Sayers, Margery

From: John Tegeris <johntegeris@gmail.com>
Sent: Sunday, October 29, 2017 10:31 PM
To: CouncilMail; Kittleman, Allan; Lazdins, Valdis; Gowan, Amy; Peter Jensen; pwood@baltsun.com; mdzwonchyk@baltsun.com; aburnett@wjz.com; Kim Dacey; srorman@sbgtv.com; bzumer@sbgtv.com; ambarnett@sbgtv.com; Ted Mariani; Rick Lober/Annette; Brent Loveless; Stu Kohn; Rob Long; Preserve Dayton; Velculescu Victor; Jeff Harp; Jeff Harp; Luv of My Life; andrew.green@baltsun.com; John Tegeris
Cc: Tim Mayer; Mike Navarre/Lynn; Bethany Hooper; Rob Bundy; Dan O'Leary; Andrew Royle; Lisa Markovitz; Susan Garber; Carol Jane Gray; Ocheltree Janet; Erin Allen; John Allen; Al Risdorfer; Bono Tony V; Paul Morris; Paul Retzbach; Colleen Retzbach; Kristin Robertson; Lora Houck; Trip Kloser; Craig Ostrom; Julius Tunji Akintade; Chelakara Shankar; James Nickel; Banwarth Dave; dave.kromer@tunnellgov.com; Sylvie Leary; Alan Schneider; Paul Shoffeitt; Mike Bucci; Robert Scales; China Williams; Katie Hester; Mike; Patricia Soffen; Joanne Heckman; <darbus37@gmail.com>; Jennifer Bush; SHARON KEENY; tilycog@comcast.net; cathydatz@yahoo.com; Richard Valentine; Belkacem Manseur; Alex Xu; Richard Taber; Phil Montag; <bstrickland@wtplaw.com>; ST Balimtas; Michael Burns; Paul Retzbach; <fernandesgj@washpost.com>; Eric Goldberg; <benabili@hotmail.com>; Rob Bovello; Paul Robertson; Michael Price; Doug Lee; Jay and Santa Bhalani; Ajay soodan; jmathew@acidd.us; Om Prakash Gupta; <jthensel61 @gmail.com>; Benjamin Lee; <joelhouck66@yahoo.com>; Ty Shrader; sdwerlinich@aol.com; Williams; Z Zhang; Brian Lehman; Lisa Valentine; Denise Howze; Hafida Manseur; Ning Hu; dianawscales@gmail.com; Richard and Susan Taber; Marisa Montag; <estrickland@offitkurman.com>; Robin Balimtas; Kathy Burns; Home; Dahna Goldberg; Michelle Meney; <jmbovello@comcast.net>; Delia Velculescu; Annette Lober; rajput31@yahoo.com; Melissa and Larry Kramer; Jyoti Gupta; <s.hensel@live.com>; Carol Werlinich; Mirra Morris; Sally Ostrom; Karen K; Laurie Lehman; kf321jump@verizon.net
Subject: CB60: School Buses and Tractor-Trailers DO NOT MIX. Kill the Bill, Not Our Children.

I personally remain astounded that even though we brought to light the two children that were struck and killed by a tractor trailer carrying 75,000 pounds of mulch while trying to board their school bus (article below), this real and terrifying risk has gone completely unacknowledged by County Executive Kittleman and by CB60 authors Sigaty and Fox. This is more than dismissive, it is downright negligent. Our concern on this matter has not been addressed by the County Executive, not by the Planning Board and not by certain members of the County Council.

The fact that no charges were filed against the truck driver, and no equipment violations were found that would have impacted the truck's ability to stop or to avoid the crash, highlights why this is a real danger. I'm reminded of what Bob Orndorff likes to tell me: "I follow the rules." All of this just furthers the point that nothing out of the ordinary has to happen for our children to lose their lives if these types of industrial actives and these types of industrial trucks are allowed on our rural roads. Simply put, school buses and tractor-trailer trucks DO NOT MIX.

We've been at this for over three years now, and I'm betting that there are certain members of the County Council who hope we'll tire of the battle and go away, but the issue highlighted in the article below tells exactly why we will NOT go away, and why we will not stop the fight until reasonable measures are in place to protect our children. This will become an election issue; I will personally see to it. Especially on issues of our children's safety, there is no gray zone, and there can be no compromise. CB60 needs major amendments, or better yet, the bill needs to be voted down.

County Executive Kittleman says CB60 will not allow for industrial mulching. Hogwash. It does and it will. Whether there are 2 acres, 5 acres or 10 acres of industrial mulching and composting throughout all RR and RC in Howard County, the

ability to truck in these materials, go through industrial processing to truck out for commercial sale, and the ability to do so with tractor-trailer/3-axle trucks, means these types of activities are limitless. Every day. Not farming. Never ending.

So we urge you again to email the architects behind CB60, County Executive Kittleman and authors Council Members Sigaty and Fox, to ask them how they justify these activities as "not industrial," and why they are willing to allow these large trucks on rural roads when our children are waiting by the roadside to board school buses every day. If you care about the safety of your children, tell the architects who own this bill that CB60 is a recipe for disaster.

And none of this is new. We all warned of this before successful passage of CB20. Here is a reference from CB20 testimony (excerpted from the Baltimore Sun article) given to the County Council in May 2014 that speaks to these real fears:

Kristin Robertson, a Dayton resident and PTA president at Dayton Oaks Elementary School, said she was concerned for the safety of children waiting for school buses along country roads. With increased truck traffic, she said, an accident *"is just a matter of time."*

Please email Council Executive Kittleman (akittleman@howardcountymd.gov) and the County Council (councilmail@howardcountymd.gov) to call for major amendments or to eliminate CB60 altogether.

Thank you for distributing to your network.

John Tegeris, PhD
President, DRPS

"CB60: Don't Defend It, Amend It!"
Your voice and your vote matter. Elections are drawing near.

http://www.nydailynews.com/news/national/tractor-trailer-carrying-mulch-kills-young-virginia-children-article-1.3014204

Tractor-trailer carrying 75,000 pounds of mulch strikes, kills two children running to meet school bus in Virginia

A tractor-trailer carrying 75,000 pounds of mulch struck and killed two young children who ran into the road to meet their school bus in Virginia.

Six kids were standing at the bus stop in Dillwyn early Thursday morning when two of them saw their bus approaching and ran across the northbound lanes.

Tori Perez, 5, was killed by a tractor trailer.

(WRIC)

The tractor-trailer was traveling northbound at the time, according to State Police Spokeswoman Corinne Geller.

The driver was coming down a hill and tried to stop, but struck 5-year-old Tori Perez and 6-year-old Jaiden Bartee.

Tori's mother Sabrina Green told the Richmond-Times Dispatch that she saw her child lying on the side of the road after hearing the accident and rushing to the scene. Jaiden was under the truck.

The two children were cousins.

Investigators look over a tractor-trailer that was involved in a tragic accident in Virginia early Thursday.

(Brian DeVasher/AP)

Tori's great aunt Barbara Rose told the newspaper she was a "joyful little child." Tori enjoyed "SpongeBob SquarePants" and "Frozen," Rose told the newspaper.

The school bus had its yellow lights flashing but hadn't come to a complete stop when the children ran across the road, according to Geller.

The bus driver "frantically began motioning to the children to stop and get back off the side of the road," Geller said.

Sabrina Green (c.), mother of 5-year-old Tori Perez, is comforted by neighbors and friends.

(Steve Helber/AP)

No charges are expected against the truck driver, who is 66.

Buckingham County Public Schools Superintendent Cecil Snead said grief counseling will be available to help students cope with what took place.

The spot in Dillwyn, Va., where two children were killed.

(Steve Helber/AP)

"All I'm asking is everyone keep Buckingham in your prayers in the upcoming days," he said.

With News Wire Services

Sayers, Margery

From: John Tegeris <johntegeris@gmail.com>
Sent: Sunday, October 29, 2017 12:04 AM
To: CouncilMail; Kittleman, Allan; Lazdins, Valdis; Gowan, Amy; Peter Jensen; pwood@baltsun.com; mdzwonchyk@baltsun.com; aburnett@wjz.com; Kim Dacey; srorman@sbgtv.com; bzumer@sbgtv.com; ambarnett@sbgtv.com; Ted Mariani; Rick Lober/Annette; Brent Loveless; Stu Kohn; Rob Long; Preserve Dayton; Velculescu Victor; Jeff Harp; Jeff Harp; Luv of My Life; andrew.green@baltsun.com; John Tegeris
Cc: Tim Mayer; Mike Navarre/Lynn; Bethany Hooper; Rob Bundy; Dan O'Leary; Andrew Royle; Lisa Markovitz; Susan Garber; Carol Jane Gray; Ocheltree Janet; Erin Allen; John Allen; Al Risdorfer; Bono Tony V; Paul Morris; Paul Retzbach; Colleen Retzbach; Kristin Robertson; Lora Houck; Trip Kloser; Craig Ostrom; Julius Tunji Akintade; Chelakara Shankar; James Nickel; Banwarth Dave; dave.kromer@tunnellgov.com; Sylvie Leary; Alan Schneider; Paul Shoffeitt; Mike Bucci; Robert Scales; China Williams; Katie Hester; Mike; Patricia Soffen; Joanne Heckman; <darbus37@gmail.com>; Jennifer Bush; SHARON KEENY; tilycog@comcast.net; cathydatz@yahoo.com; Richard Valentine; Belkacem Manseur; Alex Xu; Richard Taber; Phil Montag; <bstrickland@wtplaw.com>; ST Balimtas; Michael Burns; Paul Retzbach; <fernandesgj@washpost.com>; Eric Goldberg; <benabili@hotmail.com>; Rob Bovello; Paul Robertson; Michael Price; Doug Lee; Jay and Santa Bhalani; Ajay soodan; jmathew@acidd.us; Om Prakash Gupta; <jthensel61 @gmail.com>; Benjamin Lee; <joelhouck66@yahoo.com>; Ty Shrader; sdwerlinich@aol.com; Williams; Z Zhang; Brian Lehman; Lisa Valentine; Denise Howze; Hafida Manseur; Ning Hu; dianawscales@gmail.com; Richard and Susan Taber; Marisa Montag; <estrickland@offitkurman.com>; Robin Balimtas; Kathy Burns; Home; Dahna Goldberg; Michelle Meney; <jmbovello@comcast.net>; Delia Velculescu; Annette Lober; rajput31@yahoo.com; Melissa and Larry Kramer; Jyoti Gupta; <s.hensel@live.com>; Carol Werlinich; Mirra Morris; Sally Ostrom; Karen K; Laurie Lehman
Subject: Replacing CB20 with CB60: A Bad Deal for Howard County

Please read the article below, which appeared in the Baltimore Sun back in 2014, to be reminded that we've had the same health and safety concerns going on for 4 years now. Back then, Council Member Fox sponsored the bill that became CB20 that was passed to prohibit any industrial mulch/composting on either Howard County or MD ag preserve farmland. County Executive Kittleman, then running for his current office, was a strong supporter of what we fought hard to accomplish with the passage of CB20.

Since then, the fight has gotten harder. Council Members Fox and Sigaty, who voted for CB20, and County Executive Kittleman, have turned against us. Bottom line, CB60 will pose more health and safety risks than ever before. Also since then, DPZ has proven ineffective at enforcing CB20 zoning regulations, even with the most blatant violators of those regulations.

It is a shame that we find ourselves in a harder fight, and an unfair one, that has exhausted the communities working hard to oppose industrial mulching and composting on Howard County farmland. Unfair because this trio, along with DPZ that is unable or unwilling to enforce zoning regulations that would otherwise protect our families, has put forth a poor bill in CB60 that they claim incorrectly will not allow for industrial processing, and has been completely dismissive over our concerns for health and safety risks.

We already won this fight with passage of CB20, so why must we fight this all over again now?

It is also unfair to ask communities to fight to protect their families, when we have elected these officials into office, our Howard County leadership, to secure these protections on our behalf.

CB60 will allow for industrial mulching and composting (with food waste) given it is NOT tied to farming activities, and the mulching/composting proposed is NOT on/for/by the farm, but allows trucking off for commercial sale.

It is shameful that County Executive Kittleman, who owns CB60, and Council Members Sigaty and Fox who authored it, have placed such a burden on all of us to once again fight the same issue, but this time for even riskier legislation. They tried to sneak CB60 by us in late June and to get this passed within a month, but thankfully with your support to rise up in force/numbers we were able to get the vote tabled. In the end, unless the delay has given Council Chair Weinstein ample time to hear our concerns and call for a tougher bill through addition of major amendments to protect our families, it will all be for very little.

Please email Council Executive Kittleman (akittleman@howardcountymd.gov) and the County Council (councilmail@howardcountymd.gov) to call for major amendments to be added to CB60 that afford us the same protections for health and safety we fought hard to win together with passage of CB20 in 2014.

Thank you for distributing to your network.

John Tegeris, PhD
President, DRPS

"CB60: Don't Defend It, Amend It!"
Your voice and your vote matter. Elections are drawing near.

http://www.baltimoresun.com/news/maryland/howard/lisbon-fulton/ph-mulching-debate-story.html

In Howard mulching debate, council hears passion from both sides

Amanda Yeager, ayeager@tribune.com

May 20, 2014

Residents of rural western Howard County packed the County Council's chambers Monday night for more than three hours of testimony focused on whether large-scale mulching, firewood processing and soil processing operations are appropriate on land under agricultural preservation easement.

Wearing buttons that read "No Industry! Keep It Farm" – the "dust" in industry jumping out in bold, red letters – members of the Dayton Rural Preservation Society, formed earlier this year to fight what they see as dangerous changes to their community if these operations are allowed, offered impassioned speeches, often followed by standing ovations and cheers from the crowd.

The community group includes local farmers and non-farmers, who cite concerns about heavy truck traffic, noise and health and environmental impacts should mulching take root.

Equally impassioned were a group of farmers, many whose families have farmed for several generations, who argued against a rollback of the new regulations, which they say give them the flexibility they need to make a living. Though smaller in number – there were about two dozen visibly opposed to changing the regulations – their supporters included leaders of some local agricultural organizations.

Bob Orndorff, whose conditional use requests for mulching facilities in Sykesville and Dayton sparked the current controversy, accused DRPS members of "smear tactics" and "inflaming the public," and asked the council for "fundamental fairness."

"There's no doubt our proposal has fueled emotion and bitter debate, but I want to make it clear we followed the law," he said.

The two bills before the council would revise new regulations, added during last summer's comprehensive zoning process, that allow sawmills, bulk firewood, mulch manufacture, composting facilities and soil processing as conditional uses on land under agricultural preservation, a program by which the state or county buys development rights from a farm's owner to keep the land rural in perpetuity.

The regulations also allowed farm wineries on preserved land for the first time, but that addition has not been controversial.

One of the bills was introduced by Council member Greg Fox, a Republican who represents the west county, and would largely change the agricultural preservation regulations back to the way they were pre-comprehensive zoning. "Natural wood waste recycling" facilities – defined as a commercial facility that processes stumps, branches, leaves and the like into raw material or product – would be allowed as a conditional use, but capped at a maximum of 2 percent of the property or 1 acre.

Council members Courtney Watson and Mary Kay Sigaty have co-sponsored Fox's bill.

The other bill, introduced by the DRPS, is similar to Fox's but would remove natural wood waste recycling as a conditional use altogether.

Both bills would allow natural wood waste recycling as a permitted use in the light manufacturing zoning district and would permit composting facilities as a matter of right in heavy manufacturing zones with a solid waste overlay.

DRPS members and some other residents said mulching was an industrial-scale process that didn't belong in a rural setting.

"We advocate for legitimate farming," DRPS President John Tegeris said. "What we strongly oppose is natural wood waste recycling of any kind on ag pres or RC land. This belongs in [an industrial zone]. It does not belong in our agricultural or rural neighborhoods."

Many residents cited potential health risks of mulching as a main concern.

Victor Velculescu, an oncologist at Johns Hopkins, said the medical literature he had reviewed showed increased exposure to fungi and carcinogenic wood dust particles in areas near industrial-level wood processing facilities.

"These are not theoretical risks," he told the council. A mulching facility, he added, "would make Dayton a petri dish for health experimentation."

Leslie Long, who lives in Woodbine near an existing mulching facility, said she and her husband had developed sinusitis since mulching operations began. Her horses, she said, had other health problems, such as nasal tumors and conjunctivitis.

"My neighbors say, 'I just want it to stop, they're killing me,'" she said.

Kristin Robertson, a Dayton resident and PTA president at Dayton Oaks Elementary School, said she was concerned for the safety of children waiting for school buses along country roads. With increased truck traffic, she said, an accident "is just a matter of time."

Mark Martin, a Woodbine resident whose family has farmed in Howard County since 1956 and owns a farm in agricultural preservation, said he was also concerned about truck traffic.

He said he didn't consider mulching facilities to be a true agricultural operation. "I maintain that these mulching operations are land-clearing operations," he said.

Opponents of the bills rejected that notion.

"Agriculture is an industry – it's one of the largest in the country," said Mark Mullinix, who owns a farm in Dayton. He and his family "have been in agriculture all of our lives, and these things were put in place to help landowners in general be more profitable and stay in this county."

Mullinix, whose farm is under an agricultural preservation easement, made news in 2012 for being the first farmer to seek a termination of the easement from the state. His requests to opt out have been denied.

Dayton farmer Leslie Bauer said she thought the DRPS's protests against large-scale mulching would be a "stepping stone" for future efforts. "Today, this group is trying to stop mulching production, but what agricultural enterprise will they be trying to stop tomorrow?"

Bauer's property abuts the site of the proposed mulching operation in Dayton. She said in January that she was working with Orndorff to pave an access driveway for the site through her property.

"We have had the struggles and conflicts of dealing with our more urban neighbors while trying to run the farm," she told the council.

