NRL Report 6506

BW Decontamination With Hypochlorite

Part 1 - Exploratory Studies: Application to the STOP System

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February 28, 1967

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ABSTRACT

BW-simulant spores on Navy clothing have been killed by alkaline calcium hypochlorite solutions about 35 times faster on a laboratory scale than in previously reported field trials with military clothing under similar conditions. This decontamination process has been adopted for use in the Navy's Shipboard Toxicological Operational Protective System (STOPS).

PROBLEM STATUS

This is an interim report on the problem; work is continuing.

AUTHORIZATION

NRL Problem C08-22
Project SS 500-11749

Manuscript submitted November 3, 1966.

BW DECONTAMINATION WITH HYPOCHLORITE

Part 1 — Exploratory Studies: Application to the STOP System

INTRODUCTION

Essential components of the U. S. Navy's Shipboard Toxicological Operational Protective System (STOPS), as it is installed in USS HERBERT J. THOMAS (DD-833), include facilities for decontaminating personnel who enter the pressurized envelope from topside duty stations (1). In THOMAS' design stage, the requirement for rapid and complete decontamination of as many as several dozen men appeared to demand faster, simpler, and more effective clothing decontamination techniques than those generally available in the Fleet (2). To meet this requirement, studies in both the biological-warfare (BW) and chemical-warfare (CW) decontamination of Navy clothing were undertaken at this Laboratory.

The point of departure for the NRL STOPS studies on BW decontamination of clothing is the method given by the Bureau of Ships* Technical Manual for Fleet use (2). For non-rubber clothing, the manual specifies a standard low-temperature laundering in a 0.3-percent solution of commercial high-test calcium hypochlorite. The solution contains 0.2 percent or 2000 parts per million (ppm) of pure calcium hypochlorite or available chlorine. This method is itself based on the comprehensive 1959 report by Portner, Mayo, and Surkiewicz of the U. S. Army Biological Laboratories at Fort Detrick (3). These authors describe cooperative trials with the Quartermaster Corps on the BW decontamination of Army clothing at Fort Lee, Virginia in 1954. The Fort Lee trials explored the decontamination effectiveness of standard and modified laundry procedures, and also a simplified soaking procedure, as applied to standard military clothing items made of six different fabrics.

Either standard or modified laundry procedures, such as those used in the Fort Lee trials, for the BW decontamination of clothing would be objectionable on THOMAS, if not unacceptable, because of the extra work it would impose on the ship's laundry. In addition, the Fort Lee trials indicate that handling of BW-contaminated clothing in a ship's laundry would almost certainly result in spread of contamination to laundry operators and the laundry compartment, and possibly to other parts of the ship. Clearly, decontamination of BW-contaminated clothing in THOMAS' laundry would significantly degrade the very protection that the ship was designed to provide.

One of the Fort Lee decontamination methods did appear attractive, however, for adaptation to the STOPS situation, except for the excessive time required — about three hours. This method is a simple soaking at room temperature in an alkaline solution of hypochlorite and a detergent. The method is designated as a "pre-dip" in the Fort Lee report because it is viewed as a treatment which can precede a standard laundering in the case of clothing requiring BW decontamination. By this approach, the decontamination and laundry operations can be separated, and the laundry personnel and facilities are protected from contamination.

One of the six fabrics represented in the Fort Lee trials, a cotton-nylon mixture, was also of particular interest to the STOPS clothing decontamination studies, since the latter were to be done with a similar Navy fabric. The Navy cotton-nylon fabric had been selected

*Now the Naval Ship Systems Command.

earlier by the Naval Supply Systems Command for a developmental work-combat uniform intended for eventual Navy-wide use. The work-combat uniform was also to be given its principal Fleet trials on THOMAS. The STOPS clothing decontamination studies, therefore, were directed toward both the immediate needs of the STOP system, and the longer range needs of the entire Fleet.

The gross composition of the two cotton-nylon fabrics is identical — 50 percent each of cotton and nylon. However, in the Army fabric the fibers are segregated: the warp and filling yarns are all-cotton and all-nylon, respectively. In the Navy fabric, cotton and nylon fibers are intimately blended in both sets of yarns. The similarities in the two fabrics are believed to be sufficient to validate direct comparison of the Fort Lee and the STOPS studies.

Table 1 summarizes the results of the Fort Lee trials on the BW-decontamination effectiveness of the pre-dip process applied to cotton-nylon garments. The solution in which the garments were soaked contained sufficient calcium hypochlorite to give an available chlorine concentration of 2000 ppm. A standard Army laundry detergent was used at 0.29-percent concentration, and the fabric-to-solution ratio was 1 to 2.2 by weight. Table 2 reports the effects of the treatment on the fabric itself.

Table 1
Bacteriological Results of Fort Lee Trials on BW Decontamination of Cotton-Nylon Clothing by the Pre-Dip Procedure

Treatment* Time (hr)	Hypochlorite Concentration (%)	pH	Surviving Spores per sq cm	Surviving Spores (%)	Decontamination Effectiveness (%)
0	0.185	9.3	25900†	100	0
1	0.096	9.2	658	2.54	97.5
2	0.049	9.0	132	0.51	99.5
3	0.016	8.9	20	0.077	99.92

*Twelve lb of clothing was soaked at ambient temperature in 16-gallon garbage cans containing 3.2 gallons of water, 1.2 oz of a standard laundry detergent, and sufficient commercial 70-percent calcium hypochlorite to give an available chlorine concentration of 2000 ppm or 0.2 percent.
†Each number in this column is based on four bacteriological assays, each of which was done on a lumped sample of ten patches removed from ten locations on one set of clothing. Two sets of clothing were decontaminated together in one trial, and another two sets in a duplicate trial.

With respect to the other fabrics used in the Fort Lee trials, three general levels of effectiveness were obtained in the pre-dip process:

1. Cotton poplin and water-repellent cotton sateen were decontaminated about as readily as the cotton-nylon.

2. Cotton poplin and cotton sateen, when impregnated for chemical warfare protection, were decontaminated much more readily than cotton-nylon. A 1-hour hypochlorite soak was over 100 times as effective in decontaminating impregnated cotton garments as unimpregnated cotton or cotton-nylon items.

3. Wool shirting and wool-nylon serge were decontaminated to only a minor degree (about 50 percent) even after a 3-hour soak.

Table 2
Fabric and Color Degradation Results of Fort Lee Trials on BW Decontamination of Cotton-Nylon Clothing by the Pre-Dip Process

Treatment	Reflectometer Reading	Cotton Warp Strength (lb)	Nylon Filling Strength (lb)
None	7.5 } change† of +3.6 units(absolute)	158 } change‡ of -4.4%(relative)	86 } change‡ of +8.1%(relative)
Soak*	11.1	151	93

*Time not stated.
†U. S. Army Natick Laboratories Textile Series Report 155 of December 1955 states that a reflectance change of 2 percent absolute renders a garment unacceptable in most camouflage situations, according to Corps of Engineers recommendations.
‡The above Natick Laboratories report states that a 20-percent loss of strength in either warp or filling drastically impairs utility, and a 50-percent strength loss renders the item useless.

