

# Response of Airborne *Mycoplasma pneumoniae* to Abrupt Changes in Relative Humidity

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The effect of an abrupt change in the relative humidity on the viability of airborne *Mycoplasma pneumoniae* has been examined. When the microbial aerosols were permitted to equilibrate in air held at either low or high humidities and were then subjected to a sudden shift to a mid-range humidity, a significant loss (>90%) of the colony-forming units per liter of aerosol occurred within 8 min. In contrast, a change in the relative humidity of more than 18% in either direction from a lethal mid-range humidity noticeably decreased the rate of biological decay. Double humidity shifts (i.e., from dry to a mid-range level and then to a high humidity range) were very detrimental, with very few survivors after 8 min. These results indicate that the biological stability of airborne *M. pneumoniae* may be easily modified by a sudden change in the relative humidity, such as occurs in natural atmospheres. This increased sensitivity brought about by producing changes in relative humidity through the lethal humidity range may provide a method whereby the control of these organisms in naturally contaminated indoor air environments may be eventually achieved.

The long history of respiratory infection indicates that many microorganisms, pathogenic for man, are transmitted naturally through air. To establish the conditions under which a particular pathogen may be naturally transmitted through the air, it is necessary to examine and characterize the microorganism in natural atmospheres and under a variety of experimental conditions. Past studies of airborne microbes have largely been concerned with determining their: (i) presence and frequency of occurrence in indoor and natural atmospheres, (ii) ability to withstand laboratory procedures used to simulate natural aerosol dissemination and collection, (iii) persistence under a variety of atmospheric conditions, (iv) deposition in the upper respiratory tract of animal or human hosts, and (v) infectivity for man and selected animal species.

Few of the above studies have been conducted on *Mycoplasma pneumoniae*, even though these organisms have frequently been associated with respiratory illnesses of man (5, 14) and particularly with respiratory tract illnesses in normally crowded populations (3, 8, 9). *M. pneumoniae* has been frequently isolated from individuals

during an extended period following infection (4, 6, 10). A carrier state in healthy persons has not been recognized.

We have previously established (16, 17, 18), as have others (15), some of the atmospheric conditions under which various species of the genus *Mycoplasma* can survive for as long as 4-hr in a controlled steady-state airborne environment. If laboratory work is to be used as a model for natural disease transmission, it is essential that conditions found in natural air environments be closely simulated. This is particularly true with reference to relative humidity which is a critical parameter in airborne microbial survival. The present communication concerns experiments which simulate the variable humidity conditions to which naturally disseminated microorganisms may be subjected. The effect of abrupt changes in the relative humidity on airborne *M. pneumoniae* was determined by following the response of these organisms to humidity shifts from either low (atmospheric) or high (oral cavity) type of a natural environment to a lethal mid-range humidity level and vice versa.

## MATERIALS AND METHODS

**Organism and culture procedures.** The strain of *M. pneumoniae* and the methods used for propaga-

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tion were the same as those described previously (18).

**Aerosols.** Aerosols were produced with a modified Well's reflux atomizer containing 50 ml of the microbial suspension and a small quantity of Antifoam B (Dow-Corning). After a 15-min period of reflux atomization, the number of colony-forming units per milliliter in the spray suspensions remained reasonably constant, and 99% of the particles emitted by the atomizer were smaller than 3  $\mu\text{m}$  in diameter (18). The primary aerosols produced by the atomizer were mixed with measured volumes of filtered wet and dry air to obtain the desired relative humidity. The aerosols were then transported down a dual aerosol transport apparatus which was maintained at 27 C. A detailed description with general operational procedures for the apparatus (first prototype) has been previously reported (11, 12). This particular apparatus or aerosol chamber consisted of short sections [6 inches (15.2 cm) in diameter] of galvanized furnace pipe connected together to give an overall length of about 52 ft (15.6 m). One end of the tube was inserted a short distance into a larger [8 inches (20.3 cm) in diameter] pipe constructed similarly; the total length of both pipes was about 118 ft (35.4 m). Sampling ports were located at convenient intervals along the apparatus.

