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The 2001 Attack Anthrax: Key Observations

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Abstract

Unresolved scientific questions, remaining ten years after the anthrax attacks, three years after the FBI accused a dead man of perpetrating the 2001 anthrax attacks singlehandedly, and more than a year since they closed the case without further investigation, indictment or trial, are perpetuating serious concerns that the FBI may have accused the wrong person of carrying out the anthrax attacks. The FBI has not produced concrete evidence on key questions:

- **Where and how were the anthrax spores in the attack letters prepared?**

There is no material evidence of where the attack anthrax was made, and no direct evidence that any specific individual made the anthrax, or mailed it. On the basis of a number of assumptions, the FBI has not scrutinized the most likely laboratories.

- **How and why did the spore powders acquire the high levels of silicon and tin found in them?**

The FBI has repeatedly insisted that the powders in the letters contained no additives, but they also claim that they have not been able to reproduce the high silicon content in the powders, and there has been little public mention of the extraordinary presence of tin. All the available evidence can be explained by the hypothesis that the spore coats were silicone-coated using a tin catalyst. Chemical details are presented here.

- **Where did the anthrax spores become contaminated by a rare strain of *B. subtilis*?**

The FBI never located the source of the strain, but they never searched in the most likely places.

Once the method of preparation of the attack anthrax is understood, the questions of who made it, and where, will be rapidly resolved. The publicly-known evidence related to these questions is compiled here, with full documentation.

Introduction

On August 6, 2008 the FBI announced its conclusion that Bruce Ivins, a scientist at the US Army Research Institute for Infectious Diseases (USAMRIID), was the sole perpetrator of the 2001 anthrax letter attacks. Nine days earlier, Ivins had killed himself. The FBI's announcement met with widespread doubts among scientists and other experts who considered the evidence to be, at best, highly circumstantial. The doubts remain in spite of subsequent FBI briefings and document releases. In September 2008 the FBI issued a formal request to the National Academy of Sciences (NAS) to conduct an independent review of the scientific approaches used during the anthrax investigation. Before that review had been completed, the FBI formally concluded its investigation and released an "Amerithrax Investigative Summary" on February 19, 2010. The NAS committee issued its Report on Feb. 15, 2011, after two delays and an additional meeting at the FBI's request. When the NAS Report came out the FBI released some 10,000 pages of scientific documents, much of which consisted of plans and reports of laboratory work on unidentified, coded materials. By agreement, all the information that had been available to the NAS committee is now in the public domain. The major finding of the committee, which had no access to classified information, was "It is not possible to reach a definitive conclusion about the origins of the *B. anthracis* in the mailings based on the available [to them] scientific evidence alone"[1]. Perhaps their most celebrated finding is that "the scientific link between the letter material and flask number RMR-1029 [located in Bruce Ivins' laboratory at USAMRIID] is not as conclusive as stated in the DOJ Investigative Summary"[2].

Observations

Potential production sites of the attack anthrax

Genetic evidence from the attack anthrax itself is the prime indicator of laboratories at which genetically-matching *B. anthracis* could have been accessed for growing the spores sent in the letters. The spores were found to be Ames-strain *B. anthracis* and to include multiple colony morphotypes, some apparently unique. The latter provided the basis for four specific molecular assays that were developed for use as indicators of close relationship to the anthrax in the letters[3]. The FBI assembled a repository[4] (likely incomplete[5]) of 1070 Ames-strain *B. anthracis* samples from 17 laboratories that were identified as possessing that strain. Assays on 947 of the repository samples[6] detected samples from 10 laboratories that tested positive in one or more of the specific assays[7]. Eight samples from two laboratories were positive in all four assays: seven samples from USAMRIID (which possessed flask RMR 1029, an Ames-strain *B. anthracis* spore preparation later shown in genetic tests to be indistinguishable from the attack anthrax) and one sample from Battelle Memorial Institute (BMI) in Ohio[8] (which had been sent samples from flask RMR 1029 on May 8 and June 18, 2001)[9]. Samples with partially-matching assay results cannot be disregarded, for a variety of reasons including variability in the assay results[10], lack

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of repeat analyses, false negatives, and the fact that, prior to assay, each sample had been through two rounds of cultivation[11]. Among the eight laboratories that submitted a total of 63 samples with 1-3 positive assay results[12] are: Dugway Proving Ground, the Naval Medical Research Center, Northern Arizona University, the Canadian Defense Research Establishment at Suffield (DRES)[13], and a second sample from Battelle[14]. The submitters of the other three samples have not been revealed.

A priori, the most likely sites of production of the letter anthrax are laboratories that work with dry spores: Battelle, Dugway, and DRES, and their associated institutions and subcontractors. Battelle, for example, is well-known for its aerosol study capabilities and biodefense activities, for which dry spores are routinely needed[15]. USAMRIID, on the other hand, has always insisted that dry spores are never used in the work there. The FBI says that, prior to the attacks, no US laboratory had Ames anthrax spores in powder form[16]; however, powdered anthrax spores are known to have been produced at Dugway in the last few years before the attacks[17]. The FBI recognized that Dugway had the know-how[18], and also, the strain—Dugway had produced the bulk of the *B. anthracis* in USAMRIID's flask RMR 1029 in 1997[19]. Furthermore, Battelle has an operation at Dugway and some other government locations[20], and might have transferred material there. Also note that, according to the US Department of Justice, "Upon the receipt of RMR-1029 spores, the private research laboratory [defined earlier in the same document as "a private laboratory operated by Battelle"] was allowed to provide aliquots to other laboratory facilities for legitimate research purposes [21]".

The FBI ruled out Battelle as the source of the attack anthrax on the implicit, and unwarranted, assumptions (1) that the anthrax spore preparations in the letters must have been made covertly, and (2) made by the perpetrator(s) of the attack. They say that every minute in the "Midwest" Battelle laboratory is accounted for, and no researcher was ever alone in the laboratory; background investigations of everyone who had access to the RMR 1029 material received from USAMRIID gave unremarkable results; and the great distance of the Battelle laboratory in Ohio from Princeton, NJ, where the anthrax letters were mailed, "preclude any reasonable possibility that the mailings came from there[22]". Dugway and DRES, being much farther from Princeton than Battelle, may have been eliminated on that basis alone. However, there is no publicly available information to rule out the possibility that the anthrax spores in the letters were made somewhere in the normal conduct of authorized laboratory operations, and later acquired by the mailer(s) at the same or some other location.

The FBI has also routinely assumed[23] (3) that the attack spores were prepared during the short interval between 9/11 and the mailings of the letters on Sept. 18 and Oct. 9, 2001; but there is no publicly known evidence for that assumption. Battelle received its first shipment of material from the USAMRIID flask RMR 1029 on May 9, 2001[24]. A preparation of dry anthrax spores could have been grown at Battelle from that material any time thereafter, for some authorized (and probably classified) purpose such as vulnerability/response studies[25]; the spores could then have been provided to a distant client or other authorized person(s), who, following 9/11, decided to conduct the anthrax attacks. The same can be said of Dugway, and perhaps also of the unidentified laboratories that submitted repository samples that tested partially-positive.

The FBI has released a small amount of information on Battelle's Ames-strain *B. anthracis*. Battelle received a sample of RMR 1029 in May, 2001 and used it, in part, to test aerosol equipment[26]; they used part of the second sample, received in June, to grow a new *B. anthracis* preparation, produced at Battelle on July 17, 2001[27]. In 2002 Battelle submitted 19 samples (in duplicate) to the FBI repository, including samples of all the Ames-strain preparations it is publicly known to possess[28]. It is interesting that a document that may have contained information related to Battelle's repository samples was withheld by the FBI because of its security classification[29], and that the FBI executed a search warrant at the Battelle laboratory in 2004 to make certain that all its Ames-strain stocks were represented in the repository[30].