The results of the Fort Lee pre-dip process as applied to all six types of clothing are illustrated on a semilogarithmic graph in Fig. 1. The data for the cotton-nylon and

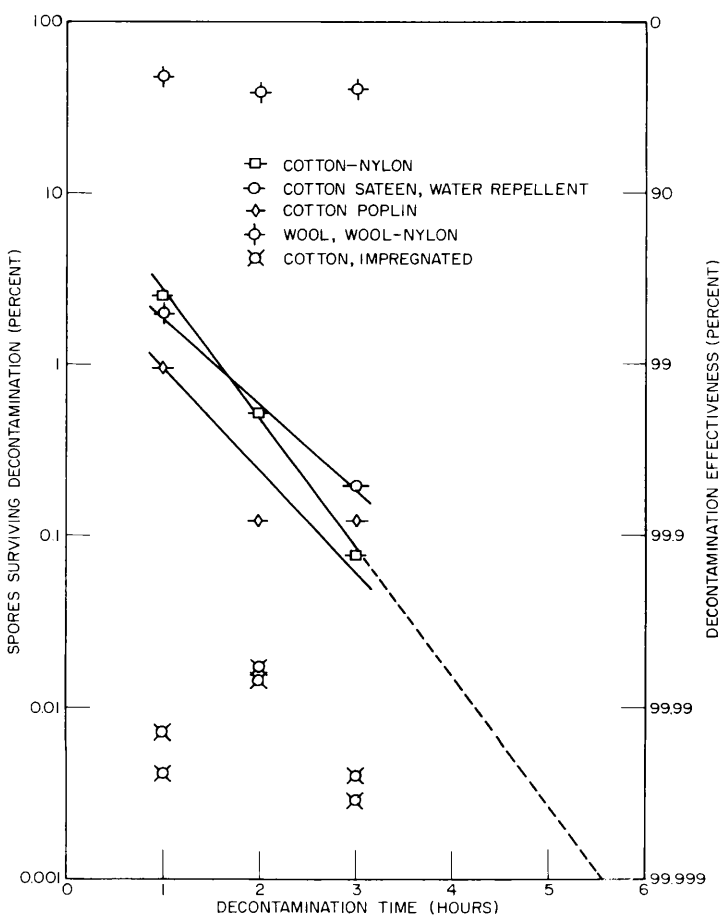

Fig. 1 - BW decontamination of clothing in Fort Lee trials using pre-dip procedure

unimpregnated cotton clothing show a marked degree of consistency in their conformity to a straight-line plot. The straight-line plots indicate a logarithmic death rate for the spores — a relatively common situation in bactericidal kinetics often designated as Chick's Law (4, p. 15 ff.). The initially accelerated death rate for the spores is unusual but not unique (4, p. 16). It could be attributed to an exceptionally rapid initial killing of the most exposed spores, i.e., those on the surface of the fabric.

Among the conclusions drawn by Portner, et al. (3) is: "Soaking clothing in chlorine solution can be recommended only as an emergency measure since this procedure causes considerable damage to fabrics." This statement is evidently based on a supplementary investigation made at the Philadelphia Quartermaster Depot, the report of which is combined with that of the Fort Lee studies. The pertinent part of these results is given in Table 2. It is clear that the fabric damage mentioned by Portner, et al. refers not to loss of fabric strength, but to the extensive bleaching indicated by the increased reflection readings. Thus, there is no reason to expect significant loss of strength as the result of a single pre-dip treatment applied to the Navy work-combat uniform. Substantial bleaching of the Navy work-combat uniform during a single decontamination treatment by hypochlorite appears also to be unlikely in view of the hypochlorite bleaching requirement incorporated in the specifications for the fabric.

EXPERIMENTAL

Materials

Bacterial Spores — The first NRL experiments used suspensions of *Bacillus subtilis* var *niger* (formerly designated *Bacillus globigii*) suspended in Freon 12 pressurized in a standard aerosol spray can.* This source was later replaced by a fresh aqueous paste supplied through the courtesy of the Army Biological Laboratories, Fort Detrick, Maryland.

Fabric — New Navy work-combat garments were washed, using "All" detergent, and rinsed in a home automatic washer. One-inch discs, or 1- by 2-inch rectangles, were then removed for the contamination and decontamination procedures.

Decontamination Solutions — Solutions generally contained 0.2-percent (ca. 0.057N) calcium hypochlorite, added as 0.3 percent of the 70-percent commercial product, and 0.5 percent of the Navy NBC decontamination detergent. See Appendix A for the significant characteristics of these materials.

Hypochlorite Neutralization — Immersion and shaking in 15 ml of a 0.4-percent (0.016N) solution of sodium thiosulfate pentahydrate was used to terminate the decontaminating action of the residual hypochlorite solution on fabric samples when they were removed from a hypochlorite bath.

General Procedures

Sterilization — All materials and equipment which might transfer unwanted contamination were sterilized by autoclaving prior to each experiment. This included glassware, fabric samples, culture media, hypochlorite neutralizer, etc.

Contamination — For each experiment an array of sterilized cloth discs (1 in. in diameter) or rectangles (1 in. × 2 in.); stapled to cardboard and autoclaved, were contaminated in one of two ways: by two single passes from a *B. subtilis* var *niger* pressurized aerosol can at a distance of 12 inches, or by an equivalent treatment in which a No. 15 Devilbiss nasal atomizer was used to spray an aqueous suspension of spores at an air

*Dispenser, Simulated Agent, BG1, 12 oz., ANM1, containing 2 g *Bacillus globigii* and 300 g Freon 12.

pressure of 20 psi. Contamination was done on the roof of the laboratory building to reduce the probability of contamination in the culture and counting laboratories.

Decontamination — In most experiments, the contaminated fabric discs were given decontamination treatments in 30-ml beakers containing 5 ml of decontamination solution. No stirring was used, except for an occasional agitation to insure initial wetting of the disc. Based on a disc weight of about 150 mg, the fabric-to-solution ratio was about 1 to 33. One departure from this typical procedure will be mentioned later.

Chemical Anaylsis — The hypochlorite-neutralizing sodium thiosulfate solution was standardized against weighed portions of potassium iodate. A 0.05 or 0.1N sodium thiosulfate solution, standardized in the same way, was used to titrate many of the decontaminating solutions in order to assure the desired initial hypochlorite concentration and to determine its decrease in concentration during the decontamination process. The pH of the decontaminating solutions was also generally checked before and after use.

Plating Technique and Counts — The surface count technique described by Miles and Misra (5) was utilized, except that the agar plates (nutrient agar (Difco) plus 0.5 percent dextrose) were dried for approximately 48 hours at 37.5°C with Petri dishes inverted and the lids closed. After the drying period, the agar plates absorbed a 0.2 ml drop within 15 to 20 minutes.

Calibrated 100-microliter capillaries ("Microcaps," Kensington Scientific Corp.) were used in conjunction with a Syringe Microburet Model No. SB2 (Micro-Metric Instrument Co.) to deliver reproducible drop volumes of 0.024 ml onto the surface of the agar from a height of about 2.5 cm. The sterilized microcapillaries were supported vertically by means of the microcapillary holder.

