LA-UR-14-21680
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Title: Detecting Bioterrorism: Is Chemistry Enough?

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Intended for: Webinar. Slides plus text in note feature (what I'm going to say).

Issued: 2014-03-13

Los Alamos
NATIONAL LABORATORY
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Detecting Bioterrorism:

Is Chemistry Enough?

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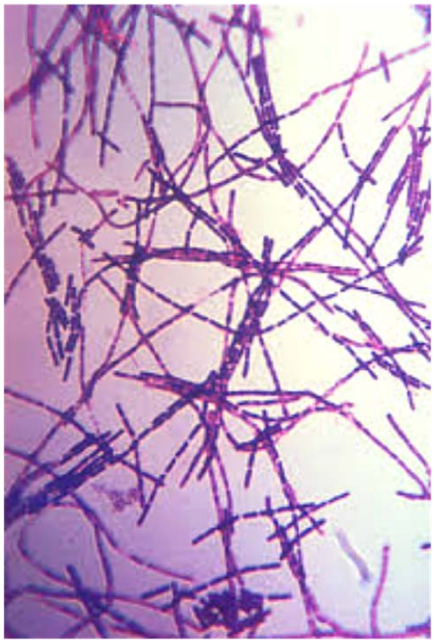
March 13, 2014

NNSA
National Nuclear Security Administration

October 2, 2001

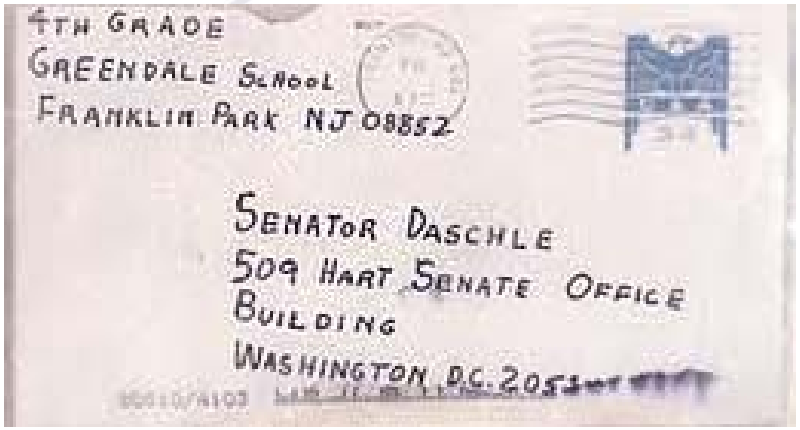
Bob Stevens. Source: Associated Press



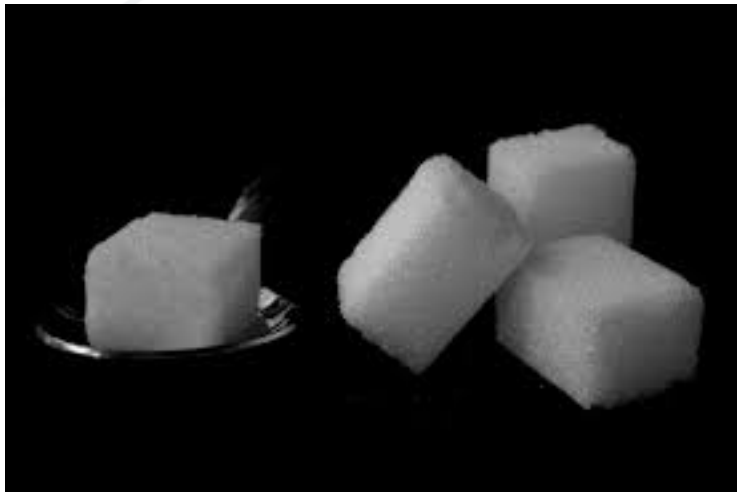
Human anthrax in the US, 1950-2000

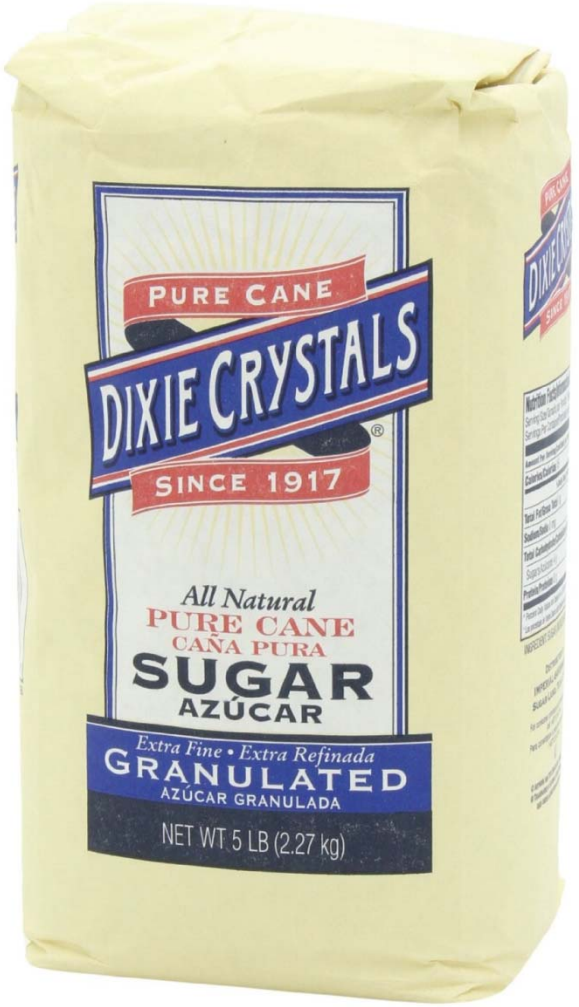
Total	State	Year (# Cases)	Comments
14	New Hampshire	1978 (2); 1966 (2); 1966 (1); 1957 (9)	Textile mills
≥ 9	North Carolina	1987 (1); 1978 (2); 1956 (≥ 5); 1953 (1)	Textile mills
5	South Carolina	1974 (1); 1960 (4)	Textile mills
5	Texas	1959 (5)	Animal outbreak
4	New Jersey	1975 (3); 1959 (1)	Gelatin plant; animal outbreak
2	California	1976 (1); 1968 (1)	Wool; animal outbreak
2	Louisiana	1971 (2)	588 animal cases
2	Pennsylvania	1961 (1); 1957 (1)	Textile mill, tannery
1	Florida	1974 (1)	Imported goatskin
1	North Dakota	2000 (1)	Animal outbreak
1	Ohio	1964 (1)	Goat hair
1	Oklahoma	1957 (1)	Animal outbreak

“Amerithrax” letters



Each letter contained less than a sugar cube of material…





So how do you protect against a whole bag?

Question: when will the next biological attack occur? And where?

