279A-WF-222936-USAMRIID - 1100

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The following investigation was conducted by Special Agent (SA) [redacted] of the federal Bureau of Investigation (FBI) on February 24, 2005:

The United States Army Medical Research Institute of Infectious Diseases (USAMRIID) Keycard Access records from 1998 through 2002 were queried for visiting foreign scientist [redacted] and visiting scientist [redacted], both from the [redacted] who have previously been identified as visiting USAMRIID circa May 1998.

All available records were searched for names containing [redacted] or [redacted] or [redacted] all queries met with negative results.

As previously reported, Bruce Ivins was the USAMRIID point of contact for [redacted] and [redacted] A query of all available records for "Ivins*" met with positive results. Records indicate Bruce E. Ivins showed keycard activity during a date range of August 01, 1998 through June 02, 2002.

[redacted]

[redacted]

FEDERAL BUREAU OF INVESTIGATION

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Date of transcription 02/25/2005

On February 18, 2005, an Internet author query on website: www.pubmed.com for [redacted] scientists, [redacted] and [redacted] met with positive results. [redacted] the following publication: [redacted] "A Novel Surfactant Nanoemulsion with Broad-Spectrum Sporicidal Activity against *Bacillus* Species," (The Journal of Infectious Diseases 1999; 180:1939-1949.

(WFO Note: Attached hereto and considered part of this document is a copy of the above-mentioned publication.)

Briefly, page [redacted] indicated [redacted] and [redacted] are [redacted] have a patent application entitled: [redacted]

Briefly, page three "Material and Methods" section indicated, "*B. anthracis* spores, Ames and Vollum 1B strains, were supplied by Bruce Ivins (US Army Medical Research Institute of Infectious Diseases [USAMRIID], Fort Detrick, Frederick, MD)...Four other strains of *B. anthracis* were provided by [redacted]

Briefly, page ten "Discussion" section indicated, "BCTP [the novel surfactant nanoemulsion] and its derivative BCTP 401 appear to have great potential as environmental decontamination agents of for treatment of exposed persons in either a military operation or terrorist attack."

Briefly, page ten "Acknowledgments" section indicated "Bruce Ivins, [redacted] . .for their technical support. [redacted] for supplying characterized *B. anthracis* strains and space at Louisiana State University..."

[redacted]

Investigation on 02/25/2005 at Frederick, Maryland

File # 279A-WF-222936-USAMRIID -\10\ Date dictated N/A

by SA [redacted]

The Journal of Infectious Diseases 1999;180:1939-1949

0022-1899/1999/18006-0024$02.00

A Novel Surfactant Nanoemulsion with Broad-Spectrum Sporicidal Activity against *Bacillus* Species

Tarek Hamouda,[1] Michael M. Hayes,[1,a] Zhengyi Cao,[1] Richard Tonda,[1] Kent Johnson,[2] D. Craig Wright,[3] Joan Brisker,[3] and James R. Baker, Jr.[1]

[1]Center for Biologic Nanotechnology and Department of Medicine, and [2]Department of Pathology, University of Michigan Medical School, Ann Arbor; [3]NOVAVAX, Inc., Rockville, Maryland

Received 10 March 1999; revised 30 June 1999; electronically published 12 November 1999.

Two nontoxic, antimicrobial nanoemulsions, BCTP and BCTP 401, have been developed. These emulsions are composed of detergents and oils in 80% water. BCTP diluted up to 1 : 1000 inactivated >90% of *Bacillus anthracis* spores in 4 h and was also sporicidal against three other *Bacillus* species. This sporicidal activity is due to disruption of the spore coat after initiation of germination without complete outgrowth. BCTP 401 diluted 1 : 1000 had greater activity than BCTP against *Bacillus* spores and had an onset of action of <30 min. Mixing BCTP or BCTP 401 with *Bacillus cereus* prior to subcutaneous injection in mice reduced the resulting skin lesion by 99%. Wound irrigation with BCTP 1 h after spore inoculation yielded a 98% reduction in skin lesion size, and mortality was reduced 3-fold. These nanoemulsion formulas are stable, easily dispersed, nonirritant, and nontoxic compared with other available sporicidal agents.

Presented in part: 98th general meeting of the American Society for Microbiology, Atlanta, May 1998 (poster A49); 38th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, September 1998 (late-breaker slide session II, LB-9); 99th general meeting of the American Society for Microbiology, Chicago, May 1999 (poster A300).

The animal experiments were approved by and performed according to the guidelines of the Unit for Laboratory Animal Medicine, University of Michigan.

D.C.W. and J.B. are employees of NOVAVAX, Inc., and have significant financial interest in the company. NOVAVAX, Inc., is the supplier of the emulsions. J.R.B., T.H., M.M.H., D.C.W., and J.B. have a patent application entitled: Methods of inactivating bacteria including bacterial spores.

Financial support: Defense Advanced Research Project Agency (contract MDA 972-1-007 of the Unconventional Pathogen Countermeasures Program).

[a] Present affiliation: Pulmonary/Critical Care Unit–Internal Medicine, University of Michigan, Ann Arbor.

Reprints or correspondence: Dr. James R. Baker, Jr., University of Michigan Medical School, 9220 MSRB-III, 1150 W. Medical Center Dr., Ann Arbor, MI 48109-0648 (jbakerjr@umich.edu).

Bacteria of the *Bacillus* genus form stable spores that are resistant to harsh conditions and extreme temperatures. Contamination of farmlands with *Bacillus anthracis* leads to a fatal disease in domestic, agricultural, and wild animals [1]. Human infection by *B. anthracis* usually results from contact with infected animals or infected animal products [2]. Human clinical symptoms include a pulmonary form that has a rapid onset and is frequently fatal. The gastrointestinal and cutaneous forms of anthrax, although less rapid, can also result in fatalities unless treated aggressively [3, 4]. *B. anthracis* infection in humans is no longer common, because of effective animal control that includes vaccines, antibiotics, and appropriate disposal of infected livestock. However, animal anthrax still represents a significant problem because of contamination of farmland. Although a vaccine is available [5] and can be used for the prevention of anthrax, genetic mixing of different strains can render it ineffective [6]. The potential consequences of the use of *B. anthracis* spores as a biologic weapon were demonstrated by the accidental release of *B. anthracis* from a military microbiology laboratory in the former Soviet Union. Seventy-seven cases of human anthrax, including 66 deaths, were attributed to the accident. Some infections occurred as far as 4 km from the laboratory [7]. Genetic analysis of infected persons revealed the presence of either multiple strains or genetically altered *B. anthracis* [8].

Other members of the *Bacillus* genus are also reported to be etiologic agents for many human diseases. *B. cereus* is a common pathogen. It is involved in foodborne diseases because its spores can survive cooking procedures. Local sepsis and wound and systemic infections have also been attributed to *B. cereus* [9].

Disinfectants and biocides (e.g., sodium hypochlorite, formaldehyde, and phenols) that are highly effective against *Bacillus* spores are not well suited for decontamination of the environment, equipment, or exposed persons because of toxicity that leads to tissue necrosis and severe pulmonary injury after inhalation of volatile fumes. The corrosive nature of these compounds also renders them unsuitable for decontamination of sensitive equipment [10–15].

Concerns about these issues have stimulated interest in new types of biocidal agents that can safely decontaminate *Bacillus* spores. We have investigated the sporicidal properties of two antimicrobial lipid emulsions. Nanoemulsions are produced by mixing a lipid-oil "discontinuous" phase with an aqueous "continuous" phase under high shear forces. The result is an oil droplet of ~400–800 μm in diameter that is able to fuse with and subsequently disrupt the membrane of a variety of different pathogens [16]. BCTP is a nanoemulsion made of soybean oil, Triton X-100 detergent, and tri-*n*-butyl phosphate in 20% water. BCTP 401 is a mixture of this emulsion and a liposome, P10. P10 is made of water, Tween 60, soybean oil, glycerol monooleate, refined soya sterols, and the cationic compound cetylpyridinium chloride. These two compounds have antimicrobial activity against enveloped viruses and bacteria through membrane disruption (unpublished data). In the current studies, we examined the ability of these emulsions to inactivate different *Bacillus* spores.

Materials and Methods

Surfactant lipid preparations. BCTP is a water-in-oil nanoemulsion, in which the oil phase is made from soybean oil, tri-*n*-butyl phosphate, and Triton X-100. Stock solutions contained 80% lipid components and 20% water. Three different preparations of BCTP, 2, 8, and 16 months old, were tested for their stability. BCTP 401 was prepared by mixing equal volumes of BCTP with P10, the latter being a liposome-like compound. P10 is made of glycerol monosterate, refined soya sterols, Tween 60, soybean oil, a cationic ion halogen-containing cetylpyridinium chloride, and peppermint oil. The average size of these nanoemulsions is in the range of 400–800 nm, as determined by laser light scatter (LS230; Coulter, Hialeah, FL). These surfactant lipid preparations were stable after boiling for 1 h or exposure to 1 *N* nitric acid or 1 *N* sodium hydroxide for 2 h. This treatment resulted in a <20% reduction in the emulsion mean particle size [16]. These solutions were stored at room temperature and were diluted before each experiment to the working dilution. All dilutions herein are in reference to the stock solution.

Spore preparation. For induction of spore formation, *B. cereus* (ATCC 14579), *B. circulans* (ATCC 4513), *B. megaterium* (ATCC 14581), and *B. subtilis* (ATCC 11774) were grown for 1 week at 37°C on nutrient agar with 0.1% yeast extract and 5 mg/L $MnSO_4$. The plates were scraped, and the bacteria and spores were suspended in sterile 50% ethanol and incubated at 22°C for 2 h with agitation to lyse the remaining vegetative bacteria. The suspension was centrifuged at 2500 *g* for 20 min, and the pellet was washed twice in cold distilled water. The spore pellet was resuspended in trypticase soy broth (TSB) and used immediately for experiments. *B. anthracis* spores, Ames and Vollum 1B strains, were supplied by Bruce Ivins (US Army Medical Research Institute of Infectious Diseases [USAMRIID], Fort Detrick, Frederick, MD) and were prepared as described elsewhere [5]. Four other strains of *B. anthracis* were provided by Martin Hugh-Jones (Louisiana State University, Baton Rouge). These strains (from South Africa; Mozambique; Bison, Canada; and Del Rio, TX) represent isolates with high allelic dissimilarity.

In vitro sporicidal assays. For assessment of sporicidal activity on solid medium, trypticase soy agar (TSA) was autoclaved and cooled to 55°C. BCTP was added to the TSA at a 1 : 100 final dilution and continuously stirred while the plates were poured. The spore preparations were serially diluted (10-fold), and 10-μL aliquots were plated in duplicate (highest inoculum, 10^5 spores/plate). Plates were incubated for 48 h aerobically at 37°C and evaluated for growth.

For assessment of sporicidal activity in liquid medium, spores were resuspended in TSB. Next, 1 mL of spore suspension containing 2×10^6 spores (final concentration, 10^6 spores/mL) was mixed with 1 mL of BCTP or BCTP 401 (at 2× final concentration in distilled water) in a test tube. The tubes were incubated in a tube rotator at 37°C for 4 h. Treatment of *B. anthracis* was done at 37°C, which promotes spore germination, and at 22°C, which does not promote spore germination [5]. After treatment, the suspensions were diluted 10-fold in distilled water. Duplicate aliquots from each dilution were then streaked on TSA and incubated overnight at 37°C; then colonies were counted. Sporicidal activity expressed as percentage of killing was calculated as follows: {[cfu(initial) - cfu(posttreatment)]/[cfu (initial)]} × 100.

The experiments were repeated at least 3 times, and the mean and SE of the percentage of killing

were calculated by use of StatView software (Abacus Concepts, Berkeley, CA). Analysis of variance tables and paired *t* test were used when applicable.

Electron microscopy. *B. cereus* spores were treated with BCTP at a final dilution of 1 : 100 in TSB by means of Erlenmeyer flasks in a 37°C shaker incubator. The spore-BCTP mixture was washed with saline and centrifuged at 2500 *g* for 20 min, and the supernatant was discarded. The pellet was fixed in 4% glutaraldehyde in 0.1 *M* cacodylate (pH 7.3). Spore pellets were processed for transmission electron microscopy, and thin sections were examined after staining with uranyl acetate and lead citrate.