As for USAMRIID, which the FBI has examined closely[31], there is no material evidence that the attack spores were made there, and no direct evidence that an individual at USAMRIID made the anthrax, or mailed

1. NAS Report "Review of the Scientific Approaches Used During the FBI's Investigation of the 2001 Anthrax Letters," Feb. 15, 2011, Summary of Committee Findings.
2. Ibid.
3. NAS Report, Chapter 5.
4. The FBI issued a subpoena in February 2002 requiring submission of samples from "each distinct *B. anthracis Ames* strain stock in your possession, which differs in source or in other parameters prescribed by the requesting agency" (no other parameters are mentioned in the documents). Both institutional and personal inventories were covered (NAS Report, p. 105; FBI "Supplemental Documents:" "Preparing and Shipping TSA Slants for *B. anthracis Ames*").
5. NAS Report pp. 104-107 and 119. The Report notes that the repository was unlikely to have been comprehensive because of uncertainty in whether all Ames-strain possessors had been identified, the lack of specificity in the subpoena protocol, uncertainties in compliance with the protocol, incomplete information on Ames-strain transfers between laboratories, the possibility that some stocks had already been destroyed in the months before the FBI sent its subpoena, and because repository collection was based on the integrity of those asked to provide samples rather than on standards of custody or evidence (which would have required that FBI agents collect the samples).
6. Eleven repository samples were not viable, and 112 samples gave inconclusive assay results, which were discarded (NAS Report, pp. 109-110). Thus, 123 samples, submitted by an unreported number of laboratories, have not been considered.
7. NAS Report, pp. 110-112. Also, note that an FBI Press Release of March 6, 2009, discussing a presentation at the 2009 American Society for Microbiology meeting in Baltimore, said that eight samples matched the genetic profile of the letter material. (http://www2.fbi.gov/pressrel/pressrel09/anthrax030609.htm).
8. FBI documents B2M10 p. 25 and B3D16.
9. FBI document B3D16.
10. See, for example *B. anthracis* assay results on 30 repeat samplings of one liquid sample (NAS Report 117).
11. See NAS Report eg. pp. 114-117 and p 107.
12. NAS Report p. 112; FBI document B2M10 pp. 25 and 104-112 (Appendix V of Report on Statistical Analysis, where the names of some laboratories are handwritten next to data on their samples).
13. A hand-drawn diagram in FBI document B3D16 indicates that Ivins sent a sample to DRES on June 21, 1998 and that an FBI repository sample from DRES tested positive for A1, negative for A3 markers; the same diagram indicates the two shipments to BMI in 2001.
14. FBI docoument B3D16.
15. For Battelle's capabilities, see www.battelle.org; and Miller J, Engelberg S, Broad W, Germs: Biological Weapons and America's Secret War (Simon and Schuster, NY, 2001), which revealed that Battelle was contracted by the Defense Intelligence Agency's Project Jefferson to reproduce a Russian vaccine-resistant strain of anthrax.

it. The FBI cannot point to specific equipment, facilities or materials that would have been needed at the site(s) where the spores were produced, or the time required for their production; in communicating with the NAS Committee, the FBI hedged on all these aspects of the production process, indicating uncertainty while seeming to know more than they were willing to reveal[32]. They did not tell the Committee the scientific rationale for the choice of the various surrogate preparations studied, and would not divulge the preparation procedures for some of the preparations. Perhaps the motive was to protect proprietary defense information; nonetheless, the lack of information raises questions about the adequacy of the investigation. The NAS Report recommended that a review should be conducted of all classified materials that are relevant to the investigation[33].

Regarding potential production sites, the FBI has not scrutinized the most significant aspects of the attack anthrax preparations; namely the silicon and tin levels and *B.subtilis* contaminant (discussed in the next two sections). On the basis of these aspects, as well as on purity, the powders in the first batch of letters (sent to the *New York Post* and Tom Brokaw) are markedly different from the powders in the two Senate letters, mailed later. These differences strongly suggest that there were two different production runs, and that, instead of making the anthrax themselves, the perpetrator(s) probably took advantage of already-existing materials that had not been made for the purpose of terrorism. The obvious differences in quality suggest that there had been a review of quality control procedures involved at some stage in the production of the powders.

Silicon and tin additives in the attack letter powders

Silicon[34] has been recognized as a "key component[35]" of the attack anthrax from the beginning[36], although there has been much controversy over its nature and purpose. The presence of silica (SiO_2) nanoparticles, classically used to increase the dispersibility of biological agents, was eventually ruled out. Six months after the attacks, an unusual chemical was said to be found in the letter spores[37], and in November 2003 the journal *Science* published an article claiming the spores contained a "polymerized glass" component –a silane or siloxane compound that "leaves a thin glassy coating[38]". This information was said to have been provided by US Intelligence to officials of two NATO countries. Richard Spertzel, former Deputy Commander of USAMRIID and Senior Biologist of the UN Special Commission for Iraq, later confirmed that this was known to the German Foreign Ministry[39]. At the end of this section we propose a process, consistent with all the known evidence, by which the attack spores may have been given a polysiloxane (silicone) coating using a tin catalyst.

Meanwhile, Dwight Adams, the Chief FBI scientist, stated at a private FBI briefing of Senators Tom Daschle and Patrick Leahy in late 2002 that the letter anthrax contained no additives, but did contain silicon which occurred naturally in the spore coat, not on the surface of the spore (the exosporium). This information was leaked by "sources on Capitol Hill"[40] but did not become fully known until Adams testified in January, 2006 in the Hatfill vs. Ashcroft et al. lawsuit. At that time Adams said, in a sworn deposition, that scientific information obtained by the FBI about the letter anthrax is too sensitive to reveal to either the public or the Senate, Congress or their staff [41].

An ongoing concern of the FBI may have been to protect what may originally, before all the leaks and speculations, have been valuable security information about anthrax spore preparation. In an August, 2006 scientific article by an FBI scientist[42], ostensibly about the procedures used to search bags of Congressional mail, a carefully-worded paragraph was inserted to imply, once again, that the attack anthrax consisted simply of spores, without additives, and was not weaponized. The article was widely touted as "the most expansive public comment on the nature of the powder by any FBI official[43]." When the scientific journal printed a reader's letter objecting to the absence of

16. Amerithrax Investigative Summary, issued by the US Department of Justice on February 19, 2010.
17. Dugway made several preparations of powdered spores from both *B. anthracis* and *B. subtilis*, for such uses as decontamination studies (Baltimore Sun, "Army confirms making anthrax in recent years: Military laboratory in Utah says powder is all accounted for," December 13, 2001; New York Times, "US recently produced anthrax in a highly lethal powder form," December 13, 2001).
18. The FBI asked Dugway to produce dry spore preparations, after the attacks, in an effort to "reverse engineer" the attack anthrax (FBI document B1M13).
19. NAS Report pp. 107-108 gives reasons why the spores produced at Dugway must have contained some or all of the *B. anthracis* genetic variants present in the final mix in flask RMR 1029.
20. Battelle News Release, "Battelle opens office in Salt Lake City to spur technology partnerships," December 18, 2001. According to a Battelle official, they are "supporting the Department of Defense on a variety of projects there."
21. US District Court, Southern District of Florida, Case number: 03-81110-CIV-Hurley/Hopkins (Stevens vs. US): Notice of Errata Correcting [DE# 153, 154-1, 155, 155-1], items 3 and 5.
22. Amerithrax Investigative Summary, (op. cit.).
23. See, for example, Amerithrax Investigative Summary (op. cit.), pp 20, 33-35, and elsewhere.
24. Amerithrax Investigative Summary (op. cit.).
25. Several government agencies were involved in projects of this kind before the anthrax attacks. See, for example, the interagency exercise codenamed "Capitol Reaction" in 1997 (William Arkin, Code Names: Deciphering US Military Plans, Programs and Operations in the 9/11 World (Steerforth Press, Hanover, NH, 2005)); and "Project Jefferson," a secret biological program with multiple sub-projects, initiated in 1998 by the Defense Intelligence Agency at Battelle (Miller J, Engelberg S, Broad W Germs: Biological Weapons and America's Secret War (Simon and Schuster, NY, 2001)).
26. FBI document B3D16, "AVA and Related Material Inventory Log" for sample no. 87-S, which is Battelle's code for the RMR 1029 sample received in June, 2001 (see description of sample 22 in the sample set submitted to the repository, B3D16).
27. FBI document B3D16. The nature and purpose of the July 17, 2001 preparation are not revealed.
28. FBI document B3D16. The Battelle repository sample that tested positive for four markers was taken directly from the RMR 1029 sample received in June from USAMRIID (FBI document B3D10). Only one other Battelle repository sample had any of the letter anthrax markers – it tested positive for two markers but negative for one, and appears to have been taken indirectly from the RMR 1029 sample they received in May 2001, which had been used up in June except for the diluted aliquot from which the submitted sample was taken (B3D16). Another Battelle repository submission was from material produced at Battelle on July 17, 2001from the June USAMRIID sample (*ibid.*); four submissions were from preparations made at Battelle in 1994, 1995, 1997 and 1999 (ibid.); no information is available on the origins of the other 12 repository samples from Battelle.
29. NAS Report, p. 148.
30. NAS Report, p. 104; the results of the search have not been revealed. Consent searches were conducted at USAMRIID and Dugway for the same purpose.

