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technical BRIEF

Results From Persistence Testing of Biological Agents Under Various Conditions

EPA investigates the persistence of biological agents under various conditions of relative humidity, temperature, and light

Introduction

Because of their potential use as weapons of mass destruction, biological agents are a significant terrorist threat. Once released, agents such as bacteria and viruses can cause disease or death in humans, animals, and plants by spreading through air, water distribution systems, and the food supply. A major attack could cause many casualties and interrupt vital civilian and government operations.

Data on how long and under what conditions agents remain viable or active influence many aspects of planning, response, containment, and recovery from biological incidents.

U.S. EPA's Homeland Security Research Program (HSRP) develops products based on scientific research and technology evaluations. Our products and expertise are widely used in preventing, preparing for, and recovering from public health and environmental emergencies that arise from terrorist attacks. Our research and products address biological, radiological, or chemical contaminants that could affect indoor areas, outdoor areas, or water infrastructure. HSRP provides these products, technical assistance, and expertise to support EPA's roles and responsibilities under the National Response Framework, statutory requirements, and Homeland Security Presidential Directives.

Following a contamination event, environmental conditions that might decrease the number of viable organisms could be implemented prior to decontamination efforts. Such pre-treatment could potentially reduce the risks of exposure, lower the costs of cleanup, and shorten the time before re-use of a facility or an outdoor area.

Persistence testing of biological agents by EPA[1]

During persistence testing, standardized samples of materials (coupons) are spiked with a biological agent or a non-pathogenic surrogate. For different intervals, coupons are exposed to various experimental conditions such as changes in temperature, relative humidity (RH), or periods of simulated sunlight. Coupons are then tested for the presence of viable organisms. Testing and analysis of data follow quality assurance protocols.

The following are important environmental factors that affect persistence, which is the length of time a microorganism or biological agent remains viable on a surface:

- Characteristics and amount of the biological agent
- Relative humidity (RH)
- Temperature (TEMP)
- Exposure to simulated sunlight
- Type of coupon material
- Porosity of material surface

[1] Note that the studies on persistence of various organisms involved differing coupon materials. These studies were performed to gather specific information on organism persistence, not to cross-compare study results. For example, the avian influenza virus study involved soil and chicken feces because these are environments of concern for that virus.

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The following disease causing microorganisms were studied:

Microorganisms	Diseases
Bacillus anthracis	anthrax
Brucella suis	brucellosis
Francisella tularensis	tularemia
Highly pathogenic avian influenza (H5N1)	HPAI H5N1 influenza
Vaccinia virus, the surrogate for *Variola major*	smallpox
Yersinia pestis	plague

As shown below, investigators found that increased temperature (TEMP), increased RH, and exposure to simulated sunlight (☼) [ultraviolet light with wavelengths of 280 to 400 nanometers (UV-A/B)], tended to decrease the persistence of some biological agents. Generally, these environmental conditions were found to decrease persistence of agents on most of the materials tested. (See references [1,2,3,4,5,6] for full reports that have details on conditions, agents, and materials.)

Summary of Tested Environmental Conditions Resulting in a Decreased Number of Viable Organisms

Biological Agent	Conditions That Decreased the Number of Viable Organisms	
Highly Pathogenic Avian Influenza (H5N1) [1]	Exposing to simulated sunlight [a] at 22°C	☼
	Increasing temperature from 4°C to 22°C [b]	⇧ TEMP
Vaccinia Virus (freeze-dried) [2]	Increasing relative humidity to > 70% at 22°C	⇧ RH
Vaccinia Virus [3]	Increasing relative humidity to > 70% at 30°C	⇧ RH
Brucella suis [6]	Exposing to simulated sunlight [c] at 22°C	☼

[a] UV-A/B – exposure was continuous
[b] Persistence was higher at low temperature (4° C), than at moderate temperature (22° C) at both low and high relative humidity
[c] UV-A/B – exposure was 12 hours on and 12 hours off

Summary of Major Results from Persistence Testing

Highly Pathogenic Avian Influenza (H5N1) [1]

Highly pathogenic avian influenza (H5N1) virus [influenza A/Vietnam/1203/2004 (H5N1, clade 1) was spiked on coupons of glass, galvanized metal, topsoil, and chicken feces. Environmental conditions were defined as low temperature (4°C), room temperature (22°C), low RH (40%), high RH (80%), and exposure/no exposure to UV-A/B.

At low temperature, low RH, and no UV-A/B, viable H5N1 was recovered from glass, galvanized metal, and topsoil on Day 13 of the 13 day test runs.

- Room temperature (at low and high RH and no UV-A/B exposure) decreased H5N1 persistence on glass, galvanized metal, topsoil, and chicken feces to less than 2 days
- UV-A/B decreased H5N1 persistence at low temperature and low RH to 4 or fewer days on glass, galvanized metal, and topsoil

Vaccinia Virus (Freeze-Dried) [2]

Vaccinia virus is used in research as a surrogate for *Variola major,* the virus which causes smallpox. One strain of vaccinia is used in the live vaccine against smallpox. Vaccinia was freeze-dried as part of the persistence investigation to emulate the conditions under which the variola virus might be shipped as part of a bioterrorism incident.

Vaccinia virus (ATCC ® VR119) was spiked on coupons of glass, galvanized metal, painted cinder block, and industrial carpet. The coupons were frozen overnight to prepare prior to being freeze-dried. Environmental conditions were defined as low temperature (10°C), room temperature (22°C), low RH (20%), and high RH (70%).