Germination inhibitors or enhancers. *B. cereus* spores (final concentration, 10^6 spores/mL) were suspended in TSB with either the germination inhibitor D-alanine (final concentration, 10 m*M*) or the germination enhancer L-alanine (final concentration, 5 m*M*) [17–19]. This suspension was then immediately mixed with BCTP (final dilution, 1 : 100) and incubated for variable intervals. Then the mixtures were serially diluted, plated, and incubated overnight. The next day, growth on the plates was counted, and the percentage of sporicidal activity was calculated.

In vivo toxicity testing. Mice were exposed to various concentrations of the different emulsions by means of different routes of administration. The highest concentrations that produced no gross or histopathologic lesions in mice were reported. Exposures included subcutaneous or intramuscular injection of 100 μL, open wound irrigation with 2 mL of the emulsions, and intranasal instillation of 25 μL/naris. The emulsions are relatively viscous when not diluted, so toxicity testing in the nares was conducted at the highest concentration that would not suffocate the animals. Three to four mice were tested for each concentration of each compound, and the experiments were repeated on at least three occasions.

In vivo sporicidal activity. Two animal models were developed to confirm the sporicidal activity of the emulsions in vivo. In the first model, *B. cereus* spores (suspended in sterile saline) were mixed with an equal volume of BCTP to a final emulsion dilution of 1 : 10. As a control, the same *B. cereus* spore suspension was mixed with an equal volume of sterile saline. Next, 100 μL of each of the suspensions, containing 4×10^7 spores, was then immediately injected subcutaneously into CD-1 mice. Nine mice were inoculated in each group, and the experiment was repeated on three different occasions.

In the second model, a simulated wound was created by making an incision in the skin on the back of the mice. The skin was separated from the underlying muscle by blunt dissection. The pocket was inoculated with 200 μL of saline containing 2.5×10^7 spores and closed by use of wound clips. One hour later, the clips were removed, and the wound was irrigated either with 2 mL of sterile saline or with 2 mL of BCTP (1 : 10 in sterile saline). The wounds were then closed with wound clips. The animals were observed for clinical signs. Gross and histopathologic examination were done when the animals were euthanized 5 days later. The wound size was calculated by the following formula: $\frac{1}{2} a \times \frac{1}{2} b \times \pi$, where *a* and *b* are two perpendicular diameters of the wound. Five mice were used in each group, and the experiment was repeated on three different occasions. Both sets of animal studies were also conducted with BCTP 401 at identical dilutions.

Results

In vitro sporicidal activity. To assess the sporicidal activity of BCTP, spores from four species of *Bacillus* genus (*B. cereus, B. circulans, B. megaterium,* and *B. subtilis*) were tested. BCTP at a 1 : 100 dilution showed 97% sporicidal activity against *B. cereus* and *B. megaterium* in 4 h (figure 1). *B. circulans* was less sensitive to BCTP, showing only an 83% reduction in spore count, whereas *B. subtilis* appeared resistant to BCTP in 4 h. The other nanoemulsion, BCTP 401, was more efficient in killing the *Bacillus* spores. At a 1 : 1000 dilution, it showed 99% killing of *B. cereus* spores in 4 h (compared with 50% with a 1 : 1000 dilution of BCTP). BCTP 401 at a 1 : 1000 dilution resulted in 96% killing of *B. subtilis* spores in 4 h, in contrast to its resistance to BCTP. Bleach diluted 1 : 100 (i.e., 0.0525% sodium hypochlorite) showed 98% sporicidal activity against *B. cereus* in 4 h. There was no significant difference in sporicidal activity against *B. cereus* between BCTP diluted 1 : 100, BCTP 401 diluted 1 : 1000, and bleach diluted 1 : 100 ($P = .23$).

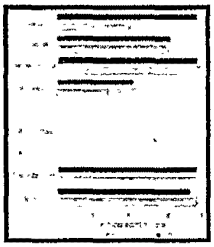


Figure 1. Sporicidal activity of BCTP against 4 different *Bacillus* species compared with that of BCTP 401 against 2 *Bacillus* species. BCTP showed significant sporicidal activity after 4 h of treatment against *Bacillus cereus, B. circulans,* and *B. megaterium* spores but not against *B. subtilis* spores. BCTP 401 showed more effective killing against *B. cereus* in 4 h and also had sporicidal activity against *B. subtilis* that was resistant to BCTP. Bleach diluted 1 : 100 was used as positive control and was comparable to BCTP or BCTP 401 at same dilutions.

Testing the stability of BCTP. Three different preparations of BCTP, stored for 2, 8, and 16 months at room temperature, were evaluated simultaneously for sporicidal activity against *B. cereus* spores to determine the stability of the emulsions. BCTP was diluted 1 : 10 and 1 : 100 for the experiments (figure 2), and there was no significant difference in the sporicidal activity of the preparations ($P = .94$ and .77).

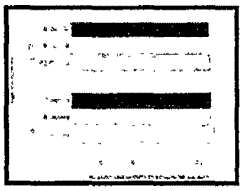


Figure 2. Comparison of sporicidal activity of 3 different preparations of BCTP aged 2, 8, and 16 months. Preparations have equivalent sporicidal activity, showing that BCTP is stable for up to 16 months.

B. cereus *sporicidal time course.* An 8-h experiment was done to analyze the time course of the sporicidal activity of BCTP (diluted 1 : 100) and BCTP 401 (diluted 1 : 1000) against *B. cereus.* Incubation of a 1 : 100 dilution of BCTP with *B. cereus* spores resulted in a 77% reduction in the number of viable spores at 1 h and a 95% reduction after 4 h. Again, BCTP 401 diluted 1 : 1000 was more effective than BCTP diluted 1 : 100 and resulted in an ~95% reduction in count in 30 min (figure 3). The improvement in killing between BCTP 401 diluted 1 : 1000 and BCTP diluted 1 : 100 was statistically significant up to the 4-h time point ($P < .05$).

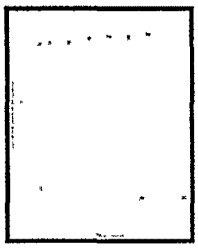


Figure 3. Time course of nanoemulsion sporicidal activity against *Bacillus cereus*. Incubation with BCTP diluted 1 : 100 resulted in 95% killing in 4 h. Incubation with BCTP 401 diluted 1 : 1000 resulted in 95% killing in only 30 min. Difference in killing between BCTP diluted 1 : 100 and BCTP 401 diluted 1 : 1000 up to 4-h point was significant ($P < .05$).

Sporicidal activity of BCTP against B. anthracis. After initial in vitro experiments, the sporicidal activity of BCTP was tested against two virulent strains of *B. anthracis* (Ames and Vollum 1B). We found that BCTP at a 1 : 100 final dilution incorporated into growth medium completely inhibited the growth of 1×10^5 *B. anthracis* spores. Sporicidal assays in fluid media, after 4 h of incubation with BCTP at dilutions up to 1 : 1000 with either the Ames or the Vollum 1B spores, resulted in 91% sporicidal activity when the mixtures were incubated at 22°C and 96% sporicidal activity when the mixtures were incubated at 37°C (table 1).

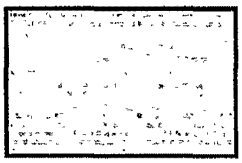


Table 1. Sporicidal activity of BCTP against 2 different strains of *Bacillus anthracis* spores as determined by colony reduction assay (% killing).

Sporicidal activity of BCTP 401 against B. anthracis. Because BCTP 401 was effective at higher dilutions and against more species of *Bacillus* spores than BCTP, it was tested against 4 different strains of *B. anthracis* at dilutions of up to 1 : 10,000 at 22°C to prevent germination. BCTP 401 showed peak sporicidal activity between ~1 : 1000 and ~1 : 5000 dilutions (table 2). It was less efficient at concentrations >1 : 100.

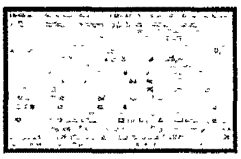
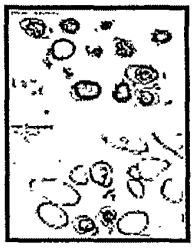


Table 2. Sporicidal activity of BCTP 401 against 4 different strains of *Bacillus anthracis* representing different clinical isolates.

Electron microscopic examination of the spores. We used *B. cereus* because it is the most closely related to *B. anthracis*. Transmission electron microscopic examination of *B. cereus* spores treated with BCTP diluted 1 : 100 in TSB for 4 h revealed physical damage to the *B. cereus* spores, including extensive disruption of the spore coat and cortex with distortion and loss of density in the core (figure 4).

Figure 4. Electron micrographs of *Bacillus cereus* spores before (*top*) and after (*bottom*) treatment with BCTP. Note uniform density in cortex and well-defined spore coat before treatment with BCTP. Spores after 4 h of BCTP treatment show disruption in both spore coat and cortex, with loss of core components.



Germination stimulation and inhibition. To investigate the effect of initiation of germination on the sporicidal effect of BCTP on *Bacillus* spores, the germination inhibitor, D-alanine [17, 18], and germination enhancer, L-alanine [19, 20], were incubated with the spores and BCTP for up to 1 h. Percentage of killing was calculated at different time points. The sporicidal effect of BCTP was delayed in the presence of 10 m*M* D-alanine and accelerated in the presence of 5 m*M* L-alanine (figure 5). All of the individual time points showed a significant difference in killing between the three treatments ($P < .002$).

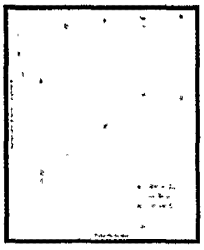


Figure 5. Effect of germination inhibition and stimulation on sporicidal activity of BCTP diluted 1 : 100 against *Bacillus cereus* spores. Sporicidal activity of BCTP was delayed in presence of 10 m*M* D-alanine (germination inhibitor) and accelerated in presence of 5 m*M* L-alanine (germination enhancer). All time points show significant difference between 3 treatments ($P < .002$).

In vivo toxicity testing. CD-1 mice injected with BCTP diluted 1 : 10 in saline did not exhibit signs of distress or inflammatory reaction, either grossly or histologically (figure 6*A*, 6*B*). Identical results were obtained when the toxicity of BCTP 401 was tested in mice subcutaneously. Intramuscular injection of the BCTP or BCTP 401 diluted 1 : 10 did not have any toxic effects in the form of inflammatory reaction, edema, or necrosis in mice. Open wound irrigation with 2 mL of the emulsions did not result in any pathologic damage. Intranasal instillation of the emulsion was less tolerable because of its viscosity; however, there was no injury from BCTP diluted 1 : 50 and BCTP 401 diluted 1 : 25. Oral administration of 10% BCTP (4 mL/kg of body weight daily) in rats for 1 week did not result in any gross or pathologic changes, and the rats maintained normal weight gain during this period (data not shown). In these tests, pathologic examination of local tissues and internal organs was done, and no abnormalities were detected.

Figure 6. Gross and histologic photographs of animals injected subcutaneously with different combinations of BCTP and *Bacillus cereus* spores. *A, B,* animals injected with BCTP alone at dilution of 1 : 10. There was no gross tissue damage, and histology showed no inflammation. *C, D,* animals injected with 4×10^7 *B. cereus* spores alone subcutaneously. Large necrotic area resulted, with average area of 1.68 ± 0.35 cm^2. Histologic examination of this area showed essentially complete tissue necrosis of epidermis and dermis, including subcutaneous fat and muscle. *E, F,* mice injected with 4×10^7 *Bacillus* spores that had been immediately premixed

with BCTP nanoemulsion at final dilution of 1 : 10. These animals showed minimal skin lesions, with average area of 0.02 ± 0.01 cm^2 (~98% reduction from lesions resulting from untreated infection with spores; $P < .002$). Histology of *F* indicated some inflammation; however, most cellular structures in epidermis and dermis were intact. All histopathology is shown at ×4 magnification.

