Rabbit is about 2 times larger than mouse.
4. *Lung compliance*: Rabbit is about 175 times greater than mouse.
5. *Pulmonary pleura size*: Thin for both mouse and rabbit.
6. *Pulmonary pleura blood supply*: Supplied by the pulmonary artery for both mouse and rabbit.
7. *Respiratory bronchiole*: Absent or single short generation for both mouse and rabbit.
8. *Lung damage threshold*: Mouse is 65 kPa for 10 min exposure duration (O'Brien and Zachary 1994). No thresholds assessed in this study.
9. *Ultrasound-induced lung damage*: A significantly greater degree of lung damage induced in mouse compared to rabbit using same scoring criteria (Table 1). Ratios of mean lung damage score values (mouse:rabbit) are 3.5 and 3.5 at 100 and 145 kPa, respectively (see Table 2).

Although the mechanical properties leading to pulmonary hemorrhage following ultrasound treatment in mice and rabbits 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 alve-

Table 4. Comparative lung morphology (blank indicates that information could not be found).

Lung property	Mouse/Rabbit	Cat/Dog	Monkey	Pig	Human
Pulmonary pleura Size ^a	Thin	Thin	Thin	Thick	Thick
Pulmonary pleura blood supply ^a	Pulmonary artery	Pulmonary artery	Pulmonary artery	Bronchial artery	Bronchial artery
Interlobular and segmental connective tissue ^a	Little, if any	Little, if any	Little	Extensive interlobular surrounds many lobules completely	Extensive, interlobular partially surrounds many lobules
Pulmonary plural lymphatics ^a	Very few	Few	Few	Extensive	Extensive
Nonrespiratory bronchiole (nonalveolarized) ^a	Several generations	Fewer generations	Fewer generations	Several generations	Several generations
Respiratory bronchiole (alveolarized) ^{a,b}	Absent or single short generation	Several generations	Several generations	Absent or single short generation	Several generations
Terminal respiratory bronchiole ^a	Ends in alveolar ducts or very short respiratory bronchioles	Ends in respiratory bronchioles	Ends in respiratory bronchioles	Ends in alveolar ducts or very short respiratory bronchioles	Ends in respiratory bronchioles
Acinus Transition Zone ^{a,b}	Abrupt	Not abrupt	Not abrupt		Not abrupt

^a Tyler and Julian (1992); ^b Pinkerton et al. (1992).

olar and capillary damage in mice and rabbits 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.

SPECULATION

It is interesting to observe that the only lung property that is even within an order of magnitude of the 3.5 mouse:rabbit ratio of mean lung damage score (item 9 above) is the mean alveoli diameter. Mice have small mean alveolar diameters ($\approx 44 \mu\text{m}$) while rabbits have larger mean alveolar diameters ($\approx 86 \mu\text{m}$). The rabbit mean alveoli diameter is intermediate between mice and man ($\approx 208 \mu\text{m}$). One might then suggest that the degree of difference in lung damage sensitivity between mouse and rabbit is a factor of 2 (because the mean alveoli diameter is a factor of 2). Therefore, assuming that the mean alveoli diameter is the principal morphological determinant for ultrasound-induced alveolar damage and hemorrhage, the mouse may be the best (most sensitive) animal model in which to demonstrate this phenomenon because this mammalian species has one of the smallest mean alveoli diameters. This is clearly a hypothesis that has yet to be proved, either biologically or physically. The only basis for this hypothesis is the similar order of magnitudes between

mean alveoli diameter and experimentally determined degree of damage for mice and rabbits.

For purposes of continued scientific speculation and extrapolation from mouse, to rabbit and then to man, let's consider the following observations between mouse, rabbit and man:

1. *Total lung volume*: Rabbit is about 100 times larger and human is about 6500 times larger than mouse.
2. *Alveolar surface area*: Rabbit is about 65 times larger and human is about 1500 times larger than mouse.
3. *Mean alveoli diameter*: Rabbit is about 2 times larger and human is about 5 times larger than mouse.
4. *Lung compliance*: Rabbit is about 175 times larger and human is about 4000 times larger than mouse.
5. *Pulmonary pleura size*: Both mouse and rabbit are thin and human is thick.
6. *Pulmonary pleura blood supply*: Both mouse and rabbit are supplied by the pulmonary artery and human is supplied by the bronchial artery. Pulmonary artery typically has a higher blood pressure than the bronchial artery.
7. *Respiratory bronchiole*: Both mouse and rabbit are absent or single short generation, and human has several generations.

Using a similar type of argument to estimate (extrapolate) to a human lung damage sensitivity as was done above for extrapolating between mouse and rabbit (degree of difference in lung damage sensitivity about

a factor of 2), the human mean alveoli diameter is about 5 times greater than mouse. Therefore, the degree of difference in lung damage sensitivity between mouse and man is estimated to be a factor of 5.

Extrapolating the 30 kHz ultrasound threshold findings of mouse to man would then suggest that the human lung hemorrhage damage acoustic pressure threshold might be in the range of 500 kPa for a 10 min exposure duration. If one were to then extrapolate in the megahertz frequency range using a \sqrt{f} dependency, the human lung damage threshold (in terms of the Mechanical Index) would be of the order of 2.5. In terms of a peak rarefactional acoustic pressure at a center frequency of 5 MHz, the derated p_r is estimated to be 5.6 MPa, with an estimated water p_r of 13 MPa (distance of 2.5 cm).

CONCLUDING REMARK

There are so many differences in the comparative morphology (Table 4) and other properties (Tables 8 and 9 in O'Brien and Zachary 1994) between the mouse and human, that this extrapolation must be considered highly speculative (and is provided here for intellectual discourse purposes only) until further experimentation is performed and understanding is available.

In summary, it is suggested that the mouse is an inadequate animal model for the evaluation of whether ultrasound has the potential for producing lung damage in humans, particularly at 30 kHz. Further, in evaluating the experimentally observed trends of lung damage between mouse and rabbit, it is suggested that there is no experimentally supportable evidence that such

damage could occur in humans unless the acoustic pressure levels were excessively high.

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