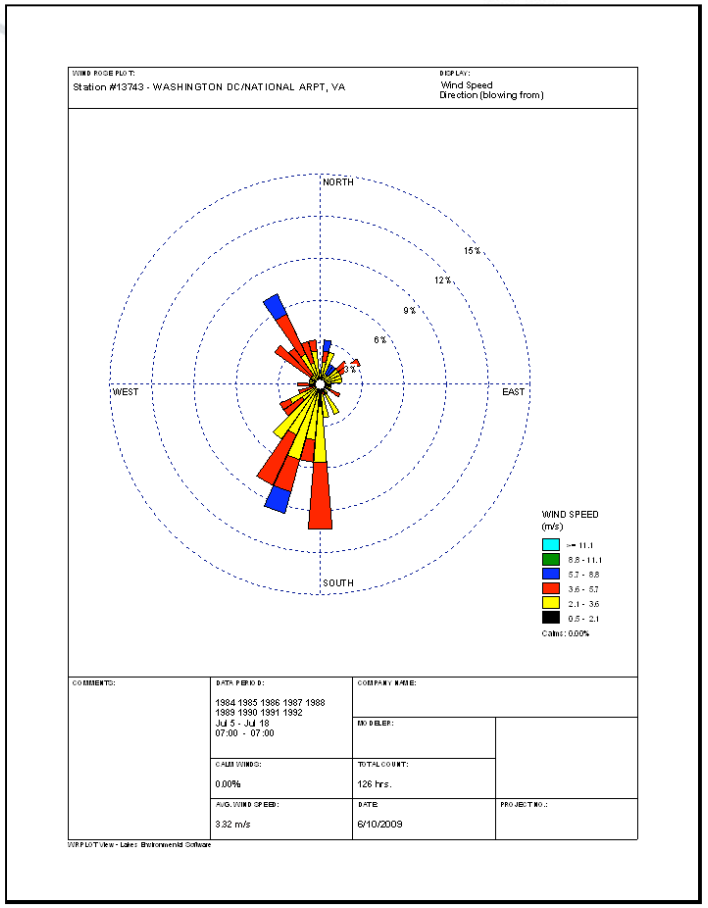
A) Within the next 10 years
B) Within the next 10-20 years
C) More than 20 years from now
D) Never; this is much ado about nothing

E) Inside the United States
F) Outside the United States

Environmental monitoring for bioaerosols

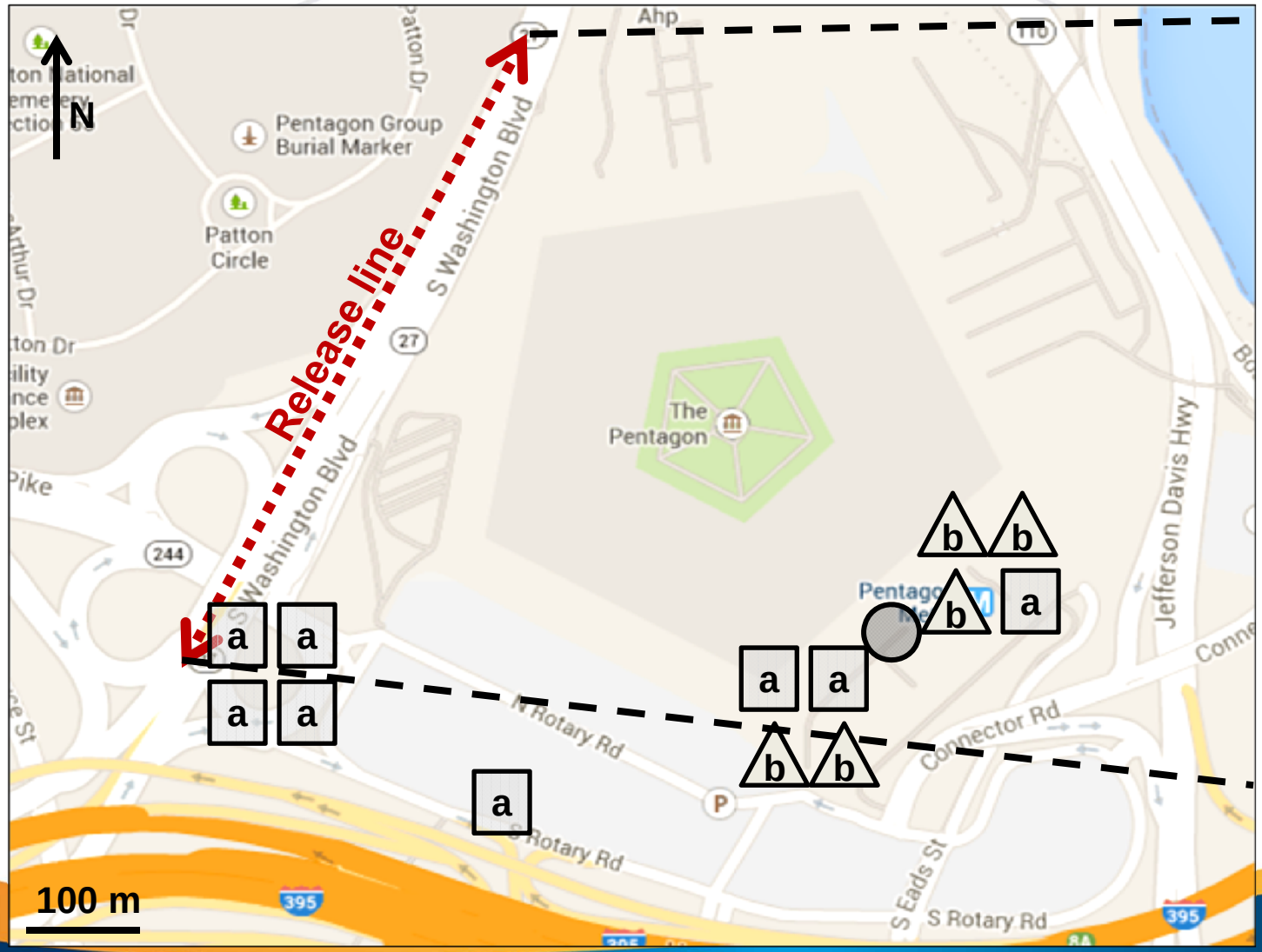
COLLECT EXTRACT ANALYZE

How do you site bioaerosol collectors?



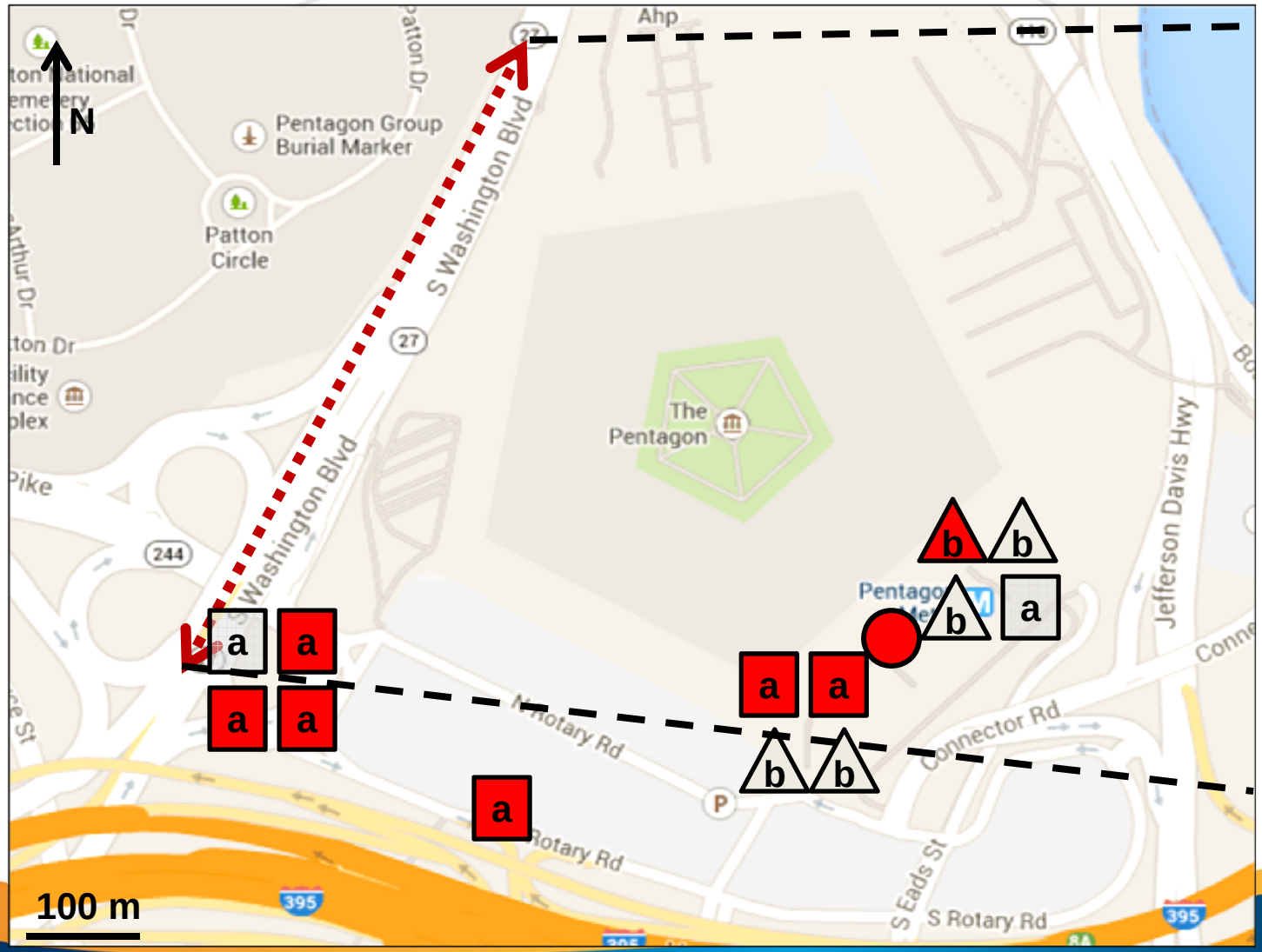
QUIC: Quick Urban & Industrial Complex Model