Four to six serial dilutions were made using 0.1-percent sterile peptone water for each disc run through the experimental procedure. From each serial dilution, three drops were deposited on the agar surface sufficiently separated to prevent coalescence. Thus the total volume plated of each serial dilution was 3×0.024 or 0.072 ml. After the drops were absorbed into the agar, the plates were immediately placed in inverted positions in the 37.5°C incubator.

Colony counts were made after 24 hours. Recounting after 48 hours showed no significant difference. The counts were made with a Quebec counter or, for higher counts (over 50/drop), by placing the Petri dishes on an opaque projector and counting the magnified image of the colonies on a white background 10 to 15 feet away.

Individual Experiments — Procedures and Results

Experiment No. 1: Decontamination with Alkaline Hypochlorite; Variations in Treatment Time, Hypochlorite Concentration, and Detergent Concentration — About 7.5×10^6 spores were applied to each of sixteen fabric discs, using two spray passes over the array with the aerosol can. This resulted in a contamination density of about 1.5×10^6 spores per sq cm. Four of these discs were control discs which were carried through the plating and counting procedures without subsequent decontamination in order to determine the degree of contamination. Two additional uncontaminated, or blank, discs were carried through the same procedure as indicators of possible inadvertent contamination in laboratory manipulations. The remaining twelve discs were used in duplicate to study decontamination treatments. Both the calcium hypochlorite and the detergent concentrations were varied as shown in Table 3. The temperature of the solution during this experiment was 26° to 27°C. No colonies were found in any of the cultures made from the twelve decontaminated discs or from the two blank discs, and the decontamination was considered to be essentially complete.

Table 3
Hypochlorite Decontamination of B. subtilis var niger Spores on Navy Uniform Clothing (Experiment No. 1)

Disc No.	Treatment Time (min)	Hypochlorite Concentration (%)	Detergent Concentration (%)	Spores Surviving (per disc)	Decontamination Effectiveness
7A,* 7B* 8A,* 8B*	0	0	0	7.5×10^6	None*
1A, 1B	15	0.2	0.5	None	Apparently Complete
2A, 2B	60	0.2	0.5	None	Apparently Complete
3A, 3B	150	0.2	0.5	None	Apparently Complete
4A, 4B	60	0.2	0	None	Apparently Complete
5A, 5B	60	0.7	0	None	Apparently Complete
6A, 6B	60	0.7	0.5	None	Apparently Complete

*Controls.

Experiment No. 2: Decontamination with Alkaline Hypochlorite — Experiment No. 2 was intended primarily as a check on the unexpectedly high decontamination effectiveness found in Experiment No. 1; also, one additional variation was added. Because of the possibility that the large fabric-to-solution ratio was strongly influencing the results in the direction of increased effectiveness, one disc was decontaminated at a fabric-to-solution ratio comparable with that used in the Fort Lee trials. This disc, after contamination, was placed in a stack with four discs above and four below. The stack was compressed, while dry, by a weight of 100 g, or about 0.28 psi. The stack was immersed in the decontaminating solution for the prescribed time, and the solution then drained away before removing the center disc. The results for all discs are given in Table 4. As in the previous experiment, decontamination was apparently complete under all conditions.

A stack of discs, identical with the above set, was saturated and the water then drained away. Based on the individual disc weights before and after saturation, the average water pickup was 115 percent, ranging from 104 to 122 percent. The average fabric-to-water ratio in the saturated discs was thus 1:2.15. This is identical with the fabric-to-water ratio used in the pre-dip process of the Fort Lee trials.

Table 4
Hypochlorite Decontamination of B. subtilis var niger Spores on Navy Uniform Clothing (Experiment No. 2)

Disc. No.	Treatment Time (min)	Hypochlorite Concentration (%)	Detergent Concentration (%)	Spores Surviving (per disc)	Decontamination Effectiveness
7A*, 7B*, 8A*, 8B*	0	0	0	9.6×10^5	None
1A, 1B	15	0.2	0.5	None	Apparently Complete
2A, 2B	60	0.2	0.5	None	Apparently Complete
3A, 3B	240	0.2	0.5	None	Apparently Complete
4A, 4B	60	0.2	0	None	Apparently Complete
5A, 5B	60	0.7	0	None	Apparently Complete
6A, 6B	60	0.7	0.5	None	Apparently Complete
16†	60	0.2	0.5	None	Apparently Complete

*Controls.
†During decontamination, this disc was in the center of a vertical stack of nine discs under a force of 100 g, or 0.28 psi.

Experiment No. 3: Decontamination with Alkaline Hypochlorite; Variations in Treatment Time, Source of Spores, and Heat Shocking — This experiment was designed primarily to test (a) the decontamination resistance of the 10-year-old Freon suspension of spores in comparison with fresh spores from Ft. Detrick, and (b) the effects of heat-shocking spores before and after decontamination. The data are presented in Table 5. It is evident that there is little or no difference produced by heat shocking before or after decontamination. However, it is also clear that the fresh spores are markedly more resistant to the decontamination process than are those in the old Freon suspension. Consequently, fresh spores were used in all subsequent experiments. The results also served to bracket the time required for complete decontamination as in excess of 2 minutes but not greater than 15 minutes. These figures apply only to the particular solutions, temperature, and other conditions of this experiment.

Table 5
Hypochlorite Decontamination of *B. subtilis* var *niger* Spores on Navy Uniform Clothing (Experiment No. 3)

Disc No.	Treatment Time (min)	Hypochlorite Concentration (%)	Detergent Concentration (%)	Spore Suspension	Heat Shock	Spores Surviving (per disc)	Decontamination Effectiveness
1-0*	0	0	0	Freon; Old	After Spraying†	9.0×10^6	None*
2-0	2	0.2	0.5	Freon; Old	After Decontam.	1.0×10^6	99.9%
3-0	15	0.2	0.5	Freon; Old	After Decontam.	None	Apparently Complete
1-N*	0	0	0	Aqueous; Fresh	After Decontam.	37×10^6	None*
2-N	2	0.2	0.5	Aqueous; Fresh	After Decontam.	21×10^6	43%
3-N	15	0.2	0.5	Aqueous; Fresh	After Decontam.	None	Apparently Complete
4-N*	0	0	0	Aqueous; Fresh	Before Decontam.	22×10^6	None*
5-N	2	0.2	0.5	Aqueous; Fresh	Before Decontam.	7.6×10^6	66%
6-N	15	0.2	0.5	Aqueous; Fresh	Before Decontam.	None	Apparently Complete

*Controls.
†Spores heat shocked after extraction from disc.

At this point, the specifications for the clothing decontamination process on THOMAS were "frozen." The key specifications were for a 15-minute soak at room temperature in a solution of 0.2-percent calcium hypochlorite (0.3 percent of the common 70-percent pure product, see Appendix B) and 0.5 percent of the Navy NBC detergent. Experimentation continued, however, with the aim of further improvements.

The U. S. Navy Supply Research and Development Facility (NAVSUPRESDEVFAC) determined that the STOPS decontamination treatment, followed by extraction, rising, and drying in the ship laundry, provided an amount of soil removal which was acceptable under emergency conditions. The report is reproduced as Appendix B.