At low temperature and low RH, viable vaccinia virus was recovered from all materials on Day 56 of the 56 day test runs.

At room temperature and low RH, viable vaccinia virus was recovered from glass, galvanized metal, and painted cinder block on Day 42 of the 56 test runs.

- High RH at room temperature decreased vaccinia virus persistence on glass, galvanized metal, painted cinder block, and industrial carpet to less than 3 days

Vaccinia Virus [3]

Vaccinia virus (ATCC® VR119), used as a surrogate for the smallpox virus *Variola major,* was spiked on coupons of painted cinder block and galvanized metal ductwork. Environmental conditions were defined as ambient RH (20°C and 40 to 70% RH), high temperature/low RH (30°C and < 40% RH), and high temperature/high RH (30°C and > 70% RH).

At high temperature/low RH, viable vaccinia virus was recovered from galvanized metal and painted concrete on Day 14 of the 14 day test runs.

- High temperature/high RH decreased vaccinia virus persistence to 9 days on painted concrete
- High temperature/high RH decreased vaccinia virus persistence to 3 days on galvanized metal

Elevated RH appeared to decrease the persistence of vaccinia virus. Other modest effects from temperature differences might have been masked by the large effects of RH, however.

Francisella tularensis* and *Yersinia pestis [4]

Francisella tularensis LVS (Battelle culture: OSU FTL361) and *Yersinia pestis* CO-92 (Battelle culture: M-YPO166) were spiked on coupons of aluminum, industrial carpet, painted joint tape

paper, and computer keyboard keys. Environmental conditions were 35 to 45% RH and 22°C for these persistence tests.

- Viable *F. tularensis* was recovered from computer keys on Day 7 of the 7 day test runs
- No viable *F. tularensis* was recovered after Day 1 on aluminum, industrial carpet, and painted joint tape paper
- Viable *Y. pestis* was recovered from aluminum and painted joint tape paper on Day 7 of the 7 day test runs
- Viable *Y. pestis* was recovered from computer keyboards keys on Day 3 and from industrial carpet at 8 hours

Bacillus anthracis [5]

Bacillus anthracis Ames and *Bacillus subtilis* (ATCC® 19659) were spiked on coupons of glass, bare pine wood, unpainted concrete, and topsoil. The bacteria were exposed to 12 hours of UV-A/B alternating with 12 hours of darkness. No clear pattern of UV-A/B exposure times and decreased viability was evident with all materials. Some viable spores of both *B. anthracis* and *B. subtilis* were recovered at 56 days on all materials.

- UV-A/B decreased the quantity of viable *B. anthracis* and *B. subtilis* recovered from glass by about 5 $\log_{10}$ after 672 exposure hours (56 days)

Brucella suis [6]

Brucella suis biotype I (Battelle BRU163) was spiked on coupons of aluminum, glass, bare pine wood, unpainted concrete, and topsoil. Not all materials were tested under all conditions. Environmental conditions were defined as low temperature (4°C or 7°C), moderate temperature (22°C), and exposure/no exposure to UV-A/B. Relative humidity was ambient, averaging from 39 to 59%.

At low and moderate temperature, without exposure to UV-A/B, viable *B. suis* was recovered from aluminum, glass, and topsoil on Day 28 of the 28 day test runs (at moderate temperature viable *B. suis* was not recovered from topsoil at day 21 or day 28).

At low and moderate temperature, with exposure to UV-A/B, *B. suis* was recovered from topsoil on the Day 14 of the 14 day test runs.

- UV-A/B at moderate temperature decreased the persistence of *B. suis*, which was recovered from glass on day 1 and from aluminum on Day 7 of the 14 day test runs

Technology Evaluation Reports Referenced

[1] Choi, Y., Rogers, J., Chappie, D., and Wood, J. 2009. *Highly Pathogenic Avian Influenza H5N1 Virus Persistence Testing and Evaluation of Liquid Decontamination Technologies.* Investigation and Technology Evaluation Report. Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-09/054.

[2] Choi, Y., Shaw, M., Rogers, J., Chappie, D., Taylor, M., Riggs, K., Willenberg, Z., and Wood, J. 2009. *Freeze-Dried Vaccinia Virus Persistence Testing and Liquid Decontamination Technology Evaluation.* Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-09/139.

[3] Stone, H., Rogers, J., Fleming, E., Choi, Y., Waugh, J., Richter, W., Taylor, M., Riggs, K., Willenberg, Z., Krile, R., and Ryan, S. 2006. *Impact of Temperature and Humidity on the Persistence of Vaccinia Virus and Ricin Toxin on Indoor Surfaces.* Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-08/002.

[4] Ryan, S. 2010. *Persistence Testing and Evaluation of Fumigation Technologies for Decontamination of Building Materials Contaminated With Biological Agents.* Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-10/086.

[5] U.S. Environmental Protection Agency. 2010. *Investigation of Simulated Sunlight in the Inactivation of B. anthracis and B. subtilis on Outdoor Materials*. Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-10/048.

[6] U.S. Environmental Protection Agency. 2010. *Persistence Testing of Brucella suis on Outdoor Materials.* Investigation Report. Washington, D.C.: U.S. Environmental Protection Agency. EPA/600/R-10/026.

Contact Information

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