APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1991, p. 2079–2084
0099-2240/91/072079-06$02.00/0

Vol. 57, No. 7

Quantitative Assessment of the Germicidal Efficacy of Ultrasonic Energy

G. SCHERBA,[1] R. M. WEIGEL,[1] AND W. D. O'BRIEN, JR.[2]*

Department of Veterinary Pathobiology[1] and Department of Electrical and Computer Engineering,[2] University of Illinois, Urbana, Illinois 61801

Received 31 January 1991/Accepted 1 May 1991

Propagated (free-field) ultrasonic energy at a frequency of 26 kHz was used to expose aqueous suspensions of bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*), fungus (*Trichophyton mentagrophytes*), and viruses (feline herpesvirus type 1 and feline calicivirus) to evaluate the germicidal efficacy of ultrasound. There was a significant effect of time for all four bacteria, with percent killed increasing with increased duration of exposure, and a significant effect of intensity for all bacteria except *E. coli*, with percent killed increasing with increased intensity level. There was a significant reduction in fungal growth compared with that in the controls, with decreased growth with increased ultrasound intensity. There was a significant reduction for feline herpesvirus with intensity, but there was no apparent effect of ultrasound on feline calicivirus. These results suggest that ultrasound in the low-kilohertz frequency range is capable to some degree of inactivating certain disease agents that may reside in water. The physical mechanism of inactivation appears to be transient cavitation.

Contamination of hospital and athletic whirlpool facilities as well as common-use spas, jacuzzis, and hot tubs by pathogens shed from users of such facilities poses a significant public health problem. A number of microorganisms have been found to withstand the water temperatures and chemical disinfectants, suggesting that chemical means are not 100% effective (8). Another possible means to decontaminate or at least control the growth of microorganisms in common-use water facilities is by the application of acoustic energy, which is investigated here.

Considerable insight has been gained through the years into the mechanisms responsible for the interaction of ultrasound with biomolecules in aqueous media (3, 4, 12). The biophysical properties of cavitation are thought to be the principal mechanism that affects the germicidal activity of ultrasound. In a liquid medium, an ultrasonic field creates cavities. The collapse of such cavities causes intense hydrodynamic shock waves, establishing intense pressure pulses which disrupt material in the vicinity of the cavity. It is these pressure pulses that are hypothesized to be destructive to microorganisms.

The purpose of this study was to evaluate quantitatively the degree to which microorganisms could be inactivated through the administration of ultrasonic energy. Aqueous suspensions of specific bacteria, fungus, and viruses were exposed to an ultrasonic frequency of 26 kHz. The pathogens were selected on the basis of their normal routes of infection (e.g., skin or intestinal tract) or their structural similarities to such agents, which would make them likely candidates to be found as contaminants of whirlpools and hot tubs.

MATERIALS AND METHODS

Ultrasound exposures. The aqueous suspension (10 ml) of each microorganism in a separate sterile, sealed Whirl-Pak bag was submerged in a degassed water-filled exposure chamber and positioned on the beam axis 1 cm from the ultrasound source. The temperature of the exposure tank water was controlled and monitored with a National Bureau of Standards calibrated thermometer at 39 ± 0.3°C for all pathogens studied. The ultrasonic frequency (26 kHz) was chosen to maximize the potential for ultrasonically induced cavitation and also to be above the frequency level of human hearing. Treatment controls consisted of similar aqueous suspensions of the various organisms and were exposed to the same water temperature conditions for the various times but not to the ultrasound.

A calibrated Naval Research Laboratory Underwater Sound Reference Detachment type F42D hydrophone (1) was used as the primary standard in this study with a calibrated voltage sensitivity at 26 kHz of −212.3 dB (re: 1 V/μPa). This probe was used in this study because its calibration is traceable to the Naval Research Laboratory's Underwater Sound Reference Detachment (Orlando, Fla.), a national standards laboratory.

The Naval Research Laboratory probe was connected in parallel to a Tektronix 466 storage oscilloscope to record the instantaneous ultrasonic pressure (p) and to a Fluke RMS voltmeter to record the root-mean-square (RMS) value of ultrasonic pressure (p_{RMS}). The temporal-peak intensity (I_{TP}) is determined from p and the temporal-average intensity (I_{TA}) is determined from p_{RMS}.

