Report to the Virginia General Assembly:

Land Applied Biosolids Epidemiological Pilot Study and Pathogen Content Analysis

Office of Environmental Health Services
Virginia Department of Health

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EXECUTIVE SUMMARY

The 2018 General Assembly appropriated $100,000 to the Virginia Department of Health (VDH) to study land-applied biosolids in Virginia, specifically health risks and pathogen content. Fifty thousand dollars ($50,000) was appropriated to conduct a pilot epidemiological study of the human health effects of land application of biosolids, following which VDH must submit its findings and a proposed design for a full-scale epidemiological study, if needed. Fifty thousand dollars ($50,000) was appropriated to perform sample testing of Class B biosolids that are land applied in Virginia to determine their pathogen content. VDH used test results to inform its epidemiological pilot study and assessment of aerosol infection risks.

VDH mailed health based surveys to addresses determined to be within 1,000 feet of sites where the Department of Environmental Quality (DEQ) received notification that Class B biosolids were going to be land applied. VDH received completed surveys and participants were put into two groups: those living within 200 feet of fields where Class B biosolids was land applied and those living between 200 and 1,000 feet of fields where Class B biosolids was land applied. VDH mailed 2,670 surveys between April and June 2019 and received a combined total of 68 responses from the two study populations. The majority (96%) of all survey participants reported that their health was fair or better with 20 (29%) reporting that they were ill or had symptoms in the past two weeks. Seven of the 20 with symptoms or illnesses reported they required medical care. The most reported symptom or illness in the past two weeks included runny nose and congestion (14), sore throat (9), and headache (8). Forty-five (66%) reported detecting biosolids odor in the past two weeks with seven reporting the level of odor as faint, 24 not providing an answer, and 38 reporting the level of odor as moderate or strong. When asked about biosolids impact on quality of life, of the 68 that provided an answer 32 (47%) reported that biosolids land application affected their quality of life.

Biosolids samples were collected from 34 application sites, four storage facilities, and two wastewater treatment facilities between March and May 2019. VDH contracted with the University of Arizona to test biosolids samples for pathogens which included seven microorganisms or classes of microorganisms. Pathogens tested included bacteria, viruses, and helminth ova. A total of 40 biosolids samples including 39 Class B biosolids and one Class A biosolids samples were sent to the University of Arizona. The biosolids treatment type identified for the 40 samples included: aerobic digestion (10), anaerobic digestion (12), lime stabilization (16), pasteurization/digestion (1), and thermal hydrolysis (1).

Fecal coliform bacteria can be used as a measure of treatment effectiveness for Class B biosolids. The Class B biosolids fecal coliform standard for effective treatment is a geometric mean of 7 samples <2,000,000 most probable number/gram (MPN/g), for biosolids producers opting to use this monitoring requirement. Five anaerobically digested biosolids from different producers had fecal coliforms greater than 2,000,000 MPN/g.

The number of samples with measurable concentration of *Salmonella* by treatment type reported: aerobic digestion (6), anaerobic digestion (8), and lime stabilization (1). There is no *Salmonella* standard for Class B biosolids; however, the *Salmonella* standard for Class A biosolids generated for unrestricted use is 3 MPN/4g. One lime stabilization treated sample *Salmonella* content was reported as 5 MPN/4g compared to six aerobic and seven anaerobically digested biosolids samples that were reported to have *Salmonella* content greater than 3

MPN/4g. Measurable concentration of pathogenic viruses were reported in five aerobic and one anaerobic digested biosolids samples. *Ascaris* was not detected in any samples.

VDH used quantitative microbial risk assessment to calculate the probability of aerosol infection by each pathogen using the laboratory results. The risk of aerosol infection was calculated using 6.5 feet, 200 feet, and 400 feet as the distance from the land application site and the measured pathogen concentrations. The aerosol risk of infection from *Salmonella* measured in collected samples was only considered elevated at 6.5 feet and 200 feet for one sample. However, there is no elevated risk to *Salmonella* if the geometric mean concentration of all samples is used in the risk calculation. The aerosol risk of infection from rotavirus and adenovirus aerosol infection at 6.5 feet from the biosolids application site was elevated. There was also an increased risk of rotavirus and adenovirus aerosol infection at 200 feet when calculating the risk using the sample with the maximum concentration. There was an increased risk of listeria aerosol infection at 6.5 feet from the biosolids application site when calculating the risk using the maximum and geometric mean concentrations of samples. There was also an increased risk of *Listeria* aerosol infection at 200 feet from the biosolids application site. There was no increased risk for any of the above pathogens at 400 feet from the biosolids application site.

The pathogen study shows that Class B biosolids generated by lime stabilization have fewer pathogens than aerobic or anaerobically generated Class B biosolids. Therefore, additional health studies to evaluate symptom onset related to pathogens do not need to include fields where Class B biosolids generated by lime stabilization is applied. The quantitative risk assessment performed using the pathogen results indicate that there is no aerosol risk to any pathogen analyzed at a distance of 400 feet from the application site. The epidemiological survey response rate was low and additional resources and employee hours are necessary to adequately study the impact on health. The survey responses received suggest that the quality of life is impacted and can be attributed to the odor.

INTRODUCTION

Class B biosolids are treated sewage that is dewatered and applied to land as a soil amendment. The treatment process reduces the pathogen content to a level that is safe for land use when land applied according to the current regulations. Class A biosolids are treated sewage where the pathogen content has been reduced to a level making them safe for direct sale to the public. Class A biosolids are also land applied in Virginia. VDH contacted universities, federal partners, and private microbiology laboratories to determine which pathogens in biosolids to analyze. VDH contracted with the University of Arizona to test 40 biosolids samples for *Salmonella*, fecal coliforms, helminth ova, somatic coliphage, enteric viruses, adenovirus, and reovirus. Samples were collected primarily from fields where biosolids were being land applied. The United States Department of Agriculture (USDA) in Mississippi agreed to test eight samples for pathogens at no cost. The USDA pathogen results were used to calculate *Listeria* risk of infection.