2005 collector siting

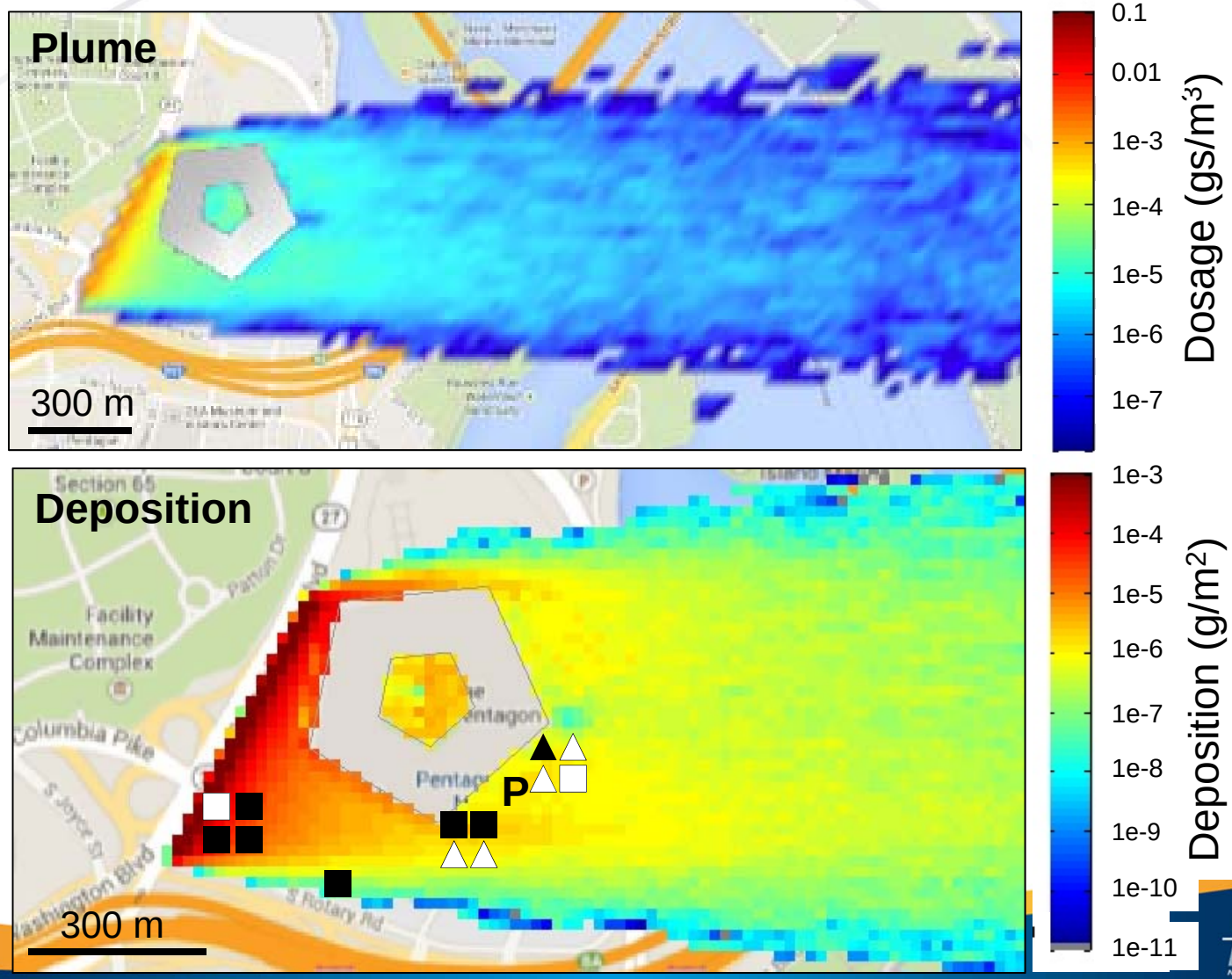


2005 results

Red = PCR-positive

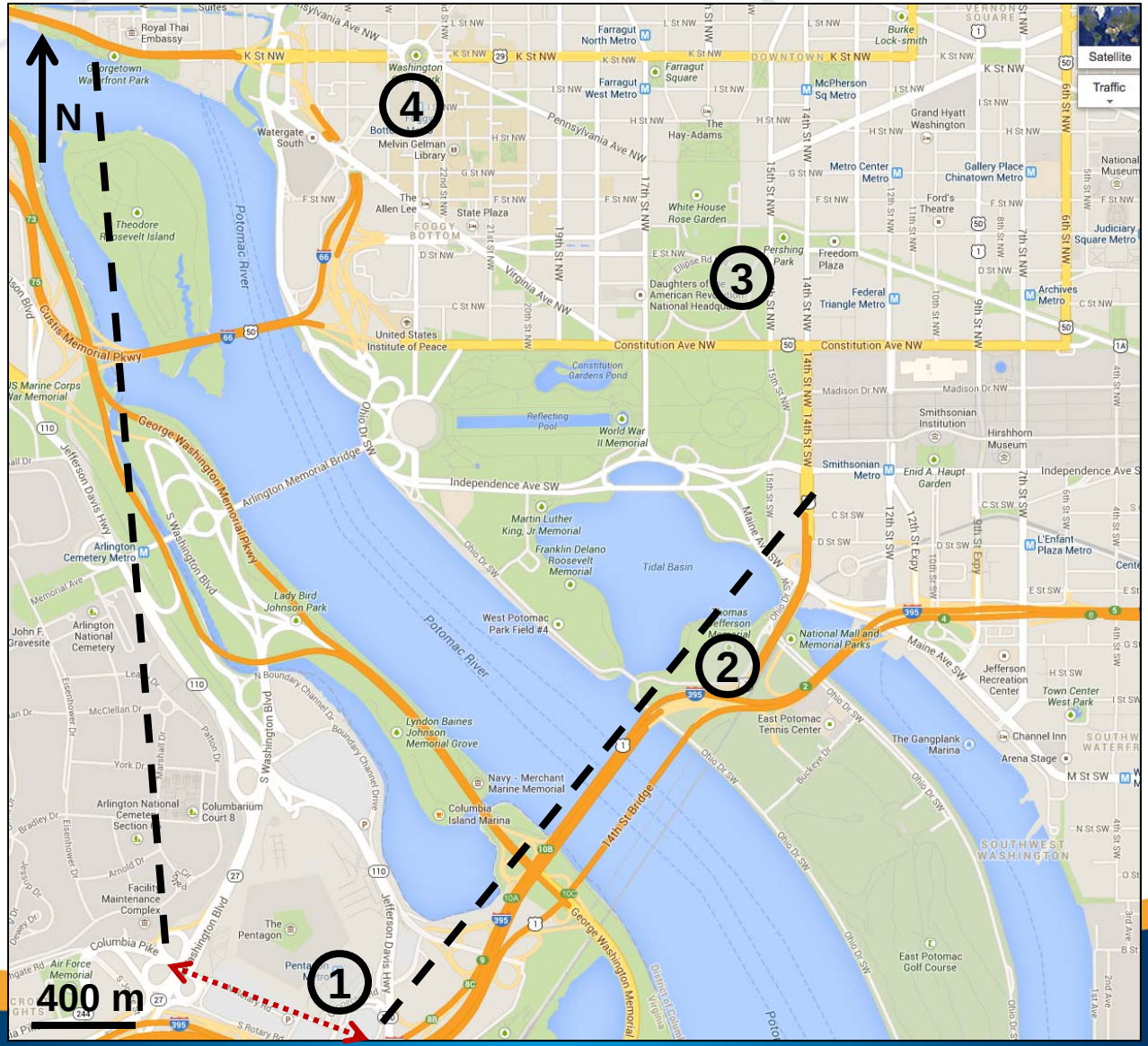


2005 modeled plume & deposition

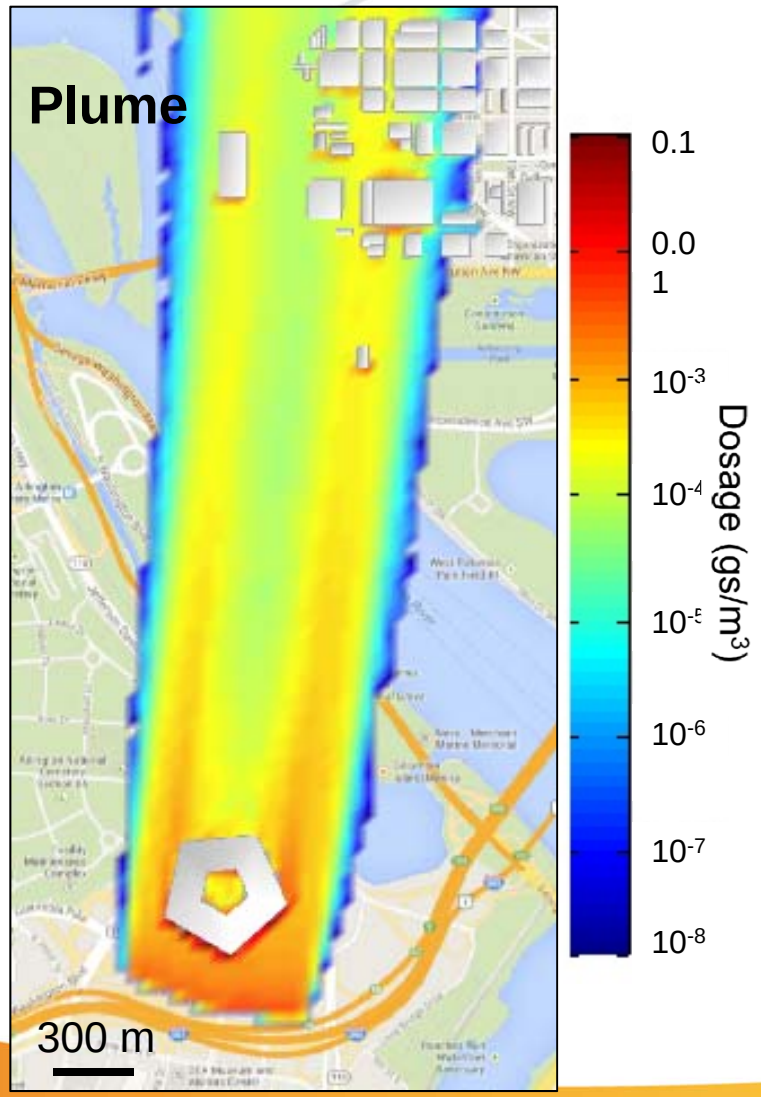


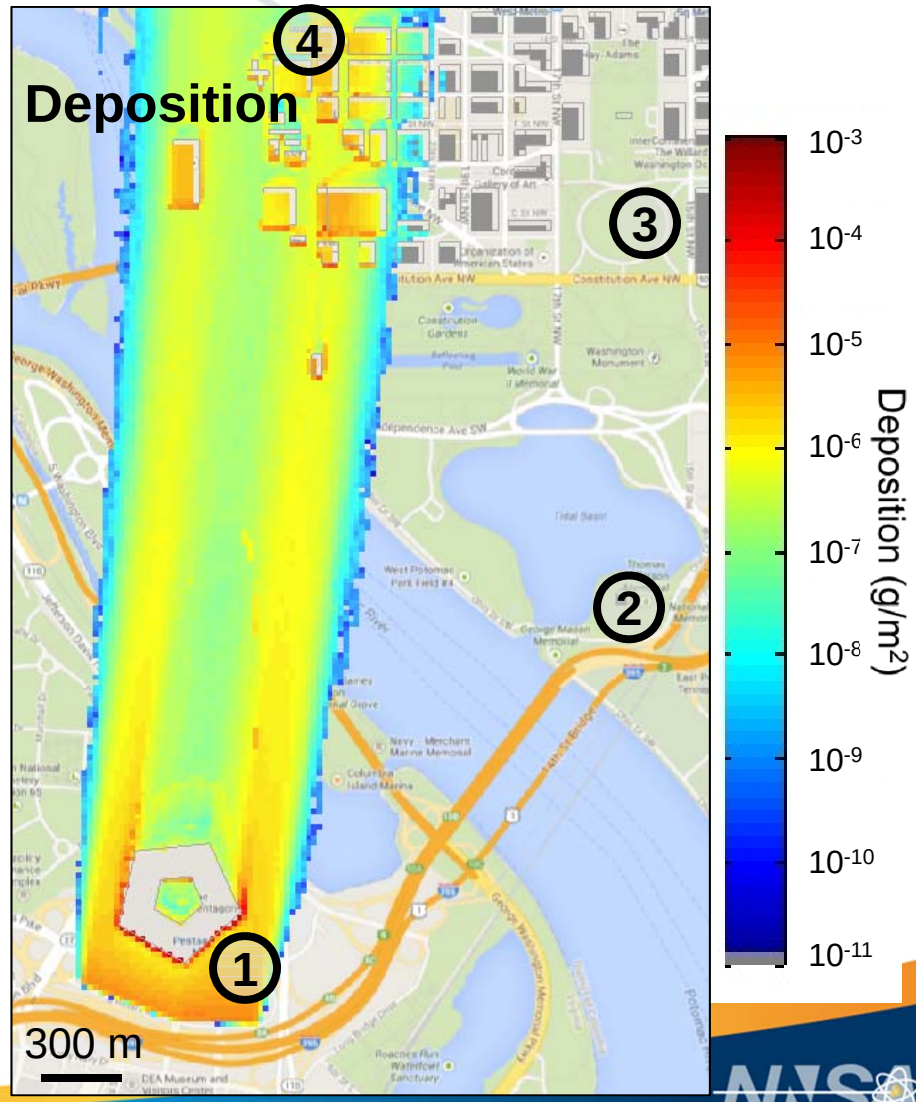
2009 collector siting

Circle = air sampler; surface samples collected in vicinity



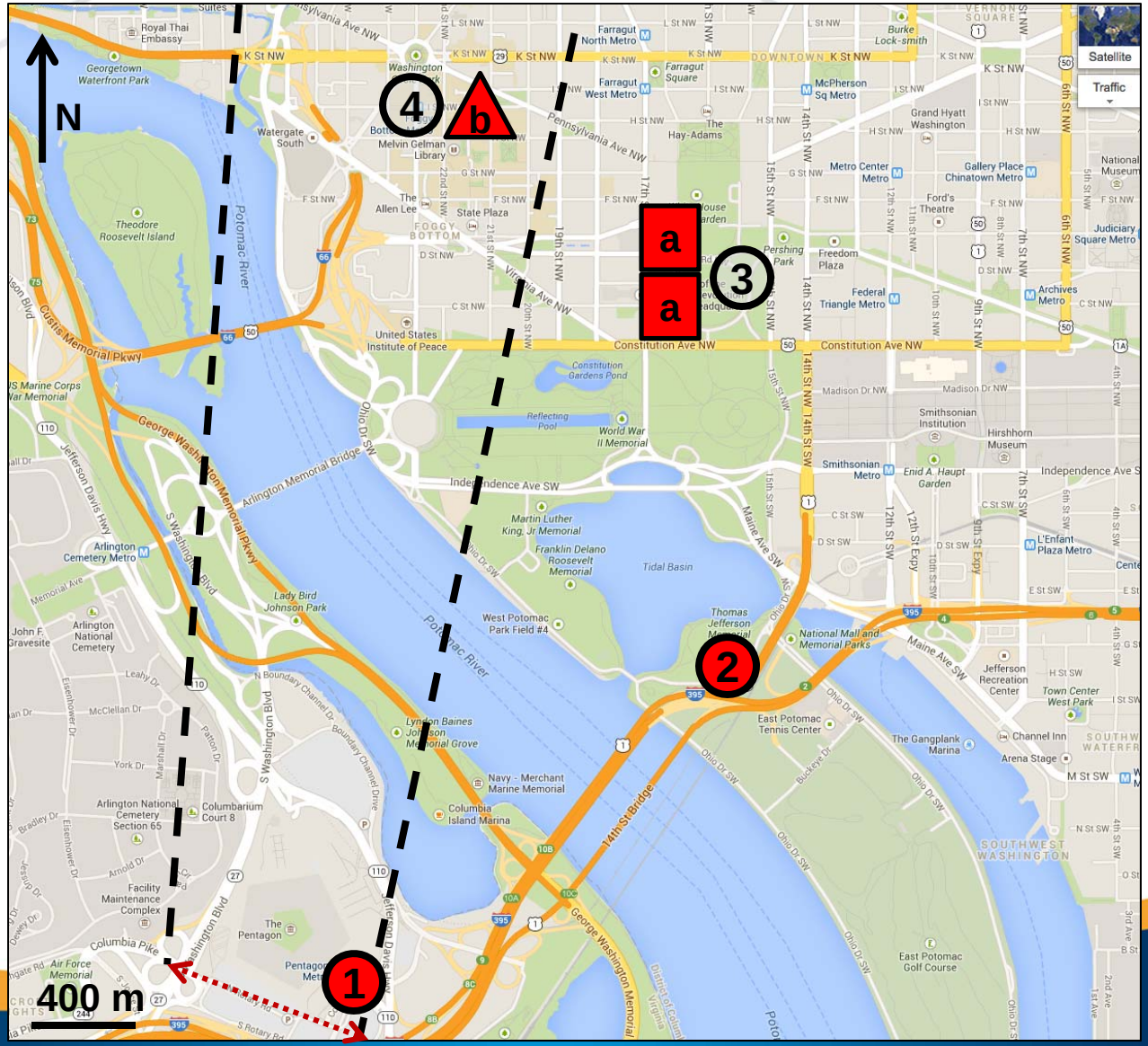