Three ultrasonic intensity values are reported, viz., the spatial-peak, temporal-peak intensity (I_{SPTP}); the spatial-peak, temporal-average intensity (I_{SPTA}); and the spatial-average, temporal-average intensity (I_{SATA}). The I_{SPTP} is the I_{TP} at the spatial location in the sound field which yields the greatest value. The I_{SPTA} is the I_{TA} at the spatial location in the sound field which yields the greatest value. The I_{SATA} is the I_{TA} averaged in space over the cross-sectional area of the specimen bag.

Three exposure levels (designated low, medium, and high) were utilized, as indicated in Table 1. These exposure levels have an absolute accuracy of ±30%; that is, the actual intensity levels were all within 30% of the stated values.

Bacteria. Pure cultures of the following organisms were obtained from the University of Illinois Microbiology Department Stock Collection: *Escherichia coli*, *Staphylococ-*

* Corresponding author.

TABLE 1. Exposure intensity settings employed

Setting	Intensity (W/cm^2)		
	I_{SPTP}	I_{SPTA}	I_{SATA}
Low	1.1	0.2	0.1
Medium	2.0	0.4	0.3
High	3.0	0.5	0.4

cus aureus, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The bacteria were cultured separately on blood agar plates. For the ultrasound exposures (both exposed and treatment controls), the microorganisms were grown separately in thioglycolate broth. The initial concentration of bacteria was diluted with sterile normal saline to between 30 to 300 colonies per plate in order to ensure accurate counts of each colony. The samples of each culture were prepared, and a total of 10 ml was placed in sterile Whirl-Pak bags for each of the exposure conditions. After exposure, each sample was plated separately and incubated for 3 days, after which the number of colonies was counted. There was a treatment control for each exposed sample. As part of the experimental design, the person exposing and assaying the specimens was blinded to the specific ultrasonic exposure condition (magnitude and duration).

Each experiment consisted of six individual plates per bacterial culture for each exposure condition: three for the treatment controls and three for the exposed samples. The resultant plate counts were averaged and computed into the formula

$$\text{initial concentration} = (\text{number of colonies/volume plated out}) \times \text{dilution factor} \quad (1)$$

in order to determine the cell concentration and to develop a linear regression model of percent killed relative to the control.

The exposure durations examined for the bacterial experiments were 1, 2, 4, 8, 16, and 32 min. For each trial, one sample was exposed for each of the durations. The number of trials per the three exposure levels was variable.

Fungus. A pure culture of *Trichophyton mentagrophytes* was obtained from the American Type Culture Collection and grown at 26°C on Emmon's modification of Sabouraud dextrose agar (25 ml per plate). Gentamicin (Sigma Chemical Co., St. Louis, Mo.) was added to the agar to inhibit bacterial contamination.

Agar plugs 1 cm in diameter were taken from the fungal culture and transferred to a sterile Whirl-Pak bag with 10 ml of sterile phosphate buffer at pH 7.0. Each of the four experiments included three temperature controls that remained at room temperature; that is, the specimens were never placed in the water. The treatment controls were placed in 39°C water. Fungal growth was evaluated for four 60-min exposure conditions. The number of samples randomly assigned to each exposure condition was as follows: temperature control, 12; treatment control, 16; medium, 14; and high, 6. After the ultrasound exposures, the fungal plugs were blotted on sterile filter paper and plated on similar agar media. The plates were then incubated at 26°C and ranked daily according to the amount of growth. The analyzer of each of the specimens was blind to the experimental condition of the specimen. A qualitative grade from 1 to 5 was assigned to each specimen, with 1 designating a complete kill (i.e., no growth) and 5 designating no kill (i.e., substantial growth). The scoring was performed daily until a steady state was achieved, which was usually within 5 to 7 days after exposure. The score at 7 days postexposure was used for analysis.

Viruses. An enveloped DNA virus, feline herpesvirus type 1 (FHV-1), and a small nonenveloped RNA virus, feline calicivirus (FCV), were selected. The viruses were propagated separately in a Crandell-Rees feline kidney cell line maintained in Eagle's minimal essential medium supplemented with 2% newborn calf serum (HyClone Laboratories, Logan, Utah) and antibiotics (100 μg of penicillin per ml, 100 μg of streptomycin per ml, and 50 μg of gentamicin per ml; Sigma Chemical Co.). Ten milliliters of a dilution of the source virus made in maintenance medium was placed in sterile Whirl-Pak bags and sealed for the ultrasound exposures (medium intensity for 60 min). The titer of infectious virus in a sample prior to (source control, held at 4°C) and after (exposed) treatment was measured by a microtitration procedure for 50% tissue culture infectious dose ($TCID_{50}$) end point determination. The $TCID_{50}$ end point was derived from the highest dilution which produced a virus-specific cytopathic effect in 50% of the quadruplicate wells of the Crandell-Rees feline kidney cell cultures inoculated, on the basis of the calculation method of Reed and Muench (9). In addition, for each experiment a treatment control also was subsequently titered.