Mike Clark, another local farmer, echoed Bauer.

"Where do you draw the line between what is industrial activity and what's not?" he said. "Our primary purpose in life is not to provide a Norman Rockwell landscape for you to gaze upon from your affluent communities."

But Dayton resident Julie Brookman said the characterization of DRPS supporters as wealthy neighbors unwilling to accept the realities of agricultural life was unfair.

"I would say probably 80 percent [of Dayton homes] are very modest homes, older homes," she said. "We are not a bunch of rich folk trying to push our weight around."

Howie Feaga, who is president of the of the Howard County Farm Bureau, said mulching, firewood processing and other operations were just another way for farmers to support themselves.

"Just like IBM, Google and Apple, we are always changing and evolving," he said. "We compete in a slim-margin business ... Those outside of agriculture have the right to do better in their field of work; why, then, would we not be allowed to do better?"

Feaga pointed out that mulching facilities would be monitored by the Maryland Department of the Environment, and said he supported 10 percent as a fair cap on the amount of land able to be used for mulching, which is about the amount that had been asked for in the Dayton conditional use request.

Lynn Moore, chair of the county's Agricultural Preservation Board, said the goal of adding extra conditional uses to preserved land during comprehensive zoning was that "we were trying to make sure the needs of the farming community were met." She said the council needed to iron out details, such as whether equipment will be part of the measurements toward any caps on uses.

"As the farms diversify and specialize, they don't always have what they need," she said, which is why she felt it was necessary to allow trucking of certain materials – such as logs or soil – to and from farms.

"It's going to be hard to address the concerns of the neighbors and the needs of the farmers and make sure they all meld together, but I think we need to come up with some kind of plan that will do that," she added.

The council is scheduled to vote on both bills on June 2. If the legislation is tabled, it could not be taken up again until after the general election in November, because of a rule that prohibits the council from voting on zoning matters between the primary and general elections.

Sayers, Margery

From: David Smith <dosmith99@gmail.com>
Sent: Saturday, October 28, 2017 8:30 PM
To: CouncilMail
Subject: Re: Work session today

Please make an effort to hear Dr. Velculescu's testimony. I am very scared for my family that CB60 is a horrible for the health and wellness of my kids.

On Oct 16, 2017 6:40 PM, "David Smith" <dosmith99@gmail.com> wrote:

> Council Members,
>
> Today's work session was the biggest, most corrupt display of leadership I have ever witnessed in my life. You all should be ashamed of yourselves. Last minute change on the meeting so the Hopkins PHD expert can't talk about the health concerns. I can't believe you can sleep at night knowing what you're doing - undermining Howard County residents to support special interests. How in your right mind do you think that industrial mulching and composting right next to families is "forward thinking"? I can't believe I am going to have to move out of my home so my kids don't have to breath wood dust filled air and drink contaminated water. It is so baffling to me. You're either purposefully jeopardizing the health and wellness of residents or your just naive and stupid to see what is going on here.
>
> I seriously hope you end this and voting NO to CB60. Maybe then you'll be able to sleep well at night..
>
> David Smith
> Dayton, MD Resident

Sayers, Margery

From: John Tegeris <johntegeris@gmail.com>
Sent: Saturday, October 28, 2017 12:15 AM
To: CouncilMail; Kittleman, Allan; Lazdins, Valdis; Gowan, Amy; Peter Jensen; pwood@baltsun.com; mdzwonchyk@baltsun.com; aburnett@wjz.com; Kim Dacey; srorman@sbgtv.com; bzumer@sbgtv.com; ambarnett@sbgtv.com; Ted Mariani; Rick Lober/Annette; Brent Loveless; Stu Kohn; Rob Long; Preserve Dayton; Velculescu Victor; Jeff Harp; Jeff Harp; Luv of My Life; andrew.green@baltsun.com; John Tegeris
Cc: Tim Mayer; Mike Navarre/Lynn; Bethany Hooper; Rob Bundy; Dan O'Leary; Andrew Royle; Lisa Markovitz; Susan Garber; Carol Jane Gray; Ocheltree Janet; Erin Allen; John Allen; Al Risdorfer; Bono Tony V; Paul Morris; Paul Retzbach; Colleen Retzbach; Kristin Robertson; Lora Houck; Trip Kloser; Craig Ostrom; Julius Tunji Akintade; Chelakara Shankar; James Nickel; Banwarth Dave; dave.kromer@tunnellgov.com; Sylvie Leary; Alan Schneider; Paul Shoffeitt; Mike Bucci; Robert Scales; China Williams; Katie Hester; Mike; Patricia Soffen; Joanne Heckman; <darbus37@gmail.com>; Jennifer Bush; SHARON KEENY; tilycog@comcast.net; cathydatz@yahoo.com; Richard Valentine; Belkacem Manseur; Alex Xu; Richard Taber; Phil Montag; <bstrickland@wtplaw.com>; ST Balimtas; Michael Burns; Paul Retzbach; <fernandesgj@washpost.com>; Eric Goldberg; <benabili@hotmail.com>; Rob Bovello; Paul Robertson; Michael Price; Doug Lee; Jay and Santa Bhalani; Ajay soodan; jmathew@acidd.us; Om Prakash Gupta; <jthensel61 @gmail.com>; Benjamin Lee; <joelhouck66@yahoo.com>; Ty Shrader; sdwerlinich@aol.com; Williams; Z Zhang; Brian Lehman; Lisa Valentine; Denise Howze; Hafida Manseur; Ning Hu; dianawscales@gmail.com; Richard and Susan Taber; Marisa Montag; <estrickland@offitkurman.com>; Robin Balimtas; Kathy Burns; Home; Dahna Goldberg; Michelle Meney; <jmbovello@comcast.net>; Delia Velculescu; Annette Lober; rajput31@yahoo.com; Melissa and Larry Kramer; Jyoti Gupta; <s.hensel@live.com>; Carol Werlinich; Mirra Morris; Sally Ostrom; Karen K; Laurie Lehman
Subject: CB60: Dr. Velculescu, MD, PhD Oct 16 Letter to County Council; Comments on Oct 16 County Council Work Session
Attachments: Butler et al., Mulch pneumonitis, 2013.pdf; Herr et al., Bioaerosols, 2003.pdf; HHS Report on Carcinogens 14th Edition Wood Dust, 2002.pdf; Mulch and Composting Health Effects Velculescu 121414.pptx; Siddiqui, Clin Infect Dis.-2007-Siddiqui-673-81, 2007.pdf

Please read the letter below that our lead medical expert, a renowned oncologist at The Johns Hopkins Medical Center, Dr. Velculescu, MD, PhD, sent to the Count Council on October 16th to defend key elements of his testimony regarding medical risks associated with industrial mulching and composting with food waste, animal mortality and manure (Type 2 feedstock). This letter was sent given that he was unable to attend the October 16th County Council Work Session due to a long standing commitment to speak that day at a medical conference in New York City.

It is important to note that he was asked to testify before the County Council on October 11, a date he was available to do so. The session was moved to accommodate Dr. Sigaty's experts, who were available on October 16. He did make himself available, however, for the October 23rd Work Session, but certain members of the County Council chose not to invite him to testify then. We have made a request to meet with members of the County Council next week and are hopeful they will grant these meetings in advance of the November 6th legislative vote for CB60.

The experts that did, indeed, testify on October 16 to refute Dr. Velculescu's prior testimony are environmental scientists and not medical professionals. While they do possess experience in their respective area of expertise, they are not qualified to comment on the medical/health risks that industrial mulching and composting with food waste, animal mortality and manure pose to families that reside in communities nearby to these facilities. As with these two environmental experts, I also possess a doctoral degree in a related field with over 25 years of related experience (dual doctorate in Pharmacology and Toxicology, owned/operated a pharmaceutical and environmental testing laboratory, now serve as a technical expert for medical countermeasure advanced development within the Office of the Secretary at the Department of Health and Human Services). However, I am also not qualified to speak to the medical risks without a medical degree.

We continue to work diligently on your behalf to bring light to the health and safety risks that CB60 will create, a bill that County Executive Kittleman supports and apparently still contends "*will not allow for industrial mulching or composting*" despite what the proposed zoning language reflects in CB60, unless major amendments are added.

From the October 16th work session, we find the following excerpt between Dr. Ball and DPZ Deputy Director Gowan representative of why we continue to push hard for amendments that protect the health and welfare of our families given the uncertainty these medical risks pose to families that live nearby to the types of facilities CB60 will allow for.

Dr. Ball: "*You said you talked with folks in the environmental community and soil conservation district, did you talk to any medical professionals, did you have any of those folks as part of the team?*"

Ms. Gowan: "*No, and I meant environmental staff with the county, not the community, environmental staff with the county and soil conservation district staff.*"

Here is Dr. Velculescu's letter:

Dear Members of the County Council and Executive Kittleman,

I am writing let you know that I would be glad to be available for any questions to health issues related to CB60. I have let Jessica Feldmark know that I would be available on October 11, 2017, the first date offered for a Council Work Session. I will also be available at the next meeting October 23, 2017, and would be glad to make myself available on other occasions. Unfortunately, I am not available on today, Monday October 16 due to my participation at a medical meeting in New York that was scheduled many months ago.

I have seen the video testimony from the Work Session of October 2, 2017 where several erroneous points were raised by Council Member Sigaty, suggesting that the references in my presentation are from occupational risks and therefore

are not relevant. This is an incorrect conclusion by Ms. Sigaty. There are indeed many health risks posed by industrial mulch and composting facilities where the bioaerosol pollution of residential outdoor air can indeed be very similar to that of occupational environments. To clarify this issue, please see items below. I am also attaching to this email my presentation from 2014 as well as several recent publications that highlight the health dangers of industrial mulch and composting operations.

1. Wood dust is a carcinogen. This is well-established as has been indicated by many national and international organizations, including the American Cancer Society, WHO, CDC, and the Department of Health and Human Services. Importantly, wood dust is a carcinogen regardless of whether it arises from wood cutting occupations or from composting activities, as indicated in the 14th Report on Carcinogens from the US Department of Health and Human Services. Please see attached document for further information.

2. Mulching and composting have health risks due to infectious agents. My presentation from 2014 (attached) included many such examples. See slides 3, 4, 5 and 23. Please also see attached example medical references from Butler and colleagues and Siddiqui and colleagues.

3. Composting can lead to toxic and carcinogenic substances. Please see slides 21 and 22.

4. Dust from mulch and composting can lead to inflammatory effects. Please see slide 24.

5. Animal mortality and waste in composting can contaminate groundwater. Please see slide 25.

6. Composting facilities have health effects on nearby communities. Please see slides 26, 27, and 28 for examples in California, Maryland and Europe of negative outcomes from such facilities on nearby residents. Please also see attached medical reference from Herr et al.

7. Infectious agents from mulch and composting facilities can pose health risks at significant distances. See slides 31 and 32. These studies detected infectious agents at up to 1000 – 2400 feet away from site.

8. Individuals living near composting sites have exposures similar to those in high risk occupations. Please see attached article by Herr et al., describing a study performed in Germany of residents near a large-scale composting site. The authors indicate "Bioaerosol pollution of residential outdoor air can occur in concentrations found in occupational environments." They also indicate "Concentrations of culturable airborne microorganisms, including molds, measured in the residential air during the study at 150 to 320 m from the composting site were 100–1000 times higher than those concentrations generally reported as natural background concentrations."

Looking forward further discussing any of these points with any of you.

Please include this email and attached documents as part of the testimony for CB60 2017.

Best regards,

Victor

Victor E. Velculescu, M.D., Ph.D.

Professor of Oncology and Pathology

Co-Director of Cancer Biology

Sidney Kimmel Comprehensive Cancer Center

Johns Hopkins University School of Medicine

1550 Orleans St., Rm 544, Baltimore, MD 21287

Phone 410.955.7033 FAX 410.502.5742

velculescu@jhmi.edu

Sayers, Margery

From: Antoinette Graziano <jpamgraz@yahoo.com>
Sent: Friday, October 27, 2017 10:43 AM
To: CouncilMail
Subject: Opposition to CB 60

I oppose CB 60 and urge you to vote against passage. Thank you
Antoinette Graziano

Antoinette Graziano
jpamgraz@yahoo.com

Sayers, Margery

From: James Palmer <jgpalmer44@gmail.com>
Sent: Friday, October 27, 2017 10:06 AM
To: CouncilMail
Subject: CB 60 Mulch Factory

I am a 50 year resident of western Howard County and I strongly oppose the mulch factory proposed via CB 60.

James Palmer
3821 Walt Ann Dr
Ellicott City 21042

Sayers, Margery

From: John Tegeris <johntegeris@gmail.com>
Sent: Friday, October 27, 2017 2:17 AM
To: CouncilMail; Kittleman, Allan; Lazdins, Valdis; Gowan, Amy; Peter Jensen; pwood@baltsun.com; mdzwonchyk@baltsun.com; aburnett@wjz.com; Kim Dacey; srorman@sbgtv.com; bzumer@sbgtv.com; ambarnett@sbgtv.com; Ted Mariani; Rick Lober/Annette; Brent Loveless; Stu Kohn; Rob Long; Preserve Dayton; Velculescu Victor; Jeff Harp; Jeff Harp; Luv of My Life; andrew.green@baltsun.com; John Tegeris
Cc: Tim Mayer; Mike Navarre/Lynn; Bethany Hooper; Rob Bundy; Dan O'Leary; Andrew Royle; Lisa Markovitz; Susan Garber; Carol Jane Gray; Ocheltree Janet; Erin Allen; John Allen; Al Risdorfer; Bono Tony V; Paul Morris; Paul Retzbach; Colleen Retzbach; Kristin Robertson; Lora Houck; Trip Kloser; Craig Ostrom; Julius Tunji Akintade; Chelakara Shankar; James Nickel; Banwarth Dave; dave.kromer@tunnellgov.com; Sylvie Leary; Alan Schneider; Paul Shoffeitt; Mike Bucci; Robert Scales; China Williams; Katie Hester; Mike; Patricia Soffen; Joanne Heckman; <darbus37@gmail.com>; Jennifer Bush; SHARON KEENY; tilycog@comcast.net; cathydatz@yahoo.com; Richard Valentine; Belkacem Manseur; Alex Xu; Richard Taber; Phil Montag; <bstrickland@wtplaw.com>; ST Balimtas; Michael Burns; Paul Retzbach; <fernandesgj@washpost.com>; Eric Goldberg; <benabili@hotmail.com>; Rob Bovello; Paul Robertson; Michael Price; Doug Lee; Jay and Santa Bhalani; Ajay soodan; jmathew@acidd.us; Om Prakash Gupta; <jthensel61 @gmail.com>; Benjamin Lee; <joelhouck66@yahoo.com>; Ty Shrader; sdwerlinich@aol.com; Williams; Z Zhang; Brian Lehman; Lisa Valentine; Denise Howze; Hafida Manseur; Ning Hu; dianawscales@gmail.com; Richard and Susan Taber; Marisa Montag; <estrickland@offitkurman.com>; Robin Balimtas; Kathy Burns; Home; Dahna Goldberg; Michelle Meney; <jmbovello@comcast.net>; Delia Velculescu; Annette Lober; rajput31@yahoo.com; Melissa and Larry Kramer; Jyoti Gupta; <s.hensel@live.com>; Carol Werlinich; Mirra Morris; Sally Ostrom; Karen K; Laurie Lehman
Subject: Impact of Mulch Manufacturing on RR and RC in Howard County

Provided below is a weblink to a comprehensive slide deck developed by a fire protection engineer with over 20 years of relevant fire services experience that walks through the fire hazards of industrial mulch manufacturing that is intended for commercial sale (not farming). It details a sampling of fairly recent mulch fires and the extent of emergency fire response in terms of personnel (up to 150 firefighters across ~3 counties), water needed (360,000 gallons or more) and duration of efforts to extinguish (10 hours to 3 days). It is important to note that some of the mulch fires listed with these estimates are less than 1 acre in size, and were obviously difficult in their own right to contain.

Again, thanks to County Executive Kittleman and CB60 authors Councilmembers Sigaty and Fox, CB60 will allow for mulching/composting in RR and RC on an industrial scale (2-10 acres) that could well exceed the acreage and, therefore, level of response needed to contain fires than what is noted above. In essence, this translates into a substantial safety risk for our families and homes if industrial mulch/compost facilities of 2-10 acres total are allowed to operate for industrial processing and commercial sale nearby to residential/rural communities.

The risks increase should a mulch fire at these facilities occur on a windy day, resulting in flying embers that could travel to nearby neighborhoods and put homes at risk. The risks also increase if these facilities are located on rural farmland where no public water exists, posing a greater threat for spread/inability to contain a mulch fire.

In Maryland alone there have been several major mulch fires in just the past two years. The threat is real. You will notice one large mulch fire from 2013 referenced in this slide deck was from the Recycled Green industrial mulch facility in Woodbine/Carroll County, which had ownership interest from Bonner who also owns Oak Ridge that continues to operate in violation to current CB20 zoning regulations. Another recent mulch fire in 2017 took place at Harvest Mid-

Atlantic and required a major fire response to contain. It should also come as no surprise that Harvest Mid-Atlantic is actually the Recycled Green facility renamed (home to the industrial mulch fire in 2013).

Please email County Executive Kittleman (akittleman@howardcountymd.gov) and the County Council (councilmail@howardcountymd.gov) and tell them that the fire risks are too great for your children, families and communities, and that you are unwilling to accept the safety risks that CB60 will allow for if major amendments are not included to keep industrial mulching for commercial sale off the farmland.

http://cc.howardcountymd.gov/LinkClick.aspx?fileticket=9N64WpWNTq8=&portalid=0

Thank you for distributing to your network.