For these experiments, the aerosols transited the dual aerosol transport apparatus at a linear rate of about 8 ft/min. To accomplish this, input air was introduced at 1.6 ft<sup>3</sup> per min, and effluent air was withdrawn at 2.85 ft<sup>3</sup> per min. A secondary air stream (1.25 ft<sup>3</sup> per min) at the same temperature as the primary air was added essentially isobarically at the junction of the large and small pipes. This resulted in a twofold dilution of the primary aerosol at this confluence point. In control experiments, the relative humidity of the secondary air stream was maintained at the same humidity as the primary aerosol. The apparent dilution ratio (defined as the particle concentration in aerosols immediately preceding dilution by the secondary air stream compared

with concentration immediately after dilution) was usually 0.59. To change the range of relative humidity of aerosols in transit, the secondary air stream was maintained at a desired humidity level different from that of the primary aerosol.

**Aerosol sampling and assay.** Aerosol samples were collected in an AGI-30 sampler (2) containing 20 ml of PPLO Broth (Difco) and a small quantity of Antifoam B. The impinger fluids were assayed within 30 min, unless otherwise noted. They were serially diluted in PPLO Broth and appropriate dilutions were plated on PPLO Agar (Difco). The plates were incubated for 7 days at 37 C, and colonies were enumerated by microscopic observation. The relative measurement of physical losses of airborne particles was made by determining the relative light scatter of the aerosol with a forward angle light scatter photometer.

## RESULTS

**Stability in the collecting fluid.** We have reported that *M. pneumoniae* should be assayed immediately after recovery from steady-state aerosols, as storage for more than 2 hr at 22 C results in an appreciable loss of colony-forming units per milliliter (18). However, the data presented in Table 1 show that *M. pneumoniae* recovered from dynamic aerosols may be quite stable in PPLO Broth when stored at 22 C for as long as 2 hr. Regardless of the aerosol transit time or humidity level tested, the number of colony-forming units in the impinger fluids remained reasonably constant throughout the test period. A decrease in viable organisms was observed only between samples taken at different aerosol transit times which reflected the combination of dilution and aerosol decay.

**Physical losses.** Figure 1A shows the rate of the physical decay of primary and diluted aerosols held in the dual aerosol transport apparatus

TABLE 1. Survival of *Mycoplasma pneumoniae* after recovery from aerosols transported down a dual aerosol transport apparatus<sup>a</sup> and stored at 22 C in PPLO Broth

Aerosol transit time	Relative humidity of aerosol	Storage time (min)					
		0	20	40	60	90	120
<i>min</i>							
0.3	26	$1.0 \times 10^4$	$0.97 \times 10^4$	$0.91 \times 10^4$	$0.83 \times 10^4$	$0.82 \times 10^4$	$0.80 \times 10^4$
6.2		$0.72 \times 10^4$	$0.74 \times 10^4$	$0.74 \times 10^4$	$0.77 \times 10^4$	$0.78 \times 10^4$	$0.71 \times 10^4$
7.0		$0.41 \times 10^4$	$0.37 \times 10^4$	$0.47 \times 10^4$	$0.35 \times 10^4$	$0.34 \times 10^4$	$0.33 \times 10^4$
14.7		$0.26 \times 10^4$	$0.28 \times 10^4$	$0.28 \times 10^4$	$0.27 \times 10^4$	$0.27 \times 10^4$	$0.26 \times 10^4$
0.3	60	$1.1 \times 10^3$	$1.1 \times 10^4$	$1.3 \times 10^4$	$1.4 \times 10^4$	$1.2 \times 10^4$	$1.2 \times 10^4$
6.2		$0.27 \times 10^4$	$0.32 \times 10^4$	$0.33 \times 10^4$	$0.31 \times 10^4$	$0.27 \times 10^4$	$0.25 \times 10^4$
7.0		$0.12 \times 10^4$	$0.13 \times 10^4$	$0.15 \times 10^4$	$0.14 \times 10^4$	$0.12 \times 10^4$	$0.13 \times 10^4$
14.7		$0.045 \times 10^4$	$0.063 \times 10^4$	$0.060 \times 10^4$	$0.044 \times 10^4$	$0.047 \times 10^4$	$0.045 \times 10^4$

<sup>a</sup> The primary aerosol was diluted with a second air stream (1.6:2.85 ft<sup>3</sup>/min ratio) at a similar relative humidity after 6.5 min of aerosol transit time.