evidence in the FBI article to support its conclusions[44], the journal's Editor invited a reply but the FBI refused.

Two years later, in connection with the announcement in 2008 that Ivins was the perpetrator of the anthrax attacks, the FBI held a science briefing in which they reiterated that the attack spores contained no additives and that silicon was naturally present in the spore coat. When asked for the percent silicon in the spores, the FBI spokesman refused to specify it[45]. A few weeks later, FBI Director Robert Mueller was asked the same question at a Congressional hearing on FBI oversight. Mueller said he was unable to answer at that time; but he eventually did so in an April, 2009 letter[46] to the Chairman of the Committee on the Judiciary, saying that the powder in the Leahy letter contained 1.4% silicon by weight. He added that insufficient material made it impossible to analyze material from the *NY Post*, Daschle or Brokaw letters. In fact, however, the FBI had known the percentage of silicon in the *NY Post* powder since October, 2002, when an FBI laboratory measured silicon content of 10.77%[47] (this was not publicly divulged until the FBI document dump of February, 2011). The FBI also analyzed the Leahy sample in 2002, and again in 2004, and found it contained 1.84 and 1.44% silicon[48].

Even earlier, measurements made by the Armed Services Institute of Pathology (AFIP) in October, 2001, when they first discovered the presence of silicon, must have been available to the FBI. AFIP released its data in response to a Freedom of Information Act request in April, 2010[49]. Using the AFIP data, one of us (SJ) calculated[50] silicon content of the order of 3% in the Daschle powder and 30% in the NY Post powder.

It was already known from earlier work that silicon can be naturally incorporated into *Bacillus* spores during their formation[51]. Consequently, the FBI contracted scientists at Sandia National Laboratory in December, 2001 to determine the location of the silicon within the spores by examining thin sections with high-resolution scanning and transmission electronmicroscopy (SEM and STEM)[52]. The Sandia findings became public in September, 2009: silicon was localized on the spore coat, "not on the spore surface," in the letter samples, as well as in surrogate samples that contained naturally-incorporated silicon[53]. The weight-percent silicon "at the spore level" was the same in the Leahy, Daschle and NY Post samples, approximately 1.5%. Sandia did not report the percentage at the bulk level[54].

Thus, the silicon content per spore in the Leahy letter, as

Sample	Al	Ca	Fe	K	Mg	Mn	Si	Sn	Na	Zn
NY Post (SPS02.88) (Q13)	158	26753	449	1008	2150	28	107700 (10.77%)	6511 (0.65%)	3993	173
Leahy (SPS02.266) Sample 1 (Q12)	220	25975	1668	934	2592	196	18441 (1.84%)	1979 (0.19%)	2940	230
Leahy (SPS02.266) Sample 2 (Q1801)	187	25987	1983	no report	2601	113	14479 (1.45%)	1959 (0.19%)	no report	221
B. subtilis (K449)	127	15380	11874	4671	3501	189	none detected	none detected	2188	169
B. subtilis with silica fluidizer (K450)	16	13369	73	12918	2039	3585	15623	none detected	23483	68

Table 1: Elements (ppm) detected by FBI labs in attack powders and simulants measured by inductively-coupled plasma atomic emission spectroscopy (ICP-OES) (data from FBI document B1M7).

31. See, for example, searches mentioned in FBI documents B3D10 and B3D15F.
32. NAS Report, Chapter 4, section 4.2, pp. 61 and 63 and section 4.3. Clearly exasperated, the Committee wrote (p. 63), "Without further specification with respect to spore preparations variables, the committee finds no scientific basis on which to accurately estimate the amount of the time or specific skill set needed to prepare the spore material."
33. NAS Report, Summary 9.
34. Terminology: Silicon is the element itself; silica (SiO_2) is the oxide form of Silicon; silane compounds are Silicon analogs of alkanes (eg $Si(CH_3)_4$, $Si(CH)_2(Cl)_2$, etc.); siloxanes are compounds containing a siloxane bond (-Si-O-Si-), eg, polydimethylsiloxane, which has equal numbers of Si and O atoms; silicones are polysiloxane polymers with organic side chains (polydimethylsiloxane is the simplest).
35. Florabel Mullick, Principal Deputy Director of the Armed Forces Institute of Pathology (AFIP), where energy dispersive X-ray spectrometry (EDX) on the Daschle spores was performed in October 2001. She is quoted in a comment on that work in The AFIP Letter, "Detecting Environmental Terrorism: AFIP's Department of Environmental and Toxicologic Pathology provides critical DoD, Homeland Defense programs," August/October 2002.
36. White House Press Briefing by Homeland Security Director Tom Ridge et al, 11:52 am EST, October 29, 2001, at which Maj. Gen. John S. Parker, Commander of Fort Detrick and of the US Military Research and Materiel Command, announced AFIP's finding of Silicon.
37. Washington Post, "Powder used in anthrax attacks was not routine," April 9, 2002; CNN.com, "Official: Unusual coating in anthrax mailings," posted 7:55 am EDT, April 11, 2002.
38. Matsumoto G (2003) Anthrax Powder: State of the Art? Science 302: 1492-1497.
39. The Wall Street Journal, "Bruce Ivins Wasn't the Anthrax Culprit," OpEd by Richard Spertzel, August 5, 2008.
40. See G Matsumoto, op. cit.
41. Dwight E. Adams, Deposition made in the Hatfill vs. Ashcroft et al. lawsuit (Case # 1:03-cv-01793-RBW, filed 8/26/03), under questioning by Hatfill's lawyer Thomas Connolly, January 11, 2006.
42. Beecher DJ (2006) Forensic application of microbiological culture analysis to identify mail intentionally contaminated with *Bacillus anthracis* spores, Appl Environ Microbiol 72: 5304.
43. Washington Post, "FBI is casting a wider net in anthrax attacks", September 25, 2006.
44. Mereish KA (who was the Chief of Biological Planning and Operations at the UN Monitoring, Verification and Inspection Commission (UNMOVIC) that inspected Iraq for biological weapons) (2007) Letter to the Editor: Unsupported Conclusions on the *Bacillus anthracis* Spores, Appl Environ Microbiol 73: 5074.
45. FBI Science Briefing on the Anthrax Investigation, August 18, 2008.

determined at Sandia (1.5%), matched the bulk silicon content in the Leahy powder, as determined by the FBI (1.4-1.8%). But for the *NY Post* letter, as the NAS Report notes, "there was a substantial difference between the amount of silicon measured in bulk [by the FBI (10.77%), and by AFIP] and that measured in individual spores [1.5%, by Sandia]. No compelling explanation for this difference was provided to the committee[55]". Neither the FBI nor the NAS committee mentioned the extra-sporular material visible in AFIP's images of the *NY Post* sample, but not in images of the Daschle sample. Extra-sporular material would be included in measurements made on bulk samples, but not in measurements using higher-resolution SEM on single spores or spore slices, the focus of the Sandia work. We will come back later to the extra-sporular material in the *NY Post* sample.