Experiment No. 4: Decontamination with Alkaline Hypochlorite; Variation in Treatment Time and Hypochlorite Concentration — This experiment was designed to explore variations in treatment time between 2 and 15 minutes, and the feasibility of a reduction in hypochlorite concentration.

The data are given in Table 6. It is shown that decontamination is only 96 percent complete after a 4-minute treatment at a hypochlorite concentration of 0.2 percent — the concentration used in all prior experiments. An 8-minute treatment, or longer, resulted in apparently complete decontamination at this concentration, however.

When the hypochlorite concentration was reduced tenfold, while the detergent concentration was held at 0.5 percent, decontamination was incomplete at treatment times of 4, 8, or 15 minutes.

As an incidental part of the experiment, two discs were included in which the fabric had been treated with the chloroamide CC-2 for CW protection.

Table 6
Hypochlorite Decontamination of B. subtilis var niger Spores on Navy Uniform Cloth (Experiment No. 4)

Disc No.	Treatment Time (min)	Hypochlorite Concentration (%)	Detergent Concentration (%)	Spores Surviving (per disc)	Decontamination Effectiveness
16,17*	0	0	0	70×10^6	None
1	4	0.2	0.5	3.0×10^6	95.7%
5	8	0.2	0.5	0	Apparently Complete
9	15	0.2	0.5	0	Apparently Complete
13	15	0.2	0.5	0	Apparently Complete
14†	15	0.2	0.5	0	Apparently Complete
15†	15	0.2	0.5	0	Apparently Complete
3	4	0.02	0.5	10.6×10^6	85%
7	8	0.02	0.5	7.8×10^6	89%
11	15	0.02	0.5	2.0×10^6	97%

*Controls.
†Fabric impregnated with CC-2 for CW protection.

Experiment No. 5: Decontamination with Alkaline Hypochlorite; Treatment of Whole Garments for Various Times in a Mockup USS THOMAS Decontamination Center — The principal purpose of this experiment was to extend the scale of the items treated from 1-inch discs to entire garments. Rectangular fabric swatches, 1 in. × 2 in., were stapled to cardboard and contaminated by spraying, as had been done previously for the 1-in. discs. After drying, some of the swatches were stapled over rectangular holes in a work-combat hood, shirt, or trousers. Other swatches were inserted in the regular pockets of trousers or shirt, or in a pocket formed in the hood by stapling. Placement of swatches in pockets was to determine the effectiveness of decontamination of spores which might be deposited in semiprotected areas of the garments. Treatment times varied from 4 to 10 minutes. Each individual garment was decontaminated in a separate 13-gallon solution using duplicates of the THOMAS' decontamination tanks. The results are shown in Table 7.

Table 7
Hypochlorite Decontamination of B. subtilis var niger Spores on Navy Uniform Cloth (Experiment No. 5)

Disc No.	Treatment Time (min)	Hypochlorite Concentration (%)	Detergent Concentration (%)	Spores Surviving (per disc)	Decontamination Effectiveness
1, 2*	0	0.2	0.5	34×10^6	None
3	4.25	0.2	0.5	0	Apparently Complete
4	4.25	0.2	0.5	0	Apparently Complete
5	5	0.2	0.5	0	Apparently Complete
6	8	0.2	0.5	0	Apparently Complete
7	8	0.2	0.5	0	Apparently Complete
8	10	0.2	0.5	0	Apparently Complete

*Controls.

DISCUSSION

Although this report is intended primarily to present the results of exploratory studies, it is desirable that major questions raised by the data be acknowledged and discussed, and answered if possible. Two such questions are:

1. Is it possible to assign numerical and realistic figures to those NRL results in which the decontamination effectiveness has been described as "apparently complete?"

2. Can a satisfactory explanation be found for the wide disparity between the decontamination effectiveness given by the Fort Lee pre-dip decontamination process and that given by the apparently similar NRL STOPS decontamination process?

Statistical Treatment of "Apparently Complete" Decontamination Effectiveness in NRL Experiments

On many occasions the NRL experiments have resulted in "apparently complete" decontamination of a fabric disc (Tables 3-7). In actual fact, however, the bacteriological assays were made on only a very small measured part (aliquot) of the 15 ml of sodium thiosulfate solution in which fabric discs were shaken to release adhering spores. Specifically, 1 ml of the thiosulfate solution was first diluted to 10 ml and, of the diluted solution, a 0.072-ml aliquot was plated onto nutrient agar for growth and counting of colonies. This means that, in effect, only 0.0072 ml of the original 15 ml, or about 1/2100, was assayed for live spores. Obviously, a considerable number of live spores could be present in the original 15 ml and yet none be present in the 1/2100 aliquot, with the result that decontamination could be "apparently complete."

Statistical treatments are available by which numerical values can be assigned to the decontamination effectiveness in such cases. When the number of samples taken for bacteriological assay is small, and the chance of finding a live spore is small, the Poisson distribution has been found by experience to represent the facts well (6). The following analysis is based on the Poisson distribution. Let λ be the average number of spores per ml of diluted thiosulfate solution, and V be the volume of an aliquot of the same solution. Then the probability P of finding <u>at least one spore</u> in V is

$$P = 1 - \exp(-\lambda V).$$

If the desired probability of a correct estimate is set at 0.95 (i.e., 95 percent, a common choice), then 0.95 is substituted for P. Since V is known, the equation can be solved for λ. When <u>no</u> spores are in fact found by bacteriological assay of the aliquot volume V, there is a 95-percent probability* that the number of spores per ml of the thiosulfate solution is less than λ. A numerical solution of the equation gives

$$0.95 = 1 - \exp(0.072\,\lambda)$$

from which

$$\lambda = 42.$$

That is, an "apparently complete" decontamination result indicates, with 95-percent probability, for the conditions stated, that there were less than 42 live spores per ml of the 1:10 diluted solution from which the aliquot was taken. Further, there were less than

*Technically, the "fiducial" probability.

$10 \times 15 \times 42$, or 6300, live spores in the original 15 ml used to detach residual spores from the decontaminated disc. If the number of spores originally deposited on the disc was 10^7, the spores surviving are less than

$$6300 \times 100/10^7 = 0.06 \text{ percent.}$$

This is equivalent to a decontamination effectiveness of greater than 99.94 percent.

Using this percentage in place of "apparently complete," the NRL decontamination results, for individual treatments of 15 minutes and less, are collected in the next to last column of Table 8, headed Single Assays. However, there is still unused information in the data. This results from the fact that most of the assays are replicates. Thus, they reinforce or tend to confirm each other. The simplest way to take advantage of the replication aspect is to change the volume of V, in the preceding calculation, to include the total volume assayed in each set of replicate assays. This has been done, and the results are shown in the last column of Table 8, headed Lumped Assays. The same data are plotted in Fig. 2, in which the arrows attached to plotted points signify "less than."