Statistical methods. The effects of ultrasound exposure on viability of bacteria were analyzed statistically by using multiple regression analysis. The dependent variable was relative percent killed with ultrasound, that is, the ratio of percent killed in the ultrasound-treated sample to the percent killed in the treatment control sample. This approach was used because it best compared the change due to ultrasound with any change if ultrasound were not used, thus providing a quantitative measure of the germicidal efficacy at time t. The independent variables were intensity (I_{SPTP}), time (exposure duration), and the intensity × time interaction. Predicted direction of association was increased killing with increased intensity and increased time. The expected interaction was a decreased slope for the regression on time with an increased intensity, representing a saturation of killing effect at earlier times under higher exposure. If the interaction term was nonsignificant, it was removed from the regression model. Goodness of fit was determined for several alternative regression models: linear (no transformations of model variables), logarithmic transformation of outcome (exponential function), logarithmic transformation of predictors (logarithmic function), and logarithmic transformation of both predictors and outcome (power function).

The fungal experiments were analyzed by the gamma coefficient (6), calculated on the two-way table of ordered categories: exposure condition (temperature control < treatment control < medium < high) versus grade (1 to 5). The predicted direction of association was decreased grade (less growth) with increased exposure.

The effects of ultrasound exposure on virus were analyzed by a paired t test, with each exposed condition compared with its paired treatment control. The outcomes analyzed were $TCID_{50}$ per milliliter in the exposed condition relative to its paired treatment control. Normality and homogeneity of variance were evaluated, with normalizing and variance stabilizing transformations applied if necessary.

Since all statistical analyses tested directional predictions, one-tailed probabilities were calculated.

RESULTS

Bacteria. Table 2 presents the results of the regression analyses for bacteria. In all cases, the linear regression model provided the best fit (that is, variable transformations were

TABLE 2. Regression analyses showing the effects of intensity and duration of ultrasound exposure on killing percentage in treated condition relative to that in treatment controls

Bacterium and parameters	Independent variable	Regression coefficient	t	P
P. aeruginosa	Intensity	0.318	7.26	<0.001
$F(2,50) = 34.00$; r^2	Time	0.021	3.07	0.018
$= 0.67$; $P <$	Intensity × time	−0.005	−1.74	0.043
0.001	Constant	−0.271	−2.68	0.005
B. subtilis	Intensity	0.558	5.05	<0.001
$F(2,21) = 15.04$; r^2	Time	0.009	2.13	0.023
$= 0.59$; $P <$	Constant	−0.664	−3.07	0.003
0.001				
S. aureus	Intensity	0.072	1.73	0.045
$F(2,51) = 5.09$; r^2	Time	0.005	2.68	0.005
$= 0.17$; $P = 0.01$	Constant	0.093	1.09	NS[a]
E. coli	Intensity	0.013	0.35	NS
$F(2,63) = 5.94$; r^2	Time	0.005	3.43	0.001
$= 0.16$; $P =$	Constant	0.132	1.86	0.034
0.004				

[a] NS, not significant.

not applied). All three exposure levels (low, medium, and high) were utilized. There was a significant effect of time for all four bacteria, with relative percent killed increasing with increased duration of exposure. In addition, there was a significant effect of intensity for all bacteria except *E. coli*, with relative percent killed increasing with increased intensity level.

The regression functions for relative percent killed versus time (by intensity) are plotted for *P. aeruginosa*, *B. subtilis*, and *S. aureus* in Fig. 1 to 3, respectively. The intensity by time interaction for *P. aeruginosa* is apparent in the faster increase in relative percent killed with increasing time (that is, steeper slope) for lower rather than higher exposure intensity. The best fit for the regression models were for *P. aeruginosa* and *B. subtilis* ($r^2 \approx 0.6$), with poorer fits for *S. aureus* and *E. coli* ($r^2 \approx 0.16$).

Fungus. The effect of 60-min ultrasound exposure on fungus growth is indicated in Table 3. The gamma coefficient calculated on these data is −0.56 ($P < 0.001$), indicating decreased growth with increasing exposure level.