The high levels of silicon found in the attack powders are extremely unusual. The largest amount that FBI contractors at Lawrence Livermore National Laboratory could find[56], in 26 pre-existing *Bacilllus* samples submitted to them by the Department of Homeland Security (DHS) and collaborating laboratories[57], was about 0.4 wt%, and the highest level they were able to achieve themselves through growth of *B. anthracis* Sterne spores in the presence of silicate (the mechanism of incorporation postulated by the FBI[58]), even in media approaching silicate saturation, was 0.3%. Similarly, the Sandia group found that

Sample**	Al	Ca	Fe	K	Mg	Mn	Si	Sn	Na	Zn	Cu	Ti
B1	2699	75829	3081	*	2844	31357	n/d	n/d	4739	3164	325	506
B2	2403	81136	2840	*	6288	24630	n/d	n/d	4057	2825	334	418
B3	1887	62745	1961	*	4510	18477	n/d	n/d	3922	2290	352	361
B4	2037	87248	2685	*	5201	23433	n/d	n/d	3356	3195	168	738
B7	1389	29024	237	*	3166	3429	n/d	n/d	5277	347	186	196
F2	1266	23687	126	*	3023	1082	n/d	n/d	5542	178	214	194
G2	1267	25411	90	*	2990	1147	n/d	n/d	3587	171	153	194
H2	1481	22329	80	*	3509	41	n/d	n/d	4625	65	12	164
I2	1371	24000	80	*	3600	19	n/d	n/d	5067	79	10	182

***B. thuringiensis israelensis* spore samples
*not reported
n/d = none detected
Other elements reported: Rb, Ba, Sr, Mo, and Pb: all values were below 12 ppm except for 2 samples with Pb between 12-20 ppm and 3 samples with Sr between 16-105 ppm.

Table 2: Elements (ppm) in *B. thuringiensis* samples prepared and processed by various methods measured by inductively-coupled plasma atomic emission spectroscopy (ICP-OES) (data from Velsko, S, *Bioagent sampling matching using elemental composition data*: *An approach to validation*, ICR:-TR-220803, Lawrence Livermore Laboratory, 2006).

46. The Mueller letter is available at http://caseclosedbylewweinstein.wordpress.com/2011/01/20/responses-to-congressman-nadlers-questions . Dr. D. Christian Hassell of the FBI later confirmed to the NAS committee that the Leahy powder contained 1.45% Silicon, and reiterated the lack of material for analyses of the other samples (D. Christian Hassell, Opening Statement, Charge to the Committee, and Background to the National Academy of Sciences Committee: "Review of the Scientific Approaches used During the FBI's Investigation of the 2001 *Bacillus anthracis* Mailings," July 30, 2009).
47. FBI document B1M7; measurements were made by the Chemistry Unit of the FBI using inductively coupled plasma optical emission spectroscopy (ICP-OES). See also NAS Report, pp. 66-67.
48. Ibid.
49. AFIP studied the Daschle and *NY Post* powders with energy-dispersive X-ray spectrometry (EDX)--scanning electron microscopy (SEM) in October 2001. Since April 2010 the AFIP data have circulated widely among scientists and the Press. The data are now accessible at https://docs.google.com/fileview?id=0B7Vzv7IcODeVZmZiYmM0ZWYtN2E0MS00Y2YwLTkxMGQtNTI5ZjMyOWQyNDQw&hl=en. The key to the sample ID numbers used in the AFIP data for the Daschle (SPS02.57.03) and *NY Post* (SPS02.88.01) samples can be found in a separate, later document at http://vault.fbi.gov/Amerithrax/Amerithrax%2010%20of%2030/view, page 112. Other samples examined by AFIP the next day, October 26, were bentonite (IV-B), sea sand, dried powder from a fermenter run (VI-B), spray dried *Bacillus thuringiesis* (VII-B), and another dried *B. thuringiensis* powder (VIII-B), all or which (except sea sand) were shown to contain silicon, aluminum and other elements.
50. The calculations were made with software for quantitative X-ray microanalysis provided by the National Institute of Standards and Technology at http://www.cstl.nist.gov/div837/837.02/epq/dtsa2/index.html.
51. Johnstone K, Ellar D, T Appleton (1980) Location of metal ions on *B. megaterium* spores by high-resolution electron probe x-ray microanalysis, FEMS Microbiology Letters 7: 97-101; M Stewart, AP Somlyo, AV Somlyo et al. (1980) Distribution of Calcium and Other Elements in Cryosectioned *B. cereus* T Spores, Determined by High-Resolution Scanning Electron Probe X-Ray Microanalysis. J Bact 143: 481-491.
52. FBI document B1M1, p. 38 and p. 54.
53. Michael J, Kotula P (2009) Sandia National Laboratories, "Elemental Microanalysis of *Bacillus anthracis* Spores from the Amerithrax Case," presentation to the NAS Committee, September 2009 (obtainable from the NAS Public Access Records Office). For more details on the Sandia work, see FBI documents B1M6 and B1M1, p. 69.
54. Michael and Kotula (op. cit.) did at one point present closely similar weight-percentages of silicon for Leahy (1.2 - 2.3%) and *NY Post* (1.2 - 1.5%) samples, but the details of their work indicate that the measurement had focused on part of an aggregate of spores in the *NY Post* sample (which, they note, "consisted mainly of larger aggregates" but also contained vegetative and sporulating cells) (FBI document B1M6, pp. 25-28); therefore, the reported silicon percentage was not representative of the bulk sample as a whole. See also the later discussion herein of "larger pieces" reported by the AFIP.
55. NAS Report, p. 6; see also p. 68, where the NAS seems confused about the data.
56. Weber P, Viani B, Davisson L, Velsko S, Lawrence Livermore National Laboratory, presentation to the NAS Committee, "Nanometer-scale secondary ion mass spectroscopy for microbial characterization," September 25, 2009 (available from the National Academies' Public Access Records Office).
57. The Lawrence Livermore group was sent *Bacillus* spore samples (6 *B. anthracis Ames*, 5 *B. anthracis* Sterne, 14 *B. thuringiensis*, and 1 *B. atrophaeus*) by DHS and collaborating laboratories; and they performed 31 growth experiments on *B. anthracis* Sterne at Livermore, attempting to manipulate silicon levels in the spores.
58. FBI Science Briefing, August 18, 2009.
59. The samples sent to Sandia by the FBI were: one from flask RMR-1029; a *B. anthracis Ames* grown in shaker flasks at USAMRIID using Leighton-Doi media; a *B. anthracis Ames* grown in the same media via fermentation at Dugway; and 6 samples labeled "evidence" by the FBI with no further description (Michael and Kotula, op cit).

the *B. anthracis Ames* spore samples sent to them by the FBI[59] (other than the attack samples) had either no silicon in their spore coats or considerably lesser amounts, and a much smaller fraction of spores that contained any silicon[60]. In surrogate samples containing silicon, according to Sandia, "the details are different."

In an effort to 'reverse engineer" the attack anthrax, the FBI had asked Dugway early on to make 36 surrogate spore preparations by various methods[61]. Bulk elemental analysis was carried out by the FBI in 2002[62] on only ten of these surrogates (none of which contained added "dispersant," i.e., silica nanoparticles). All ten were found to contain between 0.2% - 5% silicon (in bulk); four of these contained an amount of silicon in the same range as the attack samples. The NAS committee wrote that the bulk silicon analyses indicate that it is possible to prepare spores with high silicon content without adding a dispersant, but that, when analyzed for silicon in the spore coat, none of the FBI samples were similar to the attack samples with respect to either the amount of silicon per spore in the coat, or the fraction of spores containing any silicon in the coat[63]. Thus, it is possible that most of the silicon in the four samples with high silicon content was extra-sporular, like that in the *NY Post* sample[64]; there is no evidence that it was naturally incorporated. The methods of preparation of these four samples, including addition of any substances other than silica particles, were not described to the NAS[65]. The NAS Report noted that they were not provided with all of the preparation procedures used[66]; they "sought, but could not obtain, a detailed explanation of the thought process that went into selection of the DPG [Dugway] methods…it was not clear to the committee how the subset of surrogate preparation methods was selected and whether these choices were based on an understanding informed by the investigation or on other assumptions about the approach taken to produce the evidentiary materials[67]." In light of all the open questions, it is strange that a "Red Team," convened at Quantico on March 13, 2007, recommended that the FBI pursue no further research on the silicon in the letter powders[68].

