

FPE Inc

December 27, 2000

Dockets Management Branch
HFA-305
Food and Drug Administration
5630 Fishers Lane
Rm. 1061
Rockville, MD 20852

Re: Docket No. 99F-1912

To whom it may concern:

This letter is to notify the FDA of FPE's, Inc objection to 21CFR Part 21 as amended by Docket No. 99F-1912 and to request a hearing on this matter.

21 CFR Part 179 as amended by Docket No. 99F-1912 includes a requirement for turbulent flow and a Reynolds number of at least 2,200 in processes using UV irradiation to reduce pathogen content of juice. This requirement is specific to a certain application of UV technology and accordingly should not become the general rule for all possible designs.

Turbulent flow with a Reynolds number of 2200, while necessary in some designs, is not a universally applicable requirement and thus, the regulation functions as a patent as it eliminates equally effective designs.

Objection Number 1

FPE, Inc. objects to the inclusion of a Reynolds number of 2200 in the limitations section for juice products in 21 CFR Part 179.

Justification for objection number 1

The transmission of light through a liquid is governed by Beer's Law. Simplified, Beer's Law for transmission (T) is as follows $T = \exp(-\alpha * X)$ where alpha is the loss, X is the depth and the inverse of alpha is the loss length. The penetration depth falls off exponentially as a function of the coefficient of absorption for a given wavelength. For UV light with the wavelength of 253.7nm, the coefficient of absorption is very low. Calculations as well as testing demonstrate that the energy will be absorbed in approximately 1-1.5mm. The intent of the Reynolds number called for by the regulation is

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to insure turbulent flow in the process tube, thereby causing even irradiation of all parts of the juice. As stated in section II.C of the final rule, UV radiation is strongly absorbed by juice which necessitates turbulence or mixing because the depth of the juice being treated is greater than the penetration depth of the UV radiation. The treatment depth is determined by design. In this case the petitioner's design, a transparent tube, the depth may be several times the penetration depth. However, as the depth of juice being treated approaches or becomes less than that of the energy penetration depth, the requirement for turbulence is reduced or eliminated. Designs in which the layer of juice is sufficiently thin such that the UV radiation can penetrate it fully do not require Reynolds of 2200 to achieve uniform exposure.

FPE, Inc. has developed a process which utilizes this thin treatment depth. The treatment chamber is 30 inches long and is constructed of two concentric tubes, the inner of quartz glass and the outer of stainless steel. This design confines the juice being treated to a depth of less than 0.6 mm. The UV lamps are located in the inside diameter of the quartz tube. Sensors located in the juice stream at the outermost point of the treatment surface, measure the intensity of UV and automatically adjust the processing speed to compensate for variations in the coefficient of absorption. Although the Reynolds number in this tube is below 2200, all of the juice receives a uniform exposure of UV that allows for uniform and consistent 5-log or greater reduction of microorganisms in juice regardless of the optical density changes that may occur in the juice (solids, color). The rate of pathogen reduction in this design has been tested and documented by a collaboration of Dr. R. Worobo and J. Churey of Cornell University.

Request for hearing

FPE, Inc. requests a hearing to present the following information in support of objection number 1.

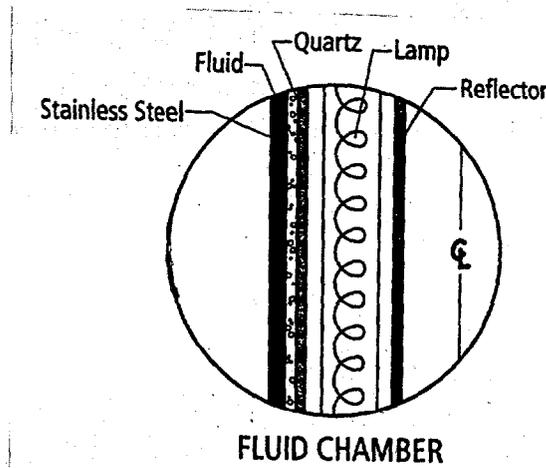
The Equipment

The process equipment is designed to expose apple cider to ultraviolet light of 254nm at levels that are lethal to *E. coli* O157:H7 organism. The unit consists of an enclosure with two compartments, one which has the electronic controls, the other with the fluid components.

