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●*Original Contribution*

RABBIT AND PIG LUNG DAMAGE COMPARISON FROM EXPOSURE TO CONTINUOUS WAVE 30-kHz ULTRASOUND

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Abstract—Previous comparative studies of ultrasound-induced pulmonary hemorrhage in mice and rabbits suggested that sensitivity to damage was species dependent (O'Brien and Zachary 1994b). In order to understand better these differences in species more analogous to the human, 74 pigs and 75 rabbits were each exposed for 10 min at 1 of 6 acoustic pressure levels (0, 145, 290, 340 [rabbits only], 460 and 490 [pigs only] kPa) at an ultrasonic frequency of CW 30 kHz. Eighteen mice were used as positive controls (10-min duration at 145 kPa). Because pig lung has numerous physiological and anatomical similarities to human lung, it was selected as the appropriate animal model for these studies. Pig lung data were compared to rabbit lung data; rabbit lung data have already been compared with mouse lung data (O'Brien and Zachary 1994a). Comparative analyses and extrapolation of these experimental data are intended to provide a better scientific basis for understanding the potential biological effects of ultrasound on human lungs since such studies will probably never be conducted with humans. Under the same exposure conditions and lung assessment criteria, mouse lung was determined to be more sensitive to ultrasound-induced damage than that of the rabbit by a factor of 3.9, the rabbit lung was more sensitive to ultrasound-induced damage than that of the pig by a factor of 3.7, and the mouse lung was more sensitive to ultrasound-induced damage than that of the pig by a factor of 14.4.

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

INTRODUCTION

The purpose of this study was to test further, with rabbits and pigs, the hypothesis that there is a species dependency in the degree of sensitivity to ultrasound-induced lung damage at an ultrasonic frequency of 30 kHz under continuous-wave exposure conditions. In a previous study with 270 mice, acoustic pressure threshold levels for producing lung damage in mice were determined from exposures to continuous-wave 30-kHz ultrasound (O'Brien and Zachary 1994a). Based on this previous study, extrapolation of the frequency dependency of the acoustic pressure threshold appeared to be consistent with that of the mechanical index (AIUM/NEMA 1992) although the mechanical index is a pulsed wave, not a continuous-wave concept. It was demonstrated that the lung damage in mice could be caused by relatively low acoustic pressure levels in the range of 65 kPa. However, 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, and particularly humans, because it was also demonstrated in another continuous-wave ultrasound study at 30 kHz 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 (O'Brien and Zachary 1994b). These sensitivity factors were estimated from the slopes of exposure–effect curves, not from threshold data, and lead to the suggestion that there is no experimentally supportable evidence that ultrasound-induced lung damage at 30 kHz could occur in humans unless the acoustic pressure levels were much higher. Since there was a suggestion about species dependency between mice

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and rabbits, albeit with only 24 mice and 16 rabbits (O'Brien and Zachary 1994b), and since it is not possible to test this on humans, the pig lung was selected because it has many morphological features more similar to human lung than to mouse and rabbit lungs. Therefore, the hypothesis that there is a species dependency to ultrasonically-induced lung damage from continuous-wave 30-kHz ultrasound has been tested with 75 rabbits and 74 pigs.

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 1 week of the time of each shipment of mice. Five- to 5 $\frac{1}{2}$-month-old (8–9 lb; 3.6–4.1 kg) New Zealand White male rabbits were obtained from Myrtle's Rabbitry, Inc. (Thompson Station, TN) and sonications were performed within 1 week of the time of each shipment of rabbits. Ten- to 12-week-old crossbred pigs (60–70 lb; 27.2–31.8 kg) were obtained from the University of Illinois College of Veterinary Medicine swine breeding farm (Urbana, IL) and sonications were performed within 2 days of the time of each delivery of pigs.

Animals were observed to be free of clinical signs suggestive of respiratory disease by visual inspection before the start of the studies and were confirmed to be free of respiratory disease at post mortem examination. Animals were provided housing, food and veterinary care according to University of Illinois and NIH guidelines.