Viruses. Table 4 presents the data for the viral study. A positive skewness in the ultrasound-minus treatment control differences was corrected by logarithmic transformation. Thus, the variable of interest with respect to inactivation effectiveness was the difference between log(virus concentration in exposed condition) and log(virus concentration in control condition). The paired t tests for differences between treatment control and ultrasound-exposed virus samples indicated a significant reduction for FHV-1 (logarithmic means: exposed, 3.21×10^2 $TCID_{50}$/ml; control, 8.41×10^3 $TCID_{50}$/ml; $t = -2.75$, $P = 0.005$) with ultrasound exposure decreasing infectious virus titer. There was no apparent effect of initial virus concentration (source virus) on inactivation effectiveness. The regression of the difference scores on initial virus concentration yielded an r^2 of 5.2% ($P = 0.23$). No significant effect of ultrasound on FCV was apparent.

DISCUSSION

E. coli, *S. aureus*, *B. subtilis*, and *P. aeruginosa* represent the various structural types of bacteria as well as likely contaminants of common-use water facilities. Gram-positive organisms (*S. aureus* and *B. subtilis*) usually have a thicker

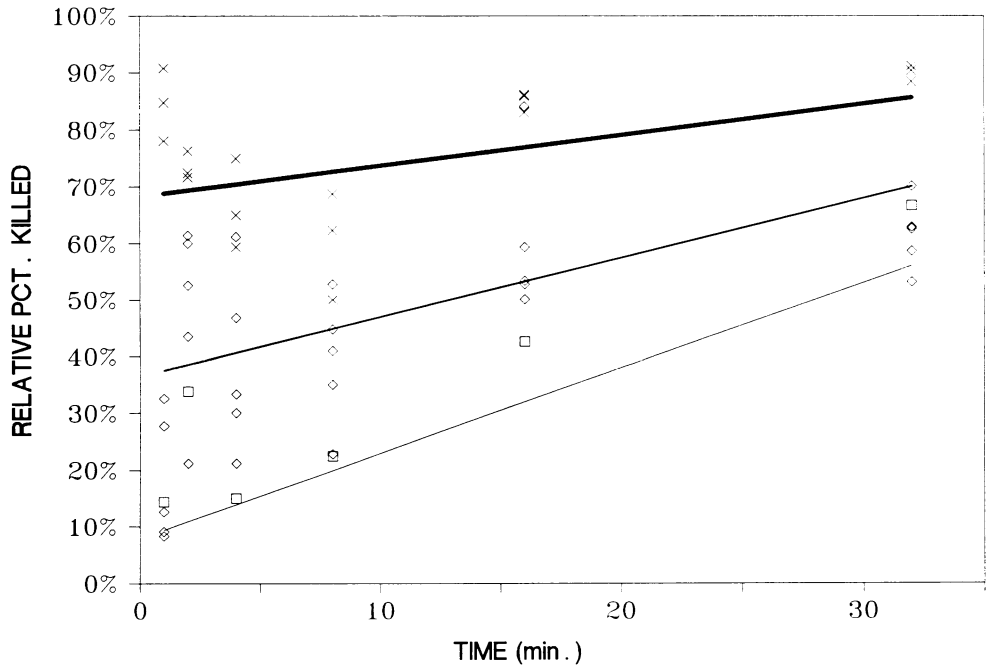

FIG. 1. Relative percent killed versus exposure time for all three exposure intensity levels of *P. aeruginosa*. Straight lines indicate the linear regression analyses for the three exposure intensity levels (thickness of lines proportional to intensity). □, low; ◇, medium; ×, high.