John Tegeris, PhD
President, DRPS

"CB60: Don't Defend It, Amend It!"
Your voice and your vote matter. Elections are drawing near.

Sayers, Margery

From: Andrea MacMurray <dustbunniez@verizon.net>
Sent: Thursday, October 26, 2017 3:53 PM
To: CouncilMail
Subject: Dayton Mulching Industry

I cannot understand, with all of the testimony and proof from the people living around the Woodbine Mulch facility, that you can even consider setting up a mulching facility in Dayton, MD. There are single family homes that will literally back up to this facility as well as newer developments

that will be right next to it. The ENVIRONMENTAL disaster that this kind of facility will cause is factual. The air, the water, the ground, the backed up traffic (which in turn will also cause more air quality issues) is not what YOU the council meant by first creating the term "farm". A farm cannot

be a mulch factory with loads of miscellaneous trash brought in, ground up to be be placed on or sold to other people' properties. There is no farming on this farm, just trash from the waysides, huge 5 ton dump trucks coming in with this trash and more going out with this material ground up.

No care as to what the material consists of, no care as to what a pile of this stuff does when it's smoking on a hot day and there is a draught, no care for the contaminated water that is seeping into the underground wells from the piles of trash that are "seasoning" and no care as to what the

people of Dayton are concerned with.

You have had experts tell you the consequences of this type of operation in a populated area. Not only does Dayton have many homes on wells, we also have an elementary school right down the road. How can you even consider this type of business in our community?????

Andrea MacMurray
4670 Ten Oaks Road
Dayton, MD 21036

Sayers, Margery

From: Victoria Stewart Moore <vstewartmo@aol.com>
Sent: Thursday, October 26, 2017 3:26 PM
To: CouncilMail
Subject: CB60 is a bad bill

Dear County Council members,

CB60 is foundationally a bad bill. Adding amendments won by make it pretty. They have a warning in the military, "Don't sh__t in your mess kit." The same is true with allowing mulching and composting of organic masses in RR and RC districts.

Western Howard County has woods, streams and rivers which border the Cheseapeake Bay. Ground water waste will not only feed into the Bay, it will leach into our well water. Odors from the rotting compost will pervade the woods where fox hunters, rider, bikers and bikers seek solace from the pollution of the cities. Why would anyone who proclaims they are protecting the environment support a bill which does just the opposite?

And, to allow farmers to sell 5% of their product just opens the door to misuse. Who's to say whether it's 5% or 25%? The country, notorious at not able or interested at monitoring such laws will look the other way This is a concoction for disaster, pitting neighbor against neighbor and tying up hours and dollars in litigation which could have been prevented.

It's time the county listened to the residents it is supposed to represent and instead of jamming bad ideas down our throats, think the issues through and weigh the good, the bad and the ugly and when the latter outweighs the former, not split the baby in half by adding amendments. Just say no!

Thank you for your support on this critical matter.

Respectfully,

Victoria Stewart- Moore
3400 Jennings Chapel Rd Woodbine

Envoyé depuis AOL Mail sur mobile

Sayers, Margery

From: Richard Tufts <tuftsdaisy@verizon.net>
Sent: Thursday, October 26, 2017 1:23 PM
To: CouncilMail
Subject: CB60

Council Members,

I am encouraged to learn of the many amendments you have added to CB60 to prevent the many loop holes folks were concerned about in the original bill.

However, I am concerned in all the many meetings on this issue, there doesn't seem to have been consideration given to the carcinogenic effects of wood dust produced by mulching. Especially as there is technology available that will mitigate it.

The purpose of my email, then, is to inquire, why you seem to have swept this under the rug and failed to consider a very important aspect of this issue.

I will greatly appreciate your response whether you intend to consider adding this preventative technology and if not why not.

Respectfully,
Richard G. Tufts
Daisy

Sayers, Margery

From: Suzanne Cotton <lrcsmc@aol.com>
Sent: Thursday, October 26, 2017 12:35 PM
To: CouncilMail
Subject: CB 60

Oppose CB 60! Protect our health, safety, property values and quality of life!

Suzanne F. Cotton
5000 Morning Star Drive
Dayton, MD 21036

Sent from my iPhone

Sayers, Margery

From: Barry Casanova <bwcasanova@msn.com>
Sent: Thursday, October 26, 2017 12:01 PM
To: CouncilMail
Subject: CB 60

After careful consideration, I have reached a decision on CB 60. It is not too often that I agree with the majority party, but I am opposed to this bill. It is not in the best interest of the residents of this country. Thanks for your consideration.
Barry W. Casanova, Esq.
Sent from my iPhone

Sayers, Margery

From: Petigara, Bhakti <Bhakti.Petigara@fda.hhs.gov>
Sent: Thursday, October 26, 2017 10:53 AM
To: CouncilMail; Kittleman, Allan
Subject: cb60

Time is winding down. CB 60 is up for vote soon. I hope you all do the right thing for the safety of the people in Howard County. Ground water contamination has been ignored completely. The right questions have not been asked to the right people to answer because you don't want to hear the answer. Any about of mulch for industrial use does not belong in ag preserve where people drink the ground water.

Please think about this hard. You don't want to hurt the health of people for the good of a few businessmen.

We elected you in because we believe in you. Don't let us down

Bhakti Petigara Harp, Ph.D
Research Chemist
Food and Drug Administration
Office of Cosmetics and Colors
5001 Campus Drive, CPK2
College Park, MD 20740-3835

Sayers, Margery

From: Ginna Rodriguez <rodriguez.ginna@gmail.com>
Sent: Thursday, October 26, 2017 10:52 AM
To: CouncilMail
Subject: akittleman@howardcountymd.gov, boe@hcpss.org, superintendent@hcpss.org

Dear County Council,

As a resident and taxpayer of Howard County, I thank you for your diligence in reviewing the APFO Bill and working to craft amendments in response to your constituents.

- Many of the amendments let me know that you are listening. I strongly support the following amendments: A, B, C, D, E1, E2, F, H (with revisions), K, S, U, X, and FF.
- There is strong potential with amendment H, if the following protections can be added: (1) protect schools by adding cap for individual capacity, and (2) add high school test now.

- I strongly oppose amendments P, Q, R, and T.

I would like to see how the amendments proposed compare to one another in terms of the number of school regions open for development. I would also like to see how they compare to what was initially approved by the APFO task force. It seems that some of the amendments proposed would accelerate the pace of development at a time when the community is asking for a slow down because we do not have adequate public facilities (e.g, Howard High School, Long Reach High School).
If Howard County is to remain one of the most desirable places to live and work, we need an updated, county-wide comprehensive plan for responsible growth paired with adequate funding from developers for infrastructure support, development, and maintenance.
I call on you, as our elected officials, to continue the necessary work to ensure that we have an APFO legislation emerge that better addresses the impacts of growth in Howard County.

Thank you,
Ginna Rodriguez
4053 Pebble Branch Road
Ellicott City MD
District 1 Resident

Sayers, Margery

From: John Tegeris <johntegeris@gmail.com>
Sent: Thursday, October 26, 2017 12:25 AM
To: CouncilMail; Kittleman, Allan; Lazdins, Valdis; Gowan, Amy; Peter Jensen; pwood@baltsun.com; mdzwonchyk@baltsun.com; aburnett@wjz.com; Kim Dacey; srorman@sbgtv.com; bzumer@sbgtv.com; ambarnett@sbgtv.com; Ted Mariani; Rick Lober/Annette; Brent Loveless; Stu Kohn; Rob Long; Preserve Dayton; Velculescu Victor; Jeff Harp; Jeff Harp; Luv of My Life; andrew.green@baltsun.com; John Tegeris
Cc: Tim Mayer; Mike Navarre/Lynn; Bethany Hooper; Rob Bundy; Dan O'Leary; Andrew Royle; Lisa Markovitz; Susan Garber; Carol Jane Gray; Ocheltree Janet; Erin Allen; John Allen; Al Risdorfer; Bono Tony V; Paul Morris; Paul Retzbach; Colleen Retzbach; Kristin Robertson; Lora Houck; Trip Kloser; Craig Ostrom; Julius Tunji Akintade; Chelakara Shankar; James Nickel; Banwarth Dave; dave.kromer@tunnellgov.com; Sylvie Leary; Alan Schneider; Paul Shoffeitt; Mike Bucci; Robert Scales; China Williams; Katie Hester; Mike; Patricia Soffen; Joanne Heckman; <darbus37@gmail.com>; Jennifer Bush; sharon.keeny@lnf.com; tilycog@comcast.net; cathydatz@yahoo.com; Richard Valentine; Belkacem Manseur; Alex Xu; Richard Taber; Phil Montag; <bstrickland@wtplaw.com>; ST Balimtas; Michael Burns; Paul Retzbach; <fernandesgj@washpost.com>; Eric Goldberg; <benabili@hotmail.com>; Rob Bovello; Paul Robertson; Michael Price; Doug Lee; Jay and Santa Bhalani; Ajay soodan; jmathew@acidd.us; Om Prakash Gupta; <jthensel61@gmail.com>; Benjamin Lee; <joelhouck66@yahoo.com>; Ty Shrader; sdwerlinich@aol.com; Williams; Z Zhang; Brian Lehman; Lisa Valentine; Denise Howze; Hafida Manseur; Ning Hu; dianawscales@gmail.com; Richard and Susan Taber; Marisa Montag; <estrickland@offitkurman.com>; Robin Balimtas; Kathy Burns; Home; Dahna Goldberg; Michelle Meney; <jmbovello@comcast.net>; Delia Velculescu; Annette Lober; rajput31@yahoo.com; Melissa and Larry Kramer; Jyoti Gupta; <s.hensel@live.com>; Carol Werlinich; Mirra Morris; Sally Ostrom; Karen K; Laurie Lehman
Subject: CB60: Groundwater Contamination, from High Microbial Activity to Elevated Heavy Metals; a Risk Not Worth Taking
Attachments: NY water report cover letter pg1.png; NY water report cover letter pg2.png; Manganese Information Sheet 4-2010[479].pdf

CB60, thanks to County Executive Kittleman and authors Council Members Sigaty and Fox, will lead to not only high microbial activity in groundwater due to compost with food waste, animal mortality and manure (aka high disease burden), but also to heavy metals contamination (aka neurotoxicity) as a result of compost processing.

Heavy metals such as manganese, as you will read in the attached fact sheet on Manganese in Drinking Water issued on behalf of the New York State Department of Health, is well documented to cause neurological disorders in adults and children, as well as developmental and behavioral abnormalities in children. There is also a wealth of data published in the medical literature to support these assertions.

Elevated levels of heavy metals, primarily manganese, were found downgradient of all 11 composting sites investigated in Suffolk County between 2011 and 2014, as noted in the letter attached here as 2 pictures, written by James Tomarken, MD, MPH, MBA, MSW, Commissioner, County of Suffolk Department of Health Services.

Please email County Executive Kittleman (akittleman@howardcountymd.gov) and the County Council (councilmail@howardcountymd.gov) and tell them to say "no" to CB60 that allows for industrial composting throughout Howard County and allows trucking out for commercial sale of compost in tractor-trailer/3-axle trucks.

Thank you for distributing to your network.

John Tegeris, PhD
President, DRPS

"CB60: Don't Defend It, Amend It!"
Your voice and your vote matter. Elections are drawing near.

January 27, 2016

Eugene Leff, Esq.
Deputy Commissioner
New York State Department of Environmental Conservation
625 Broadway
Albany, NY 12233

David Vitale, P.E.
Director, Division of Materials Management
New York State Department of Environmental Conservation
625 Broadway
Albany, NY 12233

Carrie Meek Gallagher, MS, MBA, LEED AP BD&C
Regional Director
New York State Department of Environmental Conservation
SUNY @ Stony Brook
50 Circle Road
Stony Brook, NY 11790-3409

Dear Ms. Gallagher:

Attached is a Suffolk County Department of Health Services (SCDHS) report summarizing additional groundwater sampling conducted in the vicinity of vegetative organic waste management facilities (VOWM). This "Investigation of the Impacts to Groundwater Quality from Compost/Vegetative Organic Waste Management Facilities in Suffolk County" was conducted in follow up to a prior SCDHS groundwater investigation in the vicinity of the Great Gardens/Long Island Compost facility in Yaphank, NY, results of which were released by the New York State Department of Environmental Conservation (NYSDEC) in a 2013 report titled; *Horseblock Road Investigation, Yaphank NY.*

SCDHS initiated this additional study to investigate whether groundwater impacts similar to those observed in the Horseblock Road investigation would be observed downgradient of other VOWM sites. The attached report provides the results of groundwater samples taken downgradient of eleven VOWM sites between July of 2011 and October 2014.

OFFICE OF THE COMMISSIONER
3500 Sunrise Highway, Ste. 124, PO Box 9006, Great River, NY 11739-9006
(631) 854-0000 Fax (631) 854-0108

The results of this groundwater sampling effort confirm the prior observation of elevated metals, primarily manganese, and atypical elevated concentrations of radiological parameters, in groundwater downgradient of VOWM facilities. Based on these findings, the attached report provides specific recommendations to address these groundwater concerns, including revisions to NYSDEC Solid Waste Management regulations.

SCDHS would like to acknowledge our appreciation to the Region 1 Office of the New York State Department of Environmental Conservation for their assistance, and the New York State Department of Health (NYSDOH) Wadsworth Laboratory for performing a subset of the radiological analyses of the groundwater samples.

Sincerely,

James L Tomarken

James L. Tomarken, MD, MPH, MBA, MSW
Commissioner

JLT/srg

cc: Ajay Shah, P.E., Regional Engineer, NYSDEC
Cynthia Costello, MS, MPH, CHP, Chief Environmental Radiation/Radon Section, NYSDOH
Christina Capobianco, CPA, Deputy Commissioner, SCDHS
Walter Dawydiak, PE, Director DEQ, SCDHS
Douglas Feldman, P.E. Chief, OWR, SCDHS
Andrew Rapiejko, Associate Hydrogeologist, SCDHS

COUNTY OF SUFFOLK

STEVE LEVY
SUFFOLK COUNTY EXECUTIVE

DEPARTMENT OF HEALTH SERVICES

JAMES L. TOMARKEN, MD
MSW, MPH, MBA, FRCPC, FACP
Commissioner

April 14, 2010

Manganese in Drinking Water Fact Sheet

Issued on behalf of the New York State Department of Health (NYSDOH)

Manganese is a common element in rocks, soil, water, plants, and animals. Contamination of drinking water may occur if manganese gets into surface or groundwater after dissolving from rocks and soil. It may also occur if manganese gets into surface or groundwater after improper waste disposal in landfills or by facilities using manganese.

Manganese is noticeable in tap water at levels greater than 0.05 milligrams per liter (0.05 mg/L) because the water can have a brown color and leave black deposits on bathroom fixtures or laundry. At levels exceeding 0.1 mg/L, water can have an undesirable taste or smell. The New York State (NYS) primary drinking water standard (MCL) for manganese in public water supplies is 0.3 mg/L, and was originally set based on staining and taste considerations (aesthetics). This standard is also used to provide guidance regarding the use of drinking-water from private wells.

Manganese is an essential nutrient that is necessary to maintain good health. However, exposure to too much manganese can cause adverse health effects. Since the NYS standard was promulgated, concerns have arisen about the potential health risks from exposure to elevated levels of manganese in drinking water. In 2004, the U.S. Environmental Protection Agency (EPA) issued a One-day and Ten-Day Drinking Water Health Advisories for manganese of 1 mg/L. In addition, the U.S. EPA also issued a Lifetime Drinking Water Health Advisory for manganese of 0.3 mg/L. These health advisories were issued to provide guidance to people and communities that may be exposed to drinking water contaminated with high manganese levels. The advisories are set at the manganese level in drinking water that is not expected to cause any adverse non-cancer effects over one day, ten days or a lifetime of exposure.

Because the lifetime health advisory is not meant to be a bright line between water levels that cause health effects and those that do not, exposure to levels above 0.3 mg/L are not necessarily associated with

toxicity. However, results of studies on the potential health risks of exposure to elevated levels of manganese in drinking water raise concerns about the long-term consumption of water with levels above 0.3 mg/L. In these studies, adults and children who drank water with elevated levels of manganese (average levels of 0.6 mg/L and higher) for many years (average length of exposure of 7 years and higher) seemed to have a slightly higher frequency of nervous system effects than adults and children drinking water with lower levels of manganese. Observed effects included weakness, stiff muscles and trembling of the hands (adults) and altered scores on tests of learning and behavior (children). Although the effects reported in these limited studies are not specific to manganese and might have been caused by other factors, they provide evidence (along with other studies of nervous system effects from manganese in animals and humans) that high levels of manganese in drinking water may increase the risk for health effects, particularly after frequent and long term exposure.

There is also a concern for infants fed formula prepared with water containing elevated levels of manganese. Infants may absorb more manganese than adults and may excrete less. Thus, infants have a greater potential for exposure than adults even though both groups are drinking the same water. This greater exposure and the suggestive evidence of nervous system effects in children chronically exposed to high levels of manganese from drinking water support concern about the health risks to women, infants, and children consuming water containing more than 0.3 mg/L during critical periods of development (e.g., pregnancy) or for long periods of time.

The New York State Department of Health and the Suffolk County Department of Health Services recommend that measures be taken to reduce manganese exposure when levels in drinking water are above 0.3 mg/L. The higher the level, the greater the urgency to reduce exposures. Connecting to public water or use of bottled water should be considered for drinking, cooking, and making infant formula. Another option is the installation of a water treatment system to remove the manganese, which would also address problems with staining of laundry and plumbing fixtures, and improve the aesthetic quality (taste and odor) of drinking water. If your well water is used primarily for irrigation and only occasional drinking, exposures would be reduced and the risks for health affects would be lower.