A new book[69] edited by Bruce Budowle of the FBI and others suggests a reason why the FBI may have wished to avoid the silicon question: "if the estimates of silicon concentrations in the Amerithrax spores are correct, they are not consistent with our current understanding of silica deposition, or those materials must have indeed been produced under an unusual set of conditions. If the latter were true, the silica [sic] evidence might provide a significant bound on the credible growth and production scenarios that would be consistent with the prosecution narrative in this case."

It has usually been assumed that any intentional additive containing silicon must have been intended to increase the dispersibility of the attack spores[70]. In reporting their work to the FBI the Sandia scientists wrote that "the silicon and oxygen found on the spore coat are difficult to explain as an intentional addition to the spores, mainly because of their location on the spore coat, which surrounds the spore core and is surrounded or encased by the exosporium[71]". However, the spore coat of *Bacilli*, not the exosporium, is the effective surface of the spore; it is at the spore coat, not the exosporium, that large molecules (mol. wt. about 16,000 and above) are totally excluded from the spore[72], and the coat is an important factor in spore resistance to toxic chemicals[73]. The effects that a silicon compound on the spore coat may have on spore properties, including dispersibility[74], cannot be assumed.

Presumably for this reason, an effort to determine the dispersibility of the attack spores by direct measurement was undertaken by Michael Kuhlman of Battelle Memorial Institute. Sometime between October 17-23, 2001, he measured the particle size distributions of an aerosolized Daschle sample and later, of a Leahy sample and of several *B. subtilis globigii* spore samples made at Battelle using standard methods, with no milling or other processing[75]. He found them all to be similar. The particle sizes in all cases had bimodal distributions; for the Daschle sample, surprisingly, only 0.05 % of the mass had a diameter of 2 micrometers or less, and 0.9% had a 10 micrometer diameter or less; the Leahy sample had ten times more particles in this respirable range. The NAS Report took the Battelle data to indicate that "powders with dispersion characteristics similar to those of the letter materials could be made without the addition of a dispersant[76]." However, there is reason to question whether the attack samples were in pristine condition when these measurements were carried out, or whether Battelle had autoclaved them first, which might have caused clumping.

60. J. Michael and P. Kotula, op. cit. The fraction of spores containing silicon was 76%, 66% and 65%, for the Leahy, Dashle and NY Post samples, but only 0-29% in the other samples studied.

61. NAS Report, pp. 63 ff.

62. FBI document B1M7; NAS Report, pp. 66-67; see also footnote 83.

63. NAS Report, p.78.

64. At the time the FBI chose the methods for making surrogate preparations at Dugway, the AFIP data on the *NY Post* sample were available to the FBI and it would have been sensible for them to investigate various possible explanations for that sample's unique composition.

65. NAS Report, p. 78.

66. NAS Report, p. 63.

67. NAS Report, p. 78; also p. 63.

68. Selective excerpts from an FBI communication summarizing discussions and recommendations from the Red Team review were taken from one of the FBI documents released in February 2011 and posted by Lew Weinstein on March 8, 2011: http://caseclosedbylewweinstein.wordpress.com/2011/03/08/selective-excerpts-from-fbi-communication-summarizing-discussions-and-recommendations-from-the-red-team-review/.

69. *Microbial Forensics*, 2nd edition, Edited by Bruce Budowle, Steven E. Schutzer, Roger G. Breeze, Paul S. Keim and Stephen Morse (Academic Press, 2011). The quotation is from Chapter 30, by Stephen Velsko of Lawrence Livermore National Laboratory.

70. See, e.g., NAS Report, p. 67; and FBI document B1M6, p. 37.

71. FBI document B1M6, p. 37.

72. Gerhardt P, Black SH (1961) Permeability of Bacterial Spores II. Molecular Variables Affecting Solute Permeation. J Bacteriol 82: 50-760.

73. Nicholson WL, Munakata N, Horneck G, Melosh HJ , Setlow P (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol MolBiol Rev 64: 548-572.

74. It has recently been shown that natural incorporation of silicon on the spore coat of *B. cereus* does not affect the spores' dispersibility, but it does confer acid resistance (Hirota R, Hata Y, Ikeda T, Ishida T, Kuroda A (2010) The silicon layer supports acid resistance of *B. cereus* spores. J Bacteriol 192: 111-116 However, the FBI has failed to show that the silicon in the *B. anthracis* attack spores was naturally incorporated; its chemical form and function remain to be demonstrated.

75. B2M13, Summary, pp. 148 ff.

Richard Preston's *The Demon in the Freezer*[77] describes an argument at a meeting on October 22, 2001 involving the FBI laboratory, scientists from the Battelle Memorial Institute, and scientists from the Army. The Army scientists were telling the FBI that the attack powder was "extremely rarified and dangerous," while Michael Kuhlman of Battelle "was allegedly saying that the anthrax was ten to fifty times less potent than the Army was claiming….One Army official is said to have blown up…at the meeting, saying to the Battelle man, 'Goddamn it, you stuck your anthrax in an autoclave, and you turned it into hockey pucks.'" The FBI's conclusion that the silicon content of the attack anthrax had nothing to do with its dispersibility remains unproven.

Returning to the question of the chemical form of the silicon in the attack anthrax, AFIP summarized some of its October, 2001 SEM-EDX findings as follows: "Significant findings for the SPS02.03 [Daschle] sample included the presence of silicon and oxygen, which is indicative of silica [SiO_2] or, more likely, silicates (SiO_4, etc.). Distinguishing among the various possibilities would require additional work with standard materials...The SP02.88.01 [*NY Post*] samples had regions which exhibited the same set of elements found in SPS02.03 [Daschle], but these tended to be on 'large' pieces within the sample. Many of the smaller pieces within the sample exhibited the main peak associated with silicon. It appears that silicon (not bonded to oxygen or other elements) is present in many areas of this sample[78]". When the NAS committee asked the FBI about this last point, the FBI answered that the presence of "reduced silicon" was "just an observation[79]". Exactly what AFIP meant by "not bonded to oxygen or other elements" is uncertain, but a possible guess is "not bonded to oxygen or other elements we looked for." Surely they did not mean to imply that elemental silicon was present. In any case, an explanation is needed for the fact that the *NY Post* sample contains two different chemical forms of silicon.

Since we now know from the Sandia work that the *NY Post* and Daschle spores, in isolation, are elementally indistinguishable, the "large" pieces in the *NY Post* sample that exhibited the same elements as the Daschle sample in the AFIP analyses must have been spore aggregates. The "smaller pieces," which contain most of the silicon in the *NY Post* sample, probably include cellular debris, absent in the more highly-purified Senate samples but present as extra-sporular material in the *NY Post* preparation. More on this below.

Besides silicon, an even more unusual element was found in the attack anthrax: tin (Sn). The Sandia group found tin[80] in the 10-20 nm continuous Si-O layer surrounding the spore coats of all the attack samples, but not in any of the surrogate samples they studied, including ten made at Dugway, or in samples that contained naturally-incorporated Si-O on the spore coat[81]. The presence of these unusual elements may be a fingerprint, they wrote. But the NAS committee "was never shown any evidence to indicate that this possibility was pursued further or that these discussions led to any conclusions about the source of material or production methods[82]."