a. Process Tube

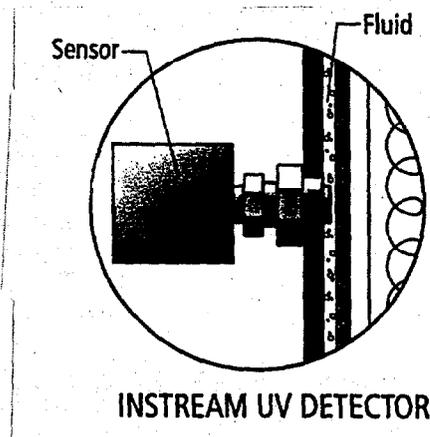
The process tube confines the apple cider between the outer stainless tube and the inner quartz tube at a precise gap for exposure to the ultraviolet lamps. The eight germicidal lamps are arranged around the inside diameter of the quartz tube. A reflector tube is

placed in the middle of the lamp array to ensure maximum radiation to the treatment area. A fan at the top of the tube pushes outside air over the lamps for cooling.



b. Sensor

The sensor is placed in the stream of cider to be treated (figure 3). This sensor is calibrated to NIST standards for the detection of Ultraviolet power at 254nm. The sensor measures the UV transmission properties of the cider and feeds this information to the computer which adjusts the flow to insure proper dosage. This information is updated every 50 milliseconds. In addition, as it is measuring the actual output power of the lamps, any correction for varying lamp power is automatically compensated for.



Flow conditions

The Reynolds Number and Turbulence

In the study of fluid mechanics, the Reynolds number plays a key role in determining whether flow is turbulent or laminar. Unfortunately, this is an imprecise science. While the

Reynolds number suggests the possibility of laminar or turbulent flow, it does not provide any guarantees.

Fluid flow can be categorized as laminar, turbulent, or transitional. Laminar flow is smooth and steady. Turbulent flow is completely rough and agitated. Transitional flow is laminar with intermittent bursts of turbulence. These bursts can not be sustained and die out naturally.

In commercial metal pipes, the accepted value for the Reynolds number where the transition to turbulence begins to occur is 2,300. This means that the flow will begin to experience intermittent bursts of turbulence at this point. Fully turbulent flow is usually not seen until the Reynolds number exceeds 4,000. It is important to note that this is merely an estimate to be used for rough design calculations. The actual Reynolds number where turbulence begins is system-specific and could be lower or higher than this. Smooth walls in the pipe, a circular inlet, and a steady inlet stream can delay the transition to turbulence dramatically. Conversely, a rough walled pipe and a rough inlet stream can cause turbulent flow to begin at a lower Reynolds number. It is also important to note that the numbers mentioned above are specific to fluid flow in a circular commercial pipe and do not apply universally to every system. The nature of the duct in which fluid is flowing can have an extreme impact on the Reynolds number where turbulence is first seen.

21 CFR Part 179 calls for a Reynolds number of at least 2,200. It is possible that in some designs flow will be transitional at this point, but this can not be assumed without taking several other factors into account. The flow may or may not contain intermittent bursts of turbulence, but while it may not be turbulent, there is sufficient mixing of the fluid in some designs to provide uniform exposure.

Microbiology

Methods and Materials

Ultraviolet treatment

Apple ciders of various apple blends were purchased from a variety of local cider producers at varying times of the year. For each test, 4 liters of apple cider were inoculated with the appropriate strain of *Escherichia coli* O157:H7. A 1% inoculum of an overnight culture, grown at 35°C in Tryptic Soy Broth (Difco, Detroit, Michigan) was used to achieve an initial 100 000 000 colony forming units/milliliter (cfu/ml) starting level of *E. coli* O157:H7. The "spiked" apple cider was passed through the FPE's UV apparatus and collected into sterile collection vessels. The treated apple cider was mixed well prior to microbiological sampling.

After each pass of spiked apple cider, the UV apparatus was purged with sterile distilled water to ensure that cross contamination with previous higher inoculum level passes did not occur. For multiple pass treatments, after an aliquot was removed for microbiological

analysis, the treated sample was passed through the UV apparatus in the same manner as the initial pass and collected into a sterile collection vessel and an aliquot subsequently removed for microbiological analysis with each successive pass.

Microbiological enumeration

The initial and treated apple cider samples were diluted in 0.1% peptone to appropriate dilutions and 1 ml volumes were plated onto Plate Count Agar (PCA) and incubated at 35°C for 24 - 48 hours. The PCA plates were counted to determine the initial *E. coli* O157:H7 levels in the "spiked" sample and the surviving *E. coli* O157:H7 after the UV treatment in the FPE apparatus.

c. Analysis of Results

The following photographs represent the typical treatments. Each photograph has three dishes, the upper left is the initial inoculation, the upper right is the results after the first pass and the bottom center is the second pass results. Each sample in the photographs is labeled with the dilution used for identification the colony populations. A statistical study of the FPE process tube was done at Rutgers University and published (ref 1) in the "Journal of Food Protection" which demonstrates the effectiveness of the design.

