

Effects of Freon-113 on the survival of bacteria

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An initial investigation was made of the bactericidal and sporicidal activity of the dry cleaning solvent Freon-113 (1,1,2-trichlorotrifluoroethane) under various conditions. Representative bacterial strains selected for this study were *Escherichia coli* (ATCC 11229), *Staphylococcus aureus* (ATCC 6538) and spores of *Bacillus globigii* (*Bacillus subtilis* var. niger). Various conditions were studied, including temperature, moisture, detergents and organic load. The results of this study indicate that there is the potential for survival of significant levels of micro-organisms in Freon-113 within the conditions evaluated. However, the bactericidal efficiency of this solvent increases significantly against vegetative forms at elevated temperature and, to a greater extent, upon the addition of detergents. Organic material present in the form of soiled fabric was observed to depress this efficacy whereas bacterial spores were virtually unaffected under all conditions evaluated.

Freon-113 (1,1,2-trichlorotrifluoroethane) is a commercially available solvent which is used in a variety of industrial and commercial cleaning applications including dry cleaning (Bartlett 1978; Ramsey 1979). Superior physical properties and toxicological characteristics of this compound in comparison with other solvents currently used for these applications are also reported by Ramsey (1979).

Previous work on the effects of 'Freon' solvents on bacteria has been reported. Playne & Smith (1983) studied Freon-113 toxicity to facultative anaerobes as determined by following gas production and found no toxicity at test concentrations of 2.5 and 25 μ l/ml of growth medium. Healy *et al.* (1974) reported inhibition in colony development, log phase growth and bacterial respiration in eight species of bacteria upon exposure to the halocarbon solvents Freon-11 (trichlorofluoromethane), Freon-21

(dichlorofluoromethane), Freon-22 (monochlorodifluoromethane) and Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane). Inhibition was found to be dependent upon the particular halocarbon used and the concentration. Complete kill of several bacterial species including *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* was reported by van Auken *et al.* (1975) upon exposure to atmospheres of 100% Freon-11 and Freon-21, while Freon-22 was found to have little if any bactericidal effects. The addition of liquid Freon-11 and Freon-21 to liquid shake cultures in this study was found to be completely inhibitory to *E. coli* and *Staph. aureus* at concentrations of 3.6-17.8 g Freon/500 ml of growth medium.

A broader study investigating the effects of Freon-113 as a bactericidal and sporicidal agent was needed to include the variables of temperature, minimum moisture, detergents and organic loads. The following study was particularly critical in the light of concerns for potential cross-contamination of clothing in a dry cleaning system where the solvent would be continuously re-used.

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Materials and Methods

CLEANING SOLVENT/DETERGENTS

Freon-113 solvent (E.I du Pont de Nemours and Co. Inc., Wilmington, DE, USA) was obtained for use in the present study along with HMC detergent and PDF detergent (Anscott Chemicals, Delaware, MD, USA) which are anionic and cationic surfactants used for water-based and grease-based types of clothing soil, respectively.

BACTERIA AND BACTERIAL SPORES

Representative bacterial strains selected for use in this study were *E. coli* (ATCC 11229) and *Staph. aureus* (ATCC 6538). These were maintained on Nutrient agar (NA; Difco) slants, and were grown overnight in Nutrient broth (Difco) at 25°C with shaking. When in the log phase these micro-organisms were harvested by centrifugation using an RC-5 Refrigerated Super-speed centrifuge (du Pont Instruments, Wilmington, DE, USA), resuspended in 0.9% sterile sodium chloride solution (NSS) and centrifuged again. The resulting bacterial pellet was suspended in 5 ml of NSS which was used as an inoculum.

Spores of *Bacillus subtilis* var. niger (*B. globigii*) were obtained from the E. Merck Company (NJ, USA). These were suspended in NSS for use in this study. Dilutions of each suspension were made in NSS and spread on NA plates to determine the total viable count.

TEST APPARATUS

The experiments were done in 150 × 25 mm glass tubes with Teflon-lined screw-top caps, secured on a wrist-action shaker (Model 75, Burrell Corp., Pittsburgh, PA, USA) set for maximum amplitude (setting of 10). Survival studies conducted at 45°C were done in a temperature-controlled waterbath (Tecom Temperature Unit, Techne, Inc., Princeton, NJ, USA).

FABRIC SWATCHES

Fabric swatches were cut from 100% bleached cotton duck (type III Army duck, 0.34 kg/m²,

mercerized and prepared for printing) into 3.8 cm² pieces for use in soiled swatch studies.