In vivo sporicidal activity. *B. cereus* infection in experimental animals had been previously used as a model system for the study of anthrax and causes an illness similar to experimental anthrax [2, 9, 21–24]. Two animal models of cutaneous *B. cereus* disease were developed to assess the in vivo sporicidal activity of BCTP. A suspension of 4×10^7 *B. cereus* spores was mixed with saline or with BCTP at a final dilution of 1 : 10 and then immediately injected subcutaneously into the backs of CD-1 mice. Mice that were infected subcutaneously with *B. cereus* spores without BCTP developed severe edema in 6–8 h. This was followed by a gray, necrotic area surrounding the injection site at 18–24 h, with severe sloughing of the skin present by 48 h, leaving a dry, red-colored lesion (figure 6*C*, 6*D*). CD-1 mice injected with *B. cereus* spores premixed with BCTP never developed such a necrotic lesion, and edema and inflammation were minimal (figure 6*E*, 6*F*). The size of the necrotic lesion in BCTP-treated mice was ~98% smaller than the necrotic lesion size in untreated mice (from 1.62 ± 0.35 cm^2 to 0.02 ± 0.01 cm^2; $P < .002$). Similar results were observed with BCTP 401 diluted 1 : 10.

In additional studies, a 1-cm skin wound was infected with 2.5×10^7 *B. cereus* spores and then closed (figure 7*A*, 7*B*). For some of the animals 1 h later, the wounds were irrigated with either BCTP diluted 1 : 10 or saline to simulate postexposure decontamination. Irrigation of experimentally infected wounds with saline did not result in any apparent benefit (figure 7*C*, 7*D*). BCTP irrigation of wounds infected with *B. cereus* spores showed substantial benefit, resulting in a consistent 98% reduction in the lesion size (from 4.84 ± 0.48 cm^2 to 0.06 ± 0.03 cm^2; $P < .001$; figure 7*E*, 7*F*). This reduction in lesion size was accompanied by a 3-fold reduction in mortality (from 60% to 20%) compared with that in experimental animals receiving either no treatment or saline irrigation. Similar results were observed with BCTP 401 diluted 1 : 10.

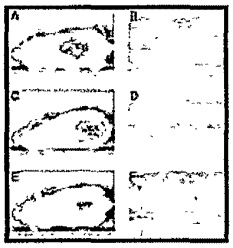


Figure 7. Gross and histologic photographs of animals with experimental wounds infected with *Bacillus cereus* spores. *A, B,* mice with experimental wounds infected with 2.5×10^7 *B. cereus* spores but not treated. Histologic examination indicated extensive necrosis and marked inflammatory response. *C, D,* mice with wounds that were infected with 2.5×10^7 *B. cereus* spores and irrigated 1 h later with saline. By 48 h, large necrotic areas surrounded wounds, with average area of 4.86 ± 0.48 cm^2. In addition, 60% of animals in this group died as result of infection. Histologic examination of these lesions indicated total necrosis of dermis and subdermis and large numbers of vegetative *Bacillus* organisms. *E, F,* mice with wounds infected with 2.5×10^7 *B. cereus* spores and irrigated 1 h later with 1 : 10 dilution of BCTP. There were small areas of necrosis adjacent to wounds (0.06 ± 0.03 cm^2), 98% reduction compared with animals receiving spores and saline irrigation ($P < .001$). In addition, only 20% of animals died from these wounds. Histologic examination of these lesions showed no evidence of vegetative *Bacillus* organisms and minimal disruption of epidermis. All histopathology is shown at ×4 magnification.

Discussion

In these studies, we demonstrated that BCTP and its derivative BCTP 401 have effective sporicidal activity against a variety of *Bacillus* spores, including *B. anthracis*. BCTP diluted 1 : 100 has a sporicidal activity against *B. cereus, B. circulans,* and *B. megaterium,* whereas 1 : 1000 is effective against *B. anthracis* in 4 h. BCTP 401, a BCTP-P10 mixture, appears to have a more rapid and broader sporicidal activity than BCTP. BCTP 401 diluted 1 : 1000 killed 95% of *B. cereus* spores in 30 min at 37°C, compared with a 70% reduction achieved by BCTP diluted 1 : 100. BCTP 401 diluted 1 : 1000 was also effective in 4 h against *B. subtilis* spores that were resistant to BCTP for up to 24 h. BCTP 401 did not show effective sporicidal activity against *B. anthracis* at dilutions of <1 : 100, contrary to the original BCTP, which showed killing at dilutions between 1 : 10 and 1 : 1000. The fact that BCTP 401 requires dilution to be effective against *B. anthracis* spores suggests that BCTP 401 needs dispersion by water to minimize its aggregation and to facilitate direct contact with spores.

Comparison of the sporicidal activity of BCTP against *B. anthracis* at 22°C, a temperature that does not promote spore germination, and at 37°C, at which germination occurs (as confirmed by microscopic examination), indicates that complete spore germination (i.e., outgrowth) is not necessary for the bactericidal activity of the emulsion. The small difference observed between the sporicidal activity at 37° C and 22°C may represent the killing of additional organisms from a few germinating spores. Sporicidal activity was also confirmed in water, a condition unsuitable for *B. anthracis* spore germination (data not shown). The sporicidal effect seems to start almost immediately and occurs within 30 min of incubation with the emulsion. Factors facilitating germination resulted in acceleration of the sporicidal activity of BCTP. Inhibition of the initiation of germination with D-alanine delayed BCTP's sporicidal activity. On the basis of these observations, we hypothesize that the sporicidal action of these emulsions occurs through initiation of germination before complete reversion to the vegetative form, leaving the spore susceptible to disruption by the emulsion. The initiation of germination could be mediated by the action of the emulsion or its components, but the emulsion appears necessary, as spores do not initiate germination in its absence. The results of the electron microscopy studies show disruption of the spore coat and cortex with disintegration of the core contents after BCTP treatment. However, the exact mechanism of killing is unclear and requires future investigation. Sporicidal activity appears to be mediated by both the Triton X-100 and tri-*n*-butyl phosphate components, because nanoemulsions lacking either component are inactive in vitro (data not shown). This unique sporicidal action of the emulsions, which is similar in efficiency to that of 1% bleach, is interesting because *Bacillus* spores are generally resistant to most disinfectants, including many commonly used detergents [15].

Animal studies demonstrated the protective and therapeutic effect of BCTP in vivo. *B. cereus* infection in experimental animals has been used previously as a model system for the study of anthrax [21, 22, 25]. The disease induced in animals experimentally infected with *B. cereus* is in many respects similar to anthrax [9, 23]. In this study, we demonstrated that mixing BCTP with *B. cereus* spores before injecting the spores into mice prevented the pathologic effect of *B. cereus*. We also demonstrated that BCTP treatment of simulated wounds contaminated with *B. cereus* spores markedly reduced the risk of infection and mortality in mice. Because the emulsion appeared to lose sporicidal activity when diluted

past 1 : 100, higher concentrations of the emulsions (1 : 10) were used for the in vivo studies to make sure they remained effective after dilution with body fluids. Other experiments show that testing BCTP 401 in mice under similar conditions demonstrated similar effects. These results suggest that decontamination of spores prior to or after exposure can effectively reduce the morbidity and mortality from *B. cereus* infection. This appeared to be a valuable application, because unlike other sporicidal agents, BCTP or BCTP 401 did not demonstrate any toxic effects, grossly or by histopathologic examination of the mice [26]. Other tests in mice showed that these emulsions are nontoxic if administered intramuscularly, intranasally, or orally, providing other potential sites for treatment.

BCTP and its derivative BCTP 401 appear to have great potential as environmental decontamination agents or for treatment of exposed persons in either a military operation or a terrorist attack. The inactivation of a broad range of pathogens, including vegetative bacteria, enveloped viruses [27] (unpublished data), and bacterial spores, combined with low toxicity in experimental animals, seems to make it suitable for use as a general decontamination agent that can be deployed even before a specific pathogen is identified. The nanoemulsions can be rapidly produced in large quantities and are stable for many months unless frozen, which causes separation of the oil and lipid phases. Undiluted, they have the texture of a semisolid cream and can be applied topically by hand or mixed with water. Diluted, they have a consistency and appearance similar to skim milk and can be sprayed to decontaminate surfaces or potentially interact with aerosolized spores before inhalation. These properties provide a flexibility that will be useful for a broad range of decontamination applications. Further studies are warranted to determine the exact mechanism of the sporicidal effect of BCTP and its derivatives, and this may lead to further improvement in formulations.

Acknowledgments

We thank Shaun B. Jones, Jane Alexander, and Lawrence DuBoise (Defense Science Office, Defense Advanced Research Project Agency) for their support; Bruce Ivins, Patricia Fellows, Mara Linscott, Arthur Friedlander, and the staff of USAMRIID for their technical support and helpful suggestions in the performance of the initial anthrax studies; Martin Hugh-Jones, Kimothy Smith, and Pamala Coker for supplying the characterized *B. anthracis* strains and the space at Louisiana State University (Baton Rouge); Robin Kunkel (Department of Pathology, University of Michigan) for her help with electron microscopy preparations; and G. Morris and A. Shih for their technical assistance with manuscript preparation.

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FD-302 (Rev. 10-6-95)

FEDERAL BUREAU OF INVESTIGATION

Date of transcription 03/22/2003

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GRAND JURY MATERIAL - DISSEMINATE PURSUANT TO RULE 6(e)

On March 19, 2003, [redacted] born [redacted] with Social Security Account Number [redacted] was interviewed at [redacted] place of employment, the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland. The interview was conducted by Inspector in Charge (IIC) [redacted] and Special Agents (SAs) [redacted] and [redacted] all of the Federal Bureau of Investigation (FBI). [redacted] provided the following information:

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1, 2, 3, 4, 6, 10, 11, 13

[redacted]

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Investigation on 03/19/2003 at Fort Detrick, Maryland

File # 279A-WF-222936USAMRIID, 279A-WF-222936-Lab Date dictated 1102 1128

by SA [redacted] IIC [redacted]

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FD-302 (Rev. 10-6-95)

FEDERAL BUREAU OF INVESTIGATION

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Date of transcription 02/24/2005

On [redacted] born [redacted] with social security account number [redacted] home address [redacted] home telephone number [redacted] cellular phone [redacted] was interviewed after completing a voluntary polygraph examination at the Frederick, Maryland offsite of the [redacted] According to the polygrapher, [redacted] The interview was conducted by US Postal Inspector (PI) [redacted] and Supervisory Special Agent (SSA) [redacted] of the Federal Bureau of Investigation (FBI). Present for a portion of the interview was FBI Special Agent (SA) [redacted] After being advised of the identities of the interviewing agents and the purpose of the interview, [redacted] provided the following information:

[redacted] is currently employed as [redacted] in the [redacted] at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Building [redacted] Fort Detrick, Maryland, phone number [redacted] was formerly known as the [redacted] supervisor is [redacted] phone number [redacted] currently shares an office with [redacted]

[redacted] began [redacted] employment at USAMRIID in [redacted] shortly after earning a [redacted] degree in [redacted] at the University of [redacted] background is limited to [redacted] has never streaked an agar plate with bacteria at USAMRIID.

[redacted] worked in [redacted] suites during the [redacted] months of employment at USAMRIID. [redacted] primarily worked on projects with [redacted] and recalls working in suite [redacted] of Building [redacted] gained access to the containment suites in [redacted] after [redacted] immunizations were complete.