UNFILTERED BLEND
Cider Mill A

Colony populations

Initial - $10^{8.36}$

Pass 1 - $10^{2.89}$

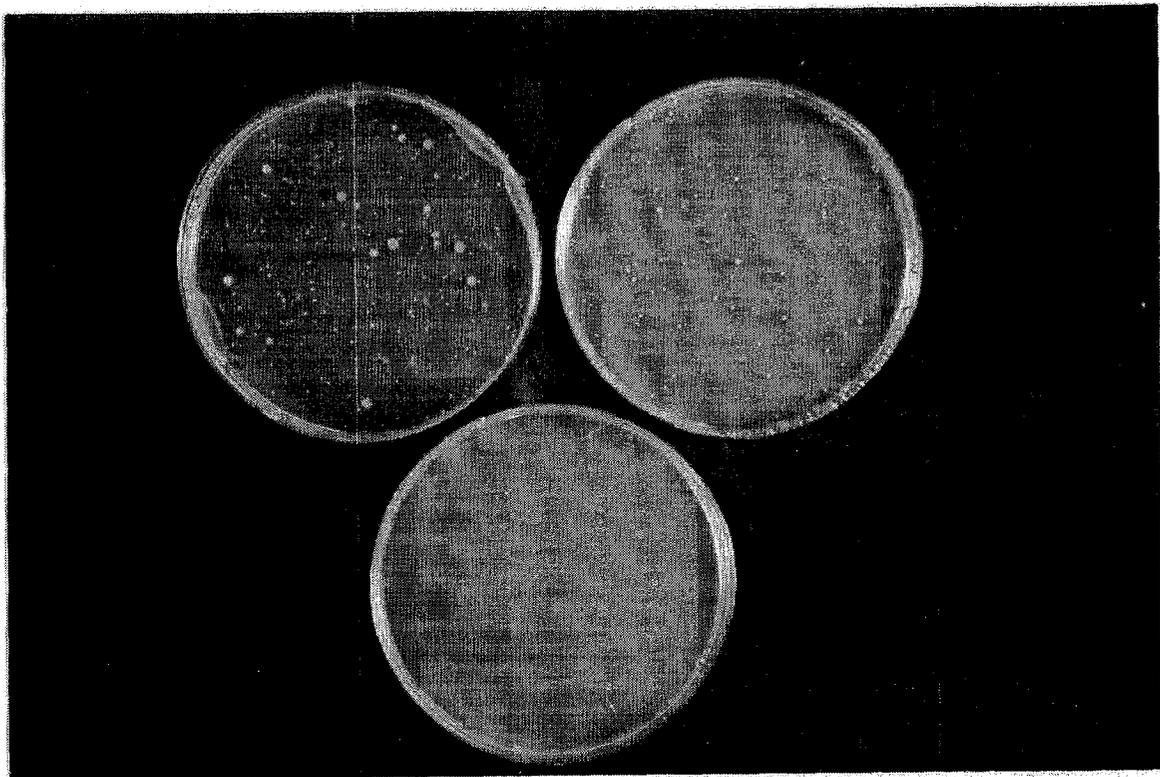
Pass 2 - 0

Examination dilution

Initial -6

Pass 1 -1

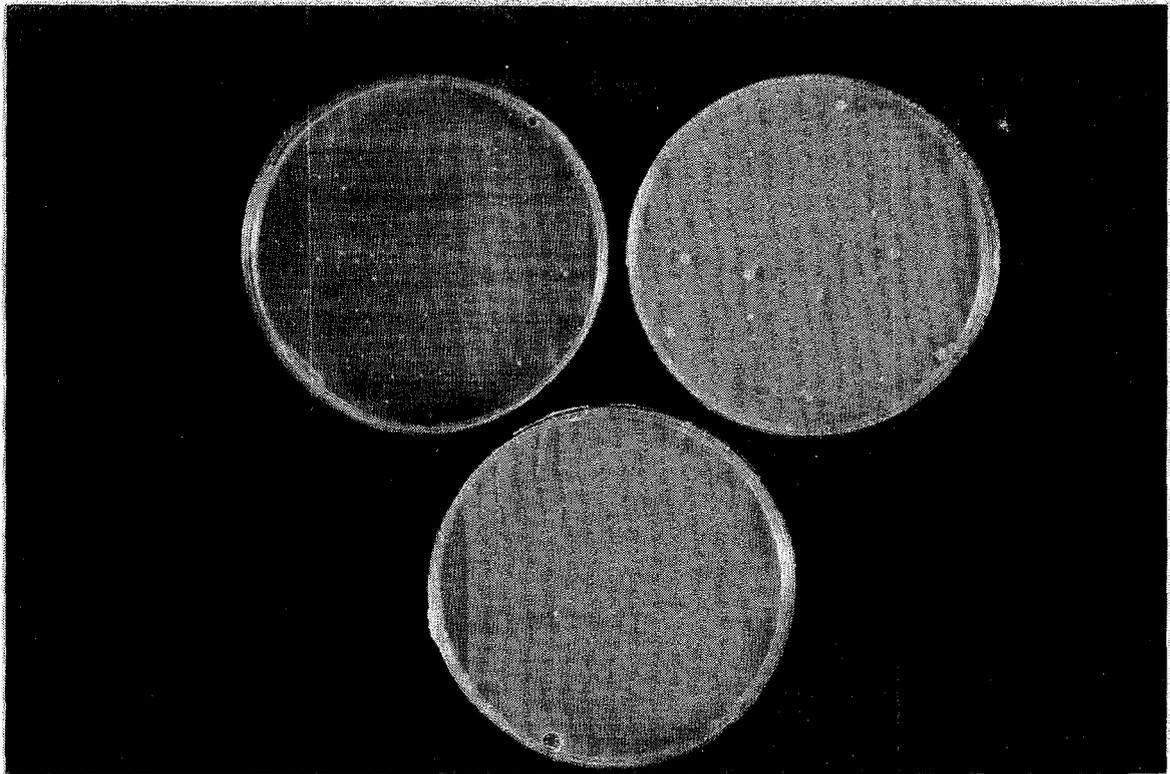