FBI documents released in February, 2011 reveal that in early 2002, the FBI laboratories also carried out extensive elemental analyses of the attack samples and of various other spore preparations, including the same ten surrogates made at Dugway. Using inductively coupled plasma optical emission spectroscopy (ICP-OES), a sensitive method, they found tin in the attack samples, but no tin in other spore preparations or in media[83]. Table 1 contains some of the FBI data. The amounts of silicon and tin appear to be related: the more of one, the more of the other. This suggests a process that involved both silicon and tin[84]. There is no tin in the simulant to which silica particles had been added, and no silicon or tin in the simulant without added silica, or in the contents of flask RMR 1029[85]. The levels of other elements are reflective of media components (which do not include tin), as can be seen by comparing Table 2, which shows elemental analyses determined by ICP-OES for nine *Bacillus* spore samples prepared by different methods[86]. Neither silicon nor tin was detectable in any of the samples. Comparing Tables 1 and 2 it is immediately obvious that the attack powders are in a league of their own as far as the elemental forensic evidence is concerned. Any meaningful reverse engineering studies would have to deliberately include compounds containing silicon and tin if there were any hope of

76. NAS Report, pp. 64-65. The Report points out that the particle distributions for the surrogate samples, unlike those for the attack samples, were only sketchily reported.

77. Richard Preston, The Demon in the Freezer (Random House, NY, 2002), p. 179. Preston also says (p. 171) that the Daschle sample was immediately autoclaved at Battelle upon its receipt from the FBI on October 17, 2001.

78. AFIPop. cit.

79. NAS Report, p. 68.

80. Michael, Kotula op. cit. They also found iron, a normal constituent of growth media, on the attack spore coats. Among the Sandia submissions to the FBI is a "Silicon Summary (DRAFT)" containing evidence from other sources that silicon and iron compounds interact and may bind together to cell surfaces (FBI document B1M6 pp. 77-81). Weber et al. (op.cit.) reported that their data for naturally incorporated silicon in spores was consistent with adsorption to hydrous ferric oxide in coat/cortex binding sites.

81. FBI document B1M1, pp 91-109 (and see page 83 for a table of elements found in various samples).

82. NAS Report 67.

83. FBI document B1M7, pp. 93-94, table containing all the data; analyses of the ten Dugway surrogates were exceptional, indicating very small amounts of tin, but the significance of these findings is uncertain because of insufficient information; all the Dugway values observed are very close to those for an undefined "STD" (B1M1, p. 83). The Dugway sample data are also presented in B1M7 pp. 27-28, where it is noted that procedural problems in analysis of Dugway samples K1662 and K1668, the two with the highest silicon and tin analyses, "likely resulted in inflated concentrations".

84. Table 1 shows that iron content does not appear to be correlated with silicon content.

85. Hassell DC, FBI Laboratory Director, FBI Press Release, March 6, 2009. Hassell confirmed the Sandia findings of silicon, oxygen, iron and tin in the Leahy, Daschle and *NY Post* letter materials, then added that "Spores from Ivins' RMR-1029 flask did not contain those elements in quantities that matched the letter spores." Publicly available FBI documents on elemental analyses do not include any data on RMR 1029.

86. Velsko S (2006) Bioagent sampling matching using elemental composition data: An approach to validation. UCRL-TR-220803, Lawrence Livermore Laboratory, 41pp.

87. Dimethyldichlorosilane is sold commercially as "Repelcote," a liquid that polymerizes to make hydrophobic coatings.

88. Colas A, Curtis J (2008) "Silicone Biomaterials: History and Chemistry & Medical Applications of Silicones, Dow Corning Corporation, Reprinted from Biomaterials Science, 2nd Edition

89. Cyclic as well as linear polymers are initially formed, with the proportion of cyclic oligomers depending on the solvent and the substituents on the silicon. Catalysis by acids or bases opens the rings and allows further linear polymerization; the length of linear polymers may depend on the catalyst concentration. Tri- or tetrachlorosilanes could be added to produce branching (Colas and Curtis, op. cit.).

90. Colas and Curtis, op. cit.

reproducing the attack powders. Using protocols that do not add silicon or tin would be quite meaningless. It is essential to learn whether the FBI labs tried to add tin to their preparations.

All the evidence in the public domain is consistent with the concept that the spore coats of the attack anthrax were silicone-coated. Silicone polymers are typically formed by hydrolysis of a silicon compound such as dimethyldichlorosilane[87] (or other silanes with similar substituents)[88], which contains no oxygen. Hydrolysis replaces the chlorine atoms with oxygen to form dimethylsilanol, which polymerizes spontaneously to form polydimethylsiloxane, containing silicon and oxygen in equal amounts. The polydimethylsiloxane[89] chains can then be cross-linked ("cured") to form a three-dimensional silicone coating for encapsulation. This step requires an organotin catalyst[90,91] such as a dibutyltin dicarboxylate[92].

A procedure of this kind can be envisioned for encapsulating *B. anthracis* spores. Silane monomers like dimethyldichlorosilane are low-molecular-weight liquids that probably can penetrate the exosporium[93], the loose-fitting membrane sac that encloses the spore. If silane monomers were added to a suspension of dry spores in an organic solvent, the silane would not contact moisture until it reached the spore coat, where residual moisture diffusing from the core inside the spore[94] would cause hydrolysis, followed by polymerization at the spore coat. The polysiloxane chains that would be formed at the spore coat could then be cross-linked to encapsulate the spore. This step would require continued diffusion of moisture from inside the spore, as well as an organotin catalyst. Organotins have low solubility in water but, like silanes, are soluble in organic solvents such as ether, carbon tetrachloride, etc[95]. The ratio of tin to silicon in the attack spores is "about right" for a tin catalyst used to produce a silicone coating, according to a chemist in the field[96].

Before contacting moisture, chlorosilane compounds react readily with various functional groups in organic materials (e.g., amino groups, alcohols, carboxylic acid groups, etc.) that would be found in cellular debris. If the cellular debris contained no moisture, the result would be "silylation" of the material, i.e., addition of a silicon atom (but no new oxygen atom) at each silylated site. The silylated material would be found in the extrasporular material such as observed in the *NY Post* sample – the "smaller pieces." Alternatively, if the cellular debris in the dried *NY Post* sample retains some moisture, chlorosilane molecules would react as they do at the spore coat, hydrolyzing and then polymerizing to form clumps of polysiloxane, a high-molecular-weight material that would not be soluble. The extrasporular material may be a mixture of these possibilities. The smaller pieces that "exhibited the main peak associated with silicon,…not bonded to oxygen or other elements" would be the silylated areas, containing Si-O-C or Si-N-C bonds, not the O-rich Si-O-Si-O bonds that form the backbone of polysiloxane polymers. These different bonds, which can be distinguished by spectroscopic methods[97], would explain the AFIP observations.

Although a process of this type is not known to have been applied to bacterial spores, methods have been developed to encapsulate biologicals with silicone polymers that confer high stability while protecting biological activity[98]. Polydimethylsiloxane has been used in some pharmaceutical coating materials for years, even though a toxic material, organotin, is employed as catalyst in its production[99].

Microencapsulation can provide protection from the environment and better dispersibility and flowability[100]. The polydimethylsiloxanes have a low surface tension that produces "very hydrophobic films and a surface with good release properties, particularly if the film is cured… surface tension is also in the most promising range considered for biocompatible elastomers[101]."

Inorganic tin compounds are genotoxic to bacteria[102]; organotins are increasingly toxic; triorganotins are used as general biocides[103]. The potential toxicity of tin compounds underlines the probability that tin was not added to the attack spores during their growth, but rather, during post-production treatment of the spores.