2009 modeled plume & deposition





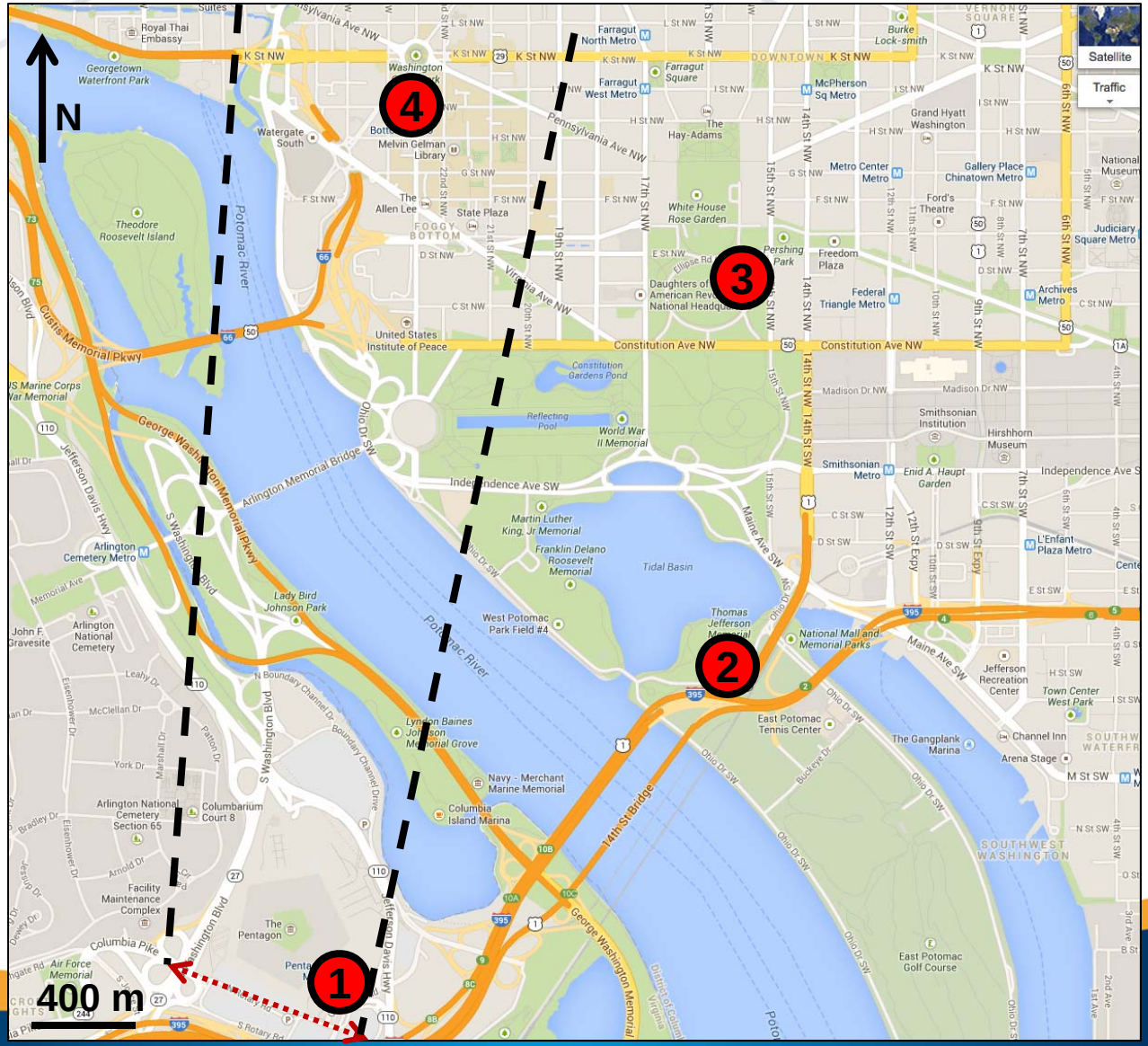
2009 results: 12 hours post-release

Circle = air sampler; square = surface samples



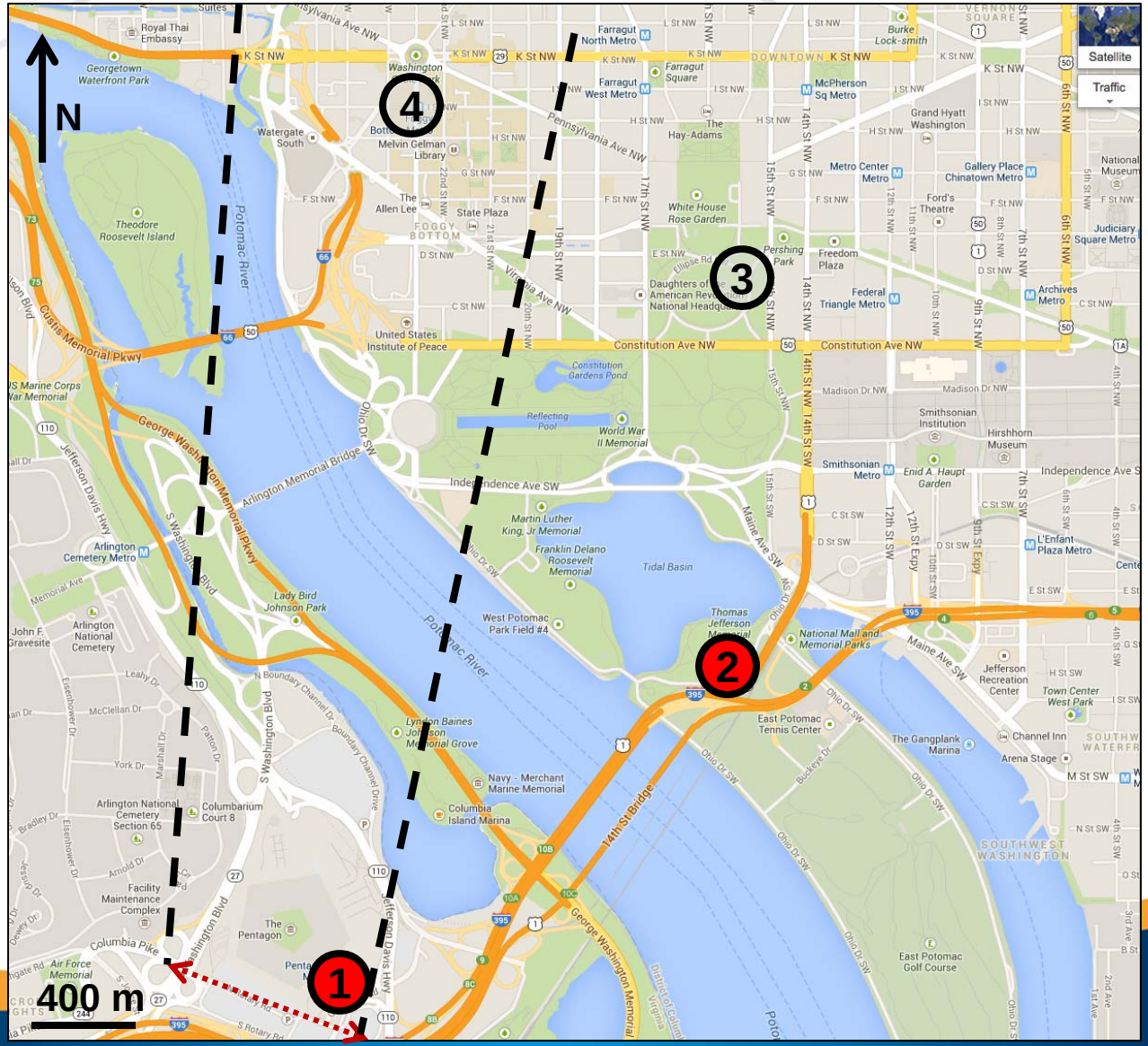
2009 results: 24 hours post-release

Circle = air sampler; no surface samples collected



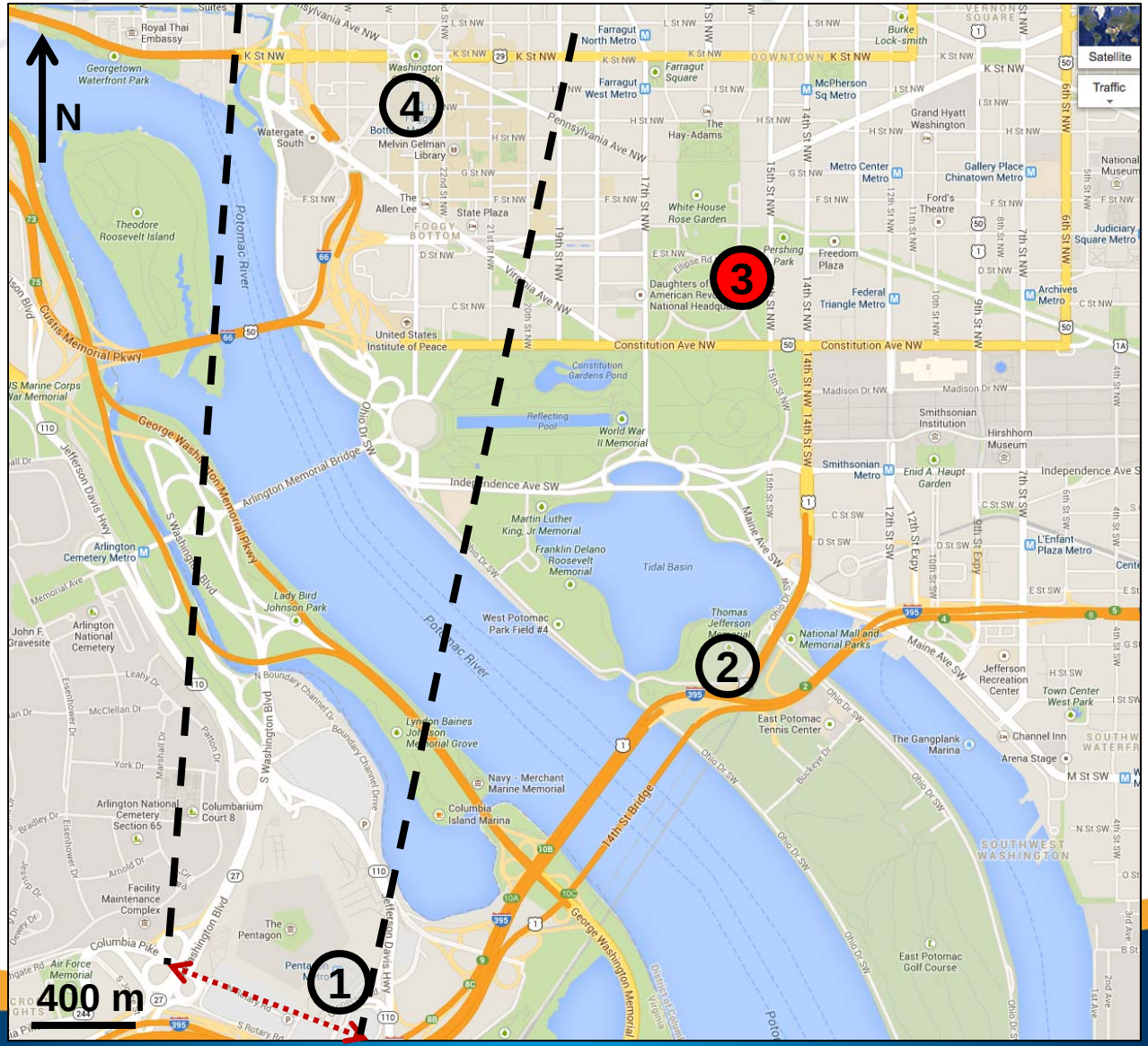
2009 results: 48 hours post-release

Circle = air sampler; no surface samples collected



2009 results: 72 hours post-release

Circle = air sampler; no surface samples collected



Question: do biological agents in the environment behave like other particles?

A) Yes; one soil-bound particle is like any other

B) No; bioagents behave differently

Summary

- Aerosol collectors in the US continuously monitor for threat agents to protect against a large-scale aerosolized biological attack
- Collectors are typically sited using transport and dispersion modeling and historic wind data
- Samples are analyzed using PCR
- Experiments and modeling have shown these systems are effective at detecting the presence of DNA
- Systems can warn public health in time to mount an effective response

But does DNA in the air mean people are infected?