Pass 2 0



UNFILTERED BLEND
Cider Mill B

Colony populations
Initial - $10^{7.99}$
Pass 1 - $10^{2.90}$
Pass 2 - 0

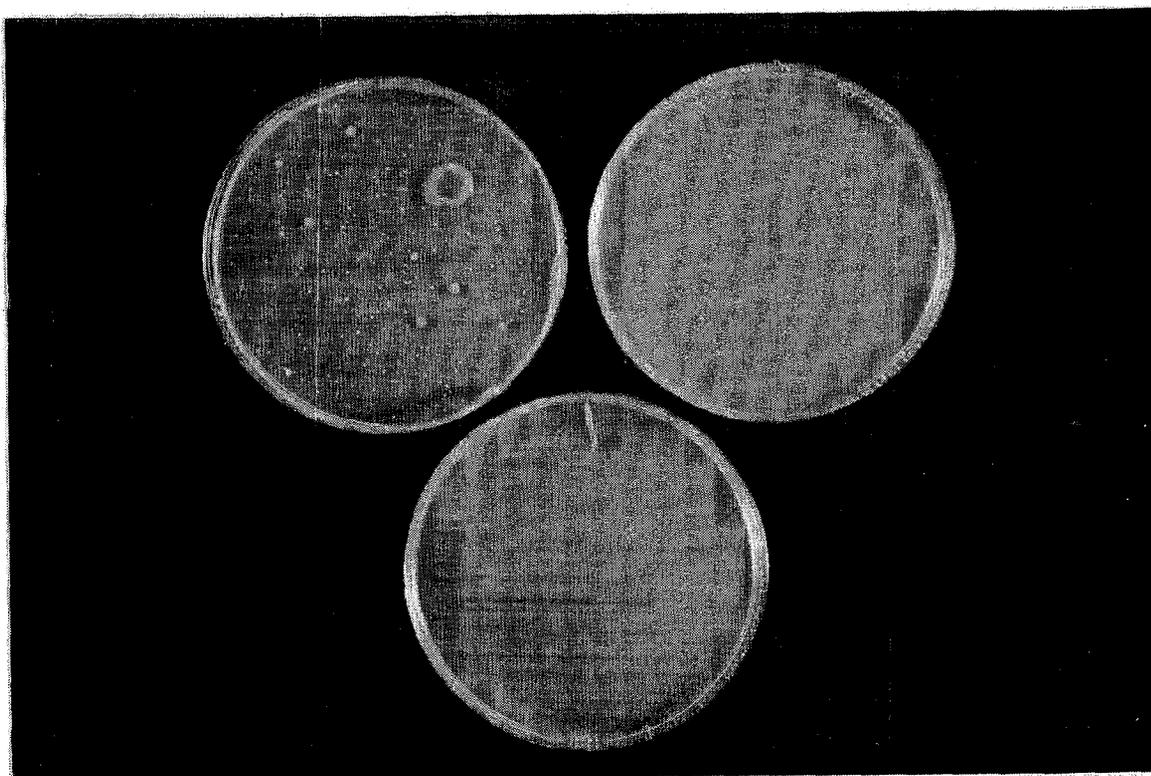
Examination dilution
Initial -6
Pass 1 -1
Pass 2 0