Mice were weighed and then anesthetized with a 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 (Neet® or Nair®) to minimize the likelihood of entrapment of air at the skin–water interface. The anesthetized mouse was then restrained in a spread-eagle position in a specially fabricated apparatus that permitted the placement of the mouse vertically in the water bath (distilled, degassed water). The ventral surface of the animal was toward the ultrasound source. A pointer attached to the ultrasound source was used to position uniformly the mouse in the calibrated sound field; the pointer was removed during animal sonication. The individual preparing each mouse for sonication (anesthetizing, depilating and placing in exposure structure) was blinded to the exact exposure condition. These procedures are similar to those used in previous studies (O'Brien and Zachary 1994a, 1994b; Zachary and O'Brien 1995).

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 (Neet® or Nair®) to minimize the likelihood of entrapment of air at the skin–water interface. The anesthetized rabbit was then restrained in a spread-eagle position that permitted the placement of the rabbit vertically in the water bath (distilled, degassed water). The ventral surface of the animals was toward 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 each rabbit for sonication (anesthetizing, depilating and placing in exposure structure) was blinded to the exact exposure condition. These procedures are similar to those used in a previous study (O'Brien and Zachary 1994b).

Pigs were anesthetized with a combination of ketamine hydrochloride (Ketamine®) (5.0 mg/kg), xylazine (Rompun®) (5.0 mg/kg) and Telazol® (10 mg/kg) administered intramuscularly. The skin surrounding the rib cage, sternum and vertebral column was clipped with an electric shaver and the hair removed with a depilatory agent (Neet® or Nair®) to minimize the likelihood of entrapment of air at the skin–water interface. The anesthetized pig was then restrained in a spread-eagle position that permitted the placement of the pig vertically in the water bath (distilled, degassed water). The ventral surface of the animals was toward the ultrasound source. A pointer attached to the ultrasound source was used to position uniformly the pig in the calibrated sound field; the pointer was removed during animal sonication. The individual preparing each pig for sonication (anesthetizing, depilating and placing in exposure structure) was blinded to the exact exposure condition.

Mice were humanely killed by cervical dislocation followed by exsanguination and dissection. Mouse lungs were placed in a petri dish that contained sterile saline and examined (blinded to the specific exposure condition) with a dissecting microscope. The rabbits were humanely killed with an overdose of carbon dioxide (CO_2) inhalation, and the lungs were dissected free from the animal. Rabbit lungs were washed free of blood and examined (blinded to the specific exposure condition) visually for hemorrhage. Pigs were humanely killed with an overdose of carbon dioxide

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

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.

Histologic evaluation required to confirm these gross interpretations.

(CO_2) inhalation, and the lungs were dissected free from the animal. Pig lungs were washed free of blood and examined (blinded to the specific exposure condition) visually for hemorrhage.

The lung damage scoring criteria were adapted from those used in previous studies (O'Brien and Zachary 1994a, 1994b) 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 (Table 1) 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.

The exposimetry procedures were identical to those reported in the mouse lung study (O'Brien and Zachary 1994a) and the mouse–rabbit lung study (O'Brien and Zachary 1994b) with the exception that the former study was conducted at 37°C and the latter study and this study were conducted at room temperature (≈22°C); there was no detectable difference in the degree of lung damage in mice between the two temperatures. Internal body temperatures were not monitored. The mice, rabbits and pigs were exposed to acoustic pressure, defined as the total pressure minus the ambient pressure (AIUM 1992), 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).

The overall acoustic pressure lateral distribution from source (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 (see O'Brien and Zachary 1994b). The mouse's thoracic area was about 2 × 2 cm, the rabbit's thoracic area was about 10 × 10 cm and the pig's thoracic area was about 22 × 30 cm. To assure that each animal was positioned at the same field location where the acoustic pressure was known, a removable metal pointer, which 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 removable 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 cm^2 square region about the pointer's tip location for mice and a 100 cm^2 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 were also performed (see Fig. 1 in O'Brien and Zachary 1994b). Within the mouse's 4-cm^2 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-cm^2 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. For the pig exposure conditions, the pig's thoracic area (about 22 × 30 cm) was greater that the source's area (11 × 16 cm).