- It only means there's DNA in the air
- Current systems often can't confirm viability
- No rapid techniques to confirm human exposure

- A recent USGS study found *B. anthracis* in 5% of soil samples collected on a north-south transect between Manitoba, Canada and Texas

Question: if you were a public health official and *B. anthracis* DNA was found in the air in your city, what would you do?

A) Nothing; it was probably from naturally occurring bacteria

B) Wait for more information; if it's really a problem, someone will show up sick soon

C) Panic! Evacuate and/or begin mass prophylaxis with antibiotics or vaccines

References

- Bales, M.E.; Dannenberg, A.L.; Brachman, P.S.; Kaufman, A.F.; Klatsky, P.C.; Ashford, D.A. Epidemiological Responses to Anthrax Outbreaks: A Review of Field Investigations, 1950-2001. *Emerg Infect Dis* **2002**, *8*, 1163-1174.
- Griffin, D.W.; Petrosky, T.; Morman, S.A.; Luna, V.C. A survey of the occurrence of Bacillus anthracis in North American soils over two long-range transects and within post-Katrina New Orleans. *Appl Geochem* **2009**, *24*, 1464-1471.
- Inglesby *et al.* Anthrax as a Biological Weapon, 2002: Updated Recommendations for Management. *JAMA* **2002**, *288*, 2236-2252.
- Garza, A.; Van Cuyk, S.V.; Brown, M.J.; Omberg, K.M. Detection of the Urban Release of a *Bacillus anthracis* Simulant by Air and Surface Sampling. *To be published in Biosecurity and Bioterrorism.*
- Layshock, S.; Pearson, B.; Crockett, K.; Brown, M.; Van Cuyk, S.; Daniel, W. B.; Omberg, K. M. Reaerosolization of *Bacillus spp*. in Outdoor Environments: A Review of the Experimental Literature. *Biosecurity and Bioterrorism*, **2012**, *10* (3), 299.

Let's go back to October 2, 2001. You could still smell the smoke rising from the September 11 attacks on the World Trade Center and the Pentagon. About 1 am, a man named Robert Stevens was admitted to JFK Medical Center in Atlantis, Florida. Mr. Stevens was the photo editor of The Sun—one of those tabloid magazines you used to see in the check-out line at the grocery store, claiming Elvis was still alive. He'd just returned, hours ago, from a vacation, where he'd been sightseeing in North Carolina with his wife and daughter. But now he was feverish and confused. Within hours, he was comatose on a respirator.

Because he deteriorated so quickly, his doctors thought he might have meningitis, so they drew spinal fluid for confirmatory testing. But the fluid looked odd under a microscope—it was full of rod-shaped bacteria, or *Bacilli*. Laboratory tests later identified it as *Bacillus anthracis*—Mr. Stevens had anthrax. On October 5, he died.

Investigators immediately began looking for the source in North Carolina. Anthrax doesn't occur very often in the US—only about 50 human cases were reported between 1950 and September 2001—but when it does, it's usually associated with agriculture or textile mills. *B. anthracis*, the bacterium that causes anthrax, lives in the soil, and can persist for decades in a hardy, drought-resistant spore form. Anthrax usually occurs in grazing animals that feed close to the soil, like cows or goats. But it also occurs in people who work closely with these animals or their hair or skin. Between 1950 and 2000, North Carolina had one of the largest incidences of human anthrax in the US, in textile mills processing goat hair.

But the investigators didn't find anything in North Carolina, because Mr. Stevens wasn't exposed there. He was exposed in the mailroom of The Sun, which had received a letter that contained powdered spores. Subsequent swipes of the mailroom were positive for *B. anthracis*, and two of Mr. Stevens' colleagues also contracted the disease.

On October 9, letters containing *B. anthracis* were mailed to two US Senators. On October 12, a similar letter was mailed to Tom Brokaw at NBC News. Additional letters were sent to ABC, CBS and the New York Post. By the end of 2001, 22 people had contracted anthrax; five had died; and over 10,000 were given precautionary antibiotics. It took more than two years to clean up the buildings impacted by the so-called "Amerithrax" attacks, and the Federal Bureau of Investigation estimates it cost more than $1 billion.

So it may seem strange when I tell you that, in the biothreat community, this was a small pretty small attack. Each letter contained less than a gram of *Bacillus*. For reference, one sugar cube is about 3 g, so the entire set of letters contained only two or three sugar cubes of material.

Former Defense Secretary William Cohen once held up a five-pound bag of sugar on national television, and announced that amount of anthrax, spread over Washington, DC, would kill more than half the city. Soviet defectors have alleged that the former USSR bioweapons program was capable of producing two tons of material per day. For reference, that's about 400 5-lb bags of sugar, or 300,000 sugar cubes. Per day.

So how DO you protect Washington, DC against an attack with a bag of anthrax, rather than a sugar cube?

Anthrax has a fairly short incubation period—between 1 and 5 days, depending on the amount of material to which one is exposed. It also has a high fatality rate. Historically, 89% of those with inhalational anthrax have died. In 2001, only five of 11 inhalational cases died, or 45%. This is a tremendous improvement in treatment, but came with a very high public health burden; if there were thousands exposed, it's unlikely we'd be able to achieve that kind of success. And the average interval between onset of symptoms and death is 3 days—that's pretty fast, especially since the early symptoms resemble those of many other diseases, including influenza, or, in Mr. Stevens' case, meningitis.

So the federal government has invested in programs to detect a large-scale attack *before* people are symptomatic. That way, public health has time to implement a very proactive response. If you can treat people with antibiotics *before* they exhibit symptoms, the survival rate for anthrax is nearly 100%. But, in order to do that, you have to detect it within hours to days of release.

One of the most common techniques used for early detection is environmental air monitoring. Both the Department of Defense and the Department of Homeland Security routinely collect high-volume air samples, which are analyzed by PCR (polymerase chain reaction) for the presence of a variety of threat agents, including *B. anthracis*. Following detection of an aerosolized agent, surface samples, such as swipes, may be collected for additional information. Although similar techniques are employed in other fields (such as air pollution monitoring), their application to the biothreat is frequently criticized for a perceived lack of "operational data" supporting their use. However, "operational data" for a real or simulated terrorist attack on an urban area are difficult to obtain. The experimental evaluations of currently fielded systems are not publicly available for security reasons, and much of the relevant open literature is from studies that are outdated or performed in non-urban settings.

Fortunately, in 2005 and 2009, the Pentagon Force Protection Agency (PFPA), which is the entity that protects the Pentagon, staged deliberate releases of a commercially available organic pesticide to assess their response protocols. The pesticide contained *Bacillus amyloliquefaciens.* Many organic pesticides contain *Bacillus* species that are closely related to *B. anthracis*. They're toxic to garden pests, but harmless to humans. Because *B. amyloliquefaciens* is morphologically and phylogenetically similar to *B. anthracis*, it can be expected to behave a lot like *B. anthracis* in the environment, and is therefore considered a reasonable simulant for studies. So these releases were good opportunities to obtain "operational data" on biothreat detection systems, and my team was funded by the Department of Homeland Security to do just that.

This slide shows how most bioaerosol detection systems work. But how do you decide where to put the collectors? Collector location is critical to system performance, and it's nontrivial to determine where they go. You could put one on every street corner, but that's not very practical. So we use atmospheric transport and dispersion modeling to evaluate collector locations.

We start by collecting information on historic winds. This is a wind rose. It's a representation of the wind speeds, directions, and frequencies based on data collected over nine years at Ronald Reagan National

Airport, which is just down the road from the Pentagon. This wind rose is for July, which is the month the 2009 PFPA release occurred. Each bar shows the direction the winds come *from*—so we can see that winds near the Pentagon usually come out of the south/southwest at this time of year.