[redacted] has worked in rooms [redacted] and [redacted] of Building [redacted] has been in a walk-in cooler located on the

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Investigation on 02/15-17/2005 at Frederick, Maryland

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File # 279A-WF-222936-USAMRIID - 1103 Date dictated N/A

by SSA [redacted] Postal Inspector

[redacted] floor of Building [redacted] stored [redacted] in this walk-in cooler in 2001 or 2002. [redacted] recalls the walk-in cooler was lined with shelves full of racks of samples from the aerosol challenge laboratories. b6 b7C b2 b7F

[redacted] has also worked in the [redacted] suite of Building [redacted] [redacted] first entered this suite with [redacted] to gain experience working in a containment suit. [redacted] assisted [redacted] with [redacted] related to [redacted] aerosol challenges. [redacted] has also worked in [redacted] outside of the containment suit at the hoodline in the "gray area" of the [redacted] suite. [redacted] recalled that [redacted] once got temporary access from [redacted] to an airlock in Building [redacted] to pick up [redacted] from [redacted] [redacted] has used [redacted]. One of [redacted] helped [redacted] with this in a [redacted] laboratory in Building [redacted]. The material resulting from the purification attempt was returned to [redacted] does not have access to Suites [redacted] or [redacted] in Building [redacted] but [redacted] may have been in the non-containment ("cold") areas of those suites to pick up material. b6 b7C

The only place [redacted] recalled seeing Ba in an odd location was in Room [redacted] of Building [redacted] This is a lab where [redacted], and [redacted] were working on a Johns Hopkins project involving the use of aerosol samples of Ba in a study of [redacted] The labeled tubes of Ba samples were stored in a refrigerator which had a sliding glass door where [redacted] had intended to place [redacted] b6 b7C b2 b7F

[redacted] worked on a number of projects at USAMRIID. One project involved [redacted] at USAMRIID. [redacted] controlled aerosol system. [redacted] b6 b7C

[redacted]

[redacted]

[redacted] was also involved in a study to determine the [redacted]

In [redacted] asked [redacted] to continue this research using [redacted] This research was funded by [redacted]

[redacted] worked on another project associated with [redacted] The goal was to [redacted]

[redacted]

[redacted] recalled that [redacted] once attended a classified meeting with [redacted] at [redacted] and [redacted] also attended the meeting.

[redacted] and [redacted] received Ba from DUGWAY PROVING GROUND (DUGWAY). DUGWAY prepared the Ba from stock that was provided to them by BRUCE IVINS. IVINS is a [redacted] and is an "odd character".

[redacted] recalled that on 9/11/2001, [redacted] was in the [redacted] unit in Building [redacted] when [redacted] observed the first plane strike the World Trade Center on television. [redacted] was at [redacted] thought initially that a light plane had struck the World Trade Center. [redacted] left [redacted] unit and began work in the [redacted] of Building [redacted] was assisting [redacted] with a [redacted] in the [redacted] room when they were interrupted by [redacted] to warn them to leave the suite immediately because the World Trade Center

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was attacked and a plane was headed for Fort Detrick. [redacted] was initially alarmed by this violation of safety protocol [redacted] later learned that [redacted]

[redacted] saw [redacted] dressed in black in the hallway near the cage washing machine. On another occasion [redacted] saw an individual whom [redacted] thought was [redacted] in a containment suit in suite [redacted] conducting [redacted] research. [redacted] or [redacted] may also have been in the suite. Sometime after [redacted] left USAMRIID, [redacted] observed [redacted] loading old hoodlines into a truck from an area outside Building [redacted] that was near the smokestacks. [redacted] could not recall the color of the truck nor any other details.

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[redacted] related that [redacted] has put forth theories to [redacted] regarding the mailing of the anthrax-laced letters. [redacted] claims USAMRIID has nothing to do with it and that a foreign government or an intelligence community mishap is responsible for the mailings. [redacted] referred to [redacted] as [redacted]

Referring to the investigation, [redacted] told [redacted] that [redacted] is a nice guy and that [redacted]

[redacted] is a [redacted] They have socialized together. [redacted]

[redacted] was [redacted] rater [redacted]

[redacted] who periodically contacts [redacted] for advice and assistance in [redacted] studies. [redacted]

has [redacted] experience and is interested in joining [redacted] visited [redacted] at [redacted], but [redacted] has not visited [redacted] Approximately [redacted] years ago, [redacted]

[redacted] USAMRIID people have voiced concerns to [redacted] about [redacted] has [redacted] experience.

[redacted] was shown a pre-stamped Federal Eagle envelope similar to those used in the mailing of the anthrax-laced letters in 2001. [redacted] has never used this type of envelope and has never seen one like it at USAMRIID.

[redacted] resides in [redacted]

In [redacted] rode on a train through New Jersey to [redacted]

In [redacted] attended a conference at [redacted] The conference was sponsored by the Center of Biomaterials.

[redacted] was less than impressed with the security at USAMRIID when [redacted] arrived there. [redacted] was very concerned about safety. [redacted] recalled that [redacted] once transported plague bacteria between buildings by the "VIP" method, which means "vial in pocket", instead of the proper packaging and

transport procedures. [redacted] never observed piggy-backing in the hot side. However [redacted] has observed piggybacking in other areas and recalled that [redacted] recently piggy-backed behind [redacted] through the front door.

The investigation of the anthrax-laced letter mailings is a sensitive issue to [redacted] but [redacted] is not sure why. [redacted] panicked feeling about the perception [redacted] associated with the facility or person responsible for the mailings. [redacted] was very concerned and [redacted] referred to the investigative and [redacted] and the impact it has had on [redacted] believes [redacted] and [redacted] does not want the same thing to happen to [redacted] was not at [redacted]

During the polygraph, [redacted] became aware of [redacted] absolutely had no involvement in the mailings.

At this point in the interview, SSA [redacted] told [redacted] thereafter provided the following information:

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[redacted]

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[redacted] knows that there are lyophilizers in cold areas of Building [redacted] at USAMRIID, but [redacted] has never used a lyophilizer at USAMRIID.

[redacted] read in the media that the mailer of the anthrax letters had probably milled the Ba. [redacted] realized [redacted] After [redacted] was initially interviewed by agents, [redacted] contacted [redacted] in [redacted] and advised [redacted] that agents may call [redacted] to verify information about [redacted]

Sometime before 2001, [redacted] contacted [redacted] regarding a project involving [redacted]

[redacted]

During the interview, [redacted] was told [redacted] was free to leave the interview at any time. [redacted] did so to use the restroom. [redacted] was offered and accepted bottled water to drink. [redacted] was told that [redacted] could take a break for lunch or bring in a lunch [redacted] had brought with [redacted] in [redacted] car. [redacted] elected to continue the interview [redacted]

SSA [redacted] explained the procedure of attempting to determine [redacted] whereabouts and activities during the windows of opportunity for the mailings of the anthrax-laced letters in 2001. [redacted] described numerous types of documentation that [redacted] might want to search for to help the investigators. [redacted] agreed to provide archived e-mails from [redacted] home and office computers, calendars, credit card records and phone bills, travel and leave records, laboratory notebooks, and any other documents that could help determine [redacted] whereabouts.

On [redacted] was re-contacted by PI [redacted] who requested that [redacted] come back into the office for a [redacted] On [redacted] brought with [redacted] several of the requested items to help with [redacted] time-line of activities during the windows of opportunity for the mailings. [redacted] SSA [redacted] and PI [redacted] talked with [redacted] to determine if any new ideas had occurred to [redacted] other than the three issues disclosed on [redacted] provided the following comments regarding the previously-discussed issues:

[redacted] concerns about the dried Bg work were not necessarily ethical concerns. [redacted] The International Convention says that as long as the work is on pathogenesis or vaccine efficacy, the work is not considered offensive in nature.

[redacted] corrected [redacted] comment that [redacted] had only been to New York City on one occasion. [redacted]

[redacted] has the [redacted] for this travel and will provide it to the investigators.

Another issue that may have been worrying [redacted] is that

[redacted]

[redacted]

[redacted] was told that [redacted] was free to leave, [redacted] provided the following in response to questions asked by SSA [redacted]

[redacted] has not trained any other individuals to use the equipment or procedures [redacted] has used to [redacted] The only training [redacted] gave was on the [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

There was a big push to do studies in the [redacted] after the mailings occurred. [redacted] was doing work for [redacted] and [redacted] is not sure if the work was classified or not.

[redacted]

[redacted] visited [redacted] in [redacted] to give a talk. [redacted] was there. [redacted] was [redacted] said [redacted] had called the FBI numerous times about the use of [redacted] in the mailed anthrax, but the FBI would not listen.

[redacted] did not host any foreign visiting scientists at USAMRIID. In [redacted] went to [redacted] to a meeting of the [redacted] with [redacted] There was a group of [redacted] Scientists from [redacted] at the meeting. The [redacted]

After thinking about it more, [redacted] cannot clearly recall if [redacted] really saw [redacted] at the truck with the hoodlines or if [redacted] remembers seeing [redacted] two times in the hallway in suite [redacted] and [redacted] socialized with [redacted], but [redacted] did not. [redacted] went to the USAMRIID library to check out a book called [redacted] It is a "really neat" book. [redacted] name was on the library checkout sheet, and [redacted] had checked out the book prior to the mailings. The book is specific to aerobiology.

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[redacted] got really upset when [redacted] found that a laboratory notebook was lost. [redacted] The missing notebook is the one which had the [redacted] in it. [redacted] was the technician who made entries in the notebook, [redacted] does not know what happened to the notebook.

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SSA [redacted] asked [redacted] about the requisition form [redacted] provided for the [redacted] had worked with. [redacted]

In regard to the original purpose of the [redacted] participated in, the anthrax spores in liquid form which were being used in aerosol challenges were [redacted] had wanted to

move toward using dried anthrax in the challenges. [redacted]

[redacted]

[redacted] could not think of any other issues potentially related to the word "anthrax" which might bother [redacted] and might have contributed to [redacted] wants to help the investigators [redacted] and will do whatever is needed. [redacted] agreed to provide additional information and documentation regarding [redacted] activities and whereabouts at the time of the mailings. [redacted] agreed to come back to the offsite for formal handwriting exemplars.

FD-302 (Rev. 10-6-95)

FEDERAL BUREAU OF INVESTIGATION

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Date of transcription 03/01/2005

[redacted] home address [redacted]
[redacted] home telephone number [redacted]
social security number [redacted] date of birth [redacted]
work address [redacted]
[redacted] work telephone number [redacted] was interviewed at the Federal Bureau of Investigation's (FBI) offsite location in Frederick, Maryland. After being advised of the identity of the interviewing agents and the purpose of the interview, [redacted] provided the following information:

[redacted]

[redacted]

[redacted]

[redacted] has conducted work for BRUCE IVINS on [redacted] at USAMRIID. [redacted]

Investigation on 02/25/2005 at Frederick, Maryland

File # 279A-WF-222936-USAMRIID-1109 Date dictated N/A

SA [redacted]

by Postal Inspector [redacted]

[redacted]

[redacted] did not recall working with any specific Ames samples at USAMRIID in [redacted] or [redacted] IVINS or [redacted] would be able to provide more information about Ames work and storage locations at USAMRIID.

[redacted]

[redacted] had no recollection of any plate count comparison study with IVINS or [redacted] for BioPort or Battelle Memorial Institute. [redacted]

[redacted] had never applied for funding from Princeton University, nor did [redacted] know anyone who attended or was employed there.

[redacted]

[redacted]

[redacted]

There was no one at USAMRIID whom [redacted] suspected of being involved in the 2001 anthrax-laced letter mailings.

FD-302 (Rev. 10-6-95)

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FEDERAL BUREAU OF INVESTIGATION

Date of transcription 03/15/2005

On March 14, 2005, [redacted] date of birth [redacted] Social Security Account Number [redacted] was interviewed at [redacted] residence, [redacted] cellular telephone [redacted] pager [redacted] by Special Agents (SAs) [redacted] and [redacted]. Also present during the interview was [redacted] of the [redacted] Resident Agency (RA). [redacted] provided assistance with the [redacted] of [redacted] computer hard drive. After being advised of the identities of the interviewing Agents and the purpose of the interview, [redacted] provided the following information:

At the outset of the interview [redacted] signed and dated a FD-941, Consent To Search Computer(s) form. A copy of the consent form is attached and the original is maintained in the 1A section of the case file. [redacted] informed the Agents that [redacted] used "password" to access the Windows 98 operating system on [redacted] computer.

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Investigation on 03/14/2005 at [redacted]

File # 279A-WF-222936-USAMRIID - 1127 Date dictated [redacted]

by SA [redacted]
SA [redacted]

FD-941 (2-26-01)

ALL INFORMATION CONTAINED
HEREIN IS UNCLASSIFIED
DATE 12-12-2008 BY 60324 UC BAW/DK/RYS

CONSENT TO SEARCH COMPUTER(S)

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I, [redacted], have been asked by Special Agents of the Federal Bureau of Investigation (FBI) to permit a complete search by the FBI or its designees of any and all computers, any electronic and/or optical data storage and/or retrieval system or medium, and any related computer peripherals, described below:

[redacted]
CPU Make, Model & Serial Number (if available)

N/A
Storage or Retrieval Media, Computer Peripherals

and located at [redacted], which I own, possess, control, and/or have access to, for any evidence of a crime or other violation of the law. The required passwords, logins, and/or specific directions for computer entry are as follows: N/A.