A calibrated NRL Type F42D hydrophone (Bobber 1969) was used as the primary standard in these experiments. The hydrophone's zero-to-peak voltage (V_{hop}) was converted into the hydrophone's zero-to-peak pressure (p_{hop}) using the hydrophone's calibrated voltage sensitivity of 37 kPa/V at 30 kHz.

As in the mouse lung experiments (O'Brien and

Table 2. Summary (mean ± standard deviation) of the results of the spot checks for the exposure intensity settings employed for the rabbit–pig lung studies.

Blue Wave Amplifier's Line Voltage V_{rms} (volts)	Hydrophone's Zero-to-Peak Pressure p_{hop} (kPa)
100	145 ± 30
150	290 ± 68
170	340 ± 73
200	460 ± 95
220	490 ± 86

Zachary 1994a) and the mouse–rabbit experiments (O'Brien and Zachary 1994b), frequent spot checks of the calibration were conducted during the study and the results were consistent. These spot checks involved placing the hydrophone at the tip of the pointer, removing the pointer and recording the hydrophone's zero-to-peak voltage (V_{hop}) for each of the Blue Wave amplifier's line voltages (V_{rms}). Table 2 lists the mean and standard deviation of p_{hop} for each V_{rms}.

The p_{hop} values reported for these experiments were the maximum p_{hop} value within either the 4 or 100 cm^2 square region for mice and rabbits, respectively, or within the entire field for the pigs, that is, between 145 and 490 kPa (Table 2). In addition, the sham exposure conditions were reported at 0 kPa.

For comparison, Table 3 summarizes the exposure conditions for three studies (those from O'Brien and Zachary 1994a; O'Brien and Zachary 1994b; and those from this study) for each of the exposure pressure levels (p_{hop}) and exposure durations.

Six exposure conditions (six acoustic pressure levels [p_{hop}] and one exposure duration) were used in order to assess the effect due only to exposure pressure level. Sixty rabbits were exposed at 145, 290, 340, and 460 kPa, and 15 rabbits were sham exposed for a total of 75 rabbits. Fifty-eight pigs were exposed at 145, 290, 460, and 490 kPa, and 16 pigs were sham exposed for a total of 74 pigs. Eighteen mice were exposed at 145 kPa for positive biological controls. All exposure durations were 10 min.

Nonparametric statistical tests were used because the assumption that the population from which the sample under observation is drawn was normally distributed was not necessary met. This resulted from the arbitrary scoring criteria (Table 1), which was 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. Should the Kruskal–Wallis ANOVA test indicate significance ($p < 0.05$), then the Dunn's multiple comparisons test, a variation of the Bonferroni test, was used to compare which means are significantly different. The Mann–Witney test is a nonparametric method used to compare the median of two unpaired groups. Linear regression analyses were used to quantify the best-fit straight line between two variables and yielded the correlation coefficient (r), which describes the amount of linear association and slope's p value which indicates the slope's significance relative to a zero slope. Statistical significance was at the 0.05 level and all statistical calculations were performed using InStat® Macintosh Version 2.0 (GraphPad Software, San Diego, CA).

Table 3. Summary of exposure conditions (acoustic pressure, p_{hop}, and exposure duration) for three lung damage studies.

	Mouse			Mouse–Rabbit		Mouse–Rabbit–Pig		
p_{hop} (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

The study denoted "Mouse" lists the exposure conditions and number of mice from O'Brien and Zachary (1994a); that denoted "Mouse-rabbit" lists the exposure conditions and number of mice and rabbits from O'Brien and Zachary (1994b); and that denoted "Mouse–rabbit–pig" lists the exposure conditions and the number of mice, rabbits and pigs from this study.