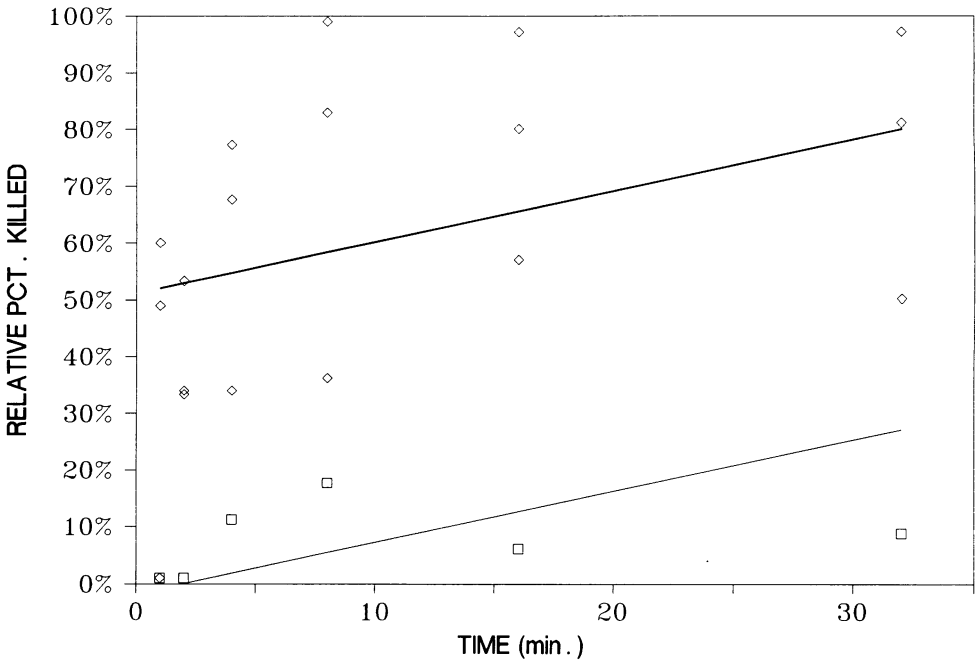

FIG. 2. Relative percent killed versus exposure time for two exposure intensity levels (low and medium) of *B. subtilis*. Straight lines indicate the linear regression analyses for the two exposure intensity levels (thickness of lines proportional to intensity). □, low; ◇, medium.

and a more tightly adherent layer of peptidoglycans than gram-negative organisms (*E. coli* and *P. aeruginosa*). However, this morphological feature did not seem to be a differentiating factor in ranking the organisms by percent killed by ultrasonic energy. Therefore, the target of ultrasonic damage may be the inner (cytoplasmic) membrane, which consists of a lipoprotein bilayer, since the structure of the peptidoglycan layer did not appear to be a factor. At this time, however, the exact nature of the damage to the inner membrane is not known.

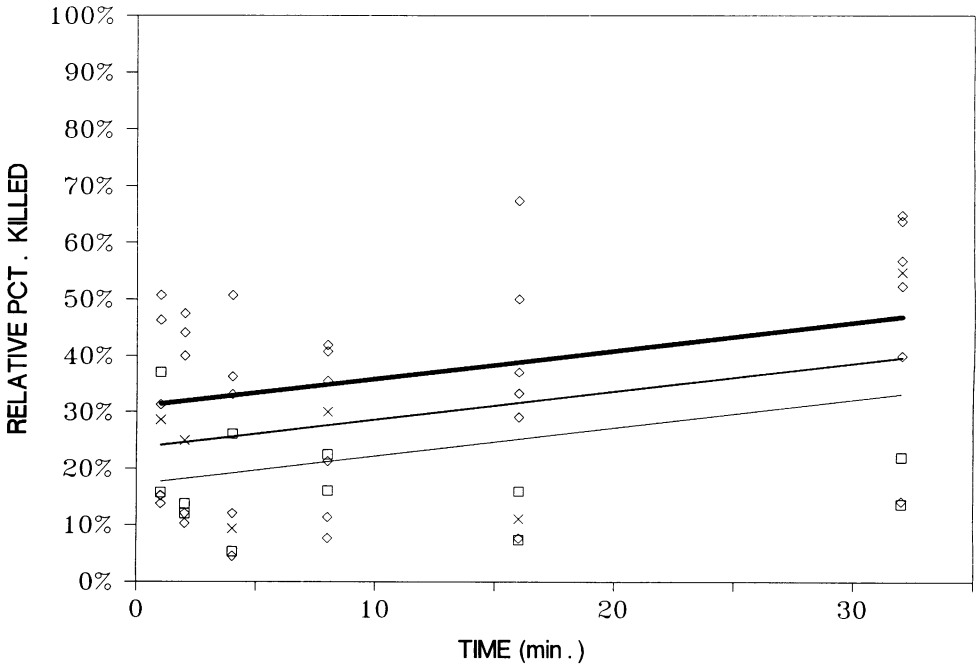

FIG. 3. Relative percent killed versus exposure time for all three exposure intensity levels of *S. aureus*. Straight lines indicate the linear regression analyses for the three exposure intensity levels (thickness of lines proportional to intensity). □, low; ◇, medium; ×, high.