UNFILTERED BLEND
Cider Mill B

Colony populations
Initial - $10^{8.35}$
Pass 1 - $10^{0.065}$
Pass 2 - 0

Examination dilution
Initial -6
Pass 1 0
Pass 2 0



Recommendations

FPE, Inc. recommends that the Reynolds number be removed from the regulation and be replaced with the requirement that the equipment design can demonstrate it can achieve the desired technical effect.

Sincerely,

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Research Note

Analysis and Modeling of the Variability Associated with UV Inactivation of *Escherichia coli* in Apple Cider

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ABSTRACT

Raw data from validation studies of UV tubes used for nonthermal pathogen reduction in apple cider underwent comprehensive statistical analysis. Data from each tube that demonstrated at least a 5-log reduction of *Escherichia coli* ATCC 25922, a surrogate for *E. coli* O157:H7, in each of three trials were used in the analysis. The within- and between-tube variability was calculated for 70 tubes. The mean log reductions of the tubes fit a Beta distribution (Kolmogorov-Smirnov test, 0.0246), and the between-replicate variability followed a logistic distribution (Kolmogorov-Smirnov test, 0.0305). These two distributions can be used together to model UV cider treatment as part of an overall *E. coli* O157:H7 in cider risk assessment. Examples of codes from @RISK and Analytica to describe these distributions, such as one would find in a quantitative risk assessment, are included.

Apple cider has been implicated as a vehicle in several *Escherichia coli* O157:H7 outbreaks since 1980, even killing one child (2, 3, 5). Although the pressers of this traditional beverage are aware of the risks posed by unpasteurized cider, the cost of thermal pasteurization equipment, estimated as high as \$185,000 (7), and its potentially negative organoleptic effects make cider pasteurization unattractive and unfeasible for most family orchards. Recent research in food microbiology has focused on nonthermal processing alternatives for cider and other ready-to-eat foods. UV treatment, already approved by the Food and Drug Administration for pathogen reduction in water (4), is a promising, lower-cost alternative to pasteurization.

One of us (R.W.W.) has been an active researcher in UV treatment of apple cider and has validated individual quartz tubes for the *CiderSure* UV pasteurizer (FPE Inc., Macedon, N.Y.). Since minor variations in the manufacture of the tubes can alter the fluid dynamics and bactericidal efficacy of UV light radiation, validation to ensure each tube meets the 100,000-fold reduction in the target pathogen, *E. coli* O157:H7, as proposed by the Food and Drug Administration (6) is prudent. All tubes used in *CiderSure* units in cider mills demonstrated at least a 5-log reduction of a nonpathogenic surrogate for *E. coli* O157:H7 in each of three trials in the laboratory; tubes that failed to meet this criterion were not sold to cider producers.

The large quantity of raw data from these validation studies presented a unique opportunity for us to accurately determine the variability of a particular component of our risk assessment. This analysis greatly improves our ability

to model UV cider treatment as part of a quantitative risk assessment.