To evaluate the health concerns of Class B biosolids, VDH conducted an epidemiological survey of residents living near fields where biosolids were land applied. The epidemiological survey was approved by the Institutional Review Board at VDH with the intent to answer: *What are potential health effects reported in relation to application of biosolids to a nearby site?* The subject selection included properties within 200 feet of the edge of a field where Class B biosolids were applied, and a second group of addresses 200 - 1,000 feet from the edge of a field where Class B biosolids were applied.

Beginning April 2019, VDH was notified by the Department of Environmental Quality (DEQ) where biosolids were scheduled to be land applied. Addresses within 1,000 feet of the application site were identified, and an invitation to take the survey was mailed to those addresses 7-10 days after DEQ notified VDH. Between April and June, 389 fields were identified and a total of 2,670 households were invited to take the survey. The survey was closed on July 31, 2019, three weeks after the last survey was mailed. Information collected included household demographics, health over the past two weeks, and any change in quality of life. Participants were given the option to complete the survey electronically, over the phone, or by using a paper version.

Quantitative microbial risk assessment (QMRA) was used to inform the epidemiological study about the pathogenicity of the tested biosolids. The USDA met with VDH staff and provided risk assessment tools that were used to determine pathogen inhalation and ingestion health risk from biosolids. VDH used conservative exposure scenarios to calculate risk of infection at 6.5 feet, 200 feet, and 400 feet, from Class B permitted fields. Calculated risk at required set back distances from dwellings (200 feet) and odor sensitive receptors (400 feet) were found to be generally low. The maximum calculated risk at 200 feet was 8.07×10^{-4} or 8 in 10,000 for the sample with the highest virus content. In no case was an elevated infection risk calculated when using 400 feet as the distance from a field where Class B biosolids were land applied.

BACKGROUND

Class B Biosolids are created through the treatment of domestic sewage sludge. Wastewater sewage is treated to reduce pathogens and chemicals that may pose a risk to human health and the environment. Pathogens in sewage include bacteria, viruses, and parasites that can cause illness. The pathogen regulations are not risk-based standards but are operational standards

intended to reduce the presence of pathogens to concentrations that are not expected to cause adverse health effects. The standards include treatment requirements, site restrictions, and monitoring requirements (National Research Council, 2002).

In the Code of Federal Regulations, Title 40 Part 503 (40 CFR Part 503) The U.S. Environmental Protection Agency (EPA) established two categories of biosolids, Class A and Class B (Standards for the Use or Disposal of Sewage Sludge, 1993). Class A biosolids have no detectable pathogens. If the levels of heavy metals are low enough and the treatment includes specific vector attraction reduction processes, Class A biosolids may be distributed as Exceptional Quality (EQ) biosolids. Land applied EQ biosolids do not require pre-approval from DEQ and may be sold to the general public.

Class B biosolids do have detectable concentrations of pathogens. Class B biosolids' less restrictive standards for disease-causing organisms require more restrictive permit limitations to protect public health and the environment. Public exposure to pathogens in Class B biosolids is mitigated by how often biosolids are applied on a field, setback distances, field use, time for re-entry, crop exclusion criteria, and pathogen content.

Class B Biosolids Pathogen Content Selection for Study

To determine the specific microorganisms to test for in Class B biosolids, VDH contacted universities, federal agencies, state partners, and private laboratories for expert advice. Pathogens in untreated sewage include bacteria, viruses, and parasites (helminths and protozoans). The consensus was to analyze biosolids for microorganisms that:

1) have established laboratory methodologies;
2) are indicators that treatment processes have been effective in reducing pathogen content;
3) are known to cause illness(es) that could inform the epidemiological study; or
4) may be an indicator for identifying other pathogens.

VDH contracted with the University of Arizona to test 40 samples. The tests included those for fecal coliforms, *Salmonella*, somatic coliphage, enteric viruses, adenovirus, reovirus, and helminths.

Indicator Microorganisms

Fecal Coliforms

Fecal coliforms are primarily composed of *Escherichia* species, but the test for fecal coliforms also detects other non-pathogenic coliforms not of fecal origin, such as *Klebsiella,* that may be present in raw sewage or introduced during treatment. Most fecal coliforms are not pathogenic, but beneficial to their host in aiding in the digestion of food and recovery of nutrients. Fecal coliforms are widely used as an indicator of pollution because they are directly proportional to fecal wastes, present in high numbers, and easier to quantify than other microorganisms. Fecal coliform analysis is used to satisfy the pathogen reduction requirement in 40 CFR Part 503 for Class A and Class B biosolids, because a reduction in it can be used as an indicator of how well the treatment process is working.

Somatic Coliphage
Somatic coliphages are viruses that infect *E. coli* and are present with great abundance in class B biosolids. Somatic coliphage is a potential indicator for human enteric viruses, since it is present in large amounts in biosolids and has similar persistence to enteric viruses. Somatic coliphage was tested to provide data on levels in Virginia biosolids for potential future use as an indicator.

Enterococci
The enterococci are a group of nonpathogenic bacteria that are part of the normal intestinal flora. Enterococci can be used as an indicator for estimating the number of *Listeria* and other bacterial pathogens present. The USDA tested eight samples for enterococci, the University of Arizona did not test for this bacteria.

Pathogens Analyzed

Salmonella
Salmonella is a genus of bacteria that includes *S. enterica*, which is capable of causing diarrheal disease. Members of the genus *Salmonella* are the most extensively studied bacteria in sewage sludge, and *Salmonella* testing is in 40 CFR Part 503 as an option to obtain Class A biosolid rating. *Salmonella* can live for more than a year in biosolids and are responsible for over one million cases of human disease in the United States. *S. enterica* is widespread enough that it is usually found in untreated sewage, and its absence in biosolids suggests disinfection methods were sufficient.

Enteric Viruses

Enteric viruses are host-specific and found in the intestinal tracts of animals. This group includes adenoviruses, reoviruses (such as rotavirus), picornaviruses (such as poliovirus and hepatitis A virus), and caliciviruses (such as norovirus). Due to the large number of enteric viruses present in untreated sewage sludge and their resilience against environmental stresses such as heat and low pH, EPA chose enteric viruses for inclusion in 40 CFR Part 503 as one of the pathogens used to evaluate successful pathogen reduction during biosolids treatment. If enteric viruses were detected in biosolids samples the sample was then tested for adenovirus and reovirus.