I have been advised of my right to refuse to consent to this search, and I give permission for this search, freely and voluntarily, and not as the result of threats or promises of any kind.

I authorize those Agents to take any evidence discovered during this search, together with the medium in/on which it is stored, and any associated data, hardware, software and computer peripherals.

[redacted]
Date

[redacted]
Signature

[redacted]
Date

[redacted]
Signature of Witness

[redacted]
Printed Full Name of Witness

[redacted]
Location

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FEDERAL BUREAU OF INVESTIGATION

Date of transcription 03/02/2005

On February 28, 2005, and March 1, 2005, [redacted], date of birth [redacted], Social Security Account Number [redacted], was interviewed at [redacted] residence, [redacted] cellular telephone [redacted] by Special Agents (SAs) [redacted] and [redacted]. After being advised of the identities of the interviewing Agents and the purpose of the interview, [redacted] provided the following information:

[redacted] in obtaining a position with the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). [redacted] characterized the working environment as friendly and professional. [redacted]

[redacted] IVINS exhibited no inappropriate behavior at the time. He was very concerned about crossing a boundary between professional and personal life. [redacted]

Investigation on 02/28/2005 at [redacted]

File # 279A-WF-222936-USAMRIID - [redacted] Date dictated [redacted]

by SA [redacted]

[redacted]

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The overall work environment in the [redacted] Division was friendly. USAMRIID personnel were both friends and co-workers. According to [redacted] a group of scientists, by definition, was an unusual group of people, thus personality conflicts were not unexpected. [redacted]

IVINS was friendly, but also conducted himself in a manner that made it clear that [redacted] IVINS was very moody, but could not be faulted for his behavior, as he "always had a heart of gold." According to [redacted] IVINS always meant well. [redacted] could not determine the cause of IVINS' mood swings.

[redacted] he did not have many friends. [redacted] felt bad that IVINS lacked friends, [redacted] recalled discussions with IVINS regarding [redacted] during [redacted] at USAMRIID.

[redacted] employment at USAMRIID ended in [redacted] of [redacted]

[redacted]

[redacted] IVINS was always a little unusual, [redacted]

IVINS discussed his own personal issues [redacted] He discussed the difficult relationship between himself and his wife, and difficulties with his teenage children. He expressed insecurities about being without friends and being perceived as strange or weird. IVINS indicated that he started to see a counselor. Issues of depression, rampant insecurities, history of psychiatric illness in his family were also discussed by IVINS [redacted] According to [redacted] IVINS was insecure personally, not professionally.

IVINS informed [redacted] that he was labeled with bipolar and schizophrenic disorders; however, the disorder labels did not fit with [redacted] so [redacted] disregarded them. [redacted] was not clear whether the labels were assigned by IVINS or his doctors. [redacted] did not recall if [redacted] was aware of these labels. [redacted] thought that IVINS had a personality disorder which did not cross into "frank psychosis." Nothing in IVINS' behavior made [redacted] worry about the technical aspects of his job performance. His condition affected his social interactions; however, his disorder did not prevent his functioning at work, nor his community involvement and work with children. IVINS was too critical of himself. He analyzed every word said to him and all of the possible meanings implied by statements directed toward him.

IVINS discussed his family background with [redacted] on occasion. He grew up in Lebanon, Ohio, where his father was a pharmacist. [redacted] did not believe that IVINS' mother worked outside of the home. [redacted] The family took trips out West to visit National Parks. IVINS visited an aunt [redacted] did not recall whether the visits were family outings. IVINS never referenced any traumatic occurrences in his life. He

indicated that he had few friends in high school and college. He mentioned that he was very awkward around women and was never "super popular." IVINS had been married only one time. IVINS attributed his mental issues to the way in which he was raised.

[redacted]

[redacted] would sometimes worry about IVINS' mood, [redacted]

[redacted] IVINS was seeing a counselor to discuss his issues. [redacted] indicated that IVINS continued to have both good and bad days, but that the counseling did quite a bit of good. [redacted] did not know the duration or extent of IVINS' counseling.

[redacted]

[redacted] IVINS' behavior was hard to describe to someone who did not know him, [redacted]

[redacted]

[redacted] IVINS did not like to talk on the telephone. [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted] did not recall specific problems or pressures associated with the [redacted] program. [redacted] indicated that tension was usually greater [redacted] around the time of an aerosol challenge, as there was a significant amount of work to accomplish within a set time frame.

[redacted] regarding contamination issues outside of the USAMRIID hot suites. [redacted] "this is what is going on at work" type of information. [redacted] recalled concern over contamination in office areas, but did not recollect specific details. [redacted] was under the impression that the whole department was involved in the swabbing of all of the office spaces. [redacted] did not remember what prompted the extensive swabbing, nor did [redacted] recall the time frame of the event. [redacted] believed that occasional swabbing outside of the hot suites was conducted as a quality control; however, [redacted] never observed such swabbing during [redacted] tenure at USAMRIID.

[redacted]

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279A-WF-222936-USAMRIID

[redacted]
verbally consented to a review of any items by the interviewing Agents during a return visit.

During the interview, [redacted] read, signed and dated an Non-Disclosure Agreement regarding the Amerithrax investigation. The form is maintained in a 1A envelope.

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FEDERAL BUREAU OF INVESTIGATION

ALL INFORMATION CONTAINED
HEREIN IS UNCLASSIFIED
DATE 12-12-2008 BY 60324 UC BAW/DK/RYS

Date of transcription 02/22/2005

[redacted] Date of Birth (DOB): [redacted] Social Security Account Number (SSAN): [redacted] work telephone #: [redacted], was interviewed, as pre-arranged, at [redacted] place of employment, room #: [redacted] Porter Street, the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland on the afternoon of February 17, 2005. After being advised of the identity of the interviewing Postal Inspector and Special Agent (SA), [redacted] provided the following information:

[redacted] provided the interviewing Postal Inspector and SA a facsimile [redacted] had received on [redacted] at approximately [redacted], from [redacted] regarding [redacted]

(WFO NOTE: Enclosed in the FD-340 attached to this communication is the above captioned facsimile.)

[redacted]

Through investigation [redacted] is described as:

LAST NAME:
FIRST NAME:
ALIAS:

POB:
[redacted]
DATE OF VISIT:
EMPLOYER:
SUPERVISOR:

[redacted]

Investigation on 02/17/2005 at Frederick, Maryland

File # 279A-WF-222936-USAMRIID-\\29 Date dictated N/A

by Postal Inspector [redacted]
SA [redacted]

279A-WF-222936-USAMRIID

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MISCELLANEOUS:

Accessed USAMRIID [redacted] Suite with [redacted] and worked with Ames strain of *Ba*.

FD-302 (Rev. 10-6-95)

FEDERAL BUREAU OF INVESTIGATION

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Date of transcription 02/18/2005

[redacted] Date of Birth (DOB): [redacted] Social Security Account Number (SSAN): [redacted] work telephone #: [redacted] was interviewed, as pre-arranged, at [redacted] place of employment, room [redacted] Porter Street, the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland on the afternoon of February 17, 2005. After being advised of the identity of the interviewing Postal Inspector and Special Agent (SA) [redacted] provided the following information:

[redacted] advised [redacted] has been [redacted] Division [redacted] for approximately [redacted] years.

[redacted] advised [redacted] was previously interviewed regarding the anthrax laced letter mailings of September and October, 2001 circa August 2003. [redacted] advised [redacted] could not recall any additional details from the August 2003 interview that would be helpful to the investigation.

[redacted]

[redacted] advised [redacted] only vaguely recalls seeing [redacted] at USAMRIID. [redacted] advised [redacted] has heard rumors that [redacted] would "come over to our laboratories" to observe research procedures unrelated to [redacted] discipline. [redacted] further advised [redacted] did not know if [redacted] had approval from either [redacted] supervisor or the [redacted] Division Investigator conducting the research to observe. [redacted] advised [redacted] could not recall what [redacted] Division laboratories [redacted] was known to frequent in particular.

(WFO NOTE: [redacted] was provided a printout of USAMRIID electronic foreign scientist records [redacted]

[redacted]

Investigation on 02/18/2005 at Frederick, Maryland

File # 279A-WF-222936-USAMRIID-1130 Date dictated N/A

by Postal Inspector [redacted]
SA [redacted]

who per USAMRIID foreign scientist records, was to have access to [redacted] lab" for [redacted] further advised [redacted] a list of all foreign scientists that visited the [redacted] Division from January 1999 to present.

[redacted]

(WFO NOTE: Enclosed in the FD-340 attached to this communication is the list of foreign visiting scientists [redacted] had provided to the interviewing Postal Inspector and SA.)

[redacted] advised [redacted]

[redacted] list of foreign scientists visiting the [redacted] Division from January 1999 to present.

(WFO NOTE: [redacted] contacted [redacted] telephonically.)

[redacted] BRUCE IVINS was possibly the USAMRIID POC for [redacted] advised, [redacted] the visit was cleared through the "front office" and the request for the visit came from "command."

(WFO NOTE: [redacted] contacted [redacted] telephonically.)

[redacted]

(WFO NOTE: [redacted] contacted BRUCE IVINS telephonically.)

[redacted] advised IVINS recalled the individual referred as [redacted] and the circumstances of [redacted] visit to USAMRIID.

[redacted] agreed to contact the interviewing Postal Inspector or SA should [redacted] recall any additional pertinent information.

Through observation and interview [redacted] is described as:

LAST NAME:	[redacted]
FIRST NAME:	
MIDDLE INITIAL:	
SEX:	
RACE:	
OCCUPATION:	
EMPLOYER:	USAMRIID
WORK ADDRESS:	Office [redacted] Porter Street Fort Detrick, Maryland
WORK TELEPHONE #:	[redacted]

Through investigation [redacted] is described as:

COUNTRY:	[redacted]
LAST NAME:	
FIRST NAME:	
OCCUPATION:	
AFFILIATION:	
DATE OF VISIT:	September 29-30, 1999
PURPOSE OF VISIT:	[redacted]
USAMRIID POC:	

Through investigation [redacted] is described as:

COUNTRY:	[redacted]
LAST NAME:	
FIRST NAME:	
AFFILIATION:	
DATE OF VISIT:	April 2000 - June 2001
PURPOSE OF VISIT:	[redacted]

USAMRIID POC:

Through investigation [ ] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
SEX:
AFFILIATION:

DATE OF VISIT: August 15 - September 30, 2000
PURPOSE OF VISIT:

USAMRIID POC:

Through investigation [ ] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: May 01 - November 01, 2000

AFFILIATION: [redacted]

DATE OF VISIT: November 23, 1999
PURPOSE OF VISIT: [redacted]

USAMRIID POC: [redacted]

Through investigation [redacted] is described as:

COUNTRY: [redacted]
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: August 16-20, 1999
PURPOSE OF VISIT: [redacted]

USAMRIID POC: [redacted]

Through investigation [redacted] is described as:

COUNTRY: [redacted]
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: August 16-20, 1999
PURPOSE OF VISIT: [redacted]

USAMRIID POC: [redacted]

Through investigation [redacted] is described as:

COUNTRY: [redacted]
LAST NAME:

FIRST NAME:
OCCUPATION:
AFFILIATION:

[redacted]

DATE OF VISIT: November 23, 1999
PURPOSE OF VISIT:

[redacted]

USAMRIID POC:

Through investigation [redacted] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
OCCUPATION:
AFFILIATION:

[redacted]

DATE OF VISIT: September 29-30, 1999
PURPOSE OF VISIT:

[redacted]

USAMRIID POC:

Through investigation [redacted] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
MIDDLE INITIAL/NAME:
AFFILIATION:

[redacted]

DATE OF VISIT: May 27, 1999
PURPOSE:

[redacted]

USAMRIID POC:

Through investigation is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: February 06, 2001
PURPOSE OF VISIT:

USAMRIID POC:

Through investigation is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: 2 weeks starting in July 2001
PURPOSE OF VISIT:

