# THRESHOLD ESTIMATES OF ULTRASOUND-INDUCED LUNG HEMORRHAGE IN ADULT RATS: ROLE OF PULSE DURATION

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## Abstract

The pressure threshold of ultrasound-induced lung hemorrhage has been estimated as a function of pulse duration (PD) in adult rats. A total of 220 10- to 11-week-old 250-gram female Sprague-Dawley rats (Harlan) were randomly divided into 20 ultrasonically exposed groups (10 rats/group) and one sham group (20 rats). The 20 ultrasonically exposed groups (2.8 MHz; 10-s exposure duration; 1-kHz PRF) were divided into four PD groups, and for each PD, there were five *in situ* (at the lung surface) peak rarefactional pressures. For PDs of 1.3, 4.4, 8.2, and 11.6 μs, respectively, lesion occurrence thresholds were 3.1, 2.8, 2.3 and 2.0 MPa. Lesion size thresholds showed similar values, thus suggesting greater likelihood of lung damage as the PD increases. A Mechanical Index of 1.9, the FDA regulatory limit of diagnostic ultrasound equipment, is equivalent to the adult rat's *in situ* peak rarefactional pressure of 4.0 MPa. All of the ED05s are less than the FDA limit.

## Introduction

The effect of exposure timing quantities (e.g., pulse duration, exposure duration, total on-time, and pulse repetition frequency) on the threshold for ultrasound-induced lung hemorrhage has been examined to a limited extent. Our study examines the role of pulse duration (PD) in producing ultrasound-induced lung hemorrhage when pulse repetition frequency (PRF) and exposure duration (ED) are held constant. Specifically, the threshold estimates of ultrasound-induced lung hemorrhage in adult rats are examined at four pulse durations.

There appears to be only one study that has reported a dependency of ultrasound-induced lung hemorrhage on PD [1]. However, there have been numerous studies that have demonstrated that ultrasound-induced lung hemorrhage can occur in mice, rats, rabbits, monkeys and pigs [2,3,4]. While the principal goal of the one PD study [1] was not aimed at assessing the dependency of ultrasound-induced lung hemorrhage on PD, its observations strongly suggested that PD influenced the pressure threshold value. Therefore, a directed study focused on whether ultrasound-induced lung hemorrhage is influenced by pulse duration is warranted.

## Methods

### *Exposimetry*

Ultrasonic exposures were conducted using one focused, 19-mm-diameter, lithium niobate ultrasonic transducer. Water-based (highly degassed water, 22˚C) pulse-echo ultrasonic field distribution measurements were performed and yielded a center frequency of 2.8 MHz, a fractional bandwidth of 12%, a focal length of 19 mm, a -6-dB focal beamwidth of 470 μm, and a -6-dB depth of focus of 2.7 mm.

An automated procedure was developed to routinely calibrate the ultrasound fields that was based on established standards [3,5,6]. A calibrated PVDF membrane hydrophone (Marconi Model Y-34-6543, Chelmsford, UK) was used. Off-line processing yielded the water-based peak rarefactional pressure. The *in situ* (at the pleural surface) peak rarefactional pressure was determined by computing the attenuation of the water-based pressure using the rat's chest wall thickness and the measured intercostal tissue attenuation coefficient at 2.8 MHz of 2.8 dB/cm.

The rats in the 20 ultrasonically exposed groups were exposed as follows: 2.8-MHz center frequency, 10-s exposure duration, 1-kHz pulse repetition frequency. The two variables were pulse duration and *in situ* (at the lung surface) peak rarefactional pressure.

### *Animals*

A total of 220 10- to 11-week-old 250±12-g female Sprague-Dawley rats (Harlan) were randomly divided into 20 ultrasonic exposure groups (10 rats per group) and one sham group (20 rats); no lesions were produced in the sham group. The 20 ultrasonic exposure groups were divided into four PD groups, and for each PD group, there were five *in situ* peak rarefactional pressures. Each of the four PD groups were designed to have the same five *in situ* peak rarefactional pressures. The individuals involved in animal handling, exposure, and lesion scoring were blinded to the exposure condition. The exposure conditions for each animal were revealed only after the final results were tabulated.

The rat exposure and analysis procedures have been described previously in detail [3]. Rats were weighed and anesthetized [ketamine hydrochloride (87.0

mg/kg) and xylazine (13.0 mg/kg)]. Hair of the left thorax was shaved, depilated and then the lung was exposed to ultrasound. Following exposure, rats were euthanized under anesthesia by cervical dislocation.