Although it is evident that the lines drawn in Figs. 1 and 2 only approximate the actual rates of decontamination, it is of interest to compare them. The times required to reduce the number of live spores by a factor of ten, or one "log," is 80 minutes for the Fort Lee procedure and 1 minute for the NRL procedure. Another comparison is the total time required to reach a decontamination effectiveness of 99.94 percent. In the Fort Lee case, it is 192 minutes; in the NRL case, 5 minutes. This is about a 35-fold improvement in decontamination time.

Table 8
Hypochlorite Decontamination of B. subtilis var niger Spores on Navy Uniform Clothing (Summary of Experiments 3, 4, and 5)

Experiment No.	Disc No..	Treatment Time (min)	Hypochlorite Concentration (%)	Detergent Concentration (%)	Decontamination Effectiveness (%)	
					Single Assays	Lumped Assays
3	2-N	2	0.2	0.5	43	-
3	5-N	2	0.2	0.5	66	-
4	1	4	0.2	0.5	95.7	-
5	3	4.25	0.2	0.5	99.94+*	99.97+
5	4	4.25	0.2	0.5	99.94+	
5	5	5	0.2	0.5	99.94+	-
4	5	8	0.2	0.5	99.94+	99.98+
5	6	8	0.2	0.5	99.94+	
5	7	8	0.2	0.5	99.94+	
5	8	10	0.2	0.5	99.94+	-
3	3-N	15	0.2	0.5	99.94+	99.984+
3	6-N	15	0.2	0.5	99.94+	
4	9	15	0.2	0.5	99.94+	
4	13	15	0.2	0.5	99.94+	

*The percentages with a "+" appended are, at a 0.95 probability, minimum figures of decontamination effectiveness. The actual effectiveness may be greater. In the case of treatment times greater than 5 minutes, the actual effectiveness almost surely is greater than the figures shown.

Review of Disparity in Effectiveness of Fort Lee and NRL Decontamination Processes

No completely satisfactory method for the BW decontamination of clothing under operational conditions is presently available to the U. S. Armed Forces. A U. S. Army Technical Manual (7) covering the subject cites the use of gaseous ethylene oxide, Carboxide, or methyl bromide; boiling in water at least 30 minutes; or soaking at room temperature for 30 minutes in 0.16-percent sodium hypochlorite. In addition, the same reference states that Quartermaster Direct Support Units will normally decontaminate clothing — presumably by the Fort Lee modified laundry process. Although it is in effect a standard for the U. S. Armed Forces, this process is not highly regarded by its developers "..... since complete sterilization is not achieved, and logistical and safety problems arise ..... Alternative treatments are not available at this time, however, to the troop commander" (3). The U. S. Air Force BW/CW Decontamination Manual (8) recommends the same procedures as does the Army. Navy shipboard clothing decontamination follows essentially the Army (Fort Lee) modified laundry method, but gaseous or vapor-phase decontaminants are not approved for use at sea (2). The Marine Corps can be expected to follow Army procedures. It is evident, therefore, that a BW clothing decontamination process which promises, even on a laboratory scale, to improve substantially the effectiveness of a widely used U. S. military process should be given serious consideration.

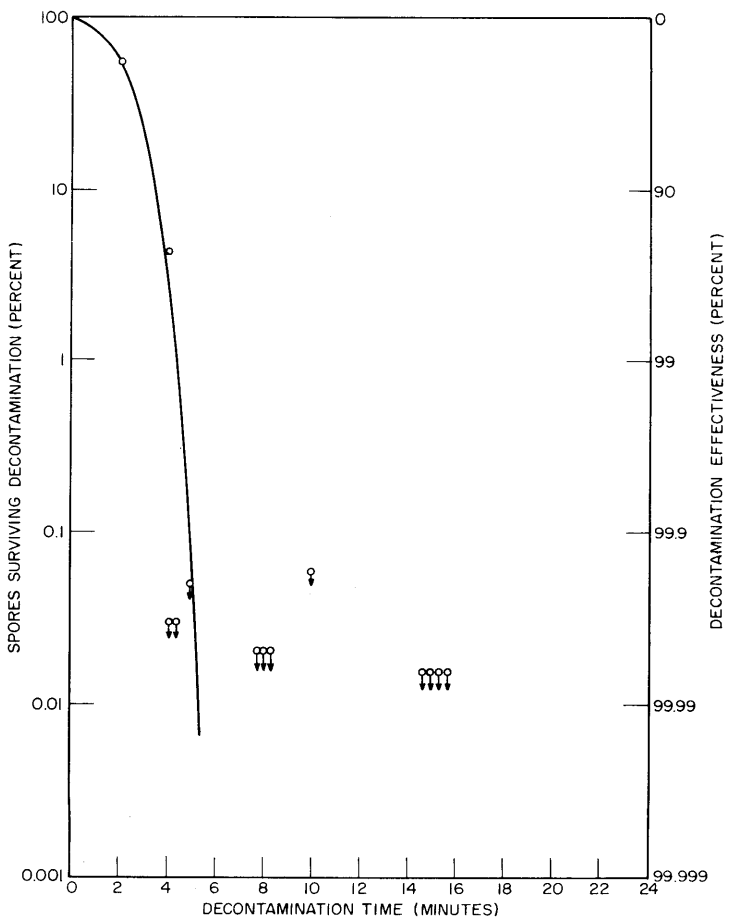

Fig. 2 - BW decontamination of clothing in NRL experiments using the STOPS procedure

There appears to be no reason to doubt the validity of either the Fort Lee or the NRL decontamination results; both are assumed to be correct for the conditions under which the work was done. However, it is also apparent that the two sets of data cannot be accepted without reservation until a satisfactory explanation can be given for the disparity between them.

A number of possible explanations for this disparity can be suggested. These include:

1. The Fort Lee clothing was preworn or soiled; the NRL fabric was prelaundered.

2. The Fort Lee clothing was contaminated with a dry, fine aerosol (all particle diameters probably less than 10 microns, based on settling time); the NRL fabric was contaminated with a wet, relatively coarse spray.

3. The degree of contamination of the Fort Lee clothing was in the range of 25,000 spores per sq cm; the NRL fabric was contaminated with about 2,000,000 spores per sq cm.

4. The Fort Lee pre-dip procedure treated 12 lb of clothing in a batch; the NRL experiments were with individual 150-mg fabric discs.

5. The Fort Lee pre-dip decontamination was done in zinc-coated garbage cans; the NRL decontamination containers were glass beakers.

6. The clothing which was decontaminated at Fort Lee was bleached significantly by hypochlorite decontamination; the change in color caused by decontamination of the Navy work-combat uniform is slight. The dye in the Fort Lee clothing therefore may have acted as a hypochlorite neutralizer by chemical reduction.

7. The temperature of the Fort Lee pre-dip solutions, while not stated, is assumed to be in the range of 10° to 25°C, based on October seasonal air and water temperatures and an indeterminate degree of heating of the vacant building used; the temperature of the NRL decontamination solution was 25° to 28°C.

8. The Fort Lee data on spore survival in Table 1 are each an average based on forty fabric samples, assayed in batches of ten samples each; the NRL data are for single fabric samples.

9. The Fort Lee clothing was worn for an hour's exercise after contamination; the NRL fabric received no comparable treatment.