Should you have questions concerning potential health impacts from exposure to manganese in your drinking water, please contact the New York State Department of Health, Bureau of Toxic Substances Assessment at **1-800-458-1158 extension 27800.**

Sayers, Margery

From: stukohn@verizon.net
Sent: Wednesday, October 25, 2017 8:53 PM
To: CouncilMail; Kittleman, Allan; Wilson, B Diane; howard-citizen@yahoogroups.com
Subject: All Key Witnesses Should Have Been Allowed to Testify at the Council's Work Session

FYI,

The controversy regarding CB60-2017 (allowing certain composting facilities and emergency natural wood waste recycling facilities as accessory uses under certain conditions in certain Zoning Districts; allowing certain natural wood waste recycling facilities and composting facilities as a use permitted as a matter of right) is bad enough, but it becomes much worse when Dr. Victor Velculescu (Co-Director of Cancer Biology and Professor of Oncology and Pathology at the Johns Hopkins Kimmel Cancer Center) an expert witness was not permitted to testify by the County Council in their Work Session. What is the reason for this decision? The Council should have no reason to deny the Concerned Citizens this opportunity so they could at least hear and receive all pertinent information to aide in their final decision. A response from the Council would be appreciated as Dr. Velculescu should have been given the chance to speak. He was available regarding speaking about possible health issues which could be impacted because of the passage of CB60 on November 6.

Sincerely,

Stu Kohn,
HCCA, President

Sayers, Margery

From: Howie Feaga <howie@merryacresfarm.com>
Sent: Wednesday, October 25, 2017 3:18 PM
To: CouncilMail; Kittleman, Allan
Cc: Ackman, Mandy; Barb Glenn; Barb Glenn; Clarks ElioakFarm; cmhudson@comcast.net; Danielle; Guy Moore; Howie Feaga; Jamie Brown; Jason Myers; Keith Ohlinger; Kenny Warfield III; Kerry Brendel; Larry Barnard; Leslie Bauer; Marc Hereth; Marc Hereth; markmaplelawn@gmail.com; Merhlyn Barnes; Merry Acres Farm; Michael Calkins; Parker Welch; Paula Linthicum; Rhonda WInkler; zek71@hotmail.com; zlevelland@gmail.com; Zoller, James
Subject: It's not a Mulch grinder !!!!

Dear Council Member's, it was brought to my attention that a picture of a combine, was sent to you all on or around October 16th, and it was being described as a NWWR Grinder. Well I can assure you, that it is in fact, a New Holland brand combine. I also know for a fact that you cannot grind wood products with that machine. It is used to harvest corn this time of the year, as well as harvest soybeans, when the proper attachments are assembled on it. These attachments are (for safety sake) not on the machine when they are moved down the road, the attachments are too wide for most of the roads in Howard County. The farmers disconnect the attachments and haul them on a cart that is specifically made to transport them from field to field. I have included a video that shows some of the hazards we face on our roads today. https://youtu.be/tm0l1n9rlzc This video was made by the Howard County Soil Conservation service. I hope that this helps you all understand that we are all trying to be safe and if you make a note of what day and time the picture was taken, the farmer was trying to move on the road, at as safe a time, as they can, by doing it on a Sunday morning at 11:30 so as not to endanger anyone. We cannot always move at the safest time of day, but we do our best. They do not, to my knowledge, make a self-propelled mulch grinder. In the future, I hope that when pictures are taken by the driver of any vehicle, of an agricultural machine, or any other so-called hazard, that the driver of that vehicle should be given a traffic ticket, for using their phone or camera while driving. That is more of a danger, then us driving and being as safe as we can, while they are trying to spread false statements for the social media. On behalf of the Ho. Co Farm Bureau, drive safe and Thank You!!!! Howie Feaga

Sayers, Margery

From: Meagan Braganca <mbraganca@verizon.net>
Sent: Wednesday, October 25, 2017 2:17 PM
To: CouncilMail
Subject: CB 60

All:

Please say "NO" to Type 2 feedstock in compost and to add major amendments to CB60 to prevent this.

Thank you
Meagan Braganca

"Now that you know, what will you do?"
-Everyone's an advocate for something-
#SinglePayerNOW

Sayers, Margery

From: Patricia Soffen <patricia.soffen@gmail.com>
Sent: Wednesday, October 25, 2017 7:21 AM
To: CouncilMail
Subject: No Type 2 feedstock in compost

County Council Members,

Studies have shown that allowing Type 2 feedstock in our neighborhood farms compost is detrimental to the health of the surrounding residents. You need to not only grow Howard County in a responsible manner, but also protect our resident's health. This is what I expected of you when you were voted to the County Council.

So, I am asking that you not only ban the addition of Type 2 feedstock in compost but also make it an amendment in CB60 to ensure that it will be banned in the future.

Thank you for your consideration,

Patricia Soffen
5310 Honey Court
Ellicott City

Sayers, Margery

From: John Tegeris <johntegeris@gmail.com>
Sent: Tuesday, October 24, 2017 11:13 PM
To: CouncilMail; Kittleman, Allan; Lazdins, Valdis; Gowan, Amy; Peter Jensen; pwood@baltsun.com; mdzwonchyk@baltsun.com; aburnett@wjz.com; Kim Dacey; srorman@sbgtv.com; bzumer@sbgtv.com; ambarnett@sbgtv.com; Ted Mariani; Rick Lober/Annette; Brent Loveless; Stu Kohn; Rob Long; Preserve Dayton; Velculescu Victor; Jeff Harp; Jeff Harp; Luv of My Life; andrew.green@baltsun.com; John Tegeris
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Subject: CB60 - Now Is Not the Time for Unsubstantiated Opinions about Groundwater Contamination

County Executive Kittleman and Council Members Sigaty and Fox keep trying to convince us that adding Type 2 feedstock to compost on our neighborhood farmland will not create health risks for our families. Contrary to their "expert" opinion, the study referenced below shows that groundwater will be contaminated by the presence of animal mortality and manure.

The fact that they are willing to take this risk of high microbial activity and increased disease burden by allowing Type 2 feedstock to be trucked in for composting is absurd. The increased disease burden translates into a higher risk of infection to everyone who's home uses well water.

Bottom line - CB60 will allow for food waste, animal mortality and manure to be added to composting resulting in leachate that will contaminate the groundwater that supplies drinking water to our homes, thereby putting our families at higher risk of disease and infection.

Please email County Executive Kittleman (akittleman@howardcountymd.gov) and the County Council (councilmail@howardcountymd.gov) and tell them to say "no" to Type 2 feedstock in compost and to add major amendments to CB60 to prevent this.

Thank you for distributing to your network.

John Tegeris, PhD
President, DRPS

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Impacts of leachates from livestock carcass burial and manure heap sites on groundwater geochemistry and microbial community structure

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Introduction

Surface water and groundwater contamination by livestock-waste-derived solutes and microorganisms and its risks to human health have long been recognized [1]. Leachates released from both mass carcass burial sites and livestock fecal waste heaps can have negative impacts on groundwater quality, and are a major public concern [2–4]. These leachates are a potential source of both conventional contaminants (e.g., chemical oxygen demand, total organic carbon, total nitrogen, total phosphorus, and solids) and biologically active contaminants (e.g., pathogens, antimicrobials and steroid hormones) that can move through subsurface environments [5].

Since 2010, major outbreaks of foot-and-mouth disease (FMD) have occurred in South Korea and a total of 3.4 million head of livestock, including 1.2 million swine, were buried in 2010–2011 [6]. Dead animals must be disposed safely to prevent issues such as odor, pathogens, and excess nutrients [7]. However, high levels of microbial contaminants, including bacteria and nitrogenous compounds, have been detected in groundwater near livestock carcass burial sites in South Korea [8, 9]. Therefore, monitoring microorganisms and groundwater quality near burial sites is essential to ensure the safety of the local public.

There has been increased concern worldwide over the effects of microbial components from livestock carcass burial and fecal waste management on groundwater quality [1, 8]. For example, pathogens and solutes presented in fecal waste can move to surrounding environments and may result in water and soil contamination. Hutchison et al (2004) reported that both fresh and stored livestock manures contained substantial proportion (8–22%) of pathogens such as *E. coli* O157:H7, *Salmonella*, and *Campylobacter*[10]. On the other hand, a previous report showed that complete animal decomposition could require more than 2 years and could generate greenhouse gases and leachates containing high levels of chemical contaminants [11, 12]. Thus, long-term leachate release may act as a continuous source of contaminants, providing growth substrates for soil microorganisms in subsurface environments [9]. The subsurface microorganisms present near livestock carcass burial sites can be classified as enteric microorganisms directly from livestock carcasses, carcass-decomposing microorganisms, or indigenous soil microorganisms [9].

To prevent groundwater contamination by harmful pathogens in the future, it is critical to track microbial sources and evolution precisely and to address animal carcasses and manure as a source [13]. However, it is difficult to identify representative microorganisms in subsurface environments using conventional culturing methods. Several studies have investigated the microbial communities of livestock burial and waste disposal sites using culture-dependent analyses [14, 15]. These studies provided evidence of the possible presence of specific microorganisms, including pathogens and indigenous soil microorganisms. However, using culture-dependent methods to detect soil microorganisms may give limited information (i.e., false-positive and false-negative results can occur) about the microorganisms present in and near livestock burial and fecal waste disposal sites. A recent study demonstrated that the composition of cultured isolates (i.e., isolation using specific growth media) selected both a small and unrepresentative share of soil microorganisms compared to that sampled using a culture-independent method, 454-pyrosequencing of total soil DNA [16].

Several studies have investigated the compositions of and changes in microbial communities in leachates from livestock carcass burial sites [17–19], but little or no effort has been expended to determine the spatial distribution of microbial taxa coupled with geochemical dynamics at livestock carcass sites. In addition, although microbial communities originating from intestinal tracts may be similar regardless of livestock species, no or little study has attempted to compare the structures of microbial communities found at livestock-derived contamination sites such as swine burial sites with those at cow manure heap sites.

Therefore, we investigated the evolution of microbial community composition and groundwater chemistry influenced by leachates from swine carcass burial and cow manure heaps along groundwater flow paths. The microbial community compositions were evaluated using culture-independent Illumina MiSeq high-throughput sequencing. The objectives of this study were to 1) understand how the leachates from livestock-derived contamination sites impact the geochemical and microbiological properties of subsurface environments; 2) identify which microbial communities are predominant in swine carcass burial site and cow manure heap sites; 3) determine which factors control the microbial community compositions around these sites, and 4) examine to what spatial distance microorganisms in leachates can transport to surrounding subsurface environments. The results of this study will improve our understanding of the transport of bacteria from livestock burial sites and manure heaps to subsurface environments and provide insights into the effective management of groundwater quality and microbial contamination.

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Materials and methods

Study area and sampling

To answer the objectives of our study, we selected two field sites influenced by livestock-derived contaminants. The swine carcass burial site (A site) and cow manure heap site (B site) examined in this study were located in the central and western regions of the Republic of Korea, respectively (Fig 1A and 1B). The cow manure heap ('D' in Fig 1B) was located outside cow sheds on a farm with 140 dairy cows, and was not a government-run waste disposal site. In January 2011, three livestock carcass burial events occurred due to an FMD outbreak in the area, resulting in the burial of 3,989 swine. Both sites were surrounded predominantly by dryland or paddy fields. The underlying aquifer systems were formed from unconsolidated colluvial materials deposited at the base of hill slopes after weathering from Jurassic granite bedrock. The hydraulic conductivities of the aquifers at the livestock burial site and livestock manure heap differed greatly according to the sediment particle size distribution. The hydraulic conductivities at the two locations were estimated at 0.33 and 0.14 m d^{-1}, respectively.

Fig 1

Study areas: livestock carcass burial site (A) and livestock manure heap site (B).

To evaluate the relationships between groundwater quality and microbial community compositions and to determine the microbial transport from the contaminant sources to surrounding environments, water samples were collected along the groundwater flow direction near the carcass burial site and manure heap, including leachate, groundwater from monitoring and background wells, and surface stream water (Fig 1). Multi-level monitoring wells (sampling sites: IA, IB, YB, and YC) with several sampling depths (IA: −5, −10, −17 m; IB: −10 m; YB: −6 m; YC: −9, −15 m) were installed at both study sites. Installation of the monitoring wells was performed in 2012 (A site) and in 2013 (B site) using a rotary drilling device. Borehole drilling was undertaken to the depth of weathered bedrock (Jurassic granite or Precambrian gneiss) underlying colluvial or alluvial sediments. The bundle of multi-level groundwater samplers [consisting of polyvinyl chloride (PVC) pipes (2.5 cm diameter) and polyethylene tubes (0.5 cm diameter) with the bottom parts slotted (15 cm long) and wrapped with stainless steel screen for water sampling] at different depths was placed into the borehole and then the monitoring wells were backfilled with excavated materials, sand and bentonite. After the completion of well installation, the monitoring wells were immediately purged by a peristaltic pump to remove mixed stagnant water. The location and sampling depth of the monitoring wells were determined by considering the general groundwater flow direction and location of the pollution sources. In other words, the monitoring wells are located downstream (very close to the pollution sources) of the carcass burials (A site) and cow manure heaps (B site) based on the groundwater flow directions in the study sites (Fig 1).

For water sampling, each well was pumped continuously at 0.5 L min^{-1} using a peristaltic pump (7523–30 Masterflex; Cole Palmer Vernon Hills, IL, USA). The samples were collected after clearing stagnant water from the wells. Well purging prior to groundwater sampling was done to remove stagnant water that may not be representative of *in-situ* groundwater quality. For this, the monitoring wells were purged approximately three times volume (electrical conductivity (EC) value also becomes constant) of water stored in the wells to avoid water quality disturbance by excessive purging. The leachate sample in the carcass burials was collected by inserting the sampling tube into the pipe which connected to the inside of the burials from the roof, and then collected the leachate by using the peristaltic pump with slow pumping rate. Between 28 October and 4 November 2013, 11 samples (eight groundwater, one surface stream, one carcass leachate, and one feces leachate) were collected. Several potentially unstable parameters, including temperature, redox potential (Eh), pH, EC, and dissolved oxygen (DO), were measured at the field sites.

The water samples were collected in capped bottles connected directly to sampling tubes and the peristaltic pump to minimize air contact. The samples were filtered through a 0.45-μm membrane filter into pre-cleaned 60-mL polyethylene bottles. For the cation analysis, samples were preserved by adding concentrated HNO_3 to keep the pH below 2. All sample bottles were filled completely and capped with elastic laboratory film to avoid air contact, and then stored at 4°C until the analysis.

For the microbial community analysis, biomass was collected by filtering the retentate from 1–5 L of water samples through a 47-mm filtration unit (Nalgene Reusable Filter Holder; Thermo Fisher Scientific Korea, Seoul, Republic of Korea) fitted with a 0.2-μm filter (Pall Gelman Laboratory, Ann Arbor, Michigan, USA). The filters were preserved in a 50-mL sterilized conical tube on dry ice until they could be stored at −20°C in the lab. Fecal waste (YH in Fig 1B) was collected using a sterilized spatula and preserved in a tube at −20°C until the experiments were completed.

Analytical methods

The temperature, pH, EC, Eh, and DO of all water samples were measured using Orion portable meters (Orion 5-star RDO Multiparameter Meters; Thermo Scientific, Beverly, MA, USA). The portable meters were calibrated and checked before the measurements. Alkalinity was determined in the field via volumetric titration using 0.05 N HNO_3 [20].

Cations were analyzed by with inductively coupled plasma-atomic emission spectroscopy (Optima 3000XL; Perkin-Elmer, Waltham, MA, USA) and anions were analyzed with ion chromatograph (DX-120; Dionex, Sunnyvale, CA, USA) at the Center for Mineral Resource Research, Korea University, South Korea. Turbidity was determined using a turbidity meter (HI93703 Portable Turbidity Meter; Hanna, Woonsocket, RI, USA). Total viable colony counts were obtained by plating diluted groundwater samples on a solid agar medium according to a method described previously [21].

Microbial community analysis

DNA extraction

Approximately one-fourth of a filter paper or 0.5 g of fecal waste was dispensed into a sterile micro-centrifuge tube. Total genomic DNA was extracted using an i-genomic Soil DNA Extraction Mini Kit (iNtRON, Seongnam, South Korea) with a bead-beating disruption apparatus according to the manufacturer's directions. The DNA concentration was quantified using a Qubit fluorometer (Invitrogen, USA) following manufacturer's instructions.

16S rRNA gene library preparation

The V4 regions of the 16S rRNA gene were amplified using F515 (`5'-GTGCCAGCMGCCGCGGTAA-3'`) and R806 (`5'-GGACTACVSGGGTATCTAAT-3'`) (Bates et al., 2011). The thermocycler conditions for the PCR amplification were 95°C for 3 min, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 5 min and holding at 4°C. The amplicons were purified using AMPure XP beads (Beckman Coulter, Brea, CA, USA). Nextera XT indexes (Illumina) were added at half reaction to remove short library fragments, including index 1 (i7) and index 2 (i5), from the population. Then, 1 μL (1/50 dilution volume) of the final product was used to verify the size of the DNA, confirming the expected size of 300–350 bp. The DNA was quantified with the KAPA Library Quantification Kit for Illumina platforms (KAPA Biosystems, Wilmington, MA, USA) or Qubit fluorometer (Invitrogen, USA) according to the manufacturer's instructions. Paired-end (2 × 254 bp) sequencing was performed at Macrogen Inc. (Seoul, Republic of Korea) using a MiSeq™ platform (Illumina, San Diego, USA).