USAMRIID POC: [redacted]

Through investigation [redacted] is described as:

COUNTRY: [redacted]
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: June 29, 2000
PURPOSE OF VISIT: [redacted]

USAMRIID POC:
DATE OF VISIT (II):
PURPOSE (II):

POC II

Through investigation [redacted] is described as:

COUNTRY:
LAST NAME: [redacted]
FIRST NAME:
AFFILIATION:
DATE OF VISIT: 4 weeks starting in June 2001
PURPOSE OF VISIT: [redacted]

[redacted]

USAMRIID POC:

Through investigation [redacted] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
AFFILIATION:

[redacted]

DATE OF VISIT: May 18-19 2000
PURPOSE OF VISIT:

[redacted]

USAMRIID POC:

Through investigation [redacted] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
AFFILIATION:

[redacted]

DATE OF VISIT: May 18-19 2000
PURPOSE OF VISIT:

[redacted]

USAMRIID POC:

Through investigation [redacted] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
SEX:
AFFILIATION: [redacted]

DATE OF VISIT: May 18-19 2000
PURPOSE OF VISIT: [redacted]

USAMRIID POC:

Through investigation [redacted] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
AFFILIATION: [redacted]

DATE OF VISIT: May 18-19 2000
PURPOSE OF VISIT: [redacted]

USAMRIID POC:

Through investigation [redacted] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
SEX:
AFFILIATION: [redacted]

DATE OF VISIT: May 18-19 2000
PURPOSE OF VISIT: [redacted]

USAMRIID POC:

Through investigation [redacted] is described as:

COUNTRY:
LAST NAME:
FIRST NAME: [redacted]

AFFILIATION: [redacted]

DATE OF VISIT: May 18-19 2000

PURPOSE OF VISIT: [redacted]

USAMRIID POC: [redacted]

Through investigation [redacted] is described as:

COUNTRY: [redacted]
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: 4 weeks starting in June 2001

PURPOSE OF VISIT: [redacted]

USAMRIID POC:

Through investigation [redacted] is described as:

COUNTRY: [redacted]
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: May 18-19 2000

PURPOSE OF VISIT: [redacted]

USAMRIID POC:

Through investigation [redacted] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: October 10-21, 2000
PURPOSE OF VISIT:

USAMRIID POC:

Through investigation [ ] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
SEX:
AFFILIATION:

DATE OF VISIT: August 16-18, 2000
PURPOSE OF VISIT:

USAMRIID POC:

Through investigation [ ] is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
SEX:

AFFILIATION:

DATE OF VISIT: April 2000 - September 2000
PURPOSE OF VISIT:

USAMRIID POC:

Through investigation is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: July 1998 - March 2000
PURPOSE:

USAMRIID POC:

Through investigation is described as:

COUNTRY:
LAST NAME:
FIRST NAME:
AFFILIATION:

DATE OF VISIT: June 21, 1999
PURPOSE OF VISIT:

USAMRIID POC:

Through investigation is described as:

COUNTRY:
LAST NAME:
FIRST NAME:

PASSPORT #:
AFFILIATION:

DATE OF VISIT: March 05-09, 2001
PURPOSE OF VISIT:

USAMRIID POC:

(Rev. 01-31-2003)

FEDERAL BUREAU OF INVESTIGATION

Precedence: ROUTINE **Date:** 03/08/2005

To: Washington Field

From: Washington Field
AMX-3
Contact: SA [redacted]

Approved By: [redacted]

Drafted By: [redacted]

ALL INFORMATION CONTAINED
HEREIN IS UNCLASSIFIED
DATE 12-12-2008 BY 60324 UC BAW/DK/RYS

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Case ID #: 279A-WF-222936-USAMRIID (Pending) - 131

Title: AMERITHRAX;
MAJOR CASE 184

Synopsis: To provide a periodic update for the ongoing project to review USAMRIID laboratory notebooks. This update summarizes information obtained from additional laboratory notebooks and folders belonging to Dr. Bruce Ivins and Ivins' research group, located in Ivins' office at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, Maryland. Notebook numbers 16 (or 3464), 1599, 4240, 4237, 3745, 3233, 3919, 1748, 2064, 3465, 3269, 1670, 3080, 3114, 2013, 1511, 3563, 3234, 1844, 1599, 1670, and folders were reviewed. Reference EC dated July 14, 2004, serial 882.

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Enclosure(s): Enclosed is a Microsoft Excel spreadsheet listing numerous laboratory notebooks and folders located in Ivins' office.

Details: Between July and October 2004, a second set of USAMRIID laboratory notebooks and folders were reviewed. Bruce Ivins and researchers in Ivins' group used this set of notebooks. Numbers were assigned by the USAMRIID library to all laboratory notebooks issued to Principal Investigators, and the folders had a handwritten title summarizing their contents. These notebooks and folders were reviewed to identify any individuals who had access to Ba Ames and were not already under investigation, previously-unknown places where Ba Ames was stored, people within USAMRIID or people and places outside USAMRIID to whom Ba Ames was distributed by this research group, and any other details of interest.

Various Standard Operating Procedures (SOPs) were copied, including an SOP for the "Production, Harvest, and

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Purification of *Bacillus anthracis* Ames Spores for Aerosol Challenge"; a March 1997 SOP prepared by [redacted] entitled [redacted] a May 1995 SOP prepared by the USAMRIID Bacteriology Division entitled [redacted] an April 1997 SOP prepared by Ivins entitled "Preparation of *Bacillus anthracis* Spores for Aerosolization"; an April 1997 SOP prepared by Ivins entitled "Quantitation of *Bacillus anthracis* Colony Forming Units"; an April 1997 SOP prepared by Ivins entitled "Preparation of *Bacillus anthracis* Spores for Testing Aerosolization Efficiency"; and a March 1997 SOP prepared by Ivins entitled "Preparation of *Bacillus anthracis* Spores for Aerosol LD50 Determination".

In the [redacted] folder, a Ba Ames Primary Subculture was shipped to Battelle in April 2004. Ba Ames "ready-to-spray" spores were shipped to Battelle between April and September 2004. Furthermore, [redacted] Ivins visited Battelle for observation and advice between May and September 2004.

Notebook #16, also labeled #3464, contained an entry by Ivins on July 27, 1994. Ivins reported that [redacted] of [redacted] was performing work with Ames spores in rabbits. Ivins also attached an August 17, 1994 graph showing a gamma-irradiation kill curve for Ba Ames strain spores. The kill curve was produced by [redacted] On or about November 22, 1994, an experiment was to be conducted to [redacted] and Ivins were to do the skin testing. Ba Ames was one of the strains used in this experiment. [redacted]

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Notebook 4240 revealed that on March 5, 2002, Ivins gave [redacted] 20 milliliters of Ames spores for a challenge with [redacted] rabbits. On or about May 8, 2003, Ames spores were also provided to [redacted]

Notebook 4237 included pages, dated in 2000, referencing RMR 1029, and provided the latest count of un-heat-shocked spores as 4.3 x 10 to the 10/milliliter. Additionally, page 23 displayed an e-mail, dated May 10, 2000, to Ivins from [redacted] This e-mail designated Ba strain identifications for isolates that [redacted] wanted to ship to Ivins. One of those strains, [redacted] is known to be Ames [redacted] A handwritten note by Ivins stated the types of Ba strains sent [redacted] but it did not appear that Ames was among them.

A folder entitled [redacted] revealed that [redacted] This plan involved studies of DNA-based immunization against anthrax. It was unclear whether the anthrax studied was the Ames strain.

Other individuals with possible access to Ba Ames not previously identified were included in a folder entitled [redacted] individuals assisted Ivins with a protocol involving the testing in guinea pigs of Vaccinia virus and Baculovirus strains, making Ba protective antigen as prototype vaccines against human anthrax. The starting date of this experiment was to be June 19, "8" [writer believes this may mean 1989], and the ending date was to be December 31, 1990. Another individual not previously identified is [redacted]

A folder entitled [redacted]

[redacted] b6 b7C

[redacted] "key personnel" working on an enhancement of anthrax vaccine efficacy with immunostimulatory oligonucleotides. It was unclear which strain of Ba was used.

[redacted] showed that on October 13, 2000, Ivins attempted to send Ba Ames for research purposes to [redacted] b6 b7C b7D This request was denied on February 7, 2001 by the United States Department of Commerce on the grounds that it would be detrimental to United States foreign policy.

Notebook 3745 contained information about RMR 1029 experiments conducted between May and December 1999. In December 1999, Ivins gave some post-aerosol challenge sera to [redacted] b6 b7C for PA determination.

Notebook 3233 contained a request made in November 1990 from [redacted] and [redacted] of [redacted] They were attempting to design a [redacted] test for Ba and asked Ivins to provide some Ames spores, germinated spores, and vegetative cells.

Notebook 3919 contained electronic mail sent on February 24, 2000 from [redacted] of USAMRIID to various other researchers at USAMRIID. [redacted] was scheduling a multi-agent vaccine study; Ames spores were used in the study.

Notebook 1748 divulged that in March 1983, concentrated Ames supernatants were given to [redacted]

Notebook 2064 contained information about a June 1987 experiment conducted by Ivins, [redacted] They were attempting to obtain some "hot" spore preparations and

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determine the virulence of some of their other spore preparations. Subsequently, Ivins attempted to increase the virulence of some "May 87" Ames spores;

Experimental Protocol written on December 8, 1993, had an objective to

Experimental Protocol written on December 8, 1993, entailed a

Experimental Protocol written on July 29, 1994, comprised an

Notebook 3465, issued September 16, 1992, included several protocols utilizing Ba Ames with as-yet unidentified individuals listed as principal investigators: Protocol written September 13, 1992, and Protocol written on May 11, 1993, included a Protocol written on December 9, 1992, included a and Protocol written on April 1, 1993, included a This notebook also contained a Standard Operating Procedure for Lyophilization of Reagents, written February 24, 1992.

Notebook 3269, issued August 27, 1990, listed an experiment Ivins performed for to determine whether anthrax spores germinate in R-medium or R-medium and 10% unheated horse serum. Ivins also gave some Ames spores to on June 9, 1992 and on September 23, 1992.

Notebook 1670, issued June 29, 1982, contained an entry written on March 3, 1983. Ivins gave Ba Ames concentrated supernatants to for LF and PA determination.

Notebook 3080, issued November 20, 1987, contained information regarding an experiment conducted on or about January 21, 1988 by and in which the two tested the Ba Aro strain as a vaccine against Ames spore challenges. On or about April 20, 1988, Ivins and

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injected some "British" Ames spores into Hartley guinea pigs. The Ames spores were obtained from [redacted]

Notebook 3114, issued May 26, 1988, contained an entry on June 29, 1988, in which [redacted] suggested that Ivins [redacted] which involved Ba Ames. This idea was suggested because Ivins deemed his plasmid isolation procedure inadequate. Subsequently, [redacted] ran Ivins' [redacted]

Seven folders (Dugway Spore Harvests #1, Dugway - 2nd. spore shipment, -3rd spore shipment, -4th spore shipment, -5th spore shipment, -6th spore shipment, and -7th Dugway Shipment) contained information about spore shipments in 1997 from [redacted] of Dugway to Ivins. Some of the information included shipment dates, what was done with the spores after receipt, and how the spores were processed at Dugway.

The "Dugway Spore Information" folder contained information entitled "Scope of Work - *Bacillus anthracis* Ames Spores", detailing the Dugway spore shipments, the last of which was to be shipped no later than June 30, 1997. Inside the folder were faxed copies of quality control assay results between April 1997 and September 1997, initialed by [redacted] Also included was a copy of the Reference Material Receipt (RMR) 1029 inventory, dated October 22, 1997.

There were multiple folders containing numerous pages of information pertaining to [redacted] experiment. This was an eight-part, long-term rabbit study using RMR 1029. The study began in April 2000 and concluded January 2002. Copies of spore preparation forms indicating plate counts and concentrations were obtained. Similar forms were also obtained in the folder labeled "Long-term Efficacy Study; 12-month rabbit study".