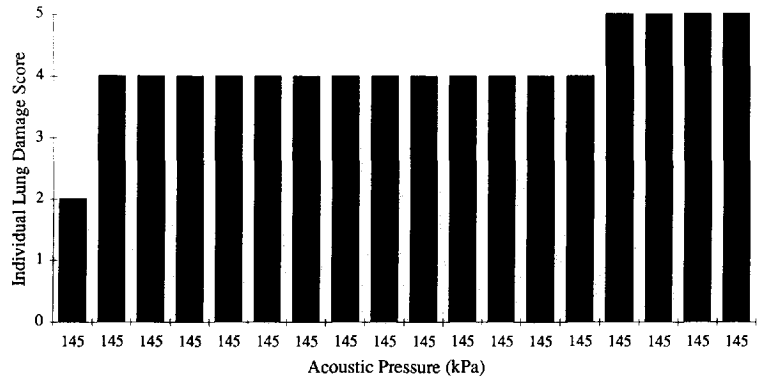

Fig. 1. Individual values of the mouse lung damage score (based on Table 1 scoring criteria) for 18 mice at an acoustic pressure of 145 kPa for a 10-min exposure duration.

RESULTS AND DISCUSSION

Figures 1, 2 and 3 show the *individual* lung damage score values (based on Table 1 scoring criteria) as a function of exposure pressure level for each of the three mammalian species of mouse, rabbit and pig, respectively. Since the mouse study represented a positive biological control (we knew what to expect from the results of experiments reported in O'Brien and Zachary 1994a, 1994b), only the 145-kPa pressure exposure level was used. The 145-kPa acoustic pressure exposure level was the level at which severe lung damage or death occurred in mice from a 10-min exposure.

Table 4 lists the mean and standard deviation of the lung damage scores for Fig. 2 (rabbit results) and Fig. 3 (pig results), respectively. Table 4 also lists the mean and standard deviation of the lung damage score values (based on Table 1 scoring criteria) for a 10-min exposure duration at an exposure pressure level of 145 kPa for each of the three mammalian species of mouse, rabbit and pig. This exposure pressure level represented the highest level for producing severe lung damage or death resulted from a 10-min exposure to mice whereas it was the lowest level used (save for the sham exposure pressure level of 0 kPa) used for the rabbit and pig exposures. It is clear that there is a marked difference in the degree of lung damage at a 10-min exposure for an exposure pressure level of 145 kPa. For the pig results, *individual* lung damage score values were 0, 0.5 and 1; for the rabbit results, *individual* lung damage score values were 0, 1, 2 and 3; and for the mouse results, *individual* lung damage score values were 2, 4 and 5. Thus, not only do the *individual* lung damage score values clearly demonstrate a marked difference between the three mammalian species, but also the mean lung damage score values show this difference.

As a function of exposure pressure level for each of the two species (rabbits and pigs), the Kruskal–Wallis ANOVA test indicated significance at the 0.0001 level. The Dunn's test compared which means are significantly different at the 0.05 level:

For the rabbit:

Sham vs. 145 kPa

Sham vs. 290 kPa

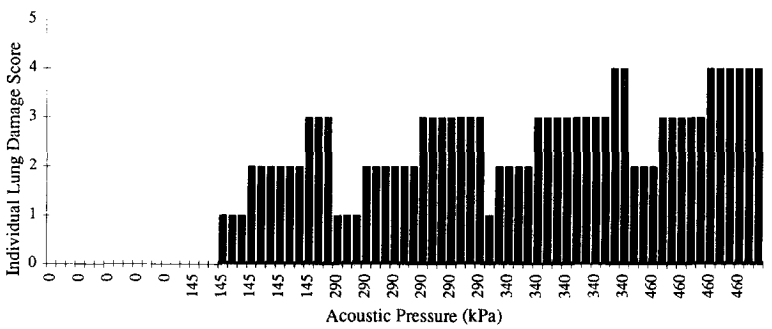

Fig. 2. Individual values of the rabbit lung damage score (based on Table 1 scoring criteria) for 75 rabbits as a function of acoustic pressure for a 10-min exposure duration.