Sequencing data analysis

The total sequencing count was 24,017, and the sequencing depth-to-average count per sample was 2,183 (S1 Table). The sequencing data were analyzed using QIIME ver.1.8.0 [22]. Sequences were clustered into operational taxonomic units at 97% similarity with the UCLUST algorithm [23]. To calculate species diversity and richness within individual samples, alpha diversity analyses (e.g., Chao1, ACE, and Shannon Index) for the statistical tests were processed using the QIIME script. To measure similarity among communities, beta diversity was analyzed, and two- and three-dimensional principle coordinate analysis (PCoA) plots were constructed. Non-metric multidimensional scaling (NMDS) analyses were also performed with the package vegan implemented in R [24], based on the microbial community information (relative abundance of each OTU detected by either MiSeq sequencing or clone library). The vector fitting of environmental variables to the NMDS ordination was determined using the vegan package with 16 major components of physical and chemical characteristics. Significance was determined based on Bray–Curtis distances and 10,000 random permutations.

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Results and discussion

Water quality changes with distance from the contaminant source and depth

The leachate (IH) from the livestock carcass burial site showed extremely high values of EC (~5,780 μS cm-1), turbidity (4,650 NTU), and major ion concentrations, but low Eh (−131 mV) and DO (0.4 mg L-1) compared to the background groundwater (ID) values of EC (100 μS cm-1), turbidity (0.1 NTU), Eh (346 mV), and DO (6.3 mg L-1) (Table 1 and Fig 2). These results suggested that the microbial decomposition of carcass biomass caused decreases in DO and Eh in groundwater. Furthermore, the carcass leachate had the highest ion concentrations, particularly Cl- (1,121 mg L-1), SO_4^{2-} (1,828 mg L-1), NO_3^- (1,965 mg L-1), and HCO_3^- (5,340 mg L-1). These ions in the leachate likely originated from carcass decomposition, as discussed below. As the water sampling distance increased from the contamination source (i.e., leachate, sample IH), EC, turbidity, Cl-, SO_4^{2-}, NO_3^-, and HCO_3^- decreased remarkably, whereas Eh and DO increased. In particular, Cl- concentrations decreased rapidly but continuously with increasing distance from the leachate source, and showed the lowest concentration in the background well. Chloride ion is a well-known tracer of groundwater and contaminant flow because it is relatively inert and is not biodegradable [25]. Therefore, our hydrochemical observations suggested that the leachate released from the swine burial site was highly diluted with ambient groundwater upon flow. Table 1 shows the results of the water quality analysis around the cow manure heap. Unlike the carcass burial site, the manure heap did not exhibit clear trends in DO, EC, and Eh levels with distance from the contaminant source ('D' in Fig 1B), likely because there were multiple manure sources around the farm. However, the surface water (i.e., runoff) sample (YG) results showed some evidence of contamination, with relatively high NO_3^-, SO_4^{2-}, and Cl- concentrations. Moreover, the groundwater chemistry around the cow sheds (YB and YC) also indicated substantial contamination with NO_3^-, SO_4^{2-}, and/or Cl-, which were much higher than those concentrations around the carcass burial site.

Fig 2

Variations in physicochemical properties and Firmicutes abundance of samples with increasing distance from the contaminant sources.

Table 1

Summary of the physical, chemical, and biological properties of the samples.

The plot of the groundwater samples in the Piper diagram showed the presence of several water types (S1 Fig). The groundwater types of samples IH (leachate) and IA4 were CO_3 type with carbonate hardness >50%, suggesting that the addition of CaO for viral disinfection at the carcass burial site increased HCO_3^- concentrations [26]. The groundwater types of samples IB3 and ID were Ca–Cl type, while those of samples YB1 and YC3 were Na–K–Cl type.

Vertical variations in the physical and chemical properties were investigated using the multi-level monitoring wells (wells IA and YC). Well IA showed decreases in temperature, Eh, DO, and Firmicutes abundance and increases in pH and EC with increasing depth (Fig 2). The physical and chemical properties of the groundwater at depths of 5, 10, and 17 m suggested that the plume of leachates most strongly affected the groundwater of IA at a depth of 17 m. Interestingly, NO_3^- concentrations in IA decreased remarkably between depths of 10 and 17 m (24.9 to 1.5 mg L-1), suggestive of NO_3^- reduction via microbial nitrate reduction [27] and simple dilution with the surrounding groundwater. This observation was supported by an increase in and predominance of nitrate-reducing microorganisms in this microbial community (see *Sulfurimonas* in section 3.3). In the case of YC, the on-site measured parameters, including DO (5.1–5.9 mg L-1) and EC (505–650 μS cm-1), were relatively similar at both measured depths (9 and 15 m), but more Na^+, Cl-, NO_3^-, and HCO_3^- and less SO_4^{2-} were observed at the depth of 15 m (Table 1). We cannot reasonably explain these differences because

samples were collected at only two depths, but it is likely that the hydrogeochemical and geological conditions at this site are vertically heterogeneous.

Comparison of microbial community compositions between the carcass burial and manure heap sites

Amplicon libraries of all samples were constructed to characterize the bacterial and archaeal communities in the carcass leachate, groundwater, manure, and surface water, and to determine community development along the groundwater flow path. To compare microbial diversity levels according to sampling location (i.e., distance of groundwater flow from the source) and between the carcass burial site and manure heap samples, rarefaction analyses were conducted with 868 randomly selected sequences per sample (S2 Fig). The steep slopes of the rarefaction curves (plots of the number of observed species as a function of the number of sequences sampled) suggested that a fraction of the species diversity in the samples remains to be discovered. However, the rarefaction curves indicated that species richness was generally higher in samples from the carcass burial site than in those from the manure heap (S2 Fig). These results suggested that the anoxic and anaerobic conditions and the leachate chemical compositions (e.g., various organic acids) at the carcass burial site might have created metabolic complexities that supported an increase in bacterial diversity [28].

The relative abundances of the bacterial phyla and genera in each sample were calculated as the percentage of sequences belonging to a particular phylum to the total 16S rRNA gene sequences recovered from each sample (Figs (Figs3 and and4,4, S2 and S3 Tables). Firmicutes, Bacteriodetes, and Proteobacteria were the major phyla in both the carcass leachate (IH; 35.6%, 15.9%, and 12.9%, respectively) and the manure heap (YH; 36.1%, 36.9%, and 23.7%, respectively) (Figs (Figs3 and and4).4). Microbes in these phyla are commonly found in animal gastrointestinal tracts [29]. Our results were similar to those of [30], which showed that the sequences matching Firmicutes and Bacteriodetes initially predominated in leachates from swine carcasses, but shifted continuously over time. Genus-level comparisons of bacterial communities between the carcass burial site and manure heap are discussed in section 3.3.

Fig 3

Bacterial community composition at the phylum level (A) and genus level (B) of the samples collected from the livestock carcass burial site.

Fig 4

Bacterial community composition at the phylum level (A) and genus level (B) of the samples collected from the livestock manure heap site.

The phylum- or genus-level components of the archaeal communities are shown in Fig 5 and S4 Table. Although the primers used in this study are known to amplify both bacterial and archaeal sequences [31], there were significantly fewer sequences related to Archaea than to Bacteria (S1 Table). Only samples containing >20 archaeal sequences are discussed hereafter. Similar to the bacterial community composition, the archaeal communities differed markedly between the carcass burial site and manure heap (Fig 5). Sequences related to the genus *Methanosarcina* dominanted in the carcass leachate (IH), whereas *Methanobrevibacter* and *Methanosphaera* were the major genera in the manure heap (YH). These genera are common rumen methanogens [32], and have been detected in paddy fields [33]. However, these genera were replaced by the archaeal genera *Methanobacterium*, *Methanosaeta*, and WCHD3-30 (unclassified) in the carcass leachate site, and WCHD3-30 (unclassified) in the manure heap.

Fig 5

Archaeal community composition at the genus level of samples collected from the livestock carcass burial and livestock manure heap sites.

Factors controlling microbial community distribution

Turbidity was extremely high in the leachate (IH) due to the high concentration of suspended particles, and was lower in samples IA and IB (Table 1). By comparison, the turbidity in the background groundwater was near zero. Suspended particles are an important component of microbial groundwater contamination because of the ability of bacteria to attach onto particulates. For example, the number of attached microorganisms on solid particles in an aquifer was reported to be one to two orders higher than that of free-living microorganisms [34, 35]. In addition, microbes associated with suspended particles had greater survivability [36]. In this study, the total colony counts at both sites generally increased with increasing turbidity (Table 1), suggesting that solid particles could be vectors of bacterial contamination in aquifers.

DO had a significant role in microbial community distribution. The weighted UniFrac distances were visualized in PCoA plots (Fig 6A and 6B). The results showed that the bacterial communities differed between samples with low DO (0.4–1.7 mg L-1) and those with high DO (5.1–11.1 mg L-1). For instance, the phylum OD1, also known as Parcubacteria, was identified in livestock carcass burial site and manure heap samples with low DO (except sample YG), suggesting that this phylum might proliferate in anoxic environments in the study area [37]. This implied that DO had an important role in the development of bacterial communities. Moreover, the bacterial community in the manure sample was similar to that in the sample with low DO.

Fig 6

Principal coordinate analysis (PCoA) and UniFrac analysis of the bacterial communities associated with the leachate, groundwater, and feces samples.

The livestock contaminant source (i.e., swine carcass versus cow manure) was another critical factor controlling microbial community distribution. An unweighted pair group method with arithmetic mean (UPGMA) tree generated from the UniFrac distance matrix (Fig 6C) showed that the bacterial communities of all samples were grouped distinctly into those from the carcass burial site and those from the manure heap site. As mentioned in the previous section, the microbial communities of the carcass leachate (IH) and manure heap (YH) samples were similar at the phylum level. However, the class- or genus-level components within Firmicutes and Bacteriodetes differed markedly between the carcass leachate (IH) and manure heap (YH) samples. Notable taxa of Firmicutes included *Syntrophomonas* (15.8%), Ruminococcaceae (unclassified genus) (4.1%), and *Clostridium* (2.4%) in the carcass leachate, while *Solibacillus* (5.5%) and Aerococcaceae (unclassified genus) (3.3%) were present in the fecal waste (Figs (Figs33and and44 and S3 Table). In addition, Bacteriodetes taxa detected in the carcass leachate (Porphyromonadaceae [unclassified genus] [6.2%], Bacteroidales [unclassified genus] [7.3%]) differed markedly from those in the manure heap (*Flavobacterium* [13.9%], Flavobacteriaceae) [unclassified genus] [9.0%], Porphyromonadaceae [unclassified genus] [5.8%]). Interestingly, the bacterial composition differed from that in a previous study [30], in which the bacterial communities in decomposing swine carcass leachates showed a predominance of uncultured *Tissierella* spp. and *Peptostreptococcus* spp. In addition, another study [38] reported that *Clostridium*, SMB53, *Prevotella*, *Treponema*, *Ruminococcus*, *Faecalibacterium*, *Enterococcus* (class: Bacilli), *Trichococcus*, *Facklamia*, *Caryophanon*, and unclassified Lactobacillales were the predominant taxa in pig intestines. These results suggest that the microbial community composition in the leachates from the carcass burial and manure heap sites may differ significantly according to regional location, livestock type, and feed type.

The chemical compositions of leachate can be an important influencing factor of microbial community composition, as it can serve as a substrate for indigenous microbial growth. For example, sequences related to sulfate-reducing bacteria (SRB) within Firmicutes (e.g., *Desulfosporosinus*) were present in the monitoring wells close to the carcass leachate (IA and IB) (Fig 3 and S3 Table). This was likely because the release of carcass decomposition products (e.g., organic carbons such as organic acids, alcohols, and cyclic hydrocarbons, sulfur, and nitrogen compounds) [39, 40] to adjacent areas improved the suitability of local environments for SRB that were not abundant in the carcass leachate or the background wells. Sequences similar to *Sulfurimonas* (class: Epsilonproteobacteria) were only observed in well IA, and they increased in abundance with increasing depth (Fig 5 and S3 Table). *Sulfurimonas* species, which are capable of sulfur (e.g., sulfide, elemental sulfur, thiosulfate, and sulfite) oxidation coupled with oxygen or nitrate reduction, are commonly identified in sulfidic environments (e.g., hydrothermal deep-sea vents), and in marine and terrestrial sediments [41]. The decrease in DO and NO3- concentrations and increase in the abundance of sequences related to *Sulfurimonas* at well IA with increasing depth suggest that *Sulfurimonas* is important to the redox process in such subsurface environments.

In a complimentary analysis, NMDS analysis using environmental variables (Table 1) and bacterial phyla (S2 Table) in the livestock burial sites were conducted to confirm whether geochemical factors affect community structure (Fig 7A). Among factors we tested, distance from source, sampling depth, temperature, pH, Eh, EC, and DO showed significant relationships with community compositions ($r2 > 0.85$, $P < 0.1$) (S5 Table). The microbial community in the samples close to the livestock burial sites was closely related to turbidity, total colony count, EC, and major ions, while that in the samples far the burial sites was correlated with relatively high DO and Eh. The result of NMDS using environmental variables and bacterial phyla in both sites together (Fig 7B) revealed that bacterial community compositions correlated significantly with several environmental variables including Eh, EC, DO, turbidity, and total colony count ($r2 > 0.56$, $P < 0.08$) and $NO3^-$, $SO42^-$, $HCO3^-$, and Cl^- ($r2 > 0.68$, $P < 0.05$) (S5 Table).

Fig 7

Non-metric multidimensional scaling (NMDS) plots of environmental variables and microbial community compositions at the phylum level in water samples collected from the livestock burial site (A) and both the livestock burial and manure heap sites (B). ...

Microbial transport potential from the carcass burial site and manure heap

Leachates from livestock carcass burial sites or livestock manure heap could cause microbial contamination of groundwater. Although the transport of bacteria from a source is dependent on various hydrogeological characteristics, including hydraulic conductivity and particle size distribution [42], microorganisms can be transported from a source to a well along a hydraulic gradient. In this study, the concentration of microorganisms (i.e., total colony counts) was positively correlated with Na+ and Cl-concentrations and somewhat correlated with SO42- concentrations. In particular, the relative abundance of Firmicutes decreased abruptly in the monitoring wells and background wells (Figs (Figs33 and and4).4). Therefore, it is critical to evaluate whether this phylum can be transported to adjacent areas via groundwater flow paths. The relative abundance of Firmicutes decreased from 35.6% to 2.0% over ~10 m from IH at the carcass burial site and from 36.1% to 4.4% over ~7 m from YH at the manure heap site (S2 Table). Some Firmicutes could be considered to have moved from the source areas to the surrounding wells. However, the occurrence of Firmicutes in monitoring wells was not explained well by their transport from the carcass leachate or manure heap to the surrounding environments because the genus-level classifications of Firmicutes taxa differed markedly between the leachates and monitoring wells. The bacterial and archaeal community compositions in each sample suggested that the transport of bacteria from the carcass burial site and manure heap to surrounding areas did not occur over meter-scale distances.

Conclusions

The physical and chemical properties of the groundwater in wells near livestock carcass burial and manure heap sites were directly influenced by the leachates. However, most of the dissolved inorganic compounds were rapidly diluted by ambient groundwater. Nitrate concentrations decrease even further due to microbial denitrification. The results of the 16S rDNA analysis showed that the genus-level microbial community compositions differed markedly between the swine carcass burial and cow manure heap sites. Turbidity, DO concentration, leachate composition, and contaminant source were the major factors controlling the microbial community distribution in the study area. The results of the community analysis supported the low probability of direct microbial transport or contamination from the carcass burial sites to surrounding environments, as the sequences related to enteric bacteria found in the leachate were not detected in adjacent wells. This study suggests that the transport of microbes from livestock carcass burial and manure heap sites to surrounding areas is unlikely over meter-scale distances but that the release of leachate results in changes in geochemical conditions that can promote the growth of specific members within microbial communities, such as SRB. This study provides insights into the effective management of groundwater quality and microbial contamination at farm and regional scales.

Sayers, Margery

From: Darren Bush <darbus37@gmail.com>
Sent: Tuesday, October 24, 2017 8:55 PM
To: Kittleman, Allan; CouncilMail
Subject: Cb 60

Please make the necessary amendments to protect our community. If this bill passes as written we will be at risk. Large trucks on small roads, health issues, groundwater contamination, etc. the list is too long.

There is even a facility in Howard county operating in violation that is still growing.

Not only does this pose health risks to citizens but once operating there does not seem to be any oversight.

Darren and Jennifer Bush
14036 Big Branch Drive
Dayton Md 21036

Sayers, Margery

From: Trip Kloser <tripkloser@verizon.net>
Sent: Tuesday, October 24, 2017 12:16 PM
To: Kittleman, Allan; CouncilMail
Subject: Council Bill # 60 (CB60) What are you thinking?

Hello Mr. Kittleman and Howard County Council people,

I want to express my deep concern about your support of Council Bill # 60 (CB60). The passage of this bill will not only have an adverse effect on the bucolic nature of the greater Dayton area, but have devastating effects on the health (airborne pollution and ground water contamination.....we are all on well water supply here) and safety for all residences.

20 ton trucks take up the entire roadway with no curb or sidewalk for barrier for pedestrian or cyclist. The existing roads are not engineered for the stress of such heavy vehicles.

With the expansion of Route 32 to 4 lanes will bring more residential traffic to an already rapidly congested road system. Large trucks don't belong in residential areas.

If this bill passes, I am very concerned about the adverse effects on all property values in zip code 21036. Lower property values = Lower Taxes to collect.

With all these negatives to the bill, I ask again "What are you thinking". I strongly urge you to drop your support for Council Bill # 60 (CB60). And to have this bill removed from consideration all together.