The thorax was opened and the thickness of each left thoracic wall (skin, rib cage, and parietal pleura) was measured using a digital micrometer. The left lung lobe was scored for the presence or absence of hemorrhage and then fixed by immersion in 10% neutral-buffered formalin for a minimum of 24 hours. After fixation, the elliptical dimensions of each lung lesion at the visceral pleural surface were measured using a digital micrometer where "a" is the semi-major axis and "b" is the semi-minor axis. The lesion was then bisected and the depth "d" of the lesion within the pulmonary parenchyma was also measured. The surface area ($\pi ab$) and volume ($\pi abd/3$) of the lesion were calculated for each animal. Each half of the bisected lesion was embedded in paraffin, sectioned at 5 µm, stained with hematoxylin and eosin, and evaluated microscopically.

### *Statistics*

Logistic regression analysis was used to examine the dependence of the lesion incidence rates on *in situ* peak rarefactional pressure and pulse duration [7]. Logistic regression estimates were transformed to yield estimates and confidence intervals for the threshold defined as an "effective dose" (ED) level of 5% (ED05) (i.e., the *in situ* peak rarefactional pressure associated with 5% probability of lesions) [3,8]. Depth and root surface area of lesions were analyzed using Gaussian tobit regression [9,10].

## Results

### *Lesion occurrence*

The logistic regression model for occurrence of lesions (Fig. 1) was highly statistically significant: the likelihood ratio chi-square for the model was 65.76 on 2 degrees of freedom, with a p-value less than 0.0001. This is strong evidence of a PD and/or *in situ* peak rarefactional pressure effect. The interaction between PD and *in situ* peak rarefactional pressure was not significant.

### *Lesion depth*

The tobit regression model for lesion depth (Fig. 2) was highly statistically significant: the likelihood ratio chi-square for the model was 90.02 on 2 degrees of freedom, with a p-value less than 0.0001. This istrong evidence of a PD and/or *in situ* peak rarefactional pressure effect. The interaction between PD and *in situ* peak rarefactional pressure was not significant.

### *Lesion surface area*

The tobit regression model for lesion surface area (Fig. 3) was highly statistically significant: the likelihood ratio chi-square for the model was 93.92 on 2 degrees of freedom, with a p-value less than 0.0001. This is strong evidence of a PD and/or *in situ* peak rarefactional pressure effect. The interaction between PD and *in situ* peak rarefactional pressure was not significant.

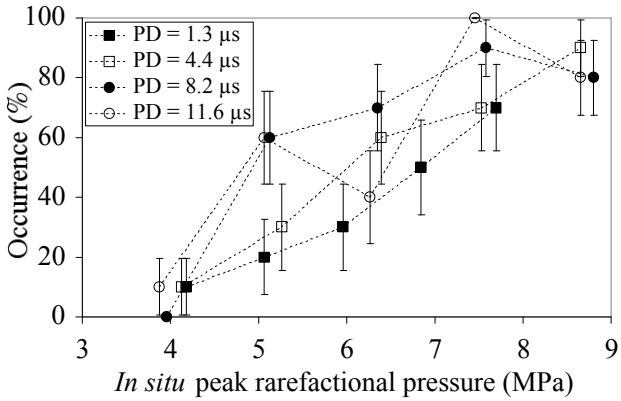


Figure 1. Lesion occurrence as a function of the *in situ* peak rarefactional pressure for four pulse durations. The dashed lines are straight lines connecting the mean values, and are intended to provide graphical guidance for the four pulse duration exposures. Error bars are the standard errors of the mean (n = 10 for each exposure condition).

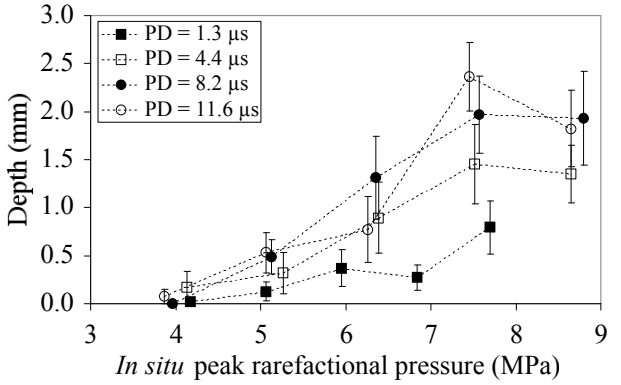


Figure 2. Lesion depth as a function of the *in situ* peak rarefactional pressure for four pulse durations. The dashed lines are straight lines connecting the mean values, and are intended to provide graphical guidance for the four pulse duration exposures. Error bars are the standard errors of the mean (n = 10 for each exposure condition).