Adenoviruses

Adenoviruses are a group of enteric viruses capable of causing gastrointestinal or respiratory infection. They are one of the viruses that can cause the common cold. In most cases infections are mild, but people with compromised immune systems can become seriously and sometimes fatally ill.

Reoviruses

Reoviruses are a group of double-stranded RNA enteric viruses, many of which cause gastrointestinal infections. Most cause mild illness, but rotavirus is capable of causing serious illness in infants and young children, and is a major cause of hospitalization for diarrheal disease in young children.

Helminths

Helminths are parasitic worms. *Ascaris lumbricoides* is the largest intestinal roundworm found in humans, and was included in this study since 40 CFR Part 503 provide standards for *Ascaris* ova in biosolids. With improved hygiene and food preparation practices *Ascaris* is now rarely found even in untreated sewage.

Class B Biosolids Sample Collection

DEQ enforces regulations on the land application of biosolids in Virginia and has regional offices across the state. Part of their enforcement involves visiting land application sites to ensure regulations are being followed. VDH coordinated with DEQ to arrange for their inspectors to collect samples at land application sites. DEQ chose fields to sample based on availability and weather conditions, while avoiding taking multiple samples from the same biosolids producer. To ensure that 40 samples could be analyzed; four samples needed to be shipped overnight and arrive at University of Arizona by Wednesday each week. Therefore, samples were collected on Monday and Tuesday each week, and if collection days were during heavy rainfall, samples were collected at storage facilities (4 samples) or wastewater treatment facilities (2 samples). One Class A biosolids sample was analyzed.

The inspectors collected samples from bulk material before it was land applied, turning over the material first to avoid collecting dry surface material. Inspectors were provided with chain of custody forms, and sampling kits for collecting and shipping each sample. If more than one sample was taken they were instructed to change gloves and use a fresh disposable scoop. Biosolids were collected in sterile one liter bottles and shipped overnight on ice to the laboratory.

Laboratory Methodology and Handling Results

A description of the laboratory method used to analyze pathogen content in biosolids is in Table 1. Many samples were reported with pathogen concentrations as less than or greater than a value. This happens when the concentration is below what the laboratory can detect or is greater than what the laboratory can report with confidence. Therefore, if a value is reported as less than a number (e.g., < 0.78), VDH would use 0.78 in this example for any calculations. This approach is conservative, health protective, and may overestimate the risk. Likewise, samples reported as greater than a number (e.g.; >500), VDH would use 500 in this example for any calculation. In this scenario the risk could be underestimated; however, there were only two instances where results were reported as greater than.

Table 1. Methods for Enumerating Microorganisms

Organism	Description
Fecal coliforms	Enumeration by EPA Method 1681: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple Tube Fermentation using A-1 medium, 2006
Salmonella	Enumeration by EPA Method 1682: Salmonella in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium, 2006.
Ascaris	Culture and enumeration by microscopy, Yanko/USEPA, 1987.

Organism	Description
Coliphages	Enumeration by EPA Method 1602 using a single agar layer (SAL) procedure with E. coli, enumeration by plaque forming units.
Enteric viruses	Enumeration by ASTM D994-89 with evaluation of cytopathic effects on BGM tissue culture, EPA Biosolids guidance document EPA625/R-92/013 Appendix H (ASTM D4994-89).
Adenovirus	Modification of ASTM D994-89, enumeration by most probable number (MPN) with confirmation by RT-PCR.
Reovirus	Modification of ASTM D994-89 with second passage in BGM cells, enumeration by most probable number (MPN) with confirmation by RT-PCR.
Enterococcus	Modification of EPA method 1600, modified for biosolids by utilization of a stomaching step of 90 seconds prior to the EPA 1600 method.
E. coli	Modified EPA method 1603 by adapting the method for biosolids, stomaching 10 g of biosolids in 100 ml physiological saline for 90 seconds prior to the EPA 1603 method.
C. perfringens	Following stomaching and heat shock at 70°C for 10 min samples were plated to CP chromoselect agar and incubated at 44.5°C for 24 h **Invalid source specified.**

Permit Regulations Intended to Protect the Public from Class B Biosolids Land Application

Pathogen content in sewage is reduced using combinations of physical, chemical, and biological processes resulting in Class B biosolids with detectable pathogens with measurable risk. Class B biosolids must be treated to meet one of three criteria:

1) a fecal coliform count of less than 2×10^6/gram (g) of dry solids at the time of disposal;
2) treatment by a process to significantly reduce pathogens (PSRP); or
3) treatment by a process that is equivalent to a PSRP.

Five processes are listed as PSRP's for Class B treatment: aerobic digestion, anaerobic digestion, air drying, composting, and lime stabilization (Standards for the Use or Disposal of Sewage Sludge, 1993). DEQ reported the following treatments used in Virginia for Class B biosolids in 2017: lime stabilization (about 54%), anaerobic digestion (about 39%), or aerobic digestion (about 7%).[1] See Table 2 for a brief description of the most commonly used treatment technology in Virginia for Class B biosolids.

[1] Bryan Cauthorn, Personal Communication October 2018.

Table 2. Processes to significantly reduce pathogens most commonly used in Virginia

Process	Description
Aerobic digestion	Aerobic digestion is a process that can be done at ambient temperatures on sludge with high water content in a period of 40 to 60 days. Unlike anaerobic digestion it doesn't require heating, but it does require air to be bubbled through the liquid to maintain aerobic conditions (making sure oxygen is present). The time required will vary between 40 days at 20°C and 60 days at 15°C.
Anaerobic digestion	Anaerobic digestion is a process that can take 15 to 60 days, depending on temperature. Sludge with a high water content is incubated in the absence of oxygen and at a temperature between 20°C and 55°C. The cooler the temperature, the longer the biosolids must be held. Typically the biosolids are heated to speed the process.
Lime stabilization	Lime stabilization relies on a large increase in pH to kill pathogens. Lime, which contains large amounts of calcium hydroxide and calcium oxide, is mixed into dewatered sludge so that the pH rises above 12 after two hours of treatment.