91. International Programme on Chemical Safety (INCHEM) :see especially "Tin and Organotin Compounds" under "Environmental Health Criteria 15."
92. Davies AG Organotin Chemistry, Vol 1 (Wiley-VCH, Weinheim 2006).
93. Nicholas Bergman, Ed., *Bacillus Anthracis* and *Anthrax* (Wiley-Blackwell, 2011), Chapter 2, "Spore Ultrastructure:" electron microscopy shows that the basal layer of the exosporium contains cavities, "likely allowing the passage of small molecules (such as germinants) but not large proteins." See also P Gerhardt and S Black, op. cit.
94. We assume that *B. anthracis* spores would have been at equilibrium at some (optimum?) relative humidity before they were suspended in the non-aqueous solvent. It has been shown that dried spores of *B. subtilis* var. niger (the strain most often used as a surrogate for *B. anthracis*) contain moisture in an amount determined by the relative humidity of their environment, when at equilibrium; when equilibrated at relative humidity of 20-40%, spores contain 5-12 % of their weight as water and are maximally stable to dry heat (Angelotti R, Maryanski J, T Butler, Peeler JT ,Campbell JE (1968) Influence of Spore Moisture Content on the Dry-Heat Resistance of *Bacillus subtilis* var. *niger*, Appl Microbiol 16: 735-745.
95. International Programme on Chemical Safety, op. cit.
96. Mike Wilson, a chemist for SiVance, a silicone products maker in Gainesville, FL, quoted in Miami Herald, "FBI lab reports on anthrax attacks suggest another miscue," May 19, 2011.
97. These methods are being used extensively in forensic science and homeland security. See: A Whitley, E Leroy, F Adar, FT-IR-Ramen Combination: The Perfect Analytical Solution for Vibrational Spectroscopists, Spectroscopy August 1, 2009
98. Gill I, Ballesteros A (1998) Encapsulation of Biologicals within Silicate, Siloxane, and Hybrid Sol-Gel Polymers: An Efficient and Generic Approach, J Am Chem Soc 120: 8587-8598; Wiemann LO , Weisshaupt P , Thum O, Ansorge-Schumacher MB (2009) Enzyme Stabilization by Deposition of Silicone Coatings, Org Process Res Dev 13: 617-620.
99. Gao Z (2004) The study of polydimethylsiloxane (PDMS) coating for controlled drug release. University of Cincinnati, UMI ProQuest Dissertation & Theses, 115 pp, 3141363; Gill I and Ballesteros A, *op. cit*.
100. Ghosh SK (ed.), Functional coatings: by polymer microencapsulation (Wiley-VCH, Weinheim 2007).
101. Colas and Curtis, op cit.
102. Pungartnik C, Viau C, Picada J, Caldeira-de-Araújo A , Henriques JAP, et al. (2005) Genotoxicity of stannous chloride in yeast and bacteria, Mutat Res 583: 146-157.
103. International Programme on Chemical Safety, op cit: toxicity is greater for algae and invertebrates than for vertebrates; toxicity for bacteria is not given. The more non-polar the organic group in the organotin, the more toxic the compound. Dibutyltin, for example, is acutely toxic to algae and invertebrates at concentrations above 5 micrograms/ml.
104. Affidavit in Support of Search Warrant, FBI Document no. 07-524-M-01, October 31, 2007, released by FBI in August 2008.
105. NAS Report, p.86. In addition, phylogenetic analysis indicated that the *NY Post B. subtilis* strain is very closely related to NRRL BD-566 from the USDA-ARS Culture Collection, which could be used as a surrogate for the former (FBI document B2M3 pp.84-95).

Whether or not the hypothesis just presented is accurate in detail, it is obvious that the attack spores must have been treated with some procedure that produced the silicon-tin signature observed on their spore coats. That signature, alone, shows that the attack spores were grossly over-qualified for their job. The FBI has adequately proclaimed and demonstrated that surrogate samples, containing no silicon or tin, can be sufficiently dangerous. Perpetrators who were inspired on 9/11 and ready to go on September 18 would not need to bother with unnecessary complications like coatings. If they could, they would take advantage of pre-existing spore preparations, regardless of their sophistication.

It would be difficult not to conclude that the spores in the attack letters were prepared for some purpose other than terrorism. Potential procedures that might be applicable for silicone coating of spores, barely touched on here, are complex, highly esoteric processes that could not possibly have been carried out by a single individual. They would require a laboratory with specialized capabilities and expertise not found at USAMRIID, in addition to the possession of the correct strains of *B. anthracis Ames* associated with flask RMR 1029.

Further analyses of the attack samples to determine the chemical forms of the silicon and tin they contain should have been a high priority for the FBI. As AFIP pointed out, "distinguishing among the various possibilities would require additional work with standard materials" using electron microscopic/spectroscopic methods. The attack powders should have been analyzed using a wider arsenal of analytical techniques, including time-of-flight ion mass spectroscopy (TOF-SIMS). Such studies can and should be done now, focusing on precisely what chemical species and chemical bonds involving silicon are present in both the spore coats and the extra-sporular material in the *NY Post* powder. Reverse engineering experiments are also essential using various tin-catalyzed silicone formulations aimed at producing coatings on *B. anthracis* spores, and testing any other hypotheses that may link silicon and tin content in the attack spores. The work needs to be undertaken both by government bodies and independently by disinterested scientists. Critical information also remains to be uncovered in records pertaining to silicon and tin compounds, their purchase, possession, use, testing, storage and so forth, at locations of interest.

B. subtilis contamination in some of the attack letter powders

A bacterial contaminant that could be an important institutional "fingerprint" was found in the attack powders in the *NY Post* and Brokaw letters, but not in the more highly purified material in the letters sent to the Senate[104], which were mailed later. The genetic sequence of the *NY Post* contaminant had 98% genetic similarity to *B. subtilis* 168[105], a standard laboratory strain, but it was not identical to any presently recognized strain of *B. subtilis*, based on assays for three genetic markers[106]. The Brokaw *B. subtilis* contaminant has never been sequenced, but its similarity to the *NY Post* contaminant was demonstrated at 23 genetic loci for which multiple PCR assays were developed[107]. An environmental sample from the office of American Media, Inc. (AMI) in Florida –the only AMI sample for which any analyses have been reported –contained bacilli that were phenotypically (i.e., observationally) indistinguishable from the *B. subtilis* in the *NY Post* and Brokaw letters[108]. Cell suspensions of the *B. subtilis* and *B. anthracis* isolates from the AMI environmental sample were prepared in 2006 and sent for genetic analysis[109], but evidently they were never analyzed; in 2011 the FBI told the NAS Committee verbally that the U.S. Attorney's Office advised that further characterization of those samples "would not be undertaken[110]." No explanation for this decision has been offered.

Samples submitted to the FBI repository by all known possessors of *B. anthracis Ames* appear to contain no *B. subtilis* contamination that perfectly matches that in the *NY Post* powder, as shown by negative results in assays for ID65[111], one of three rare markers in the *NY Post* strain[112]. An FBI subpoena, issued in 2002, had required that samples submitted to the repository must be taken from stock *B. anthracis* cultures, which are usually handled with great care to prevent contamination. Thus it is surprising that 30% of the 1070 repository samples tested positive[113] in a genetic assay for *sbo*A, a pan-*B. Subtilis-specific* marker used by the FBI as a common marker for *B. subtilis*[114]. On the basis of the ID65 results on repository samples the FBI concluded, and the NAS concurred, that the *B. subtilis* contaminant did not provide useful forensic information[115].

But this conclusion was unwarranted. Flask RMR 1029 at USAMRIID, the putative *B. anthracis* stock culture from which the

106. The FBI developed PCR assays for four genetic markers in the *NY Post B. subtilis* strain and found three of them to be rare in a survey of 72 other *B. subtilis* strains obtained from USDA and ATTC; the fourth marker was common to all *B. subtilis* strains tested (NAS Report, p. 86; FBI documents B2M4 pp. 3-10).

107. FBI document B2M2 p.146 and B2M4 3-10.

108. The environmental sample from AMI was sent to USAMRIID in June, 2005; both *B. subtilis* and *B. anthracis* in the sample were examined phenotypically and the results were reported on Dec. 22, 2006 (FBI document B1M2 pp. 80-90). The *B. anthracis* in the AMI environmental sample was found to contain morphological variants of *B. anthracis* similar to those in the letter anthrax.

