---Draft: Please do not circulate or cite---

Possible Origin of Silicon and Tin in the Leahy and NY Post Spore Material

MM/4 May 2012

Table 1 presents the levels (ppm) of tin and silicon in the bulk spore material from the Leahy and NY Post anthrax letters and in samples of *B. anthracis* Ames spores prepared at Dugway and at USAMRIID as recorded in FBI laboratory reports.

Table 1*
Tin and Silicon in Samples of *B. anthracis* Ames

Sample	Si	Sn	Sn/Si
Leahy (Q12) 26 Aug 2002 28 Jun 2004	 18,441 14,479	 1,979 1,959	 0.107 0.061
NY Post (Q13) 16 Oct 2002	 107,700	 6,514	 0.061
Dugway, 10 samples (K1662-K1671) 26 Aug 2002	 avg = 7,900 (2,000-50,000)	 avg = 86 (33-265**)	 0.011
USAMRIID 30 Oct 2003 "Burans 12"	 avg = 158 (100-265)	 not detected	 --

** Data are from FBI Laboratory Reports 020110004 (26 Aug 02); 020605001 (16 Oct 02); 031008001 (30 Oct 03); and 040624018 (28 Jun 04) as provided to the NRC as document B1M7. Analyses were done by inductively-coupled plasma optical emission spectroscopy (ICP/OES). The USAMRIID samples are from shake flask or agar cultures. The Dugway samples are from cultures produced in a 10 liter fermentor. **The calculation of this value and the next highest value (132 ppm) may be inflated owing to possibly poor sample recovery. The average for the remaining 8 samples is 57ppm.*

What might account for the relatively high levels of tin and silicon in the anthrax letter spore material, the lower but readily detectable levels in the samples of spores prepared at Dugway and the apparent lack of detectable tin in the samples of spores prepared at USAMRIID?

So far as I am aware, the only published hypothesis for the high levels of Sn and Si in the letter material is that offered by a group of independent authors who have concluded that "*all available evidence can be explained by the hypothesis that the spore coats were silicone-coated using a tin catalyst*." (1).

This memo presents a more mundane explanation of the high Sn and Si levels in the letter material: the use of a silicon-based antifoam in its production. Antifoam agents are commonly added to the medium when bacterial culturing is done under forced aeration. In contrast to the culturing of bacteria in shake flasks or on agar, bacterial culturing with forced aeration, as in a fermentor, generates unacceptable amounts of foam unless measures are taken to prevent it. An antifoam agent commonly used in bacterial culturing in fermentors is an emulsion of silica in polydimethylsiloxane. The polymerization catalyst is generally an organoplatinum or an organotin.

An elemental analysis of three silicon-based antifoams marketed by Sigma-Aldrich and specifically designated for use in fermentors is summarized in Table 2. One of these, Antifoam Y-30, described as "containing 30% active silicon" is seen to have a much higher concentration of tin than the other two, Antifoam SE-15 and Antifoam B. Expression of the results as parts per million requires comparison with standards, and is yet to be done. It is nevertheless apparent, both from what is known about the use of organotin in the production of certain antifoams and from the present analyses of antifoams that consideration should be given to the suggestion that the relatively high levels of tin in the anthrax letter materials may have resulted from their having been produced by culturing under forced aeration with a tin-containing silicon-based antifoam.

Although the above suggestion is not without forensic implications, their discussion is beyond the scope of this memo. Meanwhile, there are two simple experiments that could be done to verify its technical basis and to explore its forensic relevance to the anthrax letter attacks of 2001. (a) does culturing and sporulation of *B. anthracis* in the presence of tin-containing silicon-based antifoams yield spore preparations containing high levels of tin and silicon? If so, are the tin and silicon concentrated in the spore coats? And if so, does washing with or without prior purification by density centrifugation remove most of these elements?

Table 2*
Tin and Nickel in Antifoams Y-30, B AND SE-15

	Y-30	B	SE-15	Y-30/B	Y-30/SE-15
Sn	6,703	250	233	29	27
Ni	4,075	4,160	4,004	0.98	1.02

* *Values are counts averaged over three runs. Analysis was done for 33 additional elements (not shown). Note that Antifoam Y-30 had ca 30 times more tin than the other two antifoams. The three antifoams did not otherwise differ greatly, as illustrated for nickel. Analyses were done by inductively-coupled plasma/mass spectrometry (ICP/MS) at the Harvard Department of Earth and Planetary Sciences.*

(1) Hugh-Jones ME, Rosenberg BH, Jacobsen S (2011) The 2001 Attack Anthrax: Key Observations. *J Bioterr Biodef* S3:001. doi:10.4172/2157-2526.S3-001.