10. The laundry detergent used in the Fort Lee trials comprised a nonionic surfactant and an "inert" carrier; the NRL standard NBC decontamination detergent is a complex mixture of a nonionic surfactant with a number of "builders" or detergency aids.

11. The Fort Lee pre-dip procedure used a fabric-to-solution ratio of 1 to 2.2. The NRL ratio was generally 1 to 33.

Factors 1 through 7 above are all genuine and obvious differences between the Fort Lee and NRL studies. None of them, however, is considered to be significant enough to account for the major difference observed in the results at Fort Lee and NRL.

Factor 8 is estimated to represent a somewhat higher probability of explaining the relatively low effectiveness of the pre-dip process. For the explanation to be valid, it must be assumed that an occasional patch, of the forty accounting for each bacteriological assay reported, was decontaminated to only a moderate degree, say 50 percent. Even though the

other thirty-nine patches were 100 percent decontaminated, the average percentage decontamination would be found to be 100-(1/39 × 50) or 98.7 percent. The incomplete decontamination of an occasional patch could reasonably be related to the abnormally high fabric-to-solution ratio (factor 11), or to intimate contact with the reactive zinc (galvanized) surface of the containers used (factor 5).

Factor 9 also represents a reasonable possibility of accounting for the relatively low effectiveness of the pre-dip process. The reasoning is that some of the spores, which were originally deposited on the outer surface of the clothing, were moved to the interior of the fabric as a result of friction, flexure, etc. during the hour's exercise following contamination of the clothing. It is conceivable that, in such a location, spores would be partially protected from the action of the decontaminant and that the rate of decontamination would consequently be decreased.

Factor 10 may provide a basis for much or all of the above-mentioned difference. The standard Army laundry detergent used in 1954 is understood to have contained 15-percent liquid nonionic surfactant and 85-percent "inert" carrier, the latter being either urea or borax. A quartermaster study done by Campbell and Medde in 1955 to investigate the effects of hypochlorite on military garments evidently used the urea-containing detergent, for they report "violent foaming" when hypochlorite bleach and detergent were mixed (9). Although the reason for the foaming is not discussed by the authors, it is almost certainly the known reaction of urea and strongly alkaline hypochlorite to form both nitrogen and carbon dioxide (10) as follows:

$$2\ O{=}C(NH_2)_2 + 3Ca(OCl)_2 \longrightarrow 2N_2 + 2CO_2 + 3CaCl_2 + 4H_2O$$

or

$$O{=}C(NH_2)_2 + 3NaOCl \longrightarrow N_2 + CO_2 + 3NaCl + 2H_2O.$$

That Campbell and Medde's solution was indeed strongly alkaline follows from the fact that they used a commercial sodium hypochlorite solution (11). This solution was very probably in the commercial concentration range of 10 to 20 percent, since it was the stock solution from which distributions to 2-percent sodium hypochlorite were prepared. For such solutions, or even those of lower concentrations, to show the stability stated by Campbell and Medde — essentially no change in concentration over a 12- or 14-day period — excess sodium hydroxide is required (11).

The lack of foaming, together with the retention of available chlorine, which characterized the Fort Lee pre-dip solution are also compatible with a solution containing both hypochlorite and a urea-containing detergent, providing the solution is not highly alkaline. Various reactions are possible between hypochlorite and urea to form relatively stable compounds of reduced bactericidal activity.

Such a reaction to form dichlorourea $O{=}(NHCl)_2$ has been studied extensively by Chattaway (12-14). He states that the essential character of the decomposition of dichlorourea is hydrolysis to form carbon dioxide and monochloramine,

$$O{=}C(NHCl)_2 + H_2O \longrightarrow CO_2 + NH_2Cl,$$

followed by interaction between the resulting compounds. In strong alkali, the following reaction occurs quantitatively according to Chattaway:

$$3\ O{=}C(NHCl)_2 + 12KOH \longrightarrow 3K_2CO_3 + 2NH_3 + 6KCl + 2N_2 + 6H_2O.$$

The nitrogen is liberated with "violent effervescence," probably the situation encountered by Campbell and Medde. If pure dichlorourea is dissolved in water, the hydrolysis occurs slowly at ordinary temperature, but becomes very rapid at about 30°C, according to

Chattaway. This conclusion follows from the polymolecular order of the reaction. That is, since the decomposition involves two or more molecules of monochloramine reacting together, the lower the concentration, the slower is the rate of decomposition. In addition, in a common procedure for water purification, ammonia and chlorine are added to a water supply to form monochloramine; the latter then provides a weaker but more prolonged bactericidal action than does chlorine itself:

$$Cl_2 + NH_3 \longrightarrow NH_2Cl + HCl.$$

Subsequent to the work of Chattaway, Detoeuf (15), and Béhal and Detoeuf (16) showed that monochlorourea is readily formed by the action of chlorine on urea in aqueous solution. The compound was studied primarily as a controlled source of hypochlorous acid with which to form chlorohydrins from olefins. It is noteworthy that Detoeuf states that a neutral solution of monochloramine "reacts too slowly" to serve as a source of hypochlorous acid in his chlorohydrin synthesis (a slightly acid solution was found best).

The above summary shows that a hypochlorite bleach and urea in an approximately neutral solution can react to form monochloramine and/or monochlorourea. Either of these possesses all the titratable available chlorine of the original hypochlorite, but acts chemically and bactericidally much more slowly then the latter. The slow bactericidal action of monochloramine is well reported in the literature. Allen (17), citing Weber et al. (18), shows that the 99-percent kill time of 25-ppm chlorine against spores of B. metiens is extended from 3 minutes to 84 minutes by the addition of 18-ppm ammonia (B. metiens is a close relative of B. subtilis var niger). In other studies, Fair, et al. state "For spores of B. anthracis, dichloramine was relatively less efficient [than hypochlorous acid, HOCl], having only about 15 percent of the disinfecting ability of HOCl for a 30-minute contact period. The sporicidal efficiency of monochloramine, furthermore, was so low that its effect could not be detected in the experiments performed" (19).

It is necessary to show that the Fort Lee pre-dip solution contained enough urea, if a urea-containing detergent in fact was used, to form significant amounts of the "N-Chloro" (chlorine directly attached to nitrogen) compounds referred to above. The pre-dip solution used by Portner et al. contained 0.2-percent calcium hypochlorite and 0.29-percent detergent (3). If urea-based, the latter consisted of 0.85 × 0.29 or 0.25-percent urea. The following overall reactions

$$Ca(OCl)_2 + O{=}C(NH_2)_2 \longrightarrow O{=}C(NHCl)_2 + Ca(OH)_2$$

$$Ca(OCl)_2 + O{=}C(NH_2)_2 \longrightarrow 2NH_2Cl + CaCO_3$$

show that calcium hypochlorite and urea react in equal molar quantities, whether the product is monochlorourea of monochloramine. That is, the proportion by weight, in either reaction, is 60 grams of urea to 143 grams of calcium hypochlorite. Thus, the 0.25-percent urea which may have been present was more than enough to react with the 0.2-percent calcium hypochlorite.