MATERIALS AND METHODS

Raw, unprocessed apple cider was inoculated with *E. coli* ATCC 25922, with a target concentration of 6 to 7 log CFU/ml. This organism is a surrogate for *E. coli* O157:H7, with similar UV sensitivity (9). *E. coli* ATCC 25922 was grown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C for 18 h (to stationary phase) on a rotary platform shaker at 250 rpm. The inoculated and UV-treated apple cider was diluted in a 0.1% peptone solution, and 1-ml aliquots of appropriate dilutions were poured in duplicate on tryptic soy agar (Difco), a nonselective medium that facilitates resuscitation of injured cells. The cider was run through the *CiderSure* (FPE Inc.) according to the manufacturer's instructions. *E. coli* was double plated on tryptic soy agar and incubated at 35°C for 48 h, both before and after UV treatment, and triplicate experiments were performed with each tube. All colonies that developed on the tryptic soy agar plates were assumed to be *E. coli* 25922 to determine the most conservative levels of lethality. Raw data were entered into an Excel (Microsoft, Redmond, Wash.) spreadsheet. All the possible log reductions of each experiment were calculated. The four possible log reductions from each tube per experiment (Fig. 1), for three trials, yielded a total of 12 results from each tube. In some instances, where the duplicate platings were not recorded, fewer results were obtained. If a tube failed to achieve a 5-log reduction in any of the three trials, it was not considered for further analysis, since it was not sold to the cider industry. Seventy tubes met all passing criteria. The 12 data points for each individual tube were converted into a histogram using Excel. A mean was calculated for each tube. The correlation between each mean and its range of results was determined. The difference, in logs, between the mean and each of the 12 results was calculated. These differences of all tubes were normalized, combined, and analyzed together in

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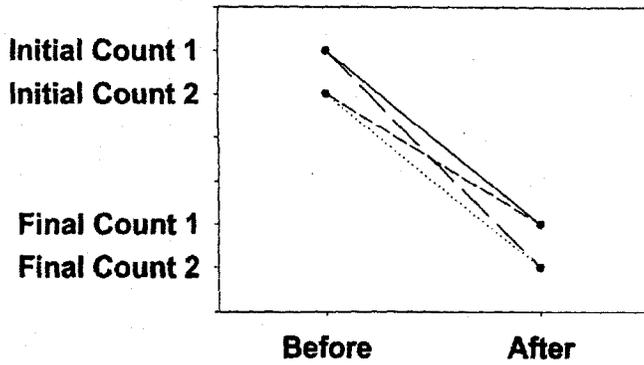


FIGURE 1. Example of the four calculable log reductions from one UV tube trial. Each log reduction is shown as a different type of line.

a histogram with a bin of 0.1 logs (the mean's bin ranged from mean - 0.05 logs to mean + 0.05 logs). All the tubes' means were analyzed with a histogram, with a bin of 0.2 logs. These histograms were fit with continuous distributions using Bestfit (Palisades Corp., Newfield, N.Y.). These distributions were used to model the efficacy of UV treatment of apple cider in @RISK (Palisades) and Analytica (Lumina, Los Gatos, Calif.) software programs. Monte Carlo simulations were run for 10,000 iterations with Median Latin Hypercube sampling.

RESULTS

The histograms for each individual tube showed a high degree of variability. In several cases, the possible log reductions were thinly spread over a 2-log range; however, histograms for other tubes centered strongly over a range of less than 0.5 log. Figure 2 shows a typical results histogram. Comparing the means of different tubes also showed a wide range, from 5 to 8.5 logs. The correlation between the mean and range of results for each tube was -0.10, showing a low interdependence of these variables. Figures 3 and 4 show the actual data points and the distributions chosen to fit them. The Beta distribution is a two-parameter model that described the mean log reductions better than 19 other continuous functions (Kolmogorov-Smirnov test, 0.0246). The variability around those means,

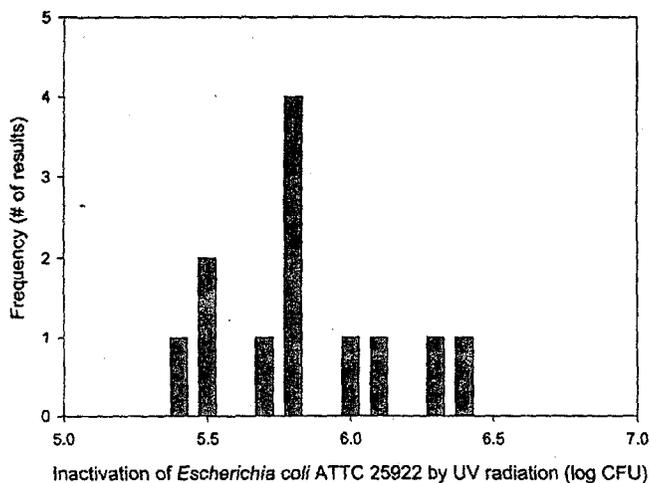


FIGURE 2. Example of 12 possible reductions of E. coli ATCC 25922 by UV radiation from validation data from one tube.