Folder [redacted] contained the previously-unknown name of [redacted] referencing a challenge [it is unclear whether this is an intramuscular or aerosol challenge] and testing two types of anthrax vaccines. Notebook 2013, issued December 6, 1985, mentioned [redacted] and [redacted] were mentioned in studies with *B. subtilis* and *B. thuringiensis*. [redacted] was consulted by Ivins in Notebook 3167, issued May 19, 1989, on using polymerase chain reaction (PCR) [redacted]

The folder entitled [redacted] had different spreadsheets indicating various information, including: agent

name, ID number, room location, principal investigator, date acquired, facility from which the Agent was acquired, etc. Another spreadsheet in the folder was labeled *"Bacillus anthracis* strain collection", and had a hand-written line on top stating "Perry's Collection" [writer believes this to be Perry Mikesell]. This spreadsheet contained information such as Ba number, name, notes, and references. However, there were no dates on this collection. A spreadsheet dated July 22, 1991 had a list of all anthrax strains in Perry Mikesell's collection. The final pages in the folder described the type and location of particular Ba strains, dated between March 2002 and April 2004.

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Notebook 1511, with entries beginning November 2, 1988, contained [redacted] It also mentioned [redacted], along with [redacted] Ivins, as having harvested [redacted] on January 12, 1989.

In Notebook 3563, issued April 8, 1993, Ivins referred to "old" and "new" batches of Ames in December 1993. Other pages also mentioned these two batches of Ames, as well as the experimental protocols.

Notebook 3234, issued March 16, 1990, was entitled [redacted] and featured [redacted] "Making B. anthracis spores for [redacted] transductants". Two pages of Ivins' notes follow, describing a strange banding pattern: [redacted] This occurrence was dated July 25, 1990.

A folder entitled [redacted] contained a copy of a memorandum dated January 4, 1993 from [redacted]

[redacted] and [redacted] were names mentioned in Notebook [redacted], issued October 20, 1983. [redacted] mentioned in relation to [redacted]

Notebook [redacted] issued October 29, 1981, mentioned [redacted] and [redacted] Ivins gave [redacted] supernatants of different Ba strains, including Ames, on June 2, 1982, in order to perform an [redacted] The notebook

also contained information on the efficacy of certain media on growth, concluding that agar was a better support matrix than agarose. b6 b7C

Notebook 1670, issued June 29, 1982, stated that on September 1 (year unknown) [redacted] (it is not known where [redacted] is employed) gave Ivins *B. thuringiensis*, *B. subtilis*, and *S. fecalis*.

The folder entitled [redacted] contained a log of the amount of [redacted] received, the amount used, and the dates of use. The log was begun October 27, 1997.

The folder entitled "RMR 1029- Highly Purified Ames Spores - $3x10^{13}$" contained a set of e-mails between Ivins, [redacted] dated between October 12, 2001 and November 9, 2001. The e-mails documented numerous individuals to whom the Ames strain was sent and the dates in which it was sent.

A folder entitled "Harvesting Spores - + GLP Spore From Dugway" contained "Acceptance Criteria Test Forms", which tested and plated RMR 1029 on March 18, 1998. The last page featured an e-mail sent from Ivins on January 17, 1997 to the following principal investigators: [redacted] In the e-mail, Ivins calculated the amount of cultures needed and the time it would take to produce enough spores for aerosol challenges of 1000 rabbits and 200 monkeys. Ivins concluded that the concentration of spores for each animal was based on what he and others in his group administered (or tried to administer) to the monkeys and rabbits in F96-16 and F96-17, or 3.0 E9. Ivins prepared 8.5 milliliters of aerosol per animal, or about 8 milliliters per tube. Ultimately, Ivins calculated that it took 13 runs to generate about 3.0 E12 Ames spores for the "current batch". Since they needed ten times that amount, it would take them 130 runs with the flasks if performed with 2 liters per run, as they currently did. Therefore, it would take 130 weeks at one run per week or 65 weeks at 2 runs per week. The total amount of culture needed to produce the spores would be 260 liters. Below the email was Ivins' handwritten note describing what was needed, when, and in what solution the spores would be delivered.

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Notebooks:	Principal Invest.	Location	Copied	Comments
12	[redacted]	Ivins' office	Some	Names not already identified as POI/Access
16 (#3464)	Bruce Ivins	Ivins' office	Some	Instances in which Ames was given to someone
10 (#3080)	Bruce Ivins	Ivins' office	Some	Shipping forms
11 (#3114)	Bruce Ivins	Ivins' office	Some	Suspicious entries (abruptly stopping around 9/01-10/01)
BOI-11 (#4241)	Bruce Ivins	Ivins' office	No	Anything with Ba 7739, AO462, BA1004, 74, 1029-1030
17 (#3465)	Bruce Ivins	Ivins' office	Some	Storage locations
15 (#3269)	Bruce Ivins	Ivins' office	Some	
1670	[redacted]Ivins	Ivins' office	Some	
2013(#8)	Bruce Ivins	Ivins' office	Some	
1914	Bruce Ivins	Ivins' office	No	
2064	Bruce Ivins	Ivins' office	Some	
1748	[redacted]/Ivins	Ivins' office	Some	
14B (#3233)	Bruce Ivins	Ivins' office	Some	
3745	Bruce Ivins	Ivins' office	Some	
3919	Bruce Ivins	Ivins' office	Some	
1599	[redacted]Ivins	Ivins' office	Some	
4240	Bruce Ivins	Ivins' office	Some	
4237	Bruce Ivins	Ivins' office	Some	
3685	Bruce Ivins	Ivins' office	No	
3167 (#13)	Bruce Ivins	Ivins' office	Some	
3234	Bruce Ivins	Ivins' office	Some	
1519	Bruce Ivins	Ivins' office	Some	
"Anthrax Toxin", 2 Nov. 88	Bruce Ivins	Ivins' office	No	
3563	Bruce Ivins	Ivins' office	Some	
3760	Bruce Ivins	Ivins' office	No	
1511	Bruce Ivins	Ivins' office	Some	
3920	Bruce Ivins	Ivins' office	No	
4420	Bruce Ivins	Ivins' office	No	
4562	Bruce Ivins	Ivins' office	No	
4103	Bruce Ivins	Ivins' office	Some	Spore equivalency calculations of Ba Zimbabwe and Ames
4306	Bruce Ivins	Ivins' office	Some	Media comparisons, effects of temp. on spore count
4281	Ivins	Ivins' office	Most	Effect of storage conditions on spore counts in suspension
	[redacted]			Spore counts on different solid media
				Percent encapsulation of spores in preps
	BioPort personnel			Pour plate versus spread plate comparison
				Percent of spores in preps that are refractile or non-refractile

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				Comments
Folders:				Names not already identified as POI/Access
118 - Adjuvant Comparison Experiment #2	Bruce Ivins	Ivins' office	No	Instances in which Ames was given to someone
115 - Live Strain vaccination	Bruce Ivins	Ivins' office	No	Shipping forms
Protocol 112 - MPL Titration/Adjuvant and Antigen Preparation	Bruce Ivins	Ivins' office	No	Suspicious entries (abruptly stopping around 9/01-10/01)
Monkey challenge - 1.5-2 year (MDPH-C)	Bruce Ivins	Ivins' office	No	Anything with Ba 7739, AO462, BA1004, 74, 1029-1030
MDPH-C Manuscript	Bruce Ivins	Ivins' office	No	Storage locations
"C" MDPH-1 - Animal Protocol - 1991	Bruce Ivins	Ivins' office	No	
Approved LF Protocol - 2004	Bruce Ivins	Ivins' office	No	
Microencapsulation Work - Protocol 114	Bruce Ivins	Ivins' office	Some	
Protocol 116 - Multikine 1	Bruce Ivins	Ivins' office	No	
117 - Adjuvant Comparison Experiment #1	Bruce Ivins	Ivins' office	No	
Matrix - III and CPG Studies	Bruce Ivins	Ivins' office	No	
Information on anthrax steering committee and progress and plans of	Bruce Ivins	Ivins' office	No	
investigators (including a. Anthrax steering committee proposal reviews -	Bruce Ivins	Ivins' office	No	
2003 and b. Anthrax progress reports)	Bruce Ivins	Ivins' office	No	
F95-09 anthrax adjuvant study in monkeys	Bruce Ivins	Ivins' office	No	
Strain information	Bruce Ivins	Ivins' office	Some	
rPA steering committee information (a. rPA research plan, user name	Bruce Ivins	Ivins' office	No	
'bivins, password docsnivis4, b. Plans for FY2003 - rPA, and c. rPA	Bruce Ivins	Ivins' office	No	
progress reports, Fall 2003)	Bruce Ivins	Ivins' office	No	
CPG in guinea pigs	Bruce Ivins	Ivins' office	No	
Anthrax spore production proposal for 2004	Bruce Ivins	Ivins' office	No	
Animal protocols	Bruce Ivins	Ivins' office	Some	
Protocol 113 - Detox and BaculoPA detox and delta-Sterne PA	Bruce Ivins	Ivins' office	Some	
Protocol 121	Bruce Ivins	Ivins' office	No	
135 - Comparison of MDPH-PA with Alhydrogel-PA	Bruce Ivins	Ivins' office	No	
Visit and Studies by [redacted]	Bruce Ivins	Ivins' office	All	
Spore studies with [redacted]	Bruce Ivins	Ivins' office	Most	
B97-04	Bruce Ivins	Ivins' office	Page 1	
2003 CPG Research Plan	Bruce Ivins	Ivins' office	All	
Strains to [redacted]	Bruce Ivins	Ivins' office	All	
In-house strain transfers of anthrax spores	Bruce Ivins	Ivins' office	No	
"Old" formaldehyde study in rabbits; animal protocol B01-11	Bruce Ivins	Ivins' office	No	
F09-02, Long-term monkey study (not yet in notebook [*Ivins' note*])	Bruce Ivins	Ivins' office	No	
Long-term efficacy study - 12 month rabbit study	Bruce Ivins	Ivins' office	Some	
117-118 data	Bruce Ivins	Ivins' office	Some	

D94-09, 0.5, 5, and 50 microgram PA vaccine in monkeys	Bruce Ivins	Ivins' office	Some	Comments
[redacted] studies	Bruce Ivins	Ivins' office	No	Names not already identified as POI/Access
[redacted] study F96-17 - Rabbits (active immunization studies)	Bruce Ivins	Ivins' office	No	Instances in which Ames was given to someone
[redacted] study F96-16 - active immunization studies - monkeys	Bruce Ivins	Ivins' office	No	Shipping forms
MDPH Potency Data	Bruce Ivins	Ivins' office	No	Suspicious entries (abruptly stopping around 9/01-10/01)
B97-05	Bruce Ivins	Ivins' office	No	Anything with Ba 7739, AO462, BA1004, 74, 1029-1030
2003 rPA Research Plan	Bruce Ivins	Ivins' office	No	Storage locations
Long-term efficacy 6-month	Bruce Ivins	Ivins' office	All	
D99-02 [redacted] (sic) spore studies	Bruce Ivins	Ivins' office	No	
Research Plans - 2001 (a. Research Plans - Jan. 2001, b. Research	Bruce Ivins	Ivins' office	Some	
Plan Review - 2001)	Bruce Ivins	Ivins' office	No	
Anthrax SOPs and SSPs	Bruce Ivins	Ivins' office	All	
SEC - 911 relief	Bruce Ivins	Ivins' office	No	
Experimental Protocols involving Ames strain 1987-1995	Bruce Ivins	Ivins' office	No	
GLP studies (spores, etc.)	Bruce Ivins	Ivins' office	No	
GLP spores	Bruce Ivins	Ivins' office	No	
Making spores for MBPI - BioPort	Bruce Ivins	Ivins' office	No	
Dugway spore information	Bruce Ivins	Ivins' office	Some	
Harvesting spores - and GLP spore information (Dugway)	Bruce Ivins	Ivins' office	No	
7th Dugway shipment	Bruce Ivins	Ivins' office	Some	
Dugway - 5th spore shipment	Bruce Ivins	Ivins' office	Some	
Dugway - 4th spore shipment	Bruce Ivins	Ivins' office	Some	
Dugway - 3rd spore shipment	Bruce Ivins	Ivins' office	Some	
Dugway - 2nd spore shipment	Bruce Ivins	Ivins' office	Some	
Dugway Spore Harvests #1	Bruce Ivins	Ivins' office	Some	
RMR 1029 - Highly purified Ames spores - 3 x 10 to the 13, safety	Bruce Ivins	Ivins' office	Some	
office registration #2432 - entry line 130, ID #7737, record 916 - agent	Bruce Ivins	Ivins' office	No	
inventory system	Bruce Ivins	Ivins' office	No	
RMR 1030 - Ames spores for F97-08 challenge	Bruce Ivins	Ivins' office	Some	
SOPs - D94-09	Bruce Ivins	Ivins' office	No	
Alternative vaccine delivery steering committee	Bruce Ivins	Ivins' office	Some	
Mucosal Immunity	Bruce Ivins	Ivins' office	No	
Battelle Spores - 2004	Bruce Ivins	Ivins' office	All	
B97-03, Strain study [redacted]	Bruce Ivins	Ivins' office	No	
B99-03 Hamster studies	Bruce Ivins	Ivins' office	No	
Spore blebbing experiments - [redacted]	Bruce Ivins	Ivins' office	No	
134 - Alhydrogel/MPL as an adjuvant for PA	Bruce Ivins	Ivins' office	No	