Thank you,

Active Voters

Trip & Karen Kloser
14113 Big Branch Drive
Dayton, MD

SPECIAL RESEARCH REPORT

BIOAEROSOLS ASSOCIATED WITH COMPOSTING FACILITIES

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COMPOST
SCIENCE & UTILIZATION

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P.D. Millner, S.A. Olenchock, E. Epstein, R. Rylander, M.D., J. Haines, J. Walker, B.L. Ooi, E. Horne and M. Maritato

SPECIAL RESEARCH REPORT

BIOAEROSOLS

An Overview

Composting has been a long accepted and practiced means of processing organic materials into useful products that are being beneficially recycled in the environment. Because of an increased number of questions about possible adverse health effects that persons might experience from living near a composting facility, the Composting Council, the U.S. Environmental Protection Agency (EPA), the U.S. Department of Agriculture (USDA) and the National Institute for Occupational Safety and Health (NIOSH) assembled, in January 1993, a group of international experts on bioaerosols, risk assessment and composting. These experts were drawn largely from regulatory (EPA and the Department of Health of the State of New York) and research (USDA, NIOSH) agencies. The composting industry, consultants, academia, and environmental groups were also represented.

During that intensive two and one-half day January meeting, the twenty five scientists and engineers reviewed, discussed, analyzed and debated the concerns, facts, and current status of the question: *"Do bioaerosols associated with the operation of biosolids or solid waste composting facilities endanger the health and welfare of the general public and the environment?"*

The workshop participants attempted to examine the full spectrum of potential bioaerosol agents and impacts, including actinomycetes, bacteria, fungi, arthropods, protozoa, and organic constituents of microbial and plant origin and not just those that might arise from the fungus *Aspergillus fumigatus*. To the best of our knowledge this is one of the first attempts at viewing the comparative health impacts of such a broad spectrum of bioaerosols from different sources of decomposing organic materials, (e.g., grass clippings, wood chips, food and household wastes, agricultural wastes, and biosolids) in the environment. As such, the report on this effort helps establish a scientifically reasoned basis for evaluation of health impacts from bioaerosols associated with the processing and handling of biologically degraded materials at composting facilities compared with other sources, and helps set the stage for future advances in knowledge about this important subject.

During the twenty one month period of time following the workshop, participants and other reviewers scrutinized a number of iterative versions of the report resulting in the following state-of-the-knowledge document entitled *Bioaerosols Associated with Composting Facilities*. Relevant data that became available during this period — such as that from the yard waste composting site study in Islip, New York — were incorporated into the report which has been edited and guided by Dr. Patricia Millner of USDA.

This state-of-the-knowledge report cites examples of individual case findings of allergic responses as well as more serious diseases that have resulted from occupational exposure to some types of bioaerosols in a wide variety of organic dusts. In spite of the fact that some types of bioaerosols can cause occupational allergies and diseases, and that some of the same types of bioaerosols are present in the air at facilities that compost organic materials, the expert participants did not find epidemiological evidence to support the suggestions of allergic, asthmatic, or acute or chronic respiratory diseases in the general public at or around the several open air and one enclosed composting sites evaluated.

Thus, in response to the question initially posed to the expert participants at the workshop, the answer that emerged was: *"Composting facilities **do not** pose any unique endangerment to the health and welfare of the general public."* The major basis for this conclusion was the fact that workers were regarded as the most exposed part of the community and where worker health was studied, for periods of up to ten years on a composting site, no significant adverse health impacts were found. In addition, the measured concentrations of targeted bioaerosols in residential zones around composting facilities showed that the airborne concentrations of bioaerosols were not significantly different from background, (i.e., as if the composting facility were not there). A likely reason that the bioaerosol levels were not significantly different from ambient is because the naturally decomposing self-heating organic matter on which these subsequently aerosolized microbes thrive are widely distributed throughout the environment.

Considering the wide range of potential respiratory responses to organic dusts, it was also the consensus of the participants that additional research be conducted to more clearly define the nature and health impacts of bioaerosols from composting facilities compared with all other environmental sources. Specifically, they recommended that assessments be made of "annoyances" and irritants at and around compost sites and that these be coupled with determinations of ambient concentrations of targeted bioaerosols from sites upwind and downwind of composting facilities and other sources that occur naturally in the environment. Furthermore, the participants recommended operational steps that could be taken at composting sites to reduce the generation and dispersal of, and consequently potential for exposure to, bioaerosols.

Reviewers: The complete text of the report, Bioaerosols Associated With Composting Facilities, has been reviewed by the following: E. Petsonsk, M.D., NIOSH, Morgantown, WV; G. Kullman, NIOSH, Morgantown, West Virginia; J.E. Parker, M.D., NIOSH, Morgantown, WV; R. Sjoblad, USEPA, Rosslyn, Virginia; J. Kough, USEPA, Rosslyn, Virginia; D. Bassett, USEPA, Washington, DC; J. Alpert, E & A Environmental Consultants, Canton, MA; J. Sherwin, NOVON/ Warner-Lambert, Morris Plains, NJ; J. Cook, National Audubon Society, Islip, NY; and C. Murray, WSSC, Silver Spring, MD. All the reviewers' comments have been included in the text.

Workshop Participants: The workshop participants included the following: J. Alpert, E & A Environmental Consultants, Canton, MA; D. Bassett, USEPA, Washington, DC; C. Cannon, Composting Council, Alexandria, VA; J. Cook, National Audubon Society, Islip, NY; J. Cookson, INET, Potomac, MD; E. Epstein, E & A Environmental Consultants, Canton, MA; J. Haines, New York State Museum, Albany, NY; E. Horne, New York State Department of Health, Albany, NY; J. Kough, USEPA, Rosslyn, VA; M. Kramer, Environmental Health Associates, Baltimore, MD; D. Lewis, NIOSH, Morgantown, WV; B. Lighthart, USEPA, Corvallis, OR; M. Maritato, ChemRisk, Portland, ME; P. Millner, USDA, ARS, Beltsville, MD; R. Monk, Composting Council, Alexandria, VA; C. Murray, WSSC, Silver Spring, MD; B. Ooi, Organic Recycling, Inc., Valley Cottage, NY; S. Olenchock, NIOSH, Morgantown, VW; R. Rylander, University of Gothenburg, Gothenburg, Sweden; J. Sherwin, NOVON/Warner-Lambert, Morris-Plains, NJ; R. Sjoblad, USEPA, Rosslyn, VA; W. Sorenson, NIOSH, Morgantown, WV; L. Stainer, NIOSH, Morgantown, WV; and J. Walker, USEPA, Washington, DC.

U.S. Department of Agriculture, U.S. Environmental Protection Agency and The Composting Council, Workshop Sponsors — November 1994.

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Bioaerosols Associated With Composting Facilities

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Composting is one of the major treatment processes used to transform wastes into agriculturally useful products. The potential health risks associated with exposure to biological aerosols (hereafter referred to as bioaerosols) generated from the processing and handling of composted organic materials are a major concern in jurisdictions evaluating existing compost installations or planning new ones. Bioaerosols of concern during composting are like those from other organic dusts and they consist of microorganisms (actinomycetes, bacteria and fungi), arthropods, protozoa and organic constituents of microbial and plant origin. Major concerns are the fungus *Aspergillus fumigatus* (AF), cell walls of gram-negative bacteria (endotoxins), β-1,3 glucans from the cell walls of fungi and mycotoxins. These biological materials are found in aerosols generated from a wide variety of organic wastes including grass clippings, wood chips, food and household wastes, agricultural wastes and sewage sludge. This report describes the dispersion of inhalable organic dust in and around composting facilities as well as the possible health effects of the microbial constituents as they relate to infection, allergy, inflammation and annoyance. Special emphasis is given to the opportunistic fungus, *Aspergillus fumigatus*, which has been the subject of specific concern in several jurisdictions involved with planning, siting and permitting of composting facilities. The role of bacterial endotoxins and mycotoxins and their association with composting and noncomposting activities/sources are also reviewed and evaluated. The common natural source exposures to AF and bacterial endotoxins in air and in organic materials and dusts are compared to the exposures at and around composting sites. In addition, this report highlights other aspects of bioaerosols that are important to the evaluation of possible health effect concerns, but which are not fully answerable at present because additional basic data are needed.

Executive Summary

Recycling of biosolids and the organic fractions of municipal solid waste is increasing because of the benefits that can arise and because the disposal alternatives, e.g., landfilling and incineration, are unpopular, too costly, or legislatively restricted. Composting is one of the major treatment processes used to transform wastes into agriculturally useful products. The potential health risks associated with exposure to biological aerosols (hereinafter referred to as bioaerosols) generated from the processing and handling of composted organic materials are an important concern in jurisdictions evaluat-

ing existing compost installations or planning new ones. Other potential health concerns associated with composting of biosolids, municipal solid wastes, and certain industrial wastes, such as plant uptake of heavy metals and worker exposure to synthetic and volatile organic and inorganic compounds, have been reviewed and evaluated by numerous other investigative teams, and are thus not part of the assessment reported here.

In January 1993, the Composting Council assisted the U.S. Environmental Protection Agency (EPA), U.S. Department of Agriculture (USDA), and National Institute of Occupational Safety and Health (NIOSH) in convening a two and one-half day workshop at which twenty five scientists and engineers reviewed, discussed, analyzed and debated the concerns, facts, and current status of the question, *"Do bioaerosols associated with the operation of biosolids or solid waste composting facilities endanger the health and welfare of the general public and the environment?"* The collaborative efforts of that workshop and subsequent reviewers' comments have led to the development of this report.

Bioaerosols of concern during composting consist of microorganisms (actinomycetes, bacteria, and fungi), arthropods, protozoa, and organic constituents of microbial and plant origin. While much public concern has focused on the fungus *Aspergillus fumigatus* (AF), workshop participants recognized that other biological constituents in compost feedstocks and compost could be of concern. Such other biological constituents have led to significant exposure effects in workers in other occupational settings where organic materials and dusts are aerosolized in large quantity and often are of greater concern where ventilation is limited. The reporting of the occupational exposures by the workshop is included as an important point of reference and is not meant to imply in any way that the levels of exposure or response from composting operations and compost will be of similar magnitude and effect.

Risks from secondary pathogens like AF, respiratory irritants, and allergenic components are the major emphasis in this document, since the risks associated with primary pathogens, like bacteria, viruses and helminth ova, have been reviewed and evaluated by others.

Significant amounts of research data on exposure concentrations and responses to airborne endotoxins (i.e., the cell walls of gram-negative bacteria), thermophilic actinomycetes, and other fungal spores in occupational settings exist. Such reports are featured in this review to provide a more inclusive (but by no means exhaustive) picture of the exposures and responses that are possible in worst case occupational settings. Limited information is available on inhalation exposures and responses to β-1,3 glucans (constituents of the cell walls of fungi) and mycotoxins, but these entities are included because they augment other response processes associated with organic dust exposure syndromes. The bioaerosols mentioned above are also found outside of the occupational setting in dust generated from a wide variety of organic wastes including grass clippings, wood chips, food/household wastes, agricultural wastes, and biosolids, even in the absence of planned, high temperature, aerobic composting.

Neighborhood exposure to bioaerosols from composting operations is generally less than occupational exposure. However, the biological constituents in commercially prepared composts are of similar type to those in homeowner and noncommercial endeavors. Hence, the potential responses that may result from inhalation of bioaerosols from composts are the same as those that can result from inhalation of a variety of other organic dusts. The responses can vary and are host- and dose-dependent, i.e., some individuals may respond to concentrations that do not affect others. The responses can range from mild cases of inflammation, to allergy, or to serious tissue or systemic infection by secondary pathogens. There are several responses that intergrade between the mild/benign and the serious extremes. Inflammation responses

can be stimulated nonimmunologically by irritants or immunologically by immune system mediators. Inflammation reactions can be mild and localized as with Mucous Membrane Irritation (MMI), or more generalized, as with Organic Dust Toxic Syndrome (ODTS), or more intense, as with Hypersensitivity Pneumonitis (HP). The intense HP responses result from respiratory exposure to extremely high spore concentrations, e.g., $10^8/m^3$, after a period of sensitization, which may consist of repeated exposures to much lower concentrations, e.g., 10^5-10^6 spores/m^3. The HP response is characterized by an allergic component as well.

Allergenic responses also involve mediators that stimulate inflammation, consequently the distinction between inflammation and allergy is less certain. Like inflammation responses, allergic responses can also present a broad range of symptoms, e.g., from mild itching, watery eyes/nose, to coughing and sneezing, to wheezing or more severe respiratory distress, as with asthma. Saprophytic fungi and pollen are well known types of aeroallergens involved in respiratory allergy and asthma. Allergic rhinitis (i.e., nasal congestion due to immune system sensitivity to allergen(s)) is a common, benign response in which a wide variety of airborne allergens have been implicated.

At present, neither minimum threshold levels nor dose-response data are available for AF, β-1,3-glucans, mycotoxins, or enzymes that stimulate inflammatory, allergic, asthmatic, or infectious processes in humans. However, individual cases that involve such agents in adverse respiratory reactions have been reported in the medical literature. These are considered as verified observations that warrant attention in the evaluation of health effects and in the design of future response studies, but they are not predictive about what will happen at the community or population level. For evaluation at that level, statistically well-designed epidemiologic study results would be needed to help establish dose-response impacts and allocate risk among the various sources of environmental exposure.

The verified health effects data, that have been observed for occupationally exposed individuals, have shown that infection (i.e., invasive growth of pathogenic microorganisms into body tissues, organs, or systems) caused by opportunistic (secondary) pathogens indigenous to organic dusts from any source is *extremely rare*, even among workers who are exposed continuously to high concentrations of various bioaerosols. When such invasive or systemic, opportunistic infections occur they usually occur in individuals whose immune defense systems are very severely compromised (functionally abnormal) because of genetic or acquired conditions. Such individuals are at risk to infection from microbes in the general environment, which contains natural sources of these organic dusts and associated microbes. Immunocompetent, corticosteroid managed asthmatics are susceptible to a specific response known as Allergic Bronchopulmonary Aspergillosis which involves the growth of *Aspergillus* species in the airways (but not the lung tissue *per se*).

None of the published or unpublished reports to which this workgroup had access either directly or through computerized databases such as Medline, Toxline, Agricola, CAB abstracts, Biological abstracts, Biosis, and CAIN, provided any dose-response data for AF, other fungi in composts, β-1,3 glucans, mycotoxins, enzymes, viruses, or bacteria in terms of inflammation, allergy, asthma, or opportunistic infections. However, based on considerable volumetric air sample data involving indoor, occupational endotoxin exposures and responses, the International Committee on Occupational Health has suggested threshold response ranges for gram-negative bacterial endotoxin as follows: 1,000-2,000 ng/m^3, organic dust toxic syndrome (ODTS); 100-200 ng/m^3, acute bronchoconstriction; and mucous membrane irritation, 20-50 ng/m^3. These endotoxin exposures are relatively high in comparison with outdoor nonoccu-

pational settings, such as in a residential community. In the absence of threshold response ranges for AF and other constituents of composts, we examined what some typical community and neighborhood exposures might be.

Published airspora* studies contain both qualitative and quantitative data confirming that the microorganisms found at compost sites, in the compost products, and their various feedstocks are also found in the ambient air environment, and, as such, should be considered as part of the total airspora. The quantitative studies show that, although concentrations may be higher at compost sites during vigorous movement of the compost, certain natural sources as may be encountered by the public during daily activities may generate very high concentrations of certain fungi and actinomycetes, notably *Aspergillus fumigatus* (AF) and *Thermoactinomyces vulgaris*. Outdoor airspora concentrations, including AF, vary seasonally; peak seasons differ by locality. Reported concentrations of AF in outdoor air range from 0-686 colony forming units (CFU)/m^3. In general, the outdoor airspora is dominated by the saprophytic fungi, i.e., *Alternaria, Cladosporium, Aspergillus*, and *Penicillium*. The concentration of thermophilic actinomycetes in outdoor air is typically so low that any concentrations above 10 CFU/m^3 strongly suggests that self-heating organic material had been aerosolized nearby the sampler site. For this reason, thermophilic actinomycetes can be regarded as indicator organisms in studies of aerial dispersion of compost bioaerosols.

Indoor air, while somewhat seasonally variable, can also contain high levels of bioaerosols dominated by *Penicillium* and *Aspergillus*, during interior housekeeping; and bioaerosols increase during indoor housekeeping activities. Indoor occupational exposures in agricultural environments where organic dusts are generated, i.e., grain elevators, barns with moldy hay, and mushroom production facilities, represent the most intense exposure situations. It is noteworthy that even these extreme exposures have not led to any increased *infectious* diseases by AF in exposed worker populations.

Bioaerosol monitoring data from several biosolids composting operations, which differ in design and feedstock, were compared to each other and to the ambient natural airspora data. The highest concentrations of aerobic bacteria, thermophilic (heat loving) fungi, and AF were detected directly on the composting sites during peak operations. As expected, concentrations of these microorganisms in air downwind from the centers of the composting sites were less than the peak values on-site, and in most cases less than or nearly similar to those at the upwind sites, (i.e., not significantly different from normal ambient exposure as if the composting facility were not present). Yard waste composting sites also produce AF and thermophilic actinomycetes aerosols, that on average are similar to those from outdoor biosolids composting facilities. There is almost no data on other bioaerosol constituents downwind of any composting site whether it be for processing biosolids, yard waste or other organic material.

To the extent that it is desirable to mitigate bioaerosols, design, siting, and operational factors are important tools. Controlling temperature and moisture of actively composting materials and stored compost and feedstocks, and timing and minimization of mechanical agitation during favorable atmospheric conditions, will abate dust and minimize the growth and proliferation of bioaerosol agents. Site enclosure, biofilters, compost scrubber piles, and site topographic and landscape design may be used in various combinations to abate bioaerosol transport downwind. Buffer distances to the surrounding community will depend on facility size, design, and operational factors. Current bioaerosol monitoring data and data from a few experimental studies

* Fungal and actinomycete spores/propagules collected from the air; identified by microscopic morphology, by in vitro culture method(s), and/or biochemical/serotaxonomic characteristics

provide the basis for estimating source strengths, and downwind concentrations associated with particular design and operational parameters. Models are helpful for predicting downwind concentrations of bioaerosols from area sources of bioaerosols, but are not overly precise.