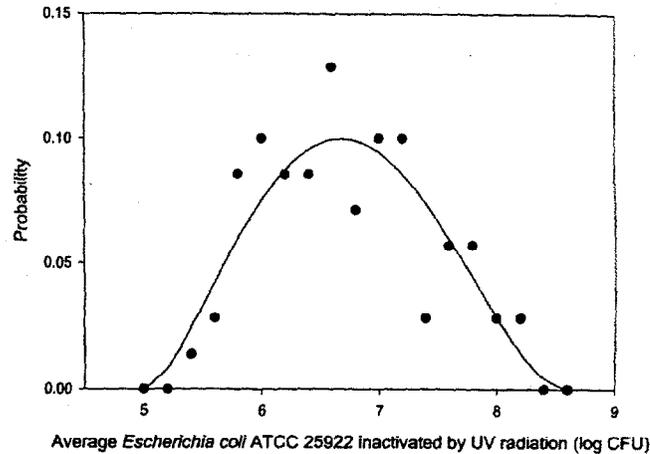


FIGURE 3. The distribution of mean log reductions for 70 tubes. The histogram of the data set is shown as dark circles, and the Beta distribution $([2.65, 2.89] * 3.6 + 5)$ is shown as the continuous line.

however, followed a logistic distribution (Kolmogorov-Smirnov test, 0.0305), which is a two-parameter, peaked version of the normal distribution.

A screenshot of the flowchartlike interface of the Analytica model is shown in Figure 5a. In Analytica, the distributions are entered in the "definition" field of each node. With @RISK, an Excel add-in, three cells needed to be coded, as shown in Figure 5b. Results of simulations of the mean's distribution alone and both distributions together are shown in both @RISK and Analytica in Figures 6 and 7. The distribution of means has the characteristic Beta distribution, but the addition of the logistic variability creates a new distribution that illustrates the wider range of possible reductions one would obtain with the CiderSure system. A tail of high log reductions (upward of 9 log) is accompanied by a small chance of a less than 5-log reduction at the other tail. By both simulations, the risk of a less than 5-log reduction with UV treatment occurs less than 0.2% of the time.

The shape of the predicted probability distributions from the simulations were similar, and any variance is due to the differing graphing routines, not a difference in the

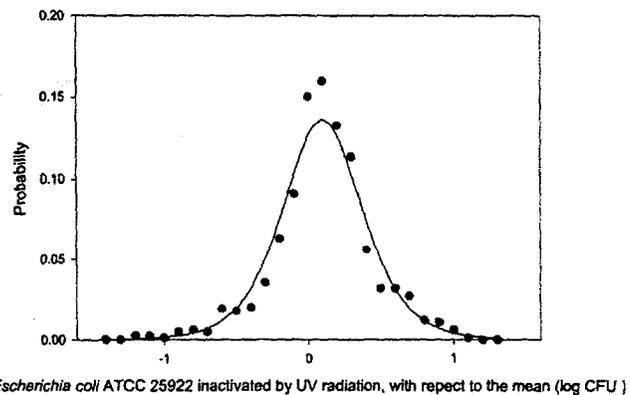
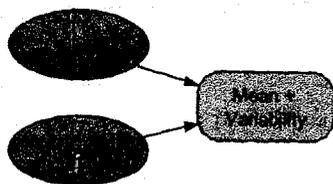


FIGURE 4. The variability around the mean for 70 tubes. The histogram of the data set, normalized so that the mean of each tube is represented as 0, is shown as dark circles, and the logistic distribution (0.0997, 0.18) is shown as the continuous line.



A

| | A | B |
|---|--------------------|----------------------------|
| 1 | Mean | =RiskBeta(2.65,2.89)*3.6+5 |
| 2 | Variability | =RiskLogistic(0.0997,0.18) |
| 3 | Mean + Variability | =B1+B2 |

B

FIGURE 5. (A) Screenshot of Analytica model. (B) The coded spreadsheet cells for the @RISK simulation.

mathematical principles the software used to perform Monte Carlo simulation. The @RISK results, however, show a troubling tendency in that the mean plus variability results (solid line) are almost always above the mean alone (dotted line). One would expect that the addition of extra variability around the mean response should produce results that would dip below the mean response a significant fraction of the time, as seen in the Analytica results (Fig. 7). We suspect that these peculiar results are due to the default graph settings of @RISK; those who use this product to conduct microbial risk assessments should be aware of this tendency.