It remains only to show that the Fort Lee pre-dip solution was not highly alkaline in pH, thus permitting adequate stability for any N-chloro compound present, in contrast with the extremely rapid decomposition of N-chloro compounds in the highly alkaline solutions used by Campbell and Medde (9). Although, as noted above, the alkalinity of Campbell and Medde's solution necessarily resulted from the excess sodium hydroxide required in their hypochlorite solution, no such source of alkalinity existed in the Fort Lee pre-dip solution.

Of the three chemicals used in the pre-dip solution, urea (assuming a urea-bearing detergent) and the nonionic surfactant are essentially neutral, while the calcium hypochlorite contains very small amounts of alkali. The overall effect was to provide a solution low in alkalinity with a pH between 9 and 10, as the authors report.

Factor 11 also appears capable of serving as a basis for explaining at least part of the difference in the decontamination effectiveness of the Fort Lee pre-dip decontamination procedure and the NRL STOPS decontamination. The rationale of this explanation is that a low fabric-to-solution ratio, such as that used at Fort Lee, affords a spore "microenvironment" which is much lower in hypochlorite concentration than is the case with the high fabric-to-solution ratio characteristics of the NRL studies. On this theory, a subnormally low hypochlorite concentration adjacent to the spores would be the result of the chlorine demand of the abnormal quantity of cloth in close proximity to the spores; it would also result in reduced rates of decontamination. In any case, however, the successful decontamination of a contaminated fabric disc resting in the center of a pile of compressed discs, as was done in NRL Experiment No. 2, appeared to minimize the significance of the above explanation in accounting for the differences between the Fort Lee and NRL results.

In connection with the microenvironment explanation, it is noted that the Fort Lee pre-dip trials did not in fact use a fabric-to-solution ratio characteristic of laundry procedures, as had been intended. Such ratios for all-cotton clothing are typically about 1 to 5, and the pre-dip trials used a 1 to 2.2 ratio. The NRL "stack" decontamination trial used the latter ratio. Although the NRL fabric stack was well saturated at the 1 to 2.2 ratio, it may be doubted whether a sizable batch of clothing can be completely and reliably saturated at the same ratio. Thus, it is suggested that the exceptionally (and unintentionally) low fabric-to-solution ratio used in the Fort Lee trials was influential in the slow decontamination rates reported.

In summary, although no certain explanation can be given for the relatively low decontamination rates of the pre-dip trials relative to the NRL STOPS experiments, we are at present inclined to believe that possible presence of urea, and the exceptionally low fabric-to-solution ratio used at Fort Lee, are the most important factors. The combined effect of all other factors is likely to have further reduced the pre-dip decontamination rates.

Comparison of U. S. Public Health Service and NRL Data

A useful perspective on the Fort Lee and NRL decontamination results is gained by considering decontamination by hypochlorite in the absence of cloth or other substrate, i.e., with bacterial spores suspended in the decontaminating solutions. Brazis et al. of the U. S. Public Health Service conducted such a study for the U. S. Navy Bureau of Yards and Docks in 1958 (20) and found, for Bacillus subtilis morphotype globigii and Bacillus anthracis (anthrax), that the rate of germicidal process was well described by an equation of the form

$$K = Ct.$$

In this equation, C is the concentration of hypochlorous acid in milligrams per liter, and t is the time in hours to kill 99.99 percent of the spores. From the B. subtilis values for K of 14 mg-hr per liter at 4°C and 3 mg-hr per liter at 22°C, it can be calculated from a plot of log K vs 1/T(°K) that the value of K at 26°C is 2.4 mg-hr per liter for B. subtilis. A value of K descriptive of the spore kill rate in the NRL experiments can also be determined. Figure 2 shows that the extrapolated time to reach a 99.99-percent kill is about 6 minutes, or 0.1 hour. The concentration of total hypochlorite is 0.2 percent or 2000 mg per liter. Of this, only a small fraction is hypochlorous acid, with the exact amount determined by the pH of the solution. The pH of the freshly prepared solution is approximately 10, but the solution is poorly buffered, and the pH gradually decreases as the small amount of calcium hydroxide, which accompanies commercial calcium hypochlorite, reacts with atmospheric carbon dioxide. It is estimated for the shorter decontamination experiments, such as those referred to in Fig. 2, that the pH would not drop below 9.5. At this pH, 1 percent of the total hypochlorite is in the form of hypochlorous acid. The concentrations

of hypochlorous acid is therefore 2000 × 0.01 or 20 mg per liter. Both C and t are now known, and the NRL value of K is calculated to be (20 mg per liter) × (0.1 hr), or 2 mg-hr per liter. For a 99.99-percent kill of B. subtilis at 25°C using a hypochlorous acid concentration of 20 mg per liter, the calculated kill time from the work of Brazis et al. would be (2.4 × 60)/20, or 7.2 minutes, versus the 6 minutes extrapolated from the NRL experiments.

It is reasonable to assume that the close correspondence between the results of the Public Health Service and NRL experiments is to a considerable degree accidental. Probable bacterial strain difference, uncertainties in pH, and differences in techniques would all be expected to contribute to variations between the results obtained by the two Laboratories. Nevertheless, it also appears reasonable to assume that the rates of kill attained in the NRL experiments approximate the rates characteristic of free spore suspensions, i.e., spores without a solid substrate. In other words, the NRL kill rates approximate the maximum rates attainable for the temperature, pH, and hypochlorite concentration used.

Another fact of considerable interest to BW decontamination is found in the paper by Brazis et al. (20). They state: "At all pH ranges and temperatures investigated, B. globigii spores appeared to be more resistant to FAC [free available chlorine] than B. anthracis spores, with the exception of pH 10.5 where the spore resistance of both species appeared to be equal." That is, a decontamination process which satisfactorily copes with B. subtilis is also capable of dealing with the most decontamination-persistant pathogenic spores, those of the anthrax bacillus.

In view of the fact that the NRL STOPS BW-decontamination process appears to be capable of killing highly resistant bacterial spores without significant interference by a cellulose-nylon substrate, it is reasonable to anticipate that the process will also be effective in the presence of other substrates, such as metal, paint, rubber, etc.

CONCLUSIONS

In a number of laboratory experiments, bacterial spores of a species highly resistant to decontamination (Bacillus subtilis var niger) on Navy work-combat fabric have been decontaminated by a hypochlorite-detergent solution in a period of time which is roughly 1/35 of the time required by the corresponding military method previously available. Plausible explanations for the improved rate of decontamination are given. The new procedure has been adopted for clothing decontamination in the STOP system.

RECOMMENDATIONS

It is recommended that work in BW decontamination with hypochlorite be continued, with the objects of:

(1) Further improvement in the process.

(2) More precise determination of effects of the process on clothing and other materials on which the process may be used.

(3) Adaptation of the process to CW-contaminated clothing.

ACKNOWLEDGMENTS

We appreciate very much the kind assistance of Mr. Burton N. Navid of the NRL Applied Mathematics Staff, and Mr. Clarence Shepherd of the Chemistry Division, in directing our attention to the particular application of the Poisson distribution to the results of our experiments.