SOILING MIXTURE

The artificial soiling mixture was composed of 50% (w/w) 'soil', 25% (w/w) motor oil and 25% generic vegetable oil. The 'soil' was prepared by mixing equal amounts of sand, dried cow manure and top soil which were all purchased from a local plant nursery. The soil was steam-sterilized at 120°C for 60 min before mixing with the oils. The soiling mixture was again steam-sterilized at 120°C for 30 min before it was applied to the experimental swatches at a rate of 100 mg/3.8 cm² swatch.

SURVIVAL STUDIES

Survival studies were made in Freon-113 with 0.1% water at room temperature and at 45°C, with and without detergents. Studies were also run with minimal water and soiled fabric swatches in the presence of detergents.

SURVIVAL STUDIES AT ROOM TEMPERATURE

A 20 µl volume of the bacterial suspension (*ca* 2 × 10⁷ organisms) was added to 20 ml of Freon-113 in each test-tube (yielding approximately a 0.1% water concentration). The tube was then immediately sealed and the wrist-action shaker was switched on. The micro-organisms were then exposed to the Freon-113 with 0.1% water at room temperature for varying lengths of time.

The bacteria or spores were retrieved from the solvent mixture by adding 10 ml of NSS, mixing on a vortex shaker (Scientific Instruments, Inc., Bahemie, NJ, USA), and holding the tube stationary to allow complete separation of the biphasic mixture. Samples from the aqueous (overlying) phase were removed, diluted appropriately, and spread on NA plates for enumeration. Preliminary studies indicated that all surviving micro-organisms were retrieved from the aqueous fraction and that no viable bacteria or bacterial spores remained in the solvent phase. It was also found that no appreciable concentration of viable micro-organisms was associated with the aqueous/organic solvent interface.

SURVIVAL STUDIES AT 45°C

Identical tests were performed at 45°C by suspending the tubes in a thermostatically-controlled waterbath. This temperature was chosen because it represents the maximum running temperature of any dry cleaning system in which this solvent is used at ambient pressure, as the boiling point of Freon-113 is 47.6°C.

SURVIVAL STUDIES WITH DETERGENTS

The micro-organisms were also exposed in similar systems containing 1.0% of the PDF or HMC detergent with all other factors held constant (the 1.0% concentration is recommended by the manufacturer). Controls were run on identical systems in which the Freon-113 solvent was replaced with NSS in order to observe bactericidal and/or sporicidal properties inherent to the detergents.

Enumeration of survivors from systems containing detergents was performed by adding 10 ml of NSS to the experimental tube, mixing and immediately withdrawing a sample for appropriate dilution and plating. In instances where extensive dilutions were not necessary, i.e. when survival was low, samples were filtered through sterile 0.45 µm cellulose nitrate membrane filters (Nalge Company, Rochester, NY, USA). The filters were then rinsed with NSS to remove residual solvent and detergent and placed on NA plates. These plates were then incubated at 37°C for 18 h for enumeration.

SURVIVAL STUDIES WITH MINIMAL WATER

For studies with minimum water, the bacterial suspension was filtered through sterile 0.45 µm, 25 mm diameter 'Zetapore Membrane' filters (AMF Cuno, supplied by Rainin Company, Woburn, MA, USA), and excess water was removed by aspiration. After aspiration, an average of 55 µl of water was found to be trapped within the membrane filter. Although this associated water does enter the experimental system, the system is considered to contain a minimal amount of water because it is not initially distributed randomly in the solvent mixture. These filters were then directly exposed to the solvent/detergent mixture for varying intervals of time and enumeration was carried out as follows. The membrane filters removed

from experimental tubes were shaken with 100 ml of NSS in dilution bottles and micro-organisms dislodged were enumerated. The number of viable micro-organisms dislodged from the membrane filter during exposure and agitation in the solvent mixture was enumerated in the same manner as tubes containing 0.1% water and detergents.

SURVIVAL STUDIES WITH SOILED SWATCHES

Studies in which the micro-organisms were applied to soiled swatches for exposure to the Freon-113 with detergent were performed in order to more closely simulate dry cleaning laundry conditions. The micro-organisms were applied to swatches at a rate of 100 µl of inoculum per swatch (approximately 1×10^8 organisms). The organisms were counted by treating the soiled swatch and solvent mixture in the same manner as described for filters and solvent in systems with minimal water.