Several conclusions reached by the working group included:

1) The general population is not at risk to systemic (i.e., whole body, generalized, as in circulatory, lymph etc.) or tissue infections from compost associated bioaerosol emissions.

2) Immunocompromised individuals are at increased risk to infections by various opportunistic pathogens, such as *A. fumigatus*, which occurs not only in compost but also in other self-heated, organic materials present in the natural environment.

3) Asthmatic and 'allergic' individuals are at increased risk to responses from bioaerosols from a variety of environmental and organic dust sources, including compost. *A. fumigatus* is not the only or even the most important bioaerosol of concern in assessment of risk for ODTS, MMI, and HP (extrinsic allergic alveolitis) associated with exposure to dust from organic materials. The amounts of airborne allergens that sensitize and subsequently incite asthmatic or allergic episodes cannot be defined with current information available, especially given the wide variation in host sensitivity, the numerous sources of natural environmental exposure, and the diversity of constituents and bioaerosols. Prospects for such precise definition are limited in the short-term because of these factors.

4) In spite of the fact that some types of bioaerosols can cause occupational allergies and diseases, and that some of the same types of bioaerosols are present in the air at facilities that compost organic materials, available epidemiological evidence does not support the suggestions of allergic, asthmatic, or acute or chronic respiratory diseases in the general public at or around the several open air and one enclosed composting sites evaluated.

Hence, the answer that emerged to the question posed at the beginning of the workshop is: *"Composting facilities* ***do not*** *pose any unique endangerment to the health and welfare of the general public."* The major basis for this conclusion is the fact that workers were regarded as the most exposed part of the community and where worker health was studied, for periods of up to ten years on a composting site, no significant adverse health impacts were found. In addition, in most cases the measured concentrations of the targeted aerobic bacteria, thermophilic (heat loving) fungi, and AF bioaerosols in residential zones around composting facilities showed that the airborne concentrations of bioaerosols were not significantly different from background, (i.e., as if the composting facility were not there). A likely reason that the bioaerosol levels were not significantly different from ambient is because the naturally decomposing self-heating organic matter on which these subsequently aerosolized microbes thrive are widely distributed throughout the environment.

5) Occupational exposure to bioaerosols on composting sites may be significant, depending on the circumstances at the site, operational characteristics, and worker proximity. Compost site workers are clearly more exposed to compost bioaerosols than are the surrounding populations. However, as already stated, worker populations at such facilities thus far have not shown any significant differences in overall body or respiratory fitness as compared to nonexposed persons. On the other hand, adverse health effects have been observed in a few workers at such commercial facilities as those for producing mushrooms or processing wood chips and bark. This suggests that future worker studies should include systematic assessments for Mucus Membrane Irritation, Organic Dust Toxic Syndrome ODTS, HP (extrinsic allergic alveolitis), and related disorders in low, chronic exposure situations, i.e., situations in which exposures

of 10^4-10^5 CFU/m^3 are generally present.

6) Because of continuing public concern, and because of the wide range of potential respiratory responses to organic dusts, additional study would be helpful to further verify this apparent lack of adverse health impact from composting facilities. Two kinds of studies (epidemiological and annoyance studies) would be helpful for defining potential impacts of bioaerosols from any source, composting or otherwise. Epidemiological studies would help define dose-response relationships and if carefully planned and conducted could perhaps document clearly any negative health effect impacts on a community population near a compost site. Such epidemiological studies are expensive and difficult and have not yet been conducted around composting facilities. If such epidemiological studies are conducted, they should include nonsubjective measurements like pulmonary function measurements, serology to antigens from compost bioaerosols and microbial serotypes in the exposure environment, full medical histories of individuals, and other measures appropriate to quantitate irritant responses to organic dusts.

7) Annoyance studies are much easier to conduct; they can and have yielded useful information at far less cost. If carefully planned and conducted in communities near compost sites and coupled with environmental measurements of actual exposures, these studies can help document annoyance as related to the presence or absence of bioaerosols and other factors such as malodor, irritation, malaise, noise, visual concerns, and traffic. The procedures for assessment of annoyance are available and could be valuable to community impact evaluation processes because they offer a systematic mechanism for recording observations (olfactory or otherwise), corroboration, correlation, and interpretation. Augmentation of annoyance studies with a limited number of nonsubjective measurements could help in the separation of correlation into cause and effect.

Introduction and Purpose

In recent years, as composting* has gained increasing acceptance as a process for transforming significant portions of municipal solid waste into stable, agriculturally useful organic material, waste management planners have had to balance their evaluation of technological solutions against concerns about public health and safety. Many of the concerns associated with other waste management practices also have been raised in conjunction with the currently expanding compost industry. For example, citizens have resisted construction of waste management facilities in their neighborhoods because of concerns about possible impacts on property values (due to aesthetics), increased neighborhood traffic, noise, odor, water pollution and airborne dust.

Without doubt, odor issues have been the foremost public concern associated with planned or operational composting facilities. However, citizens groups recently have raised concerns about possible health effects associated with inhalation of airborne dust transported from nearby facilities. Like other organic dusts, airborne dust from compost operations contains materials of animal, vegetable and microbial origin; airborne dust is readily generated during standard processing operations.

This report focuses on the dispersion of inhalable organic dust in and around composting facilities as well as on the possible health effects of the microbial constituents as they relate to infection, inflammation, allergy and annoyance. Special emphasis will

* Composting is a controlled, aerobic, high temperature, biological decomposition process which converts solid organic matter into a humus-like mixture through the growth and activity of mixed populations of actinomycetes, other bacteria, and fungi that are indigenous to the various organic wastes that are composted (Golueke, 1992).

be given to the opportunistic fungus, *Aspergillus fumigatus* and to bacterial endotoxins, and mycotoxins, and their association with composting and noncomposting activities/sources. The common natural source exposures to AF and bacterial endotoxins in air and in organic materials and dusts will be compared to the exposures at and around composting sites. In addition, this report will highlight other questions that are important to better understand the possible health effects issues which are not fully answerable at present because additional basic data and information are needed.

Composting: Scope and Process

The U.S. Environmental Protection Agency (USEPA, 1989) has challenged communities in the U.S. to reduce and recycle at least 50 percent of its municipal solid waste by the year 2000, and many states have established similar recycling goals. Most states consider composting of yard trimmings and food scraps as a form of recycling and are, therefore, implementing composting plans to achieve recycling goals. This reflects recognition that as much as 40 percent of household "waste" consists of compostable food scraps and yard trimmings. As a result, composting has increased, and so have some of the perceived and actual problems associated with the activity. Additional information about the scope of the composting industry and various technical approaches are described and referenced in the Appendix to this report.

Compost Bioaerosols: Characteristics and Properties

By the very nature of the product, compost contains large amounts of organic materials of biological origin. When processed properly, i.e., with minimal odor production, the product will contain less than 20 percent moisture and be easily friable. In fact, the lower water content and workable texture are some of the advantages of the material for horticultural and agronomic uses. During ordinary handling of the organic feedstocks (leaves, wood chips, grass clippings), composts and screened products, some of the biological components can be released into the air. General descriptions of a variety of these components are provided below.

Procedures for appropriate collection, measurement and quantification of bioaerosols vary according to the environment being sampled, but the most reliable and useful methods are volumetrically based, i.e., collection of particles and molecules present in a known quantity of air. Thus, results are reported in terms of milligrams (mg) of the specific material/m^3 of air. In the case of viable microorganisms, results are reported as colony forming units (CFU)/m^3 of air. A colony forming unit is a microbiological term used to refer to a microbial colony that grows on nutrient agar in a petri dish; the colony may have started from one or more (as in a tight cluster) spores, hyphae, bacterial cells or organic particle(s) in which the microbe(s) reside. In the case of bacterial cells and viruses, death rate and inactivation are sensitive to fluctuations in the air temperature, moisture, UV radiation and temporal and spatial distance from the emitting source (Gregory, 1973). Spores are typically more resistant to these variables.

General Definition, Characteristics and Properties

Compost bioaerosols may contain actinomycetes, other bacteria, fungi, arthropods, protozoa and organic constituents of feedstock materials (Table 1). Microbial products include endotoxin, microbial enzymes, β-1,3-glucans and mycotoxins. The focus of this report will be on inhalable microbial particles and their constituents as

they relate to infection, inflammation, allergy and annoyance. Although many different types of fungi occur in composted materials and various ones may serve as potential allergens, the primary focus of concern has centered on *Aspergillus fumigatus* because it can also be involved with infection under special circumstances which are described in the Responses section.

Aspergillus fumigatus

A. fumigatus, one of the most prevalent Aspergillus species, has been isolated from soils worldwide including Antarctica and all other continents. It has been isolated from temperate and tropical zone soils, humus, and from extreme habitats such as drilling cores from Japan at 1,800 to 2,100 foot depths, deserts, caves and mines. It is associated with soils of numerous crop plants and is reported from bird's nests, bird droppings, chicken roosts, dung of cattle and horses, hay, fodder, corn, straw, grass and compost (Domsch *et al.*, 1980), from refrigerator and bathroom walls (Wyngaarden and Smith, 1988), and from building ventilation systems in which molds have had a chance to grow (Seabury *et al.*, 1973).

It is also one of the most frequently found fungal species in airspora surveys. It is able to grow and survive over a wide range of temperatures (12-50°C), relative humidities and substrates (Millner, 1985). Some of its physical features include typically columnar conidial ('spore') heads which macroscopically appear drab, olive green and velvety to floccose. Individual spores (conidia) are small (2 µm in diameter) and can be carried for some distance by very light wind currents; if inhaled, the spores can enter the lungs (Gregory, 1973).

TABLE 1.
Compost feedstocks as sources of bioaerosols.

	Most Important Biohazards							
				Enteric	Allergenic			Other Specific
Feedstock Category	AF	Endotoxin	TA	Bact/Vir.	Fungi/	Bact/	Mites	Biohazards
Yard waste (including grass clippings, brush, leaves, wood chips)	+	+	+	-	+	+	+	Allergenic pollen, terpenes, resins, lectins, phenols toxalbumin.
Food/Household waste (MSW)	+	+	-	+	+	+	+	
Food Processing Winery waste (grapemarc) and cheese	+	+	+	+	+	+	+	
Fisheries (shellfish)	+	+	+	+	+	+	+	Allergens of crustaceans
Agricultural Wastes (cotton gin & textile waste, garden wastes sugarcane/pineapple)	+	+	+	+	+	+	+	Deep mycoses pollen, terpenes, resins, lectins
Biosolids	+	+	-	+	+	+	+	
Animal Wastes								
Carcasses	-	+	-	+	-	-	-	
Manures	-	+	-	+				
Barnyard manure	+	+	+	+	+	+	+	

+ = present; - = not present

Bacterial Endotoxin

Bacterial endotoxin is the chemically complex portion of the outer layer of cell walls of gram-negative bacteria. Endotoxins are very heat stable, lipopolysaccharide-protein complexes (Rietschel *et al.*, 1993) that are released into the environment during cell growth and after the cell dies (Windholz *et al.*, 1976; Bradley, 1979). In the airways, intact (living or dead) bacterial cells can be engulfed by macrophage cells which process them and release the endotoxins (Duncan *et al.*, 1986). Endotoxins increase the activity of macrophages which leads to a series of inflammatory events described below (Burrell and Rylander, 1981; Rylander and Snella, 1983).

Gram-negative bacteria and their endotoxins are ubiquitous; they are found in the soil, water and in other living organisms throughout the world. Organic dusts are a common source of airborne endotoxins (Olenchock, 1990). The first reports on endotoxins in organic dusts dealt with dust in cotton mills (Cavagna *et al.*, 1969), followed by studies from the UK (Cinkotai, 1976) and Sweden (Rylander *et al.*, 1985). Endotoxin was detected in water from contaminated humidifiers (Rylander and Haglind, 1984) and then in a variety of agricultural environments. However, they can also be found in many other environments, including office buildings and libraries where humidification systems are used (Dutkiewicz *et al.*, 1988).

Agricultural environments have been the most frequently evaluated because worker exposure to large amounts of organic dust and its constituents is a frequent and commonly reported factor associated with the onset of pulmonary symptoms. Thus, endotoxins have been measured in organic materials and dusts generated in very diverse agricultural environments, such as occur during handling of stored grains, silage, hays, straw and animal bedding material (Olenchock *et al.*, 1990a); composted wood chips (Olenchock *et al.*, 1991) and stored timber (Dutkiewicz *et al.*, 1992); tobacco; baled cotton (Olenchock *et al.*, 1983); mushrooms, including production materials such as manure, compost and spawn (Olenchock *et al.*, 1989); swine confinement units (Donham *et al.*, 1989) and poultry confinement and processing facilities (Lenhart *et al.*, 1990); horse and dairy cow barns (Olenchock *et al.*, 1992; Siegel *et al.*, 1991). In other industrial settings endotoxins have been measured in association with humidifiers, air conditioners, cooling towers and other water associated processes (Dutkiewicz *et al.*, 1988; Rylander *et al.*, 1978; Flaherty *et al.*, 1984); dusts generated during processing of cotton, wool and flax (Castellan *et al.*, 1987; Kennedy *et al.*, 1987; Ozeami *et al*, 1987; Rylander and Morey, 1982); waste disposal (Nersting *et al.*,1991; Sigsgaard *et al.*, 1990), sewage and sewage composting (Mattsby and Rylander, 1978; Lundholm and Rylander, 1980, 1983); animal feed production (Smid *et al.*, 1992a,b); and biotechnology (Olenchock, 1988; Palchak *et al.*, 1988).

Glucans

Fungal cell walls contain the polymer β-1,3-glucan which is a polysaccharide composed of glucose units joined by β-1,3-linkages. This polysaccharide is also found in cereals (barley and oats) and in certain bacteria. In the lung, β-1,3-glucans depress macrophages (Rylander, 1993b) which compromises their normal reactions to other agents such as endotoxins and antigens (Fogelmark *et al.*, 1991; COD Report, 1991d,1993).

Mycotoxins

Mycotoxins are toxic metabolites of fungi that may be present in mycelium, excreted into the substrate or found in spores (Sorenson *et al.*, 1987; Wicklow and Shotwell, 1983). In general, mycotoxins are heat stable, nonpolar, low molecular

weight compounds (MW<1000) (Wyllis and Morehouse, 1977). Mycotoxins may be cytotoxic, mutagenic, teratogenic, carcinogenic and/or immunotoxic (Ciegler *et al.*, 1981) and certain mycotoxins have been shown to be acutely toxic to alveolar macrophages (Gerbarick and Sorenson, 1983; Gerbarick *et al.*, 1984; Sorenson *et al.*, 1985; Sorenson *et al.*, 1986) and in the lung (Sorenson *et al.*, 1982).

Mycotoxic fungi are ubiquitous and have been found and quantified in stored grains (Olenchock *et al.*, 1990b; Parker *et al.*, 1988), silage (Dutkiewicz *et al.*, 1989; Morey *et al.*, 1989), hay (Shen *et al.*, 1990) and straw (Shen *et al.*, 1990). Workers are known to be exposed to aflatoxin during harvesting of corn (Burg *et al.*, 1982) and aflatoxin has been measured at 143 ppb in airborne dust around combine harvesters (Popendorf *et al.*, 1985). However, the health effects which may result from ingestion or inhalation of aflatoxin by workers is unknown (Merchant, 1982).

Organic Dust: Exposure Effects

Considerable information is available on human health effects which result from exposure to microbes and other biological components in organic dusts. Inhalation is the major exposure route; consequently, the effects on the lung have received the greatest attention. Some reports demonstrate, however, that systemic effects may also occur, either as a result of ingestion of material cleared from the lungs or through release of bioactive substances from the cells of the lung into the blood. These cellular reactions are part of the normal response of the human system to biological and biochemical agents.

Early reports of the respiratory effects of organic dusts and associated microbes emphasized the situations where symptoms occurred (e.g., farmers lung and grain handlers' and pigeon breeders' diseases). It is now realized that these different lung diseases* associated with organic dust inhalation are similar and that they can be classified according to pathogenic mechanisms (Rylander and Peterson, 1990).

Inflammation

Inflammation in the tissue exposed to airborne microbes can be caused by several components associated with microbes. Bacterial endotoxins from gram-negative bacteria, proteases, water soluble forms of fungal β-1-3-glucans and mycotoxins are the most studied of such agents; others are probably also present.

Inflammation responses to inhaled organic dust can range from relatively mild, almost benign, to severe. Inflammation responses are nonimmunologically mediated, although the symptoms can resemble the same that are manifested in immunologically mediated responses such as allergenic processes. Numerous observations of individuals in various occupational settings have been accumulated and suggest a continuum of responses. These responses are summarized by general and specific types in Table 2, along with their major symptoms, and some of the references which provide details of each response type.

Rylander (1993a) noted that repeated exposures to organic dusts in occupational settings can cause *mucous membrane irritation* (MMI), which is characterized by irritation (itching and watering) in the eyes, nose and throat (Richerson, 1990). The under-

* "Disease" was defined by Campbell *et al.* (1979) as the sum of the abnormal phenomena displayed by a group of individuals in association with a specific set of characteristics by which they differ from the norm in such a way as to place them at a biological disadvantage. Organic Dust Toxic Syndrome and Hypersensitivity Pneumonitis fulfill these criteria. Mucus Membrane Irritation and the intermittent symptoms (cough and sputum) associated with grain dust exposures may or may not fulfill the criteria (Richerson, 1990).