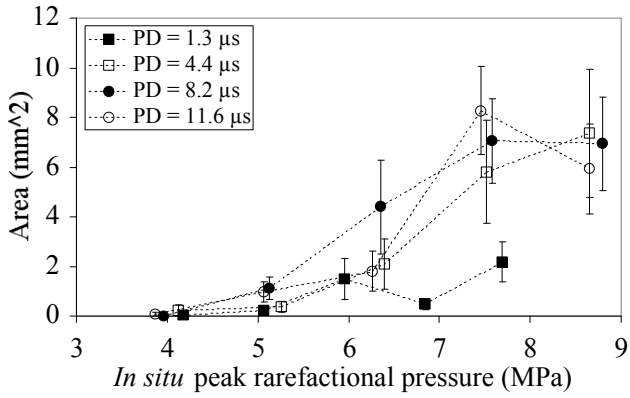


Figure 3. Lesion area as a function of the *in situ* peak rarefactional pressure for four pulse durations. The dashed lines are straight lines connecting the mean values, and are intended to provide graphical guidance for the four pulse duration exposures. Error bars are the standard errors of the mean (n = 10 for each exposure condition).

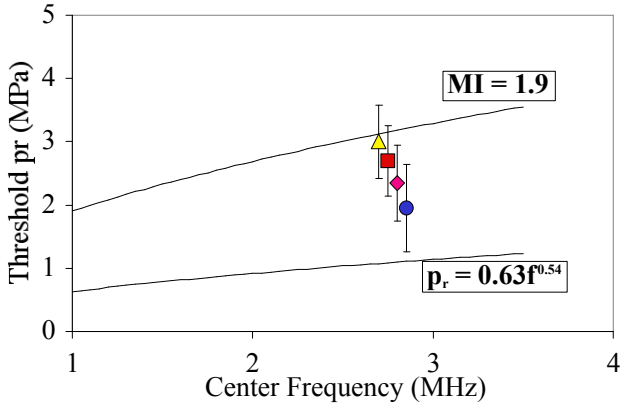


Figure 4. Pressure threshold as a function of frequency. The upper MI = 1.9 curve represents the FDA regulatory limit [11] for diagnostic ultrasound equipment. The lower curve is the summary equation from the AIUM Consensus conference [2] that is based on the best available understanding of the threshold for ultrasound-induced lung hemorrhage as of August, 1998. The four ED05 data points (±standard errors) are the results reported herein at 2.8 MHz (spatially separated on the figure for clarity) and show that as the pulse duration increases, the ED05 threshold decreases.

## Discussion

The main purpose of this study was to estimate the pressure threshold in terms of the ED05 level of ultrasound-induced lung damage as a function of pulse duration (PD) in adult rats. The ED05 level is the *in situ* peak rarefactional pressure associated with 5% probability of lesions. This purpose was accomplished by experimentally determining exposure-effect dependencies (Figs. 1-3).

There were statistically significant trends in the occurrence, depth and surface area of lesions as pulse duration increased. These upward trends in occurrence and size of lesions translate into downward trends in the ED05 thresholds as PD increases (Fig. 4). However, for lesion occurrence, depth and surface area, the four ED05 threshold values were not statistically significantly different. It is conjectured that the ED05 thresholds are at the boundaries of the experimental levels and thus estimated with considerable uncertainty compared with the trends in occurrence, depth and surface area within the experimental range.

The same decreasing trend was noted for the Mechanical Index (MI) thresholds because the MI is directly proportional to the derated peak rarefactional pressure. The MI thresholds (1.5, 1.3, 1.1 and 0.9) were based on the ED05 occurrence thresholds (3.1, 2.8, 2.3 and 2.0 MPa, respectively) so this trend is also significant. Note that all of the MI threshold values are less than 1.9, the FDA regulatory limit [11] for the study reported herein. An MI of 1.9 is equivalent to the adult rat's *in situ* peak rarefactional pressure of 4.0 MPa.

What is particularly interesting is that while there was an almost 10-fold increase in the temporal-average ultrasound exposure level as the PD increases from 1.3 µs to 11.6 µs, the four ED05 occurrence and size thresholds were not significantly different, although there was a significant trend. Because the PRF (1 kHz) and ED (10 s) were held constant, the total on-time, and thus the temporal-average intensity, increased by a factor of 8.9 (11.6 µs/1.3 µs). But near the ED05 occurrence and size threshold levels, the temporal-peak exposure quantities were about the

same (2.0-3.1 MPa; with standard errors around ±0.6 MPa). This observation suggests that there is no need to consider a pulse duration dependency as part of an FDA or safety-based guideline that is based on ultrasound-induced lung hemorrhage.

In summary, this study appears to be the first to report on the effects of ultrasound-induced lung damage as a function of pulse duration (PD) for any species. Based on the overall exposure-effect observations, there appears to be a significant and monotonic effect for lesion occurrence, depth and area. However, the uncertainty is too great to determine if there is a significant ED05 threshold effect.

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