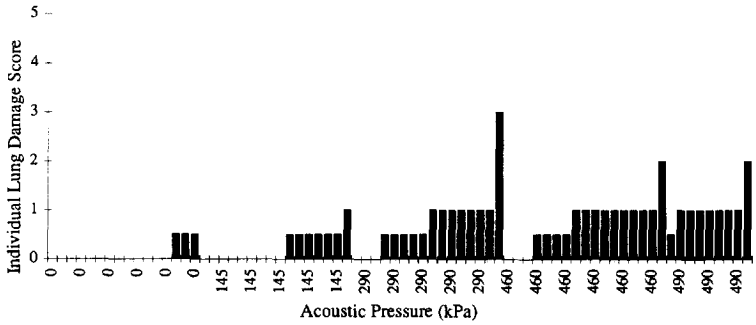

Fig. 3. Individual values of the pig lung damage score (based on Table 1 scoring criteria) for 74 pigs as a function of acoustic pressure for a 10-min exposure duration.

Sham vs. 340 kPa
Sham vs. 460 kPa
145 kPa vs. 460 kPa

For the pig:

Sham vs. 290 kPa
Sham vs. 460 kPa
Sham vs. 490 kPa
145 kPa vs. 460 kPa
145 kPa vs. 490 kPa

Linear regression analyses of lung damage score values vs. acoustic pressure levels for the two species yielded:

$$\text{score} = 0.0069\ \text{pressure} + 0.26 \quad r = 0.81$$
$$p < 0.0001 \quad \text{rabbits, n} = 75 \quad (1)$$

$$\text{score} = 0.0018\ \text{pressure} + 0.091 \quad r = 0.57$$
$$p < 0.0001 \quad \text{pigs, n} = 74 \quad (2)$$

Both regressions are highly significant with a much greater slope for rabbits compared with pigs by a factor of 3.8 (0.0069/0.0018) demonstrating the difference in degree of sensitivity to lung damage between these two species.

To compare the results from the three mouse studies, the original records from the previous mouse experiments (O'Brien and Zachary 1994a, 1994b) were re-evaluated and scored based on the criteria for the lung damage score values of this study (Table 1). Table 5 lists the mean and standard deviation lung damage score values for mice from all three experiments (based on the scoring criteria of Table 1), which were exposed for a 10-min duration. The individual lung damage score values for mice for the three studies (based on the scoring criteria of Table 1) show essentially the same distribution of 4s and 5s with one 2. The distinction between scores of 4 and 5 is that the lungs are damaged to the same extent but 5 represents the mouse died *during* the 10-min exposure procedure. Comparison of the lung damage score values of the

Table 4. Mean ± standard deviation of the lung damage score based on the scoring criteria listed in Table 1 for a 10-min exposure duration.

Exposure Zero-to-Peak Pressure p_{hop} (kPa)	Score for 10-Min Mouse Exposure	Score for 10-Min Rabbit Exposure	Score for 10-Min Pig Exposure	Mann–Whitney or Kruskal–Wallis ANOVA p Value
0		0.0 ± 0.0 (15)	0.1 ± 0.2 (16)	*
145	4.1 ± 0.7 (18)[a,b]	1.6 ± 1.1 (15)[a]	0.3 ± 0.3 (16)[b]	0.0001
290		2.3 ± 0.8 (16)	0.8 ± 0.7 (16)	0.0001
340		2.7 ± 0.8 (15)		
460		3.2 ± 0.8 (14)	0.8 ± 0.5 (17)	0.0001
490			1.1 ± 0.4 (9)	

Number in parenthesis indicates the number of animals for that exposure condition.

* It is not possible to analyze because one of the group's standard deviation is zero (all of the observations were the same value).

Significant at the 0.05 level as determined by the Dunn's test is denoted by matched superscripts for the 145-kPa exposures because the ANOVA yielded a significant difference ($p < 0.05$) in the means.

Table 5. Mean ± standard deviation of the lung damage score values based on the scoring criteria listed in Table 1 for a 10-min exposure duration.