The risk of infection from aerosol exposure to pathogens in Class B biosolids remaining after treatment is mitigated by setback distances and time restrictions. Setback distances are required between occupied dwellings and fields where biosolids are applied. The setback can be decreased if the homeowner requests the reduction and signs a waiver. This distance from homes may vary depending upon the location of the resident's well, and in some cases, whether there are occupants in the home that have submitted an extended (increased) setback form signed by their physician. There are also mandatory setbacks from property lines, roadways, wells, water bodies, streams, and environmentally sensitive areas such as rock outcrops and sinkholes. Some setback distances may vary depending upon whether or not the biosolids are incorporated (worked into the soil). DEQ allows waivers only for dwelling and property line setbacks; the property owner cannot waive environmental restrictions such as wells or areas next to streams. Table 3 lists the minimum setback distances that apply to public health as evaluated using QMRA in this report.

Table 3. Minimum setback distances that apply to protecting public health

Feature adjacent to land application area	Minimum setback distance to land application area (feet)(1)
Occupied dwelling	200 (2), (3), (4)
Odor sensitive receptors (without injection or same day incorporation)	400 (4)
Odor sensitive receptors (with injection or same day incorporation)	200
Property lines	100 (3), (5)
Property lines of publicly accessible sites (6)	200

(1) In cases where more than one setback distance is involved, the most restrictive distance governs.
(2) The setback distance to occupied dwellings may be reduced or waived with the written consent of the occupant and landowner of the dwelling.
(3) DEQ shall grant to any landowner or resident in the vicinity of a biosolids land application site an extended setback of up to 200 feet from their property line and up to 400 feet from their occupied dwelling upon request from their

physician based on medical reasons. In order for an extended setback request to be granted, the request must be submitted to DEQ in writing on a form provided by DEQ. A request must be received by DEQ no later than 48 hours before land application commences on the field affected by the extended setback, and communicated by DEQ staff to the permittee no later than 24 hours before land application commences on the field affected by the extended setback. DEQ may extend a setback distance within 48 hours of land application if requested by the Virginia Department of Health in connection with the landowner or resident's physician.

(4) Setback distances may be extended beyond 400 feet where an evaluation by the Virginia Department of Health determines that a setback in excess of 400 feet is necessary to prevent specific and immediate injury to the health of an individual.

(5) The setback distance to property lines may be reduced or waived upon written consent of the landowner.

(6) Publicly accessible sites are open to the general public and routinely accommodate pedestrians and include, but are not limited to, schools, churches, hospitals, parks, nature trails, businesses open to the public and sidewalks. Temporary structures, public roads or similar thoroughfares are not considered publicly accessible.

Source: Department of Environmental Quality

Calculating Aerosol Infection Risk Using Quantitative Microbial Risk Assessment (QMRA)

To assess the risk of aerosol exposure to pathogens in Class B biosolids samples VDH used QMRA which uses exposure assumptions to model risk of infection. QMRA uses what is known about pathogen infectivity, human anatomy and physiology, human behavior, and air transport mechanisms of pathogens to quantitate risk of infection. The most up-to-date information on QMRA dose-response modeling and variables for each pathogen can be found on the QMRAwiki at http://qmrawiki.canr.msu.edu.[2]

The risk of infection from aerosol exposure to biosolids was evaluated using a model developed by John Brooks of USDA (Brooks, McLaughlin, Gerba, & Pepper, 2012). The risk was evaluated at three distances, 6.5 feet, 200 feet, and 400 feet from the site of land application and the exposed individual. The required setback distance from an occupied dwelling is 200 feet and DEQ may grant the larger setback distance of 400 feet if petitioned. The exposure at 6.5 feet is the exposure a farmer or land applier might be exposed to, and would not be a typical neighboring residential exposure. Because land application may take place over several days, an exposure duration of 24 hours per day for 7 days a year was used. The model uses downwind transport simulation of aerosolized pathogens previously published, and constant wind conditions (Paez-Rubio, et al., 2007; Brooks, et al., 2005). An inhalation rate of 0.83 cubic meters per hour was used, which is a typical breathing rate for a working adult, approximately the 75th percentile for a young adult, and a conservative estimate of residential exposure. Some particles that are inhaled may get trapped in the upper airway and are later swallowed. VDH conservatively used 50% as the amount of aerosol inhaled that is then swallowed. The probability of infection by each pathogen was calculated using the dose-response equations and variable values currently recommended on QMRAwiki for each pathogen.

Participant Selection for Epidemiological Study

VDH designed its epidemiological study to collect health information on Virginia residents living near fields where Class B biosolids land application was scheduled. Questions used in other epidemiological health studies were incorporated into the health survey (Wing, Lowman, Keil, & Marshall, 2014) (Khuder, et al., 2007) (Damascus Citizens for Sustainability, 2013).

[2] QMRAwiki was created by microbiologists from multiple universities including Michigan State and Drexel, and recommended as a source of information by USDA.

VDH also considered complaints made to DEQ when developing the survey. The average number of citizen complaints to DEQ from 2008 to 2012 was 143 per year with the most complaints (203) received in 2008. From 2013 to 2018 DEQ received and average of 33 biosolids land application complaints per year. The complaints include odor, health concerns, truck traffic, and threat to surface or groundwater with the majority of complaints pertaining to odor.[3]

Participants in the study were able to select from a list of health effects experienced in the past two weeks that included nausea, difficulty breathing, eye, nose, and throat irritation, and skin infection and provide additional information in a comment box on the survey. VDH also asked about odor, perception of risk associated with biosolids, and biosolids' impact on quality of life. In addition to this VDH also collected demographic information on the survey. Finally, the epidemiological study was approved by VDH's Institutional Review Board.