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133 - Diethanolomine PA versus Ammonium Acetate PA	Bruce Ivins	Ivins' office	No	Comments
132 - Efficacyof different emulsions and PA in guinea pigs	Bruce Ivins	Ivins' office	No	Names not already identified as POI/Access
128 - Encapsulated live vaccine trial with [redacted] strains	Bruce Ivins	Ivins' office	All	Instances in which Ames was given to someone
PASSIMM3.LIT	Bruce Ivins	Ivins' office	No	Shipping forms
ACTIMM2 - Experiment with [redacted]	Bruce Ivins	Ivins' office	No	Suspicious entries (abruptly stopping around 9/01-10/01)
OLDMPL.126 - Efficacy test of MPL + PA in Emulsion from June 1992	Bruce Ivins	Ivins' office	No	Anything with Ba 7739, AO462, BA1004, 74, 1029-1030
Freezing/Storing/Lyooplizing (sic) buffer for PA Storage 125	Bruce Ivins	Ivins' office	No	Storage locations
Protocol/124 - PA and Emulsion I MPL	Bruce Ivins	Ivins' office	No	
123 [redacted] live strains	Bruce Ivins	Ivins' office	All	
122 [redacted] Microencapsulated PA	Bruce Ivins	Ivins' office	No	
PASSIMM2 Expt c [redacted]	Bruce Ivins	Ivins' office	No	
Protocol 119 - Multikine 2	Bruce Ivins	Ivins' office	No	
Comparison of MDPH-PA with Alhydrogel and PA	Bruce Ivins	Ivins' office	No	
MPL and PA - Protection/Info. to Art	Bruce Ivins	Ivins' office	No	
Vaccinest Vaccine Candidates Reports	Bruce Ivins	Ivins' office	No	
Presentation to vaccine working group - Oct. '92	Bruce Ivins	Ivins' office	No	
Passing out CR4 and Processing	Bruce Ivins	Ivins' office	No	
PA1, PA2, PA7	Bruce Ivins	Ivins' office	No	
Aro-1 and Aro-2	Bruce Ivins	Ivins' office	No	
Monkey protocol bacteremias	Bruce Ivins	Ivins' office	No	
RAB-GP Rabbit study and G. pig study	Bruce Ivins	Ivins' office	No	
Summary statistical data for massive computation	Bruce Ivins	Ivins' office	No	
BAWG Talk - 1999; ASM 1988	Bruce Ivins	Ivins' office	No	
Survivors 0/2 PBS, 9/10 Alhydrogel-PA, 9/9 MDPH-AVA, D94-01	Bruce Ivins	Ivins' office	No	
Adjuvants Used in Anthrax Vaccine Preclinical Studies and Vaccine	Bruce Ivins	Ivins' office	No	
Clinical Trials	Bruce Ivins	Ivins' office	No	
KIL-1, G. pig immunization c Aro-strains	Bruce Ivins	Ivins' office	No	
Protocol 137 - Immunization c live and irradiated delta-Sterne (pPA102) spores	Bruce Ivins	Ivins' office	No	
136 - LT stimulation by different anthrax vaccines	Bruce Ivins	Ivins' office	No	
2002 Research Plans	Bruce Ivins	Ivins' office	No	
Immunization with PA fragments and other experiments c [redacted] JD-374	Bruce Ivins	Ivins' office	No	
Vaccine Efficacy Studies-Protocols 98-102; Protocol 103 - PA titration	Bruce Ivins	Ivins' office	No	
Aro Experiments c [redacted]	Bruce Ivins	Ivins' office	No	
B90-03, 1990 Animal Protocols - G. pig immunization/LD505	Bruce Ivins	Ivins' office	No	
Mouse safety of Aro KIL-2	Bruce Ivins	Ivins' office	No	
B00-03 [redacted] Experiments (Parts 1-7)	Bruce Ivins	Ivins' office	Some	

FD-302 (Rev. 10-6-95)

FEDERAL BUREAU OF INVESTIGATION

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ALL INFORMATION CONTAINED
HEREIN IS UNCLASSIFIED
DATE 12-12-2008 BY 60324 UC BAW/DK/RYS

Date of transcription 01/29/2005

DOB home telephone number , was interviewed at the Federal Bureau of Investigations Office, . After being informed of the identity of the interviewing Agents and the purpose of the interview, provided the following information:

is currently employed with area. resides at the above address with

was assigned to the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). Within USAMRIID, primarily worked as a in the within the

believes may have been involved in challenges using the Ames strain of Bacillus anthracis (Ba) , but could not be sure since most test samples were not marked with particular strain information, nor was it a common practice for the Principle Investigators (PI), Doctors Bruce IVINS and , to inform the testing individuals of sample strain types. If used Ames strain Ba, believes more than likely worked with it in rooms and

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could not remember ever working for, or with,

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could not remember ever working with Ames strain Ba made at or by Dugway Proving Grounds (DPG), Utah.

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believes worked with Ba conducting challenge studies in "hotside" rooms and

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Before October 2001, stated most everyone on were involved in conducting aerosol

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Investigation on 01/28/2005 at

File # 279A-WF-222936-USAMRIID -1160 Date dictated

by SA
SA

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challenges in the hotside, but after October 2001, most of the [redacted] were stripped away to conduct testing on unknown mail samples. [redacted] stated that on many occasions, [redacted] and IVINS would be present during the challenges, but not during all of these experiments.

For challenge experiments, [redacted] and other [redacted] would begin preparation for testing the week prior to the actual start of testing by decontaminating the work areas. Normal decontamination procedures involved the use of paraformaldahyde to decontaminate all work areas. At the start of testing, [redacted] received all samples and animals into the hot area and transported them to the test area. For challenge experiments, agent samples were placed into all-glass impingers (AGI) and aerosolized into the test animal cages. [redacted] would regularly collect air samples for later plating, to determine the quantity of agent the animals were exposed to during the testing. [redacted]

[redacted] remembers using anti-foam in aerosol challenges, but could not remember its brand-name or whether it contained silicone. Anti-foam was added to the AGI to keep materials inside the nebulizer from bubbling over.

[redacted] could not provide any information concerning added materials since solutions for challenges were pre-mixed before being provided to the testers.

[redacted] knowledge of any substitutes for anti-foam used in these aerosol challenges.

[redacted] conducted tests for [redacted] BRUCE IVINS, [redacted] during [redacted] employment at USAMRIID, but had no knowledge of them adding anti-foam or olive oil to their challenges. [redacted] was already gone from USAMRIID when [redacted] started working there.

[redacted] believed there was spore materials left over after many of these challenges, [redacted]

At the completion of the challenge, the remaining materials would be poured back into the original test tube and placed into the autoclave for destruction.

To the best of [redacted] knowledge, no one ever asked [redacted] for, or took, any of the left over material or plates out of the hot area.

[redacted]

[redacted] Trash was the only item from the hot area which was taken to the basement for additional autoclaving.

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[redacted] believed the only access points for the basement included one stairwell and one elevator. [redacted] never observed anyone carrying autoclave bags out of the basement. As stated previously, [redacted] did not work at USAMRIID while [redacted] was there. Therefore, [redacted] could not comment on whether [redacted] was ever around Building [redacted]

[redacted] observed what [redacted] believed to be plates containing Ba during late 2002, but could not be sure whether they were Ames strain or not.

[redacted] had no knowledge of olive oil being used in any testing or mixtures during his tenure at USAMRIID. [redacted] may have observed a container of olive oil on the cold side, but could not provide any time frame on when [redacted] remembered seeing a quart-sized, green-tinted bottle with some type of label. [redacted] believed this bottle was located in the [redacted] suite, possibly suite [redacted] but could not provide any further details about the bottle. [redacted] believed the bottle to contain olive oil only because it looked visually like a bottle of olive oil, but [redacted] never verified the bottle's contents.

[redacted] could not provide any information about the use of Bertolli brand olive oil in testing while [redacted] worked at USAMRIID.

Phosphate Buffered Saline (PBS) was purchased by the case from a company called Gibco and came in 10X concentration solutions. PBS was then diluted down with distilled water for each experiment. [redacted] had no knowledge of the addition of oil to the PBS or the use of anti-foam in relation to the silicon content of the PBS.

Any documentation about whether anti-foam was used or not in challenges would be located in laboratory SOPs at USAMRIID.

[redacted] was not involved in any of the Ames challenges in November 1997, and did not observe [redacted] in Building [redacted] during that same time period.

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[redacted] had no knowledge of anyone at USAMRIID keeping left over challenge spore preparations and could not speculate on who might do such a dangerous thing.

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[redacted] could not provide any further information which might be related to the theft of Ames strain Ba from USAMRIID or to other aspects of the mailings.

Ba was delivered to [redacted] for challenge testing in double bagged, plastic 10 milliliter (ml) Falcon tubes.

[redacted] believed the liquid Ba samples were mixed by the laboratory assistants for the testing primary researcher. [redacted] observed Dr. IVINS mix samples on several occasions. All leftover samples were destroyed by double autoclaving. Testing samples were stored outside of the lab before testing, inside of the challenge hoods in test tube racks, and placed inside of autoclave bags at the completion of the challenges. Samples were always labeled with concentration information.

FD-302 (Rev. 10-6-95)

FEDERAL BUREAU OF INVESTIGATION

Date of transcription 04/06/2005 b6 b7C

Pursuant to the AMERITHRAX investigation, a trash cover search regarding items discarded by BRUCE IVINS, was conducted on 04/01/2005 and 04/05/2005, at his residence, [redacted] Military Road, Frederick, Maryland. The items were discarded by IVINS at curbside for Frederick Department of Public Works (DPW) pickup on regularly scheduled trash pickup. The trash route for his residence was scheduled for Tuesday and Friday pickup. b6 b7C b2 b7E

[redacted]

[redacted] There was no trash placed at the curb at [redacted] Military Road. Two trash pails were observed in the driveway, adjacent to the residence, but it was unknown whether or not they contained garbage. b2 b7E b6 b7C b2 b7E

[redacted] Five plastic bags were collected from the residence of IVINS [redacted]

[redacted] b6 b7C

Investigation on 03/31/2005 at Frederick, Maryland

File # 279A-WF-222936-USAMRIID -1174 Date dictated 04/06/2005

by SA [redacted]
SA

Item 1: Papers consisting of advertisements and information for an orchestra and events relating to the orchestra.

Item 2: Information packet titled, "Friends Welcome" and 8.5" by 11" map of a resort community named Bethany Bay, with writing on the map.

Item 3: Several printed e-mails sent to various recipients from [redacted] regarding an orchestral quartet.

Item 4: Manila envelopes with names of orchestral quartet members printed on white labels.

Item 5: One ziploc bag with brown residue, one empty box of gelatin mix, and one receipt from Lee Nails, 1700 King Fisher, Frederick, Maryland.

FEDERAL BUREAU OF INVESTIGATION
FOIPA
DELETED PAGE INFORMATION SHEET

No Duplication Fees are charged for Deleted Page Information Sheet(s).

Total Deleted Page(s) ~ 32
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Page 89 ~ b6, b7C
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Page 165 ~ Duplicate
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