Acoustic Pressure p_{hop} (kPa)	Score for 10-min Mouse Study from O'Brien & Zachary, 1994a	Score for 10-min Mouse Study from O'Brien & Zachary, 1994b	Score for 10-min Mouse Study from this study	Score for 10-min Mouse Studies (all three combined)
0	1.3 ± 0.9 (15)	0.0 ± 0.0 (4)		1.1 ± 1.0 (19)
145	4.9 ± 0.3 (15)	5.0 ± 0.0 (10)	4.1 ± 0.7 (18)	4.6 ± 0.6 (38)

Number in parenthesis indicates the number of mice for that exposure condition. It was not possible to statistically analyze the score value differences at the same acoustic pressure level because one group's standard deviation is zero (all of the observations were the same value) at each level.

three mouse studies shows considerable agreement for the 145-kPa acoustic pressure level; it was not possible to statistically analyze because one of the group's standard deviation for each acoustic pressure level was zero.

To compare the results from the two rabbit studies, the original records from the previous mouse–rabbit experiments (O'Brien and Zachary 1994b) were re-evaluated and scored based on the criteria for the lung damage score values of Table 1. Table 6 lists the mean and standard deviation lung damage score values for rabbits from both studies (based on the scoring criteria of Table 1), which were exposed for a 10-min duration. The individual lung damage score values for rabbits for the two studies (based on the scoring criteria of Table 1) show essentially the same distribution of 0's, 1's, 2's and 3's. Comparison of the lung damage score values of the two rabbit studies showed considerable agreement for the 145-kPa exposure pressure level where the Mann–Whitney's p value was 0.43.

At the 145-kPa acoustic pressure level, the three mouse studies (Table 5) and the two rabbit studies (Table 6) showed considerable agreement and thus are combined.

Table 7 lists the mean and standard deviation for the combined (by species) lung damage score values for mice, rabbits and pigs from all three studies for an acoustic pressure level of 145 kPa for an exposure duration of 10 min (Fig. 4). These observations suggest that ultrasound-induced lung damage is a strong function of species (mouse vs. rabbit vs. pig) as indicated by these 145-kPa exposure pressure level results. Also, linear regression analyses of lung damage score values vs. all of the acoustic pressure levels for the three species from all three studies yielded (based on Table 1 scoring criteria):

$$\text{score} = 0.026\ \text{pressure} + 0.84 \quad r = 0.81 \quad p < 0.0001 \quad \text{mice, n} = 132 \quad (3)$$

$$\text{score} = 0.0066\ \text{pressure} + 0.40 \quad r = 0.78 \quad p < 0.0001 \quad \text{rabbits, n} = 91 \quad (4)$$

$$\text{score} = 0.0018\ \text{pressure} + 0.091 \quad r = 0.57 \quad p < 0.0001 \quad \text{pigs, n} = 74 \quad (5)$$

All three of these regressions are highly significant with a much greater slope for mice compared with rabbits by a factor of 3.9 (0.026/0.0066), with a much greater slope for rabbits compared with pigs by a factor of 3.7 (0.0066/0.0018) and with a much greater slope for mice compared with pigs by a factor of 14.4 (0.026/0.0018) demonstrating the difference in degree of sensitivity to lung damage between these three species.

Table 6. Mean ± standard deviation of the lung damage score values based on the scoring criteria listed in Table 1 for a 10-min exposure duration.

Acoustic Pressure p_{hop} (kPa)	Score for 10-min Rabbit Study from O'Brien & Zachary, 1994b	Score for 10-min Rabbit Study from this study	Mann–Whitney p Value	Score for 10-min Rabbit Studies (both studies combined)
0	0.0 ± 0.0 (2)	0.0 ± 0.0 (15)	*	0.0 ± 0.0 (17)
145	2.0 ± 1.2 (7)	1.6 ± 1.1 (15)	0.43	1.7 ± 1.1 (22)

* It is not possible to analyze because one of the group's standard deviation is zero (all of the observations were the same value).

Table 7. Mean ± standard deviation of the lung damage score values based on the scoring criteria listed in Table 1 for a 10-min exposure duration.