Participants for the epidemiological study were selected using DEQ's geodatabase of field locations and notification requirement between permit holders and DEQ prior to applying biosolids. Class B biosolids permit holders must provide the DEQ 24-hours written notice before applying biosolids. DEQ shared this information with VDH to identify sites where biosolids were scheduled to be delivered. Between April 1 and June 30, 2019, DEQ shared all permit holder written notices with VDH. Next, VDH used DEQ's geodatabase and Virginia Geographic Information Network (VGIN) to identify addresses within 1,000 feet of the sites where permit holders notified DEQ biosolids were going to be land applied. A total of 2,670 of addresses were identified and mailed an invitation to participate in the epidemiological study.

Postcards were mailed approximately 7-10 days after land applications were scheduled to begin for each site. This was done to capture reported health effects that were associated with the recent land application and reduce recall bias. It was also done in order to capture any delays in spreading that may occur at larger sites where land application may occur over several days. The participant receiving the survey invitation had until the end of July 2019 to complete it. The first survey invitation was mailed April 1, 2019, and the last survey invitation was mailed on July 8, 2019.

The epidemiological survey tool, REDCap, from Vanderbilt University, was used to create the survey and collect responses. A unique identification (ID) number created using ArcMap (10.6.1) was generated for every address within 1,000 feet of where biosolids are permitted to be applied throughout Virginia. REDCap was then used to assign a unique survey code for each ID number. The survey code was attached to a postcard that was mailed to residents, inviting them to participate in the epidemiological study. The survey code allowed the residents from each household access to REDCap's online survey. A phone number was provided for the respondent to call VDH with questions about the survey and to allow residents the opportunity to request a paper or phone survey as an alternative to the online version.

DEQ receives reports from land applicators after Class B biosolids are land applied that include the source and treatment type of the biosolids applied, total acres, dry tons applied, field location,

[3] Neil Zahradka Personal communication September 2019.

and date of application. This information was shared with VDH and used to group survey participants into two groups: those living within 200 feet of applied field, and those living within 200-1,000 feet of applied field.

FINDINGS

Pathogen Content of Class B Biosolids Samples by Treatment Type

The University of Arizona analyzed the pathogen content in 39 Class B biosolids, and one Class A sample that were generated using either aerobic digestion, anaerobic digestion, lime stabilization, pasteurization digestion, or thermal hydrolysis.

Aerobic digestion

Nine Class B biosolids samples that were analyzed for pathogen content were from generators that use aerobic digestion.

- All samples contained detectable concentrations of fecal coliforms (geometric mean: 25,825 MPN/g, high 307,000 MPN/g, and low 113 MPN/g);
- Six samples contained detectable concentrations of *Salmonella* (geometric mean: 5 MPN/4 g, high 29 MPN/4 g, and low <0.75 MPN/4 g);
- All *Ascaris* were reported as < 1 ova/4 g;
- Seven samples contained detectable concentrations of somatic coliphages (geometric mean: 159 plaque forming units per gram (PFU/4 g), high 4,120 PFU/ 4 g, and low < 4 PFU/ 4 g); and
- Five samples had detectable concentrations of enteric viruses (geometric mean: 1 MPN/4 g, high 11 MPN/4 g, and low < 0.92 MPN/4 g). Viruses found in the sample with the highest concentration were reported as adenovirus and reovirus. The other four samples with enteric viruses were reported to have reovirus.

Anaerobic Digestion

Twelve Class B biosolids samples that were analyzed for pathogen content were from generators that use anaerobic digestion.

- All samples contained detectable concentrations of fecal coliforms (geometric mean: 185,102 MPN/g, high 8,960,000 MPN/g, and low 114 MPN/g);
- Eight samples contained detectable concentrations of *Salmonella* (geometric mean: 11 MPN/4 g, high 23,000 MPN/4 g, and low < 1.03 MPN/4 g);
- All *Ascaris* were reported as < 1 ova/4 g;
- Eleven samples contained detectable concentrations of somatic coliphages (geometric mean: 103 PFU/4 g, high 99,500 PFU/ 4 g, and low 3 PFU/ 4 g); and
- One sample was reported to have the enteric virus, reovirus, at a concentration of 5.2 MPN/4 g. Enteric viruses were not detected in the other eleven samples.

Lime Stabilization

Sixteen Class B biosolids samples that were analyzed for pathogen content were from generators that use lime stabilization.

- Ten samples contained detectable concentrations of fecal coliforms (geometric mean: 9 MPN/g, high 400 MPN/g, and low < 0.63 MPN/g);
- One sample contained detectable concentration of *Salmonella* (5.1 MPN/4 g);
- All *Ascaris* were reported as < 1 ova/4 g;
- Six samples contained detectable concentrations of somatic coliphages (geometric mean: 16 PFU/4 g, high 1,450 PFU/ 4 g, and low < 3.64 PFU/ 4 g); and
- Enteric viruses were not detected in any samples.

Pasteurization/Digestion

One Class B biosolids sample that was analyzed for pathogen content was from a generator that uses pasteurization/digestion. The detectable concentration of fecal coliforms was 7,120 MPN/g. *Salmonella*, *Ascaris*, somatic coliphages, and enteric viruses were reported as not detected.

Thermal Hydrolysis

One Class A biosolids sample that was analyzed for pathogen content was from a generator that uses thermal hydrolysis. The fecal coliforms content was 2,180 MNP/g, and *Salmonella Ascaris*, somantic coliphages, and enteric viruses content were reported as not detected.

Calculated Risk of Infection From Aerosol Exposure to Pathogens in Class B Biosolids

VDH used QMRA to calculate the risk of infection from aerosol exposure using the pathogen concentrations determined by University of Arizona and USDA. In many situations it is impossible to eliminate all risk, so the level of risk that is acceptable must be determined. Based on current practice the acceptable limit of risk according to the USDA regarding infection risk from biosolids exposure is 1 in 10,000, or 10^{-4} (J. Brooks, personal communication, September 16, 2019).

Ascaris

Ascaris was not detected in any of the samples, which is consistent with the fact that human infection by this organism is now rare, and it is difficult to find *Ascaris* ova even in untreated sewage. Since *Ascaris* was not detected in any biosolids samples, risk calculations were not done for this organism.