TABLE 3. Summary of four 60-min exposure fungus studies

Exposure condition	No. of samples assigned to grade[a]:				
	1	2	3	4	5
Temperature control		2		3	7
Treatment control		2	3	6	5
Medium	2	5	4	2	1
High	2		3		1

[a] Grade 1 represents no growth (complete kill), and grade 5 represents substantial growth (essentially no kill).

The 21 temperature control fungal samples showed more growth than the 16 treatment control samples. This presumably reflects the fact that the temperature control samples remained at room temperature (about 22°C), whereas the treatment control samples were at 39°C for 60 min. Thus, heat alone affected the degree of fungal growth in that it had a slight inhibitory effect. The medium- and high-exposure groups showed a marked reduction in fungal growth compared with that of the treatment control group. Thus, ultrasound alone affected the degree of fungal growth at 39°C.

FHV-1 was chosen on the basis of its subfamily classification, *Alphaherpesvirinae*. This subfamily also contains the human herpesviruses, herpes simplex virus types 1 and 2, that can cause cutaneous vesicular lesions as well as oral and urogenital tract infections. FHV and the human herpesviruses share the same morphological and physiochemical characteristics. The experimental results indicate that the experimental conditions chosen did significantly reduce viral infectivity and that there was equal effectiveness for all initial titers. These results most likely reflect ultrasonic destruction of the viral envelope, an essential structure for infectivity. An envelope consists of cellular membrane with inserted virus-coded glycoproteins. These virus-specific glycoproteins act as ligands for viral adsorption to cell surface receptors. Therefore, any destruction of the envelope abrogates viral infectivity, essentially inactivating the virus. As a result, enveloped viruses tend to be labile, since this outer lipoprotein bilayer structure can be easily destroyed.

Caliciviruses were originally classified in the *Picornaviridae* family because of their seemingly structural similarities to members of this family, such as poliovirus. These viruses have since been reclassified as a separate family, *Caliciviridae*. However, the structure, the size, and the ease of propagation of FCV made it a useful model for small, nonenveloped viruses such as poliovirus, a potential water contaminant. The FCV proved refractive to the inactivating effects of the ultrasound treatment. In this case the viral ligands used for attachment to cell surface receptors are components of the viral outer protein coat, the viral capsid. The capsid structure is not as easily destroyed as a viral envelope. This is reflected by the fact that nonenveloped viruses are usually more resistant to environmental influences than are enveloped viruses.

When ultrasound is propagated through aqueous suspensions such as those in this study, the potential for alteration of these materials exists. There are two mechanisms which are known to alter biological systems, viz., thermal effects and cavitation.

Theoretical and experimental studies have clearly demonstrated that the thermal mechanism is insignificant in terms of producing a temperature rise in biological systems around an ultrasonic frequency of 26 kHz at various intensity levels employed. Consider the example in which the ultrasonic frequency is 26 kHz, the ultrasonic absorption coefficient (α) is 0.0004 neper/cm, and the I_{TA} is 5 W/cm^2. The average rate of heat generation per unit of volume per unit of time (Q) and the maximum rate of temperature rise assuming no heat lose (dT_{max}/dt) expressions and values are

$$Q = 2\alpha I_{TA} = 0.004 \text{ J/cm}^3 \cdot \text{s} \quad (2)$$

$$dT_{max}/dt = Q/C_h = 0.00096°\text{C/s} \quad (3)$$

where C_h is the medium's specific heat (4.18 J/cm^3 · °C).

The value of dT_{max}/dt is approximately valid for short exposure times, that is, up to a few seconds. For longer