<sup>b</sup> Expressed as colony-forming units per milliliter.

at 26% relative humidity. A mean rate of 1.5% per min was observed in both the large and small pipes, and a humidity change produced by dilution of the primary aerosol at the confluence point had no demonstrable effect on these rates. In subsequent experiments in which the range of relative humidities was near the mid-range or saturated level, the rate of physical losses increased concomitantly with the increase in humidity up to a maximum rate of 4.2% per min at a relative humidity of 91%. In most instances, the observed apparent dilution ratios were about 0.59; they deviated from this value only when the humidity of the diluted aerosol was significantly higher or lower than that of the primary aerosol. Such changes in the relative humidity appear to affect the light scatter measurements, possibly because of changes in the size of the airborne particles (7). However, these small variations in the apparent dilution ratios did not suggest significant physical losses resulting from the humidity shifts.

**Biological effect of aerosol dilution and shift in relative humidity.** The data presented in Fig. 1B show the results of control experiments in which primary and secondary aerosols were both held at 26% relative humidity. The observed biological dilution ratio (defined as the number of viable bacteria per liter immediately before dilution as compared with the number immediately after dilution) was about the same as the apparent dilution ratio, which indicated that no biological loss occurred as a consequence of aerosol dilution. The dynamic humidity-death ratio (defined as the extent of biological loss with time observed

in primary aerosols as compared with a similar loss in diluted aerosols) was about equal to 1.0. These results indicate that the rates of biological decay before and after aerosol dilution were similar. In contrast, the number of colony-forming units per liter sharply decreased after an abrupt change in humidities from 26 to 55% (Fig. 1C). Since the physical losses or fallout were irrelevant to the overall total decay (defined as the combined total of the physical and biological losses as estimated by the viable counts at various aerosol transit times) observed, this and the increased rates of decay subsequently found may, in practicality, be regarded as reflecting biological effects. Apparently, some organisms were killed instantaneously with the humidity shift, as the biological dilution ratios were frequently much less than the apparent dilution ratios. The observed dynamic humidity-death ratios ( $<0.12$ ) indicated that airborne *M. pneumoniae* already equilibrated at a dry humidity level was quickly inactivated by rapidly shifting the humidity to a mid-range level.

When humidity in the dual aerosol transport apparatus of both primary and diluted aerosols was held at about 91% relative humidity, the biological and apparent dilution ratios were quite similar (Fig. 2A). However, if the humidity was suddenly shifted at the confluence point from wet to a mid-range humidity (i.e., from 84 to 57% relative humidity), the number of viable organisms per liter (Fig. 2B) rapidly decreased. Under these conditions, the effect of aerosol dilution was not detrimental, but the rate of biologi-

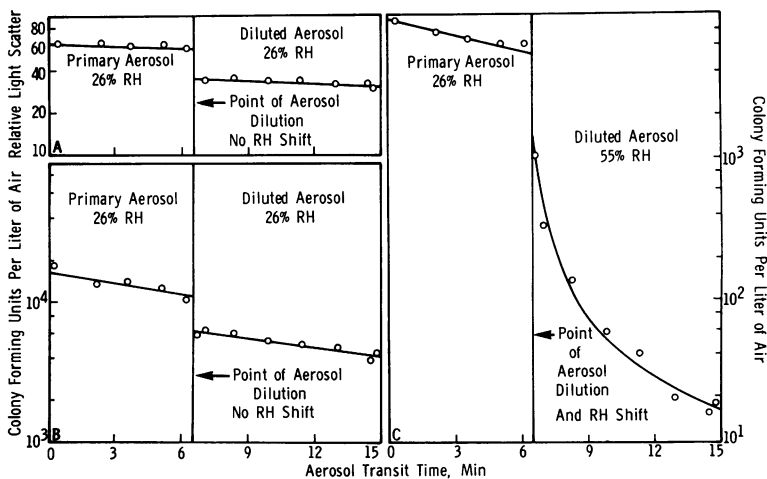


FIG. 1. Effect of diluting aerosols of *Mycoplasma pneumoniae*, controlled at 27 C and a low relative humidity, with a second air stream at the same or higher humidity. (A) Physical decay curves of airborne particles before and after aerosol dilution. (B) Biological decay curves of airborne bacteria before and after aerosol dilution. (C) Biological decay curves of airborne bacteria subjected to a sudden change in relative humidity from 26 to 55%.