Salmonella

Salmonella was detected in only 15 out of 39 samples. The analysis of risk of infection by *Salmonella* was complicated by one sample with a *Salmonella* count 386 times higher than the next highest sample. Infection risk exceeded the acceptable limit of 1 in 10,000 for the highest sample at distances of 6.5 feet and 200 feet (see **Table 3**). All other samples fell below the acceptable risk limit at all ranges. For the sample with the highest *Salmonella* count, the risk of infection from aerosol exposure was 1.90×10^{-4} at a distance of 200 feet, which exceeds the acceptable risk level, but fell to 1.14×10^{-6} at 400 feet. Risk modeling was based upon experiments in the literature (McCullough & Eisele, 1951).

Table 3. Risk of Infection from Aerosol Exposure, Salmonella

	MPN/4 g dry weight	Risk of Infection at Different Distances		
		6.5 feet	200 feet	400 feet
Minimum	0.730	8.55×10^{-7}	6.04×10^{-9}	3.61×10^{-11}
Maximum	23000	**2.64×10^{-2}**	**1.90×10^{-4}**	1.14×10^{-6}
Geometric Mean	3.30	3.87×10^{-6}	2.73×10^{-8}	1.63×10^{-10}

Values in bold exceed 1 in 10,000 risk of infection. MPN: most probable number

Adenovirus and Reovirus

Six samples out of 39 contained detectable infectious enteric viruses. All six of these were positive for reovirus, and one of the six was positive for both reovirus and adenovirus. The infection risk for aerosol exposure to these samples was calculated.

For reoviruses, the risk was calculated assuming the reovirus detected was rotavirus, a major cause of gastroenteritis in children (see **Table 4**). For the sample positive for both adenovirus and reovirus, the risk of infection by rotavirus was calculated conservatively assuming the sample contained only rotavirus, and infection risk was calculated based upon the literature (Ward, et al., 1986). For all samples positive for enteric viruses the risk of infection by rotavirus exceeded the acceptable risk limit at 6.5 feet from the application site, but it only exceeded the acceptable risk limit at 200 feet for one sample, the sample that was positive for both adenovirus and reovirus and had the highest viral content of the samples. At 400 feet the risk estimate for infection by rotavirus was below the acceptable risk limit for all samples.

Table 4. Risk of Infection from Aerosol Exposure, Rotavirus

	MPN/4 g dry weight	Risk of Infection at Different Distances		
		6.5 feet	200 feet	400 feet
Minimum	0.920	**9.45×10^{-3}**	9.85×10^{-6}	5.88×10^{-8}
Maximum	10.8	**1.03×10^{-1}**	**1.16×10^{-4}**	6.91×10^{-7}
Geometric Mean	1.16	**1.18×10^{-2}**	1.24×10^{-5}	7.39×10^{-8}

Values in bold exceed 1 in 10,000 risk of infection. MPN: most probable number

Risk for infection by adenovirus was calculated similarly, assuming that the sample with the highest content that contained both reovirus and adenovirus contained only adenovirus, and using data from the literature (Couch, Cate, Douglas, Gerone, & Knight, 1966). The detection limit was used for all other samples reported as less than. Infection risk was projected to exceed the acceptable limit at 200 feet for the sample with the highest concentration (see **Table 5**).

Table 5. Risk of Infection from Aerosol Exposure, Adenovirus

	MPN/4 g dry weight	Risk of Infection at Different Distances		
		6.5 feet	200 feet	400 feet
Minimum	0.920	**9.69×10^{-3}**	6.88×10^{-5}	4.11×10^{-7}
Maximum	10.8	**1.08×10^{-1}**	**8.07×10^{-4}**	4.82×10^{-6}
Geometric Mean	1.16	**1.21×10^{-2}**	8.63×10^{-5}	5.16×10^{-7}

Values in bold exceed 1 in 10,000 risk of infection. MPN: most probable number

Listeria

Listeria was not tested directly, but estimated based upon enterococcus counts determined by USDA, and risk estimates carried out based upon literature values (Golnazarian, Donnelly, Pintauro, & Howard, 1989). Only eight samples were tested for enterococcus, and only five of those had detectable counts. Based on previous research a ratio of 948 enterococci per 1 *Listeria* bacterium was used to estimate the *Listeria* content of the biosolids samples (Pepper, Brooks, Sinclair, Gurian, & Gerba, 2010; Garrec, Picard-Bonnaud, & Pourcher, 2003). One sample was estimated to have a risk of infection higher than the acceptable risk limit at 200 feet, though several exceeded the range at 6.5 feet (see **Table 6**). *Listeria* counts are estimated based upon enterococci counts for a small number of samples using a ratio from the literature that has not been validated with Virginia biosolids, so they are a preliminary estimate only.

Table 6. Estimated Risk of Infection from Aerosol Exposure, Listeria

	Estimated MPN/4 g	Risk of Infection at Different Distances		
		0-6.5 ft	200 ft	400 ft
Minimum	0.0100	4.87×10^{-6}	3.44×10^{-8}	2.05×10^{-10}
Maximum	52.7	**4.68×10^{-2}**	**3.43×10^{-4}**	2.05×10^{-6}
Geometric Mean	0.300	**2.74×10^{-4}**	1.94×10^{-6}	1.16×10^{-8}

Values in bold exceed 1 in 10,000 risk of infection. MPN: most probable number

Epidemiological Survey Results

The top five counties with the most responses to the survey were Culpeper (21), Goochland (12), Augusta (11), Rockbridge (8), and Orange (7). The top five counties with the most land application by acres applied were Culpeper (4,475 acres), Essex (3,141 acres), Westmoreland (3,066 acres), Louisa (2,645 acres), and Caroline (2,524 acres). The top five counties receiving the most invitations to participate in the survey were Hanover (308), Spotsylvania (196), Caroline (190), Louisa (176), and Culpeper/Fauquier (150 each). The survey response rate was less than 3%. A health study conducted by Khuder *et.al* had a higher response rate; however, they mailed selected participants invitation up to three times to take the survey. Their response rate was 50% after the third mailing and 42% after second mailing to houses in the exposed group. A study by Wing *et. al* employed 17 interviewer to conduct door-to-door interviews in study areas that reported recent biosolids land application. The door-to-door interview was done following a mailing notifying that a study was being conducted. The researchers reported that 74% of invited residents agreed to participate.