Acoustic Pressure p_{hop} (kPa)	Score for 10-min Mouse Exposure	Score for 10-min Rabbit Exposure	Score for 10-min Pig Exposure	Kruskal–Wallis ANOVA p value
145	4.6 ± 0.6 (43)[a,b]	1.7 ± 1.1 (22)[a,c]	0.3 ± 0.3 (16)[b,c]	0.0001

Significant at the 0.05 level as determined by the Dunn's test is denoted by matched superscripts when the ANOVA yielded a significant difference ($p < 0.05$) in the means.

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 are much higher, that is, higher than those employed herein.

Consider these observations between mouse, rabbit, pig and man [see Tables 8 and 9 in O'Brien and Zachary (1994a) and Table 4 in O'Brien and Zachary (1994b)]:

(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.

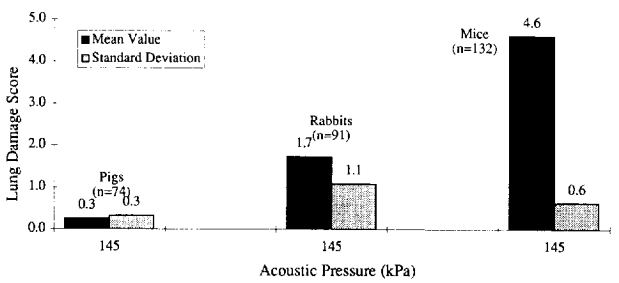

Fig. 4. Mean and standard deviation lung damage scores (based on Table 1 scoring criteria) of 132 mice, 91 rabbits and 74 pigs from the species combined data for O'Brien and Zachary (1994a, 1994b) and this study at an acoustic pressure of 145 kPa for a 10-min exposure duration.

(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 (Table 1). Ratios of lung damage scores are:

mouse:rabbit—3.9
rabbit:pig—3.7
mouse:pig—14.4.

It is beyond the scope of these experiments to determine which of these lung properties might be the principal determinant affecting ultrasonically-induced lung damage. However, the trends of each of these lung properties suggest that human lungs could be the least sensitive to ultrasound-induced lung damage.

In summary, this study in combination with the previous studies (O'Brien and Zachary 1994a, 1994b; Za-

chary and O'Brien 1995) demonstrate that the hypothesis has been validated, that is, there is a species dependency in the degree of sensitivity to ultrasound-induced lung damage. However, there are certain cautions to be exercised with these results. The input quantitative data of lung damage scores were acquired be one highly-trained observer blinded to the specific exposure conditions. It is not known to what extent another highly-trained observer would have scored the lungs using the same scoring criteria (Table 1). Also, the extensive statistical evaluations should not be construed to indicate a high degree of accuracy. The statistical evaluations were principally used to assess differences among groups and trends. Therefore, the absolute observations have not been tested, only the relative observations.

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REFERENCES

AIUM: Acoustic output measurement and labeling standard for diagnostic ultrasound equipment, Maryland, AIUM Publications Department, American Institute of Ultrasound in Medicine, 1992.

AIUM/NEMA: Standard for real-time display of thermal and mechanical indices on diagnostic ultrasound equipment. Maryland, AIUM Publications, American Institute of Ultrasound in Medicine, 1992.

Bobber RL. Underwater electroacoustic measurements. Washington, DC, Underwater Sound Reference Division, Naval Research Laboratory, US Government Printing Office, 1969.

O'Brien WD, Jr, Zachary JF. Mouse lung damage from exposure to 30 kHz ultrasound. Ultrasound Med Biol 1994a;20:287–297.

O'Brien WD, Jr., Zachary JF. Comparison of mouse and rabbit lung damage from exposure to CW 30 kHz ultrasound. Ultrasound Med Biol 1994b;20:299–307.

Zachary JF, O'Brien WD, Jr. Lung hemorrhage induced by continuous and pulse wave ultrasound in mice, rabbits, and pigs. Vet Path 1995;32:43–54.