109. FBI document B1M2, pp. 83 and 84.

110. NAS Report, p. 46.

111. Repository screening for *B. subtilis* (FBI document B2M4 280-359; see 358). The results would have been stronger if all three rare markers had been assayed.

112. FBI document B2M4 pp. 3-10.

113. FBI document B2M4 280-359, repository screening for *B. subtilis*.

114. FBI document B2M4 pp. 3-10: FBI Chemical-Biological Sciences Unit, "SOP for *B. subtilis* Analysis by single plex Real Time PCR."

115. NAS Report, Summary p. 5.

116. FBI Science Briefing on the Amerithrax Investigation, August 18, 2008. The NAS Report (Summary, p. 5) states their opinion that the scientific evidence is consistent with RMR 1029 as the source of the letter anthrax, but not conclusive.

117. FBI document B2M1 p. 21, Report from Novozymes Biotech, Inc. They compared the *B. subtilis* contaminant from the letter spores to *B. licheniformis*, a species that is widely distributed in dust. Novozymes found that the two strains were somewhat similar phenotypically, but differed in several ways, including sporulation propensity.

118. Gibbons HS, Broomall SM , McNew LA, Daligault H, Chapman C, et al. (2011) Genomic Signatures of Strain Selection and Enhancement in *Bacillus atrophaeus var. globigii*, a Historical Biowarfare Simulant, PLoS ONE 6: e17836, published on line March 25, 2011

119. FBI documents B2M4 pp. 261-440 and B2M2 pp. 146-156.

120. NAS Report pp. 47-49; FBI document B1M3 includes *B. anthracis* assays on identified environmental samples from locations along the paths of the attack letters.

spores in all the attack letters were derived and the source of the more interesting repository samples contains no *B. subtilis* of any kind[116]. Because the letter spores were probably contaminated during growth or processing, the repository of stock *B. anthracis* cultures was not an ideal place to look for a contaminant of the letter spores. The *NY Post B. subtilis* strain is an extraordinarily efficient sporulator, which suggests that it may readily persist in the environment[117]. It is noteworthy that "military" strains that originated at USAMRIID and Dugway for use as *B. anthracis* simulants have evolved over time to become hypersporulators[118]. The FBI should have collected environmental and other samples from all laboratories that were potential production sites of the letter spores—i.e., laboratories known or suspected to possess *B. anthracis* similar to the attack anthrax.

FBI documents released in February 2011 contain assay results on about 400 samples consisting mainly of swabs, etc., grouped under the titles "Environmental Samples" and "Location Searches[119]." The samples are not identified. Some or all of them may have originally been collected for *B. anthracis* analysis along the suspected paths of the letters and at contaminated worksites[120], perhaps including some at USAMRIID. The samples were not assayed for *B. subtilis* until 2007-2008, when the procedures became available. A few of the 400 samples were found to contain *B. subtilis*, but none of them was ultimately shown to contain B. subtilis identical to that in the *NY Post* letter. The assay history of these samples raises questions about the reliability of the procedures used, however[121].

Because of the various inconsistencies that have been encountered in the *B. subtilis* assays, a re-evaluation of the assay procedures is needed; future work should preferably be based on full genome sequencing. Since *B. subtilis* has been shown to possess high genetic variability[122], perfect agreement with the *NY Post* strain should not be sought. For this reason it would also be desirable to re-examine some of the repository samples.

There is no evidence that relevant samples were ever collected at Dugway, Battelle or other potentially suspect sites. Samples of microbial production runs that took place in the appropriate time frame at those locations, and environmental samples, especially in areas involved in production and other activities with *Bacillus* species (aerosol testing, for example), should have been collected and examined for the presence of *B. subtilis* resembling the *NY Post* strain. Some environmental sampling may still be worthwhile today, and samples of stock cultures and products, as well as records of past activities, should still be available.

Next steps

The NAS Report, in the words of the *Washington Post*, "is not satisfying - nor is it conclusive[123]". Many scientists who contributed to the investigation have expressed reservations about the FBI's conclusions[124]. Senator Patrick Leahy, a target of the anthrax letters and Chairman of the Judiciary Committee, says he has "extreme doubts" about the case; "I've expressed those concerns to the FBI, and this report adds to those concerns[125]". Senator Chuck Grassley, the ranking member of the Judiciary Committee, says the NAS report "shows that the science is not necessarily a slam dunk. There are no more excuses for avoiding an independent review and assessment of how the FBI handled its investigation in the anthrax case[126]." Legislation[127] to establish a Congressional commission for that purpose has been reintroduced by Rep. Rush Holt[128], a scientist and Princeton professor who represents the area in New Jersey where the attack letters were mailed.

The National Academy of Sciences was the right body to examine the scientific issues in the anthrax case, and the scientific issues were the right target. Once the method of preparation of the attack anthrax is understood, the questions of who made it, and where, will be rapidly resolved. But the NAS committee was held back by its dependence on the FBI for information and sponsorship. To quote the *Washington Post* again, the committee "should not be blamed for nitpicking over the test results; that are essentially what it was tasked to do by the FBI, which commissioned its report[129]." The committee had no access to classified information[130], and they met with a number of impediments in trying to interpret the data they were given[131]. Answers to questions posed to the FBI for clarification, they wrote, "were sometimes minimal or terse, or were deflected as … beyond the purview of the committee despite the committee's explanation that the questions were of a scientific nature[132]". Clearly, the committee's purview should have been broader.

Critical scientific questions, some of which have already been indicated in this paper, must be answered before the anthrax case can be laid to rest. That will require scientific expertise and political neutrality, ideally with full access to all that the FBI knows, and with the resources to commission additional work at other laboratories if the existing scientific information is inadequate. Indeed, further scientific investigation may be the only way to bring the facts of the case to light.

121. See the *B. subtilis* assay data on "Environmental Samples" and "Location Searches" in FBI document B2M4 and B2M2. The contorted assay history demonstrates that errors can arise from mutation, inhibition, degradation, low % *B. subtilis* in the presence of *B. anthracis*, sub-optimal annealing temperature or other assay conditions, etc.

122. FBI document B2M3 pp. 84-95, Genetic Diversity and Phylogenetic Characterization of *B. subtilis*.

123. Washington Post, Editorial: "Answers in 2001 anthrax attacks are still elusive", February 17, 2011.

124. See comments by Claire Fraser Liggett, who led the team at the Institute for Genomic Research in genetic analysis of the attack spores, and Paul Keim, the anthrax expert at Northern Arizona University who identified the attack spores and helped develop molecular tests (quoted in "Anthrax Redux: Did the Feds Nab the Wrong Guy?" by Noah Shachtman in *Wired*, March 24, 2011. Two former chiefs of bacteriology at USAMRIID during Ivins' time there, Russell Byrne (1998-2000) and Gerard Andrews (2000-2003), have testified under oath that Ivins did not have the skills to make the attack anthrax (quoted in the Houston Chronicle, "Widow of anthrax victim doubts FBI's conclusion," May 19, 2011). See also, comments by Jeffrey Adamovicz, who oversaw Ivins' work at USAMRIID (quoted in Frederick News-Post, "One year after FBI closes Ivins case, doubts still linger," February 20, 2011, and others at USAMRIID (Palm Beach Post, "Doubt of anthrax suspect's role resurfaces in lawsuit," April 30, 2011).

125. Washington Post, "Sen. Leahy on anthrax case: 'It's not closed," February 16, 2011. According to the Washington Post, Leahy has studied the case and has been given access to any file he wanted.

126. "US Sen. Grassley: Response to National Academy of Sciences Amerithrax report", February 16, 2011.

127. HR 720.

128. Holt R "Following National Academy Report, Holt Calls for Congressional Anthrax Commission: NAS Report Makes Clear There Are Still Questions to be Answered and Still Lessons to be Learned," February 15, 2011.The bill was first introduced in 2008.

129. Washington Post, Editorial, op cit.

130. NAS Report, p. 8.

131. NAS Report, pp. xviii, 3, 25-27.

132. NAS Report 26.