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Appendix A

CALCIUM HYPOCHLORITE AND NBC DETERGENT

Calcium hypochlorite for Navy shipboard use is described in Federal Specification O-C-114(a) and amendments. Some of its applications and properties are discussed in NRL letter report 6140-144A:GHF:lf of July 15, 1964 (Unclassified). The specification material is a commercial product which is 70-percent pure calcium hypochlorite. Since pure calcium hypochlorite is 99.2-percent "available chlorine," the 70-percent calcium hypochlorite also contains essentially 70-percent available chlorine. The stability of calcium hypochlorite in the currently used plastic 6-oz and 3-3/4-lb plastic containers is excellent as long as water vapor is rigorously excluded. A 0.2-percent solution of calcium hypochlorite can be prepared for about 3 cents per gallon if the 6-oz container is used; the cost of 0.5-percent NBC detergent in the same solution raises its gallon cost by 1.6 cents per gallon. The term HTH should not be used as a generic name for 70-percent calcium hypochlorite; it is a registered trademark of the Olin Chemical Company.

The NBC decontamination detergent, which is described in Military Specification MIL-C-7907A(WP), is used in solution with calcium hypochlorite for BW and CW decontamination aboard ship. It was originally developed by the Bureau of Naval Weapons for radioactive decontamination and was adopted for all-purpose NBC decontamination in Chapter 9770 of the Bureau of Ships Technical Manual (2). The composition of the NBC detergent is 10-percent nonionic surface-active agent and 90-percent builders and other detergency promoters. It is supplied only in 50-lb pails, in which it is 100 percent stable.

Appendix B

SOIL REMOVAL BY STOPS DECONTAMINATION PROCEDURE

In Reply Refer To: 13:AR:jb
3960/80-02-001-00-1
10/12/65

From: Officer in Charge, NAVSUPRANDFAC, Bayonne, N. J.
To: Director, U.S., Naval Research Laboratories (6140)
Washington, D. C., 20390

Subj: Soil removal from work clothing using the decontamination method of the STOP System

Ref: (a) Meeting at NRL on 25 Aug 1965
(b) Instructions for BW/CW Decontamination in the STOP System 6140-175 A:GHF:ef of 13 Aug 1965

1. As agreed during reference (a), this Facility determined whether the hypochlorite/detergent Decontamination Solution, with subsequent rinsings (section 3.30 of reference (b)) removed enough soil from representative sets of work clothing to yield an acceptable level of cleaning. The sets included: (a) 50% nylon/50% cotton sateen trousers and 50% nylon/50% cotton twill shirt, (b) 100% cotton denim trousers and 100% cotton chambray shirt, (c) Polaris coverall, (d) XXCC3-impregnated 50% nylon/50% cotton sateen trousers and XXCC3-impregnated 50% nylon/50% cotton twill shirt. To complete the work clothing load cotton undershirts, shorts and socks were added to the sets.

2. Naturally available soils from the local machine shop were uniformly rubbed into the fabric of the basic garments. The left sleeves (shirt area) and left legs (trouser area) of each set were soiled with one fluid ounce, per application, of used motor crankcase oil. The right sleeves and right legs of each set were soiled with one fluid ounce, per application, of clean auto lubricating grease. The shirt backs and trouser seats were soiled with one ounce, per application, of damp, non-oily, clay-like dirt. These soils applied on the garments were "aged" for a period of 24 hours at room conditions.

3. The Decontamination Solution was prepared as described (with room temperature water) by section 4.00 of reference (b). The sets of soiled clothing were wet out, drained and transferred to plastic bags. It was determined that the approximate quantity of solution retained per clothing set was at least 3/4 gallon (for the synthetic blend sets), and no more than 1 gallon (for the cotton sets). The solution-soaked clothing was allowed to stand for 30 minutes. Subsequent extraction time was 5 minutes. The non-impregnated sets of clothing were rinsed twice (5 minutes each at 160°F., then 140°F) at the appropriate water level, extracted and dried at 160°F. The XXCC3-impregnated set of clothing was rinsed twice (5 minutes each at 90°F., then at tap temperature) at the appropriate water level, extracted and dried at 140°F.

4. The regular non-impregnated garments, rinsed at the higher temperatures, were fairly clean but visually showed limited spotting. (Used oil stains were essentially completely removed. Surface grease was completely removed but minor residual discoloration remained. Surface dirt was completely removed but minor brown discoloration remained.) The XXCC3-impregnated garments, rinsed at the required low temperatures, were not quite as clean as the regular garments and visually showed definite spotting. (Surface oil was removed but residual dark stains remained. Surface grease was removed but definite

residual discoloration remained. Surface dirt was removed but brown residual discoloration was noticed.) Original levels of XXCC3 chemical impregnation were not significantly changed (trousers: before - 0.70, after - 0.69 mg Cl^+/cm^2; shirt: before - 0.29, after - 0.28 mg Cl^+/cm^2) by the decontamination process.

5. Since reasonable cleanliness of work clothes results from the use of the decontamination procedure of the STOP System, it is considered a practical method to employ during emergency situations. Standard washing conditions can be used later when conditions return to normal.

C. E. FULTON

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Security Classification

DOCUMENT CONTROL DATA - R & D

Security Classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY *(Corporate author)* Naval Research Laboratory Washington, D. C. 20390	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP

3. REPORT TITLE

BW DECONTAMINATION WITH HYPOCHLORITE. PART 1 - Exploratory Studies: Application to the STOP System

4. DESCRIPTIVE NOTES *(Type of report and inclusive dates)*

This is an interim report on the problem; work continues.

5. AUTHOR(S) *(First name, middle initial, last name)*

G. H. Fielding, W. H. Echols, and R. A. Neihof

6. REPORT DATE February 28, 1967	7a. TOTAL NO. OF PAGES 26	7b. NO. OF REFS 20
8a. CONTRACT OR GRANT NO. NRL Problem C08-22 b. PROJECT NO. SS 500-11749 c. d.	9a. ORIGINATOR'S REPORT NUMBER(S) NRL Report 6506 9b. OTHER REPORT NO(S) *(Any other numbers that may be assigned this report)* None	

10. DISTRIBUTION STATEMENT

Distribution of this document is unlimited.

11. SUPPLEMENTARY NOTES None	12. SPONSORING MILITARY ACTIVITY Dept. of the Navy (Naval Ship Systems Command)

13. ABSTRACT

BW-simulant spores on Navy clothing have been killed by alkaline calcium hypochlorite solutions about 35 times faster on a laboratory scale than in previously reported field trials with military clothing under similar conditions. This decontamination process has been adopted for use in the Navy's Shipboard Toxicological Operational Protective System (STOPS).

DD FORM 1 NOV 65 1473 (PAGE 1)

S/N 0101-807-6801

Security Classification

14 KEY WORDS	LINK A ROLE	LINK A WT	LINK B ROLE	LINK B WT	LINK C ROLE	LINK C WT
CLOTHING (Protective)						
DECONTAMINATION						
CALCIUM HYPOCHLORITE						
BIOLOGICAL WARFARE						
SHIPBOARD TOXICOLOGICAL OPERATIONAL PROTECTIVE SYSTEM (STOPS)						