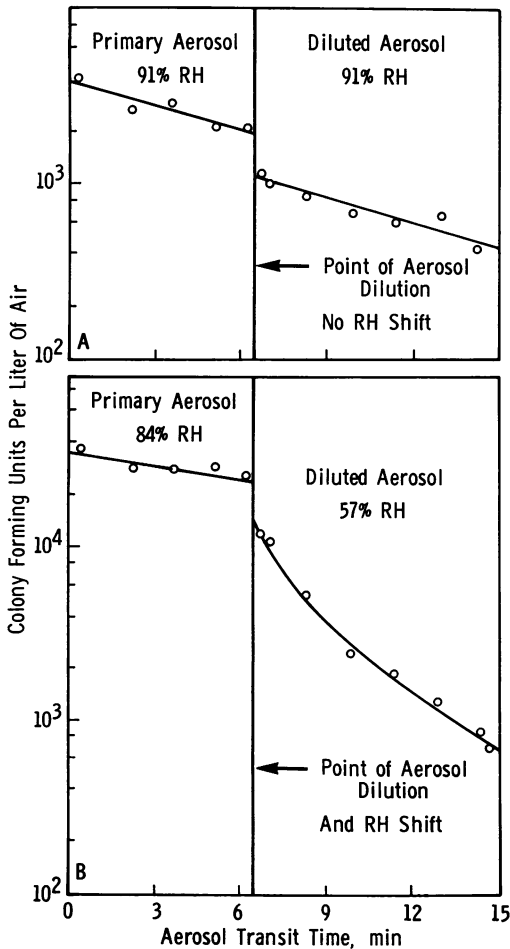


FIG. 2. Effect of diluting dynamic aerosols of *Mycoplasma pneumoniae*, controlled at 27 C and a high relative humidity, with a second air stream at the same or lower humidity. (A) Biological decay curves of airborne bacteria before and after aerosol dilution. (B) Biological decay curves of airborne bacteria subjected to a sudden change in relative humidity from 84 to 57%.

cal decay sharply increased and dynamic humidity-death ratios were as low as 0.11.

When airborne *M. pneumoniae* was subjected to a rapid shift from a lethal mid-range to less deleterious humidity levels (Fig. 3 and 4), less than 10% of the survivors were rendered non-viable during the ensuing 8 min after such shifts, and dynamic humidity-death ratios around 3.0 were frequently found. This indicates a greatly improved stability for the surviving bacteria shifted from a lethal mid-range relative humidity to a more stable humidity level.

The response of airborne *M. pneumoniae* to a double shift in atmospheric humidity was exam-

ined. The aerosolized bacteria at supportive relative humidities (i.e., 26 or 83%) were subjected to a rapid shift to a lethal mid-range level in the previously described manner. After this

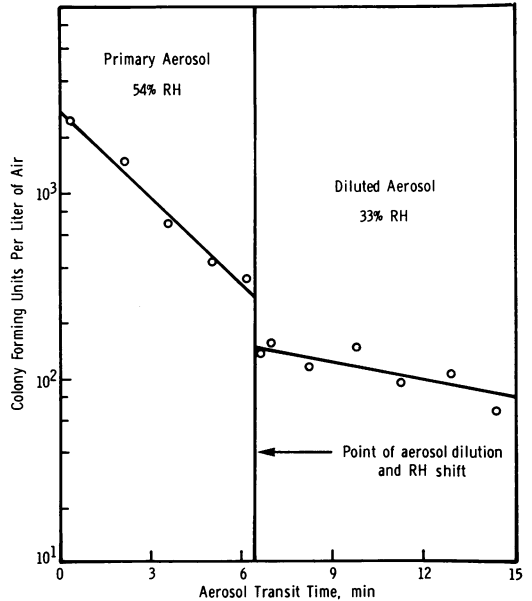


FIG. 3. Survival of airborne *Mycoplasma pneumoniae* after an abrupt change from a lethal mid-range to a more stable dry relative humidity.