TABLE 4. Summary of results for ultrasound-treated and control virus samples

Virus and treatment[a]	Titer ($TCID_{50}$/ml) of sample:									
	1	2	3	4	5	6	7	8	9	10
FHV-1										
None	1.28×10^6	7.2×10^5	7.2×10^5	4.0×10^5	4.0×10^5	2.24×10^5	2.24×10^5	2.24×10^5	1.28×10^5	1.28×10^5
E	4.0×10^5	4.0×10^5	4.0×10^5	2.24×10^5	7.2×10^5	2.24×10^5	4.0×10^2	2.24×10^4	1.28×10^3	0
C	2.24×10^5	7.2×10^5	1.28×10^6	4.0×10^5	7.2×10^5	2.24×10^5	7.2×10^4	4.0×10^4	1.28×10^4	4.0×10^4
None	7.2×10^4	7.2×10^4	7.2×10^4	5.6×10^4	4.0×10^4	2.24×10^4	2.24×10^4	2.24×10^4	4.0×10^3	7.2×10^3
E	7.2×10^1	0	2.24×10^4	2.24×10^5	2.24×10^1	2.24×10^1	0	0	4.0×10^3	2.24×10^3
C	1.3×10^2	1.28×10^4	2.24×10^4	7.2×10^4	2.24×10^4	1.28×10^4	1.28×10^4	2.24×10^3	7.2×10^3	2.24×10^3
None	7.2×10^3	7.2×10^2	7.2×10^2	4.0×10^2	4.0×10^2	4.0×10^2	1.28×10^2	7.2×10^1		
E	2.24×10^1	0	2.24×10^1	0	0	0	0	0		
C	2.24×10^3	7.2×10^2	2.24×10^2	7.2×10^2	4.0×10^2	0	2.24×10^3	0		
FCV										
None	4.0×10^4	1.28×10^5	4.0×10^4	1.28×10^5	2.24×10^3	2.24×10^4	4.0×10^4	7.2×10^3	2.24×10^2	2.24×10^3
E	7.2×10^3	1.28×10^5	1.28×10^4	7.2×10^4	4.0×10^2	7.2×10^3	2.24×10^3	4.0×10^3	2.24×10^1	1.28×10^2
C	1.28×10^4	4.0×10^4	7.2×10^4	7.2×10^4	7.2×10^2	4.0×10^3	1.28×10^4	1.28×10^4	7.2×10^1	1.28×10^2
None	1.28×10^3	1.28×10^3	4.0×10^2	4.0×10^2	2.24×10^2					
E	2.24×10^2	0	2.24×10^1	7.2×10^1	0					
C	2.24×10^2	4.0×10^2	0	2.24×10^1	4.0×10^1					

[a] None, source virus held at 4°C; E, ultrasound exposure (medium, 39°C); C, control (unexposed, 39°C). All exposures were for 60 min.

exposure times, heat removal processes inhibit the rate of temperature rise. Therefore, ultrasonically induced thermal effects can be discounted as a cause responsible for altering bacteria, fungi, and viruses under the exposure conditions employed in this study.

Cavitation refers to ultrasonically induced activity occurring in a liquid or liquidlike solid material that contains bubbles or pockets containing gas or vapor. Cavitation can affect a biological system by virtue of a localized temperature rise, mechanical stress, and/or free radical production, all of which are initiated by a nonthermal mechanism.

Cavitation can be discussed in terms of two general categories, viz., transient cavitation and stable cavitation. Transient cavitation connotes a relatively violent activity (bubble collapse) in which hot spots of high temperature and pressure occur in very short (of the order of microseconds) bursts at highly localized locations in the sonicated medium. These bursts may be accompanied by localized shock waves and/or by the generation of highly reactive chemical species.

In contrast, the much less violent form, stable cavitation, is associated with vibrating gaseous bodies. The nature of this form of cavitation consists of a gaseous body that remains spatially stabilized within but not necessarily because of the ultrasound field and, because of the ultrasound field, oscillates or pulsates. When such volumetric oscillations are established, the liquidlike medium immediately adjacent to the gas bubble flows or streams (termed microstreaming). Microstreaming has been shown to produce stresses sufficient to disrupt cell membranes.

There is no known literature which reports findings of effects on suspensions of microorganisms from propagated (free-field) ultrasound in the low-kilohertz (10- to 100-kHz) frequency range, that is, under the experimental conditions reported here. Both transient and stable types of cavitation need to be considered in order to gain an understanding of what cavitationlike activity might be responsible for the killing of aqueous suspensions of bacteria, fungus, and virus.

In propagated (free-field) ultrasound fields, transient cavitation activity occurs more easily at lower ultrasonic frequencies (low-kilohertz frequency range) compared with that at ultrasonic frequencies in the low-megahertz frequency range (1 to 10 MHz) in aerated and degassed media in vitro (5). At a frequency of 26 kHz, threshold intensities (I_{TP}) have been reported to be near 0.1 and 1 W/cm^2 for degassed and aerated media, respectively (5), which are both less than the I_{SPTP} used in this study.

Three different types of exposure procedures have been used to provide controlled exposures which simulate stable cavitationlike activity, viz., longitudinally vibrating needle, transversely oscillating wire, and transverse oscillations at a stationary needle in a propagated ultrasound field, all of which have been carefully reviewed (7). The controlled exposure procedures report their exposure amplitudes in terms of the needle or bubble displacement. It is possible, therefore, to assume that the displacement represents the free-field particle displacement and to extrapolate, via theory, to a localized ultrasonic intensity (I_{TP}) for the purpose of comparison to the literature.