We input the wind data into models that simulate the movement of particles in urban environments. I'm going to show you a movie that comes from a model called QUIC—that stands for the Quick Urban & Industrial Transport Model. This model is specifically designed to show us how particles transport and deposit in areas with a lot of buildings, where the buildings impact the airflow. If you've ever been in a big city, and come around a corner and gotten hit by a wind you didn't know was there, you've experienced how the buildings channel airflow in interesting ways. This movie shows a release in downtown Boston, and you can see how the buildings spread the particles out.

One important consideration for transport and dispersion models is particle size. Larger particles tend to fall out of the air relatively quickly, producing a lot of deposition but not a very substantial downwind plume. Smaller particles, in contrast, stay in the air a long time. They don't produce as much deposition, but they do produce a sizeable downwind plume area. Fortunately for us, in 2005, the PFPA release had a rather large particle size—around 150 microns—while the 2009 release had a much smaller particle size, with the majority of the particles in the 1-10 micron range.

We put the particle size information into the QUIC model, along with the historic winds, and generated what we thought would be the downwind impact areas. This slide shows the 2005 release line—that's the red line along the road to the west of the Pentagon—and the projected downwind impact area. The impact area is a combination of the projected plume and the projected deposition. The 2005 release had a large particle size, so the downwind impact area was projected to be fairly small, with a lot of the agent deposited within the facility boundaries. We sited one aerosol collector by the Pentagon Metro Station, and collected post-release surface samples at the locations shown as by the squares and triangles. The surface samples were collected both above and below ground, in the subway station. The below-ground swipes are shown by triangles, and the above-ground by squares.

I should mention that we collected background samples prior to the release. Since *Bacilli* are naturally occurring, and since *B. amyloliquefaciens* is an approved pesticide, there was a possibility of finding it in the area before the release. We collected samples all over the Washington, DC metro area over a period of a few years, and all background samples were negative against our PCR assay, which was custom-developed for these experiments by Lawrence Livermore National Laboratory.

Air and surface samples were collected between 6 and 40 hours post-release in 2005. There's a range in that timeline because there was a tremendous snowstorm shortly after the release, so we collected the air sample at 6 hours, and the surface samples after the snow died down. And it was interesting, because there was a lot of snow, and snowmelt, in the final surface samples. But here are the results, and you can see that the air sample was positive, as were seven of 13 surface samples, including one surface sample collected below-ground, in the subway.

These figures show the plume and deposition maps using the actual data collected at the time of the release. The top figure is the plume, and the bottom is the deposition. And the results map reasonably

well with our predictions. The surface sample results—seven of 13 positive—may not sound very good, but deposition is a tricky process, and there's no guarantee the deposition is as homogenous as our models predict, so you're sampling for something you can't see, and you don't know exactly where it is. This is compounded by our current surface sampling techniques, which produce only about a 20% recovery efficiency for biological agents. These two factors, combined, mean that negatives are usually expected, except in cases of white powders, where you have a very clear sampling target.

The 2009 release was very different. The particles were nearly all within the respirable range—that is, between 1 and 10 microns—so the models predicted there would be very little deposition, but a fairly sizeable plume extending well into the metro area. Based on the expected winds, we again identified the projected downwind impact areas—the plume area plus the deposition area—and this time, we sited four air collectors which we ran out to 72 hours post-release. We also collected surface samples at 12 hours post-release, but not that many, because, again, we didn't expect a lot of deposited material because of the particle size.

Because the 2009 results are really very, very interesting, I'm going to spoil it a bit by showing you the plume and deposition maps using the data collected at the time of the release first. That's the plume, on the left, and the deposition on the right. And you can see that the actual release should have headed largely to the north, if the only factor impacting its spread was the wind.

But here are the results at 12 hours post-release. And you can see that the collector at the facility is positive, as we'd expect. And we're seeing some deposition up north—site four is Foggy Bottom metro, and site 3 is near the White House—but what's going on down here, at the Jefferson Memorial? That collector is positive, and that's not explained by the winds. But remember I told you earlier that buildings impact airflow in cities? Well, so do rivers and traffic. And we have both here, which probably explains how some of the material was directed to the northeast, toward the Jefferson.

At 24 hours post-release, all four of the air collectors are positive. And that's great news, because if this had been a real event, public health would have been notified in time to mount an effective response campaign before people in the city started showing up at emergency rooms.

And at 48 hours post-release, we've still got a positive on the collector at the Pentagon, and the one at the Jefferson. That's not necessarily good news, because we changed these filters at 12 and 24 hours. So this means that, between 24 and 48 hours post-release, the agent was still floating around. It was probably reaerosolized by traffic—again, this is the 14th Street bridge, and it's a major commuter roadway for people who work in the city.

Fortunately, by 72 hours, things have calmed down a bit, and we've only got one positive, at the location near the White House. But this raises an interesting question. Particles reaerosolize. You only have to be in the mid-west or western US during one major duststorm to realize that. There have been a lot of studies of blowing soil, to better understand erosion. And there have been a lot of studies on reaerosolization of radionuclides because of concern of fallout from nuclear testing. And we know that radionuclides in the environment are usually bound to soil, so their resuspension is often modeled using the same equations used for just soil.

But there's no good consensus in the biothreat community as to whether you can use data from other particles, like soil or radionuclides, to model reaerosolization of biologicals. Is a biological particle in the environment, where it's likely bound to other things such as soil, just a particle like any other, or is it special, because it has surface proteins and other functionality? What do you think?

I'd like to wrap up my part of this webinar with a brief summary of what we discussed today, plus one more question for all of you. There is a lot of concern in the biothreat community, and in the federal government, about a large-scale aerosolized attack. Because of that, we've implemented environmental monitoring programs that use aerosol collectors to continuously monitor for the presence of threat agents in the air. Air samples are usually analyzed using PCR, which is one of the most effective analytical techniques we have for identifying DNA. Experiments and modeling have shown these systems are effective, and can warn public health of an impending crisis in time to mount an effective response. But.

Does DNA in the air mean people are infected? Nope. It means there's DNA in the air. With PCR, we often can't even confirm the agent was viable when it was collected, especially if it's a more fragile threat agent, like *Yersinia pestis*, which causes plague. And there are no good, rapid, high-throughput tests for assessing exposure in a population.

So public health has to extrapolate from DNA on a filter to an appropriate response. If you were a public health official, and I told you there was *B. anthracis* DNA in the air in your city, what would you do? If you remember back to my third slide, anthrax does occur naturally in the US. In fact, a recent study performed by the US Geological Survey found that about 5% of soil samples collected on a north-south transect between Manitoba and Texas contained *B. anthracis*. And this is a big gap in our science. There's no rapid way to differentiate between a naturally occurring agent and one that has been deliberately released, and there's no rapid way to determine whether people are infected.

If it was me, I'd probably wait for more information. And that's a problem, because, as we discussed earlier, anthrax is very difficult to treat once people are symptomatic. As chemists, this is a problem we need to fix. We need better ways to determine viability in environmental samples, and we need rapid methods for determining whether people have been exposed to a pathogen before they develop symptoms. These are two areas of active research for the Departments of Defense and Homeland Security, and now, when you read their research calls, I hope you'll understand why.

In conclusion, I've cited a number of references in this study, and I'd like to point you toward them if you're interested. The Bales paper contains the information I presented on outbreaks of anthrax in the US between 1950 and 2001. The Griffin paper is the USGS survey of prevalence of *B. anthracis* in soils. The Inglesbly paper is one of the most prominent reviews of anthrax as a disease, and is great if you're interested broadly in bioterrorism. And the other papers are mine. The study I presented today will be published in the March/April edition of *Biosecurity and Bioterrorism*, and will be open access, as are most of my papers because I believe this information needs to be shared so we can improve the nation's defenses against terrorism. And now I'm happy to take questions from our esteemed moderator!