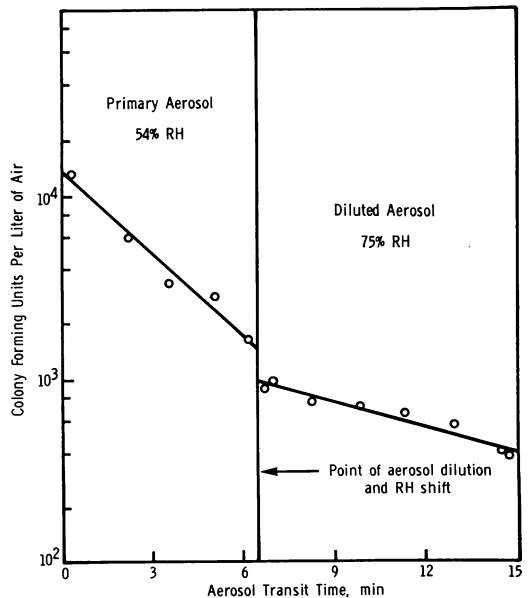


FIG. 4. Survival of airborne *Mycoplasma pneumoniae* after an abrupt change from a lethal mid-range to a more stable wet relative humidity.

TABLE 2. Effect of one and two shifts in the relative humidity on survival of *Mycoplasma pneumoniae* in dynamic aerosols<sup>a</sup>

Transit time after shift 1	Colony-forming units per liter of aerosol			
	Experiment I		Experiment II	
	Per cent relative humidity (before shift, 26%)		Per cent relative humidity (before shift, 83%)	
	Shift 1 (to 55%)	Shift 2 (to 90%)	Shift 1 (to 57%)	Shift 2 (to 88%)
<i>min</i>				
0.2	$1.0 \times 10^3$	$0.13 \times 10^3$	$12.0 \times 10^3$	$2.7 \times 10^3$
0.5	$0.32 \times 10^3$	$0.050 \times 10^3$	$10.0 \times 10^3$	$2.0 \times 10^3$
1.8	$0.13 \times 10^3$	$0.024 \times 10^3$	$5.2 \times 10^3$	$0.89 \times 10^3$
3.3	$0.055 \times 10^3$	$0.011 \times 10^3$	$2.5 \times 10^3$	$0.40 \times 10^3$
4.8	$0.030 \times 10^3$	$0.005 \times 10^3$	$1.8 \times 10^3$	$0.25 \times 10^3$
6.4	$0.022 \times 10^3$	$0.003 \times 10^3$	$1.2 \times 10^3$	$0.17 \times 10^3$
7.9	$0.017 \times 10^3$	$0.002 \times 10^3$	$0.84 \times 10^3$	$0.11 \times 10^3$
8.2	$0.016 \times 10^3$	$0.002 \times 10^3$	$0.78 \times 10^3$	$0.097 \times 10^3$

<sup>a</sup> Primary aerosols were allowed to equilibrate to atmospheric conditions for about 6 min; they were then diluted with a second air stream of a different humidity level, which caused an abrupt shift in the relative humidity. As a function of the aerosol transit time after the first shift, a second shift in humidity was imposed immediately before collection of the sample.

first shift, the aerosol samples collected at various stations downstream from the point where the shift occurred were briefly transported through a water-saturated air atmosphere before impingement. This final shift-up in humidity was made possible by the use of a humidifying device as described previously (13) and effectively shifted the aerosol sample from the existing humidity range to >85% relative humidity. The results of these experiments are presented in Table 2 and show that, whereas the first shift from either extreme of the humidity range to a mid-range level was quite detrimental, a second shift from this lethal range to a high humidity level resulted in an even greater loss of viable cells. It is also apparent that the longer the delay before the second shift in the relative humidity, the more pronounced was the effect following that shift. This suggests that the injurious effects of the first humidity shift became progressively more severe as time progressed and that the injury inflicted by the second shift was compounded upon the first and was sufficient to inactivate most of the airborne organisms.

## DISCUSSION

In previous experiments (18), it was shown that *M. pneumoniae*, held in steady-state aerosols at 27 C and at <25 or 90% relative humidity, is quite stable, whereas *M. pneumoniae* held at this temperature but at mid-range humidities is unstable. The general susceptibility of an aerosol of various species of *Mycoplasma* to detrimental effects of moisture, at certain critical humidity

levels and not at others, is reminiscent of observations on many other bacteria (1) and suggests that the unique properties of these bacteria do not afford them any greater persistence in air than most other bacterial species.