In all experimental systems, disinfection was considered sufficient when an observed reduction in survival exceeded six log cycles (Anon, 1984). All survival curves are plotted as log survivors/ml vs time in minutes and represent averages taken from two, three or four replications of each experiment.

Results

EXPOSURE TO FREON-113 WITH 0.1% WATER

Numbers of viable micro-organisms, in logarithmic growth phase, remaining after contact with Freon-113 for increasing lengths of time are given in Fig. 1 at both room temperature and 45°C. Aside from the observed increase in bacterial kill over time at the elevated temperature with *E. coli* and *Staph. aureus*, it is evident that cells of *E. coli* are much more susceptible to destruction in systems containing Freon-113 with 0.1% water than are *Staph. aureus* or spores of *B. globigii*.

EXPOSURE TO FREON-113 WITH 0.1% WATER AND DETERGENTS

The survival of organisms after addition of detergents at a 1.0% concentration to Freon-

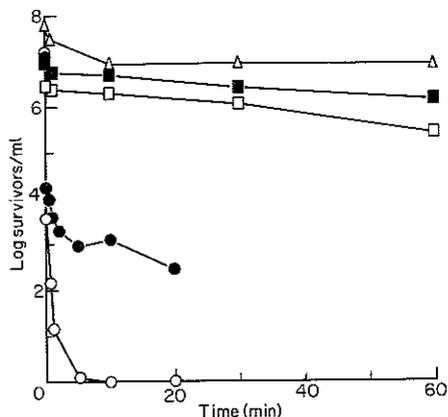


Fig. 1. Survival in Freon-113 with 0.1% water. ●, *Escherichia coli* at room temperature; ○, *E. coli* at 45°C; ■, *Staphylococcus aureus* at room temperature; □, *Staph. aureus* at 45°C; △, *Bacillus globigii* spores at 45°C.

113 with 0.1% water at 45°C is shown in Fig. 2. *Escherichia coli* was effectively reduced at a rapid rate in systems containing either one of the two detergents. However, a residual number of these cells (approx. 1×10^1 or less) were observed to persist throughout the remainder of these exposures.

Numbers of *Staph. aureus* were significantly reduced when either of the two detergents were employed. Complete kill in populations of this organism was seen within 1 min of exposure to

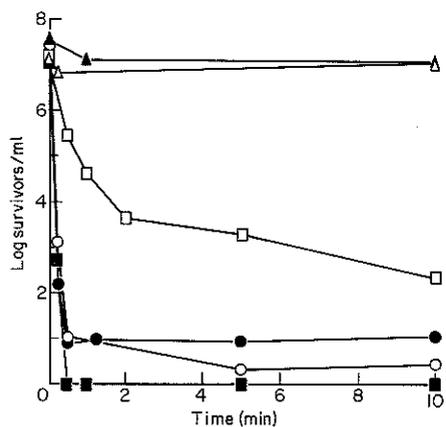


Fig. 2. Survival in Freon-113 at 45°C with 0.1% water and 1.0% detergent. ●, *Escherichia coli* with HMC detergent; ○, *E. coli* with PDF detergent; ■, *Staphylococcus aureus* with HMC detergent; □, *Staph. aureus* with PDF detergent; ▲, *Bacillus globigii* spores with HMC detergent; △, *B. globigii* spores with PDF detergent.

systems containing HMC detergent whereas a significantly reduced rate of kill occurred when PDF detergent was used.

Little reduction in viability was observed when spores of *B. globigii* were exposed in these systems in the presence of either detergent.

EXPOSURE TO FREON-113 WITH DETERGENTS AND MINIMAL WATER

In systems of minimal water where each bacterial species was applied to and exposed upon nylon membrane filters, both *E. coli* and *Staph. aureus* were observed to have similar rates of survival (Fig. 3). Variations in survival appeared to be dependent upon the detergent employed. Experiments with the HMC detergent resulted in increased rates of kill over similar systems where PDF detergent was used.

Exposure of the bacterial spores again resulted in no significant reduction over the length of exposure.