DISCUSSION

The lack of a strong correlation between the mean and the range of results around the mean validates using two independent distributions to model the mean and variability around that mean. It is not surprising that the variability around the mean followed a logistic distribution. The biological significance of normallike distributions has been shown anecdotally and by probability theory (8). The logistic distribution is also known to describe subsets of data of other distributions very well (1), which is the case with variability around the mean.

The Beta distribution, on the other hand, does not have biological significance in describing the distribution of means; it is commonly used when there are insufficient data (1). However, the data set used for modeling was artificially truncated, because tubes that showed a less than 5-log reduction were not used in the analysis. So, the fact that there were no continuous data from a natural process could account for the lack of mechanistic explanation for the Beta distribution's fit. Since our objective was to accurately model the UV process for risk assessment, trying to force these data to fit a normal distribution would be less useful than describing the phenomenon with another, more complex distribution.

The high and low log reduction tails from the @RISK

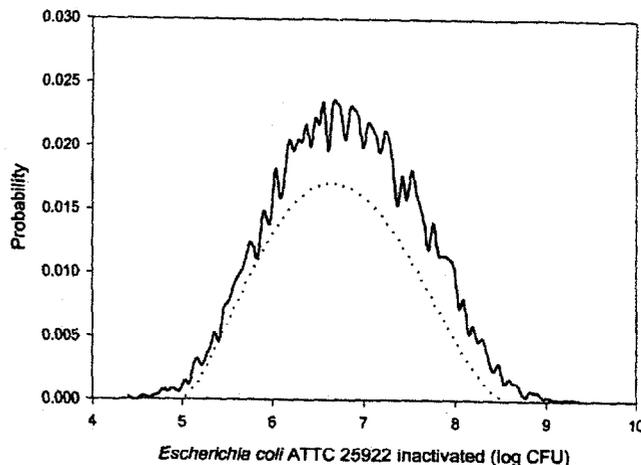


FIGURE 6. Results of @RISK simulations (10,000 iterations). The dotted line is the result of the mean alone, and the solid line is the outcome of using the mean and variability together.

and Analytica simulations are probably an accurate reflection of the performance of this machine rather than an artifact of simulation. The small percentage of failure to achieve the target log reduction is only quantifiable through the rigorous analysis and simulation used to study the system; a similar low occurrence of failures occurs with all machine and natural processes. Because no step in food processing is ever 100% consistent, it is important to acknowledge the variability and chance associated with the processes. A highly variable process with a high mean bacterial reduction is less useful than one with a slightly lower mean kill but a more narrow range of variability, which is less likely to fail. One way to improve the consistency of a 5-log reduction would be to make the criteria for a saleable tube more strenuous; if only tubes with higher than a 5.5-log reduction were passed, the probability of failing to reach the 5-log mark would drop significantly.

It is always better to know the variability associated with a process; however, this has not traditionally been a focus of food microbiology research. For example, it is common knowledge among cider producers, regulatory

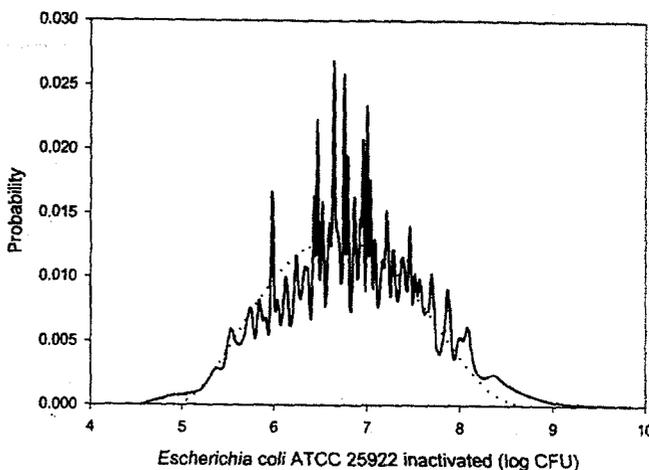


FIGURE 7. Results of Analytica simulations (10,000 iterations). The dotted line is the result of the mean alone, and the solid line is the outcome of using the mean and variability together.

agencies, and scientists who study cider that flash pasteurization of apple cider at 160°F for 6 s yields a 5-log reduction of *E. coli* O157:H7 (7); however, estimates of the variability necessarily associated with this process are unknown or at least unpublished. In this emerging age of risk assessment, it becomes imperative to quantify variability not only to formulate better predictive models but also to separate uncertainty from variability as much as possible. Uncertainty calls for further research, whereas variability can be quantified and accepted or spur new actions that will limit its range.

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