The number of viable bacteria per liter of aerosol found at the first sample station (17-sec aerosol transit time) is indicated on the ordinate axis (Fig. 1 to 4), where the counts varied from 0.27 to  $3.6 \times 10^4$  per liter. A similar 10-fold variation in the viable counts of the atomizer fluids was also observed. When we produced four aerosols on a given day from a single bacterial suspension, the spray fluid counts were within a threefold differential, and initial aerosol recoveries, irrespective of the humidity range tested, never varied more than fourfold. Hence, these variations at the ordinate axis reflect primarily our techniques in the production and handling of *M. pneumoniae*. We have found that the quality of the horse serum used is a major factor in this regard. However, regardless of differences in the initial number of airborne bacteria recovered, the effects of the shifts in relative humidity on the biological properties of airborne *M. pneumoniae* were the same. The present report indicates that the biological properties of airborne *M. pneumoniae* in dynamic aerosols may be demonstrably affected by sudden changes in the atmospheric humidity, such as might occur after their discharge into various atmospheric environments or when inhaled into an animal or a human respiratory system. Airborne *M. pneumoniae* already equilibrated at a

supportive dry or wet humidity range was shown to be highly sensitive to the effects of atmospheric moisture immediately after an abrupt change to a mid-range humidity. In contrast, a change in either direction from a lethal mid-range humidity produced a marked reduction in the biological decay rate, and a double humidity shift, from a stable to lethal and then to a wet humidity range, was even more detrimental.

These effects are particularly significant in instances in which >1,000 colony-forming units per liter of air survived in a diluted aerosol for 8 min after confluence, whereas only about 10 colony-forming units were present at this time if the dilution was to a lethal relative humidity.

A simple explanation for these results is not readily apparent. The death of microorganisms in air may be attributable to water movement out of the airborne droplet and hence out of the intracellular microenvironment (19). This rapid loss of water presumably results in one or more of the following: (i) the weakening and ultimate collapse of certain cellular structures; (ii) the concentration of droplet or intracellular constituents to toxic levels, with cellular disruption resulting from this or osmotic shock; or (iii) the inactivation of vital cellular functions imbalanced by a disproportionate amount of water throughout the cell. However, our results are not easily explained on the basis of irreversible inactivation from collapsed cellular structures, as a quantitative change in atmospheric humidity may further sensitize or even stabilize the airborne organism.

Undoubtedly, the organisms were exposed to external osmotic shock during the initial concentration of medium constituents in airborne droplets after their generation and desiccation and possibly during humidity shifts in the dual aerosol transport apparatus. However, whether they were also subjected to any additional internal osmotic pressure changes, which may be a mode of killing, has not been established. It seems unlikely that lysis occurred, in that the effect was directly related to the direction of the humidity shift.

Instead, the airborne cells appeared to be quite sensitive to any change in the moisture content of the air atmosphere. This suggests that the biological properties of these organisms, particularly when they are exposed in naturally changing atmospheres, are easily modified. The extent of the modification depends on the direction of the shift and on the number of times the cells are subjected to a changing atmosphere. Thus, the stability of airborne *M. pneumoniae* at a particular sampling time is dependent on a number of factors.

The data shown in Table 1 indicate that factors

contributing to cellular inactivation in dynamic aerosols, even at a lethal mid-range humidity, do not include appreciable cellular degradation while in the collecting fluid.

It is particularly interesting that airborne *M. pneumoniae* can be shifted from a severely detrimental relative humidity to one that provides greater stability with a resulting decrease in the rate of decay. However, to say that the prior experience of the organisms at atmospheres of various humidity levels is without effect on the ultimate survival of the organism does not agree with the data reported for the double shifts. Rather, the results of this work indicate that the effects of various humidity levels to which an organism is exposed may be cumulative and that the stresses placed on the organism shifted through a number of changes in the relative humidity may prove lethal, although survival at any one of the humidity levels by itself may be good.

The present study has clearly shown that inactivation of most airborne *M. pneumoniae* by shifts from either low or high to mid-range humidities is accomplished in <8 min. This time is well within the normal air recycling times one could obtain by conventional air conditioning equipment. Thus, it is possible that the killing effects observed may have an application in air hygiene. What effects the methods of natural aerosol generation and suspending menstua, as found in the discharge from the human oral cavity, may have on survival and infectivity of airborne microbes subjected to such humidity shifts are not known. However, the effect of these factors on the epidemiology of such microbially contaminated atmospheres must be known before one can determine the practicality and application of changes in relative humidity in disease control.

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