Demographics of Participants Living within 200 Feet of a Field Where Biosolids Were Applied

VDH identified nine surveys from participants that lived less than 200 feet from a field where Class B biosolids were confirmed to be land applied during the study period. The participants completing the survey were five males, three females, and one preferring not to answer. The average age of the participants were 59 years with the youngest and oldest age reported as 35 and 81 years, respectively. The majority of the participants (8) were white with one preferring not to answer. Eight reported their ethnicity as non-Hispanic with one preferring not to answer. The top response for primary occupation included four choosing other or preferring not to answer

followed by two selecting healthcare and social services. Seven out of the nine participants reported attending college or having completed a degree from a college or university.

Eight participants reported living in their current residence for more than three years with three reporting living in their current residence for more than 20 years. The majority of participants (8) owned their home and all listed private water as the home's source of potable water. Only one participant reported living with one or more children under the age of 18 and two participants reported living with someone over the age of 65. All but three participants reported living with at least one other person.

Summary of Health Responses for the Participants Living within 200 Feet of Land Applied Class B Biosolids

Participants were asked to describe their health as very good, good, fair, bad, or very bad. One participant reported their health as fair and the others reported their health as good or very good. When asked if they had a chronic health condition, three answered yes and when asked about smoking history, four of the participants responded that they previously smoked. Survey participants were asked to select any chronic health conditions they had. Participants reported the following chronic health conditions: cancer (1) hypertension (1), asthma (2) and gastrointestinal illness (1).

VDH asked participants about illnesses or symptoms requiring medical care in the past 2 weeks, as well as the symptoms. Two participants completing the survey reported having illness or symptoms in the past two weeks. One participant reported having excessive cough, runny nose, difficulty breathing, and a headache. The same participant sought medical care and was diagnosed with allergies. The second participant reported having a runny nose and sore throat but did not seek medical care.

Summary of Health Responses for Household Members Living within 200 Feet of Land Applied Class B Biosolids

Participants were asked if members of the household living with them had a chronic health condition. Three reported that household members had chronic health conditions, three reported no, and three did not provide an answer. The following household member chronic health conditions were reported: arthritis (1), chronic obstructive pulmonary disease (1), diabetes (2), heart disease (1), and allergies (1). When asked if any household members were sick or had symptoms of illness three reported yes, three reported no and three did not provide an answer. The three symptoms provided for household members were shortness of breath, croup, and sneezing. Two of the three with symptoms or illnesses sought medical care. One was diagnosed with upper respiratory infection, and the other was diagnosed with allergies from the smell.

Demographics of Participants Living 200 to 1,000 Feet of a Field Where Biosolids Were Applied

VDH identified 59 surveys from participants that lived 200 to 1,000 feet from a field where Class B biosolids were confirmed to be land applied during the study period. The participants completing the survey were 33 males and 26 females. The average age of the 59 participants was 59 years with the youngest and oldest age reported as 30 and 86 years, respectively. The

majority of the participants (53) were white with two blacks and four preferring not to answer. Fifty-two reported their ethnicity as non-Hispanic and seven preferred not to answer. The top response for primary occupation included 25 choosing "other" or "prefer not to answer" as their response. This was followed by eight selecting healthcare and social services and six selecting agriculture, fishing, hunting, and mining as their primary occupation. When asked about the highest level of education, six reported having a high school degree or equivalent, and one preferred not to answer. The remaining participants reported education beyond high school including attending college or having completed a degree from a college or university.

Fifty-six participants reported living in their current residence for more than three years of which 22 reported living in their current residence for more than 20 years; and three reporting living in their current residence for less than one year. The majority of participants (57) owned their home and 55p participants listed private water as the home's source of potable water. Fourteen participant reported living with one or more children under the age of 18 and 22 participants reported living with someone over the age of 65. All but four participants reported living with at least one other person.

Summary of Health Responses for the Participants Living 200 to 1,000 Feet of Land Applied Class B Biosolids

Participants were asked to describe their health as very good, good, fair, bad, or very bad. Two participant reported their health as bad and eight reported their health as fair. The rest reported their health as good or very good. When asked if they had a chronic health condition, 15 answered yes and one preferred not to answer. Forty participants responded that they never smoked, four responded they currently smoke, 14 previously smoked, and one preferred not to answer. Survey participants were asked to select any chronic health conditions they had. When asked about chronic illnesses 15 participants reported the following chronic illnesses: arthritis (4), asthma (3), cancer (2), chronic obstructive pulmonary disease (1), diabetes (1), gastrointestinal illness (3), hypertension (4), anxiety (3), and others (6) reported as mastocytosis IBS, fibromyalgia, multiple sclerosis, charcot-marie-tooth disease, hypothyroidism, celiac disease, asbestosis, and seasonal allergies.

VDH asked participants about illnesses or symptoms requiring medical care in the past 2 weeks, as well as the symptoms. Eighteen participants completing the survey reported having illness or symptoms in the past two weeks. Participants could chose more than one illness or symptom. Illnesses and symptoms reported: excessive cough (8), runny nose, congestion (12), sore throat (8), difficulty breathing (5), loss of appetite (1), nausea (3), headache (7), fever of chills (2), itchy or burning of the eyes, nose, or throat (9), Three participants' symptoms or illnesses were severe enough for them to seek medical care. The medical provider's diagnosis was: infection due to asthma induced by allergies (1), chronic obstructive pulmonary disease (1), and common cold or upper respiratory infection (1).

Summary of Health Responses for Household Members Living within 200 to 1,000 feet of Land Applied Class B Biosolids

Participants were asked if members of the household living with them had a chronic health condition. Fifteen reported that household members had chronic health conditions, 39 reported no, and five did not answer. The following household member chronic health conditions were

reported: arthritis (6), asthma (3), cancer (1), chronic bronchitis (1), chronic obstructive pulmonary disease (2), diabetes (2), gastrointestinal illness (2), heart disease (1), hypertension (4), mental health (3), and four others reported as cardiac arrhythmia (1), and Henoch-Schönlein purpura (1). When asked if any household members were sick or had symptoms of illness in the past two weeks, thirteen reported yes, 41 reported no, and five did not answer. Three of the 13 that experienced illness or symptoms sought medical care. The diagnosis reported by those seeking medical care was one common cold or upper respiratory infection, once Folliculitis (rash comes and goes every four years), and one strep throat.