Theoretical and experimental studies (2, 10, 13) have demonstrated that temporal-peak particle displacement amplitudes must exceed 18 μm to cause blood hemolysis under stable cavitation conditions. This translates to a local ultrasonic intensity (I_{SPTP}) of around 500 W/cm^2. By using an oscillating wire procedure with a relatively sensitive biological model (11), serotonin was released from human platelets at a calculated intensity of more than 70 W/cm^2.

These preliminary experiments suggest that ultrasound in the low-kilohertz frequency range has some efficacy in inactivating some disease agents that may reside in water. Since transient cavitation can occur at intensity levels consistent with those used in this study, this would suggest that transient cavitation is the physical mechanism responsible for affecting the microorganisms. The stable cavitation mechanism would appear to require much higher intensity levels for such effects.

Complete decontamination of common-use water facilities is virtually impossible to achieve unless extreme conventional measures are taken. Therefore, at least the control of the growth of waterborne pathogens is in the public health interest. The use of ultrasound in the low-kilohertz frequency range as a method to obtain such effects has not been investigated previously. Our quantitative analysis indicates that some degree of an ultrasound-induced germicidal effect can be obtained against certain pathogens that might be found in common-use water facilities. However, absolute definitive answers have not been achieved in these preliminary experiments. Therefore, additional quantitative studies will be required to define more fully the exact exposure conditions which might ensure complete germicidal efficacy as well as the specific organisms that may be susceptible.

ACKNOWLEDGMENTS

We thank T. Croix and S. Drysdale for assistance with virus production and titration, L. Cerglione for assistance with the fungus experiments, R. Staack for performance of the exposures, and A. Helm for providing the bacteria.

This work was partially supported by Swen Sonic Corporation, Davenport, Iowa.

REFERENCES

1. **Bobber, R. J.** 1969. Underwater electroacoustic measurements. Underwater Sound Reference Division, Naval Research Laboratory, U.S. Government Printing Office, Washington, D.C.
2. **Coakley, W. T., and W. L. Nyborg.** 1978. Cavitation; dynamics of gas bubbles; applications, p. 77–159. *In* F. J. Fry (ed.), Ultrasound: its applications in medicine and biology. Elsevier Science Publishing, Inc., New York.
3. **Dunn, F., and W. D. O'Brien, Jr. (ed.).** 1976. Ultrasonic biophysics. Dowden, Hutchinson & Ross, Inc. Stroudsburg, Pa.
4. **El'piner, I. E.** 1964. Ultrasound: physical, chemical and biological effects. Consultants Bureau, New York.
5. **Esche, R.** 1952. Investigation of vibratory cavitation in liquids. Akust. Beih. **4**:217–218. (In German.) (Translated in F. Dunn and W. D. O'Brien, Jr. [ed.], Ultrasonic biophysics, p. 124–125. Dowden, Hutchinson & Ross, Inc., Stroudsburg, Pa.)
6. **Goodman, L. A., and W. H. Kruskal.** 1954. Measures of association for cross classifications. J. Am. Stat. Assoc. **49**:732–764.
7. **Miller, D.** 1988. A review of the ultrasonic bioeffects of microsonation, gas-body activation, and related cavitation-like phenomena. Ultrasound Med. Biol. **13**:443–470.
8. **Nerurkar, L. S., F. West, M. May, D. L. Madden, and J. L. Sever.** 1983. Survival of herpes simplex virus in water specimens collected from hot tubs in spa facilities and on plastic surfaces. JAMA **250**:3081–3083.
9. **Reed, L. J., and H. Muench.** 1938. A simple method to estimate fifty percent endpoints. Am. J. Hyg. **27**:493–497.
10. **Rooney, J. A.** 1970. Hemolysis near an ultrasonically pulsating gas bubble. Science **169**:869–871.
11. **Williams, A. R.** 1974. Release of serotonin from human platelets by acoustic microstreaming. J. Acoust. Soc. Am. **56**:1640–1643.
412. **Williams, A. R.** 1983. Ultrasound: biological effects and potential hazards. Academic Press, Inc., New York.
13. **Williams, A. R., D. E. Hughes, and W. L. Nyborg.** 1970. Hemolysis near a transversely oscillating wire. Science **169**:871–873.