TABLE 2.
General relationships between types of responses, specific designations, and symptoms resulting from inhalation of dust from organic materials*

Type of Response	Specific Designated Response	Major Symptoms	Dusty Organic Materials**/(Endotoxin)	References with Summaries
Inflammation (Nonimmunologically Mediated Specific IgE's Normal)	Mucous membrane irritation (MMI)	Irritation (itchy, watery) of eyes, nose, and throat, including dry cough; no elevated IgE	Cotton, compost and grain dust/(0.02-0.05)	Richerson, 1990, 1993
	Acute bronchoconstriction	Contraction of bronchial smooth muscles resulting in reversible narrowing of the airways; acute decrease in FEV_1 after exposure	Cotton, grain, animal and compost dust /(0.01)	Castellan *et al.*, 1987
	Chronic bronchitis	Increased secretion of mucus with productive cough persisting over time	Wood, grain, cotton, tea dust /(0.1-0.2)	Kilburn, 1986
	Toxic pneumonitis (organic dust toxic syndrome)	Influenza-like with fever, chills, muscle and joint pains, fatigue, headache	Cotton, grain, animal dust 'sick' buildings, mycotoxin/(1-2)	Rylander and Peterson, 1990, COD, 1991 b, c
	Nonallergic asthma (irritant receptor type)	Chest tightness/ pressure; pulmonary eosinophilia; specific IgE's normal	Grain, wood, green/dried plant and coffee bean dust	Salvaggio *et al.*, 1986
Allergy (Immunologically Mediated; Specific IgE's)	Hypersensitivity pneumonitis (allergic alveolitis)	Granulomatous pneumonitis, often with radiographic evidence of lung fibrosis when exposures are repeated frequently	Moldy hay & straw, thermophilic actino-mycetes/fungi, amoebae, animal proteins, humidifier reservoirs	Fink, 1986 Pepys, 1969 Edwards *et al.*, 1976
	Allergic asthma	Chest tightness/ pressure, pulmonary eosinophilia	Grain, Wood, green/dried plant and coffee bean dust	Salvaggio *et al.*, 1986
	Allergic rhinitis	Itchy, watery eyes, sinuses and throat; sneezing; histamine release	Pollen, fungal spores, proteins, enzymes, insect debris	Salvaggio *et al.*, 1986: Burge, 1985, 1990
Infection	Necrotizing pneumonia -invasive, systemic	Abnormal x-rays/CT scans; isolation of microbes from normally sterile tissues or body fluids (see Appendix for Invasive Aspergillosis)	Specific bacteria and fungi	Denning and Stevens, 1990, Meeker *et al*,1991, Zuk *et al.*,1989
	Allergic bronchopulmonitis -noninvasive, nonsystemic	Microgranulomas on chest x-rays; dyspnea 4-6 hrs after exposure to agent; decreased FEV_1; microbe-specific IgE in serum (see Appendix Case Definition of ABPA as example)	Aspergillus and possibly other microbes	Stevens, 1992

*See text for descriptions and details of symptoms. Distinctions between certain manifestations of inflammation and allergy are unclear (e.g., allergic vs. nonallergic asthma and MMI vs. allergic rhinitis) without data on the presenting symptoms, clinical tests and patient history, including exposure circumstances.

** Some of the reported dusty organic materials, and *proposed* threshold levels ($\mu g/m^3$) for endotoxin (COD) 1991, b,c) where data exist.

lying mechanism involves an increase in secretion of inflammatory mediators. MMI can develop after several weeks of exposure to rather low levels of the causative agent. Persons with MMI and who also develop an increased reactivity in the airways may develop bronchoconstriction, i.e. a narrowing of the airways and respiratory deficit. It is not yet known whether continuous MMI in connection with high exposure levels will progressively develop into *chronic bronchitis*, characterized by excess mucus production, increase in mucous glands and changes in the rheological properties of the mucus. Chronic bronchitis usually develops after several years of exposure to probably relatively high levels of the inciting agent. No data are available for microorganisms, but for air pollutants, several $\mu g/m^3$ or more are required to initiate chronic bronchitis.

In the lung after inhalation exposure, the initial stage in the inflammatory response is the activation of macrophages. These cells secrete a series of inflammatory compounds, some of which are chemotaxic and cause the migration of neutrophils from the blood into the lung tissue (Henson and Murphy, 1989). The neutrophilic invasion together with fluid leaking from the capillaries causes a *toxic pneumonitis*, which if severe enough will be recognizable clinically as the organic dust toxic syndrome (ODTS), a nonallergenic process (do Pico, 1986; Von Essen *et al.*, 1990). This reaction, which occurs within hours after exposure, is clinically recognized by fever, influenza-like symptoms and fatigue. Examples of ODTS are pulmonary mycotoxicosis (Emanuel *et al.*, 1975; May *et al.*, 1986), grain fever (Malmberg *et al.*, 1988; Malmberg and Rask-Andersen, 1988) and mill fever (Trice,1940).

Reports on cases with *acute haemorraghic pneumonitis* (Cresia *et al.*, 1990) have been related to extremely high, acute exposure levels of spores ($10^{9\text{-}10}/m^3$). Whether this was caused by mycotoxin or β-1-3-glucan in the spores is not known. ODTS is caused by a single peak exposure which may be of short duration. *Proposed* threshold levels are 10^9 spores/m^3 and 1 µg endotoxin/m^3 (COD, 1991b; Rylander, 1987). Data on acute alveolitis, pneumonitis or ODTS caused by AF do not exist, but experience from animal experiments (Fogelmark *et al.*, 1991) suggests that the threshold for this species could be 10^7-$10^8/m^3$, i.e., lower than for other common spores such as conidia of *Penicillium*.

A particular form of granulomatous pneumonitis, *hypersensitivity pneumonitis*, was first observed among farmers and later in workers in a variety of moldy environments (Lacey and Crook, 1988; Richerson, 1983, 1993). The disease requires a *chronic* exposure to molds and/or thermophilic actinomycetes which cause a lymphocytosis in the airways without clinical symptoms. This response has been referred to as extrinsic allergic alveolitis (Edwards and Al-Zubaidy, 1977). In connection with exposure to very high spore concentrations, on the order of $10^9/m^3$ or more, an acute disease with fever and respiratory impairment can develop. It is unclear whether this is a specific reaction or an ODTS caused by endotoxin contamination on the fungus spores, mycotoxin, or some other constituent of the fungi. Chronic, low level exposures to airborne fungi, such as that which occurs in barns with moldy hay, may in rare cases lead to fibrosis which is observable on lung radiographs (Richerson, 1993; Lacey and Crook, 1988); such exposures can range from 10^5-10^7 spores per cubic meter. Each exposure pattern can be exclusively related to a particular form of disease.

Allergy

The precise distinction between inflammation and allergic disease is by no means clear. Recent evidence from cell toxicology demonstrates that the same inflammatory cells are involved in both processes and that asthma is largely an inflammatory disease (Henson and Murphy,1989). Allergy in a strict sense describes the immunologic reaction to a small amount of material in the environment, after a period of exposure which sensitizes specialized cell systems. Organic dusts contain many antigenic materials, including fungal β-1-3-glucans, gram-negative bacterial endotoxins, pollen and various proteins, all of which are potent modulators of the immune system and may enhance or depress the reaction to allergens (antigens).

Asthma is characterized by an intensive reaction to very small amounts of the agent to which sensitization has taken place, although nonimmune-mediated sensitivity to unspecified irritants can elicit asthmatic episodes in some individuals. During the asthmatic attack, substances which cause inflammation are liberated from ac-

tivated cells and a chronic stage of inflammation with the same characteristics as described above develops. An elevated, short-time exposure can cause an acute asthmatic attack, toxic pneumonitis. A long-term exposure, but not necessarily at high levels, is required for sensitization.

Persons at risk are those with a genetic predisposition to react to allergens in the environment, i.e., to become sensitized to natural products. Approximately, six percent of U.S. citizens have asthma (NCHS, 1992). In occupational exposures, it is now known that several host factors have been recognized as contributory to such responses as grain dust induced lung disease. These susceptibility factors include atopy, smoking, intermediate alpha-1-antitrypsin deficiency and nonspecific bronchial responsiveness (Chan-Yeung *et al.*, 1992). Viral infections also may be involved in susceptibility to HP although more research is needed to confirm this finding (Cormier *et al.*, 1994).

The most common fungal allergen sources are the saprophytic microfungi, e.g., *Mucor, Rhizopus, Cladosporium* and *Aspergillus*. For two of the common molds regarded as the main allergenic fungi, threshold concentrations for evoking allergic symptoms are estimated to be 100 *Alternaria* spores/m^3 and 3000 *Cladosporium* spores/m^3 (Gravesen, 1979). No data are available for Aspergilli, but it is reasonable to estimate that sensitization can occur at similar concentrations.

Infection

Infection caused by airborne microbes in organic dusts can occur either from human pathogens or from organisms that are not true pathogens but invade the tissue of particularly sensitive individuals. Infection due to pathogens in organic dusts is rare even among highly exposed workers. Studies have been made on persons exposed to biosolids in wastewater treatment facilities in the U.S. and in Sweden (Clark *et al.*, 1983, 1984; Lundholm and Rylander, 1980, 1983). No indications of an increased risk of infection were found except under extreme exposure conditions such as when a person who did not know how to swim fell into a sewage water tank and inhaled large amounts of the water.

Among the organisms that are not primary but rather secondary pathogens, AF is of particular interest. It is often referred to as a secondary or opportunistic pathogen because it colonizes and infects persons who are immunocompromised or otherwise debilitated by a preexisting medical condition. AF is a common organism in the general environment; levels up to several hundred/m^3 have been reported. Because the spores are easily inhaled and expectorated, the isolation from sputum cannot be considered diagnostic for disease caused by aspergilli (Pepys *et al.*, 1959). The nearly universal occurrence of circulating *Aspergillus* antibodies (Bardana *et al.*, 1972a,b) reflects the ubiquity of this fungal antigen in the environment. Several investigators have found AF antibodies in healthy persons (Jameson, 1969; Pepys, 1969; Reed, 1978). Furthermore, commercial house dust extracts used for allergy testing contain antigens common to AF (Bardana, 1974). Positive serology to AF is thus not, in and of itself, diagnostic of an adverse health response to AF antigen but rather a marker of exposure.

The lung has a large capacity to defend itself against microorganisms and even very high numbers of inhaled spores may not cause any effects. Occasionally, AF may cause widespread invasion of the tissue. These events require that normal defense mechanisms be compromised, e.g., after severe viral infections or because of drug treatments that suppress the immune system. Reactions to AF are based on the prior health status of the individuals as follows:

Immunocompetent

Healthy individuals with normal lungs typically tolerate even very high numbers of inhaled spores without succumbing to infection. The exact nature of the defense mechanism is not known but cellular and mechanical defenses are probably involved. Healthy individuals with abnormal lungs, e.g. with pulmonary cavities from tuberculosis or sarcoidosis, can have aspergillus colonies in the cavities or airways (see Appendix II, ABPA, and Table 2). The prognosis of such cases depends on the general health and pulmonary status of the patient (Glimp and Bayer, 1983).

Immunocompromised

If the immune defense system in humans malfunctions, there is increased risk of infection. Persons receiving immunosuppressive drugs, high levels of corticosteroids or antibiotics, or who have hematologic malignancies or suffered trauma/burns have a decreased immunity and are in this category. In such persons, AF from the general environment may colonize the lung airways, initially growing saprophytically with formation of "fungus balls" or aspergillomas (= mycetomas). There is a strong antibody response to this growth, but clinical symptoms may be absent for many years. If the balance between immune defense and growth is further distorted, invasive growth may take place, and this leads to a generally fulminant and fatal situation.

Immunodeficient

Persons particularly at risk to infection from common pathogens are those with a genetically determined impairment of the immune system. These impairments "are termed immunodeficiency diseases and afflict, in varying degrees, approximately one in every 500 U.S. citizens." (NIAID, 1991, p.13). Acquired immunodeficiency disease (HIV/AIDS) also increases a person's risk to a variety of primary and secondary pathogens.

General Effects and Reactions

Exposure assessment is further complicated by the nature of the contaminants. The same types of microbes found in composting facilities also are present in the normal environment, sometimes in high concentrations. Levels of up to 4.5×10^5 fungal spores/m^3 were reported in studies of domestic interiors in the UK (Hunter *et al.*, 1988). Consequently, it is extremely difficult, if not impossible, to conclude that measured levels at particular sites cause specific types of *noninfectious* disease in individuals unless more information is available about the antigen sensitivity of those individuals.

Annoyance Reactions

Annoyance can be defined as a feeling of displeasure with an environmental factor, which is known or believed to have an influence on health (Borsky, 1971).

The extent of annoyance in a population can be studied using standardized questionnaires via interviews or mail. The investigation is generally presented as a survey on environmental conditions in general; questions are posed on a variety of possible sources of annoyance, e.g., noise from road traffic, air pollution, presence of industry, and general satisfaction with the environment on the site. Assessing the extent of annoyance in populations around composting plants would be appropriate in evaluation of their impact on the population. In this approach, the extent of annoyance in a pop-

ulation is not determined by the number of complaints, since complaints are dependent upon many factors unrelated to exposure, such as the persistence in accessing the appropriate authorities to deal with the matter, evaluation of the impact of registering complaints and community responsiveness to issues. Thus, this approach can assist with objective assessments. In addition, dose-response relationships can be obtained by studying respondents chosen from areas with different exposure levels. These can be calculated or measured. A nonexposed population would be included for reference. This approach appears not to have been used yet at compost facilities.

In Sweden, health authorities have used 10 percent very annoyed as a threshold for emissions in densely populated areas. When the extent of annoyances exceed 10 percent, requests are made to decrease the emissions (Rylander, pers. comm.).

Exposures: Nonoccupational

Current Perspectives on Airborne Exposure to Potential Allergenic and Pathogenic Fungi, Especially Aspergillus fumigatus

The air that we breathe is a very complex environment filled with constantly changing microorganisms, parts of microorganisms and organic molecules. The amounts and kinds of biological particles can change several orders of magnitude with wind, precipitation and season. Air contains spores of most of the thousands of known species of fungi and bacteria, most of which are not associated with the composting process. The natural environment of outdoor and indoor air contains all of the bioaerosols that are of concern in composting; they are present in variable amounts. *Aspergillus fumigatus* and thermophilic actinomycetes are components of this airspora (environment) and their natural occurrence is recorded in surveys cited herein.

In support of a comparative approach to exposure assessment, a review is presented herein on published reports of ambient levels of fungi found in both outdoor and indoor environments, with emphasis on AF. Exposure assessment involves measuring the actual or potential exposure of humans to harmful agents. Exposure to aerosols of composting materials and particularly of microorganisms represents a complicated pattern with regard to duration and concentrations. An acute (temporary) exposure to a very high concentration can be present for a very short time period, followed by considerably lower concentrations or no exposure at all during the following time periods. A chronic (long-term) exposure can last for several weeks or years. When the exposure is within or below the average range of background concentrations found in the natural environment, compost bioaerosols do not constitute additional exposure. In this review, only reports of volumetrically measured airspora are included.

Avocational exposures to many of the bioaerosols outlined in this report can potentially occur through a variety of activities. Some common activities that provide for potential exposure include lawn mowing, gardening, home landscaping and potting of household plants. Walking through an arboretum or nature trail likewise causes exposure to airborne fungi and bacteria. Environmental sample concentrations for AF and thermophilic actinomycetes obtained using Andersen viable particle samplers as previously described (Millner *et al.*, 1980) are shown in Tables 3 and 4.

Aspergillus fumigatus is the primary species of concern because of its predominance in the composting process and its multiple potential health effects, but *Paecilomyces variotii* Bain, *Mucor pusillus* Lindt, *Humicola grisea* var. *thermoidea* Cooney and Emerson, *Humicola lanuginosa* (Griff and Moubl) Bunce and other thermophilic species are found both in self-heating situations in the natural environment as well as at composting facilities (Cooney and Emerson, 1964; Kane and Mullins, 1973).

TABLE 3.
Seasonal counts of viable *Aspergillus fumigatus* particles in air in the Washington, D.C. Metropolitan Area during 1979-1980.

Site	Seasonal Counts (CFU/m^3)			
	Fall	Winter	Spring	Summer
Lawn				
during mowing	1	5	2	0
with mulch	75	2	6	686
under trees	3	0	5	4
of hospital	2	0	0	0
of park	8	4	24	2
Wooded Area				
arboretum	4	1	6	136
nature trail	56	0	10	8
road side	1	5	2	3
Agricultural				
corn field	1	0	0	4
barn	2,070	105	352	5,550
barnyard	44	0	35	4
poultry coop	21	93	2,060	6
mushroom house	88,700	740,000	580,000	67,100
brush pile	1	1	25	5
Refuse				
municipal dump	6	2	0	5
supermarket dumpster	2	0	0	12
Greenhouse				
potting room	868	1,350	1,070	9,810
low humidity	NS	11	312	1
high humidity	NS	0	152	2
Pool — indoor				
Library-stacks	171	0	0	0
Attic	NS	1	1,160	125
Zoo-birdhouse	5	0	42	2
Boiler room	30	38	1	1
Reference Sites				
School playground	6	1	12	9
University parking lot	7	1	2	4
Shopping center	11	1	7	3

A. fumigatus is one of the most commonly found aspergilli and has been isolated from soils worldwide including temperate and tropical soils, humus and even from some extreme habitats such as subsurface drilling cores (1,800 to 2,100 ft. depths), deserts, caves and mines (Domsch *et al.*, 1980). It is associated with the soils of numerous crop plants and is cultured from bird's nests, bird droppings, chicken roosts, dung of cattle and horses, hay, fodder, corn, straw, grass and compost (Domsch *et al.*, 1980).

Airborne concentrations of AF in natural environments are reported in numerous studies in conjunction with emission tests at composting facilities, around hospitals