Quality of Life Responses from Participants Living within 200 Feet from Land Applied Class B Biosolids

To understand how Class B biosolids land application impacts quality of life VDH asked questions about odor, change in daily activity, and perception of biosolids. Participants were asked about odor. Six reported detecting an odor in the past two weeks. When asked about the level of odor three of the six described the level as strong and the other three described it as moderate. The description of the odor included "Aoue [sic] milk, very pungent, rotten, like biosolids, and like manure." Five participants responded that the smell caused them to change their daily activity. Daily activities reported to be impacted: letting children play out doors (2), open house or car windows (4), host outdoor gatherings (5), dry laundry outdoors (2), walk outdoors (4), exercise outdoors (4), garden or work outdoors (4), cook and eat outdoors (4), and sit outside (5). The five participants that reported that their daily activity was impacted also answered how long outdoor activities were impacted. The most and least number of days outdoor activities were affected was ten and five, respectively, with one participant not providing a response.

VDH asked the participants if they perceived biosolids as harmful to health and three responded yes, two responded no, three responded I don't know, and one did not provide an answer. Participants were asked to rank their knowledge of biosolids and biosolids application with one being no knowledge and 100 being extensive knowledge. The average ranking was 46 and the lowest and highest ranking reported was 15 and 80, respectively. Lastly, VDH asked if a setback distance had ever been requested and eight responded no and one did not answer the question.

Quality of Life Responses from Participants Living 200 to 1,000 Feet from Land Applied Class B Biosolids

To understand how Class B biosolids land application impacts quality of life VDH asked questions about odor, change in daily activity, and perception of biosolids. Participants were asked about odor. Thirty-nine reported detecting an odor in the past two weeks. When asked about the level of odor 20 of the 39 described the level as strong, 12 described it as moderate, and 7 described it as faint. Thirty-six participants provided a description of the odor. The descriptions were all interpreted as negative except possibly one or two. The descriptions included earthy, like a sewer, nothing compared to hog or turkey litter, chemical in nature, horribly unpleasant, normal for sludge, dry and musty, sulfurous, nasty, and awful. Twenty-seven participants responded that the smell caused them to change their daily activity. Daily activities reported to be impacted: letting children play out doors (9), open house or car windows (26), host outdoor gatherings (18), dry laundry outdoors (9), walk outdoors (20), exercise outdoors (17), garden or work outdoors (18), cook and eat outdoors (19), and sit outside (23).

Other activities impacted that were provided by the participants were Easter egg hunt, swimming, writing on porch, and fishing. The average number of days that 20 of the 27 participants whose activities were impacted was 15 and the least and most number of days provided was three and 99, respectively. Six participants did not provide number of days activities were impacted by land application of biosolids.

VDH asked the participants if they perceived biosolids as harmful to health and 23 responded yes, 22 responded no, and 14 responded I don't know. Participants were asked to rank their knowledge of biosolids and biosolids application with 1 being no knowledge and 100 being extensive knowledge. The average ranking was 52 and the lowest and highest ranking reported was one and 100, respectively. Lastly, VDH asked if a setback distance had ever been requested and three responded yes, 55 responded no, and one did not answer the question.

CONCLUSIONS

The concentrations of pathogens and indicators found in Class B biosolids generated by lime stabilization was less than concentrations found in aerobic or anaerobically digested generated Class B biosolids.

Quantitative microbial risk assessment calculations showed that at 6.5 feet from the application site, the greatest risk of aerosol infection was from enteric viruses, rotavirus and adenovirus.

Quantitative microbial risk assessment calculations showed that at 200 feet from the application site only the sample with the maximum concentration of each pathogen exceeded a 1 in 10,000 aerosol risk of infection.

Quantitative microbial risk assessment calculations showed that at 400 feet from the application site none of sample concentrations of pathogen exceeded a 1 in 100,000 aerosol risk of infection.

The response rate to the epidemiological study was low compared to biosolids health studies from other researchers.

The majority of participants living within 200 feet and between 200 and 1,000 feet of where biosolids were land applied reported being able to detect odors and that land application impacted their quality of life.

The number of participants living within 200 feet and between 200 and 1,000 feet of where biosolids were land applied that reported as having health symptoms or family members having health symptoms in the past two weeks was more than 20%. However, more responses would be needed to determine the statistical significance.

RECOMMENDATIONS

The pathogen study shows that future epidemiological studies designed to assess illnesses onset by exposure to pathogens should be done at residences living within 400 feet of Class B biosolids application sites that were generated by aerobic or anaerobic digestion. This is based on the fecal coliforms analysis results, which show lime stabilization to be more effective. Also, there was no elevated calculated risk of aerosol infection at 400 feet.

If future epidemiological studies are designed to evaluate odor and its effects on health and impact on quality of life then all land applications sites regardless of the methods used to generate Class B biosolids should be included in such study. Such study should also include surveying residents living greater than 1,000 feet from land where Class B biosolids are applied.

Repeat survey mailings to participants or door-to-door interviews are necessary to increase survey participation. A larger response rate is necessary to assess the impact of Class B biosolids on health.

Generators of aerobic and anaerobically digested biosolids should report pathogen content of Class B biosolids to the Department of Environmental Quality until biosolids generated by facilities using these methodology can meet the pathogen content found in Class B biosolids generated by lime stabilization methods.

The Department of Environmental Quality should increase buffers between occupied dwellings and biosolids land application sites to 400 feet. These finding should be shared with the Department of Labor and Industry and the Department of Environmental Quality.

To conduct a more thorough follow-up health study the Virginia Department of Health would need to employ a minimum of two 40 hours/week contract staff in the central office during periods of land application, an assigned vehicle, student interns or staff at local health departments, funds to cover the cost of printing and mailing, and funds to cover overnight in-state travel lodging and per diem for a minimum of 30 trips. The estimated minimum cost is $250,000.

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