EXPOSURE TO FREON-113 WITH DETERGENTS ON SOILED SWATCHES

Exposure of the vegetative bacterial populations to experimental systems containing detergents after application to soiled swatches is presented in Fig. 4. Rates of bacterial kill were seen to be significantly reduced in these systems when

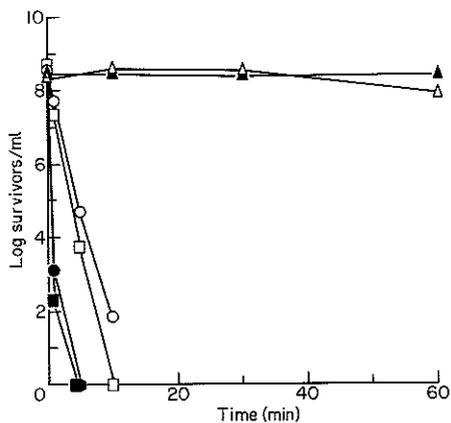


Fig. 3. Survival in Freon-113 at 45°C with minimal water and 1.0% detergent. ●, *Escherichia coli* with HMC detergent; ○, *E. coli* with PDF detergent; ■, *Staphylococcus aureus* with HMC detergent; □, *Staph. aureus* with PDF detergent; ▲, *Bacillus globigii* spores with HMC detergent; △, *B. globigii* spores with PDF detergent.

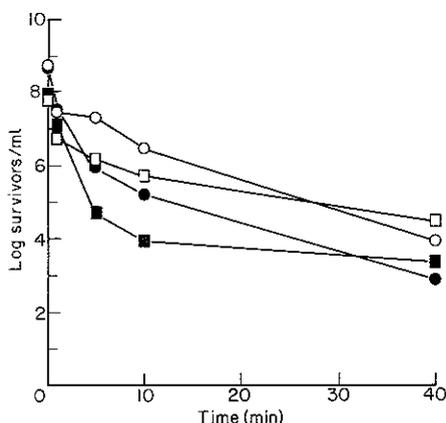


Fig. 4. Survival in Freon-113 with 1.0% detergent when exposed upon soiled swatches. ●, *Escherichia coli* with HMC detergent; ○, *E. coli* with PDF detergent; ■, *Staphylococcus aureus* with HMC detergent; □, *Staph. aureus* with PDF detergent.

compared with those containing detergents where the micro-organisms were not applied to soiled swatches. As was found in exposure studies in systems of minimal water, rates of kill were found to be dependent upon which detergent was employed, with the HMC-type yielding the greater reductions.

Discussion

A review of the results presented reveals that only under some of the conditions studied was there sufficient reduction (six log cycles) in survival of the representative micro-organisms. Table 1 summarizes the survival figures over the range of conditions evaluated. Sufficient reduction in *E. coli* occurred within 1.25 min of exposure in Freon-113 with 0.1% water at 45°C only, in contrast to *Staph. aureus* and *B. globigii* which appeared much less sensitive in this system. This difference in survival may be

related to the relative resistance of spore walls and differences in cell wall compositions of *E. coli* and *Staph. aureus*. The cell wall of *E. coli* is known to contain an abundance of lipoproteins and lipopolysaccharides (Stanier *et al.* 1986) which may render the cell wall of this micro-organism particularly susceptible to the non-polar Freon-113 solvent.

When detergents were added to these systems a significant increase in kill in both *E. coli* and *Staph. aureus* is observed with a six log reduction being reached within 0.5 min in *E. coli* with both detergents and in *Staph. aureus* only where the HMC detergent was used.

A sufficient level of disinfection occurred in systems of minimal water within 5–10 min of exposure of both *E. coli* and *Staph. aureus* when HMC and PDF detergents were used. In soiled swatch studies this efficiency was not observed, however, illustrating that the presence of soiled clothing will reduce the bactericidal efficiency of analogous laundry systems.

The results of these studies indicate that there is the potential for survival of significant levels of micro-organisms in virtually 100% Freon-113. The addition of detergents significantly increased the bactericidal efficiency of the solvent systems against vegetative micro-organisms. These vegetative organisms, however, survived particularly well in the presence of soiled fabric. In addition, bacterial spores were virtually unaffected by treatment under all conditions evaluated.

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Table 1. Time in minutes in which a six log reduction in survivor numbers is obtained under the conditions described

	0.1% water, no detergent		0.1% water and detergents		Minimum water and detergents		Soiled swatch study	
	Room temp.	45°C	HMC	PDF	HMC	PDF	HMC	PDF
<i>Escherichia coli</i>	—	1.25	0.5	0.5	5.0	10.0	—	—
<i>Staphylococcus aureus</i>	—	—	0.5	—	5.0	10.0	—	—
<i>Bacillus globigii</i>	—	—	—	—	—	—	ND	ND

—, Six log reduction not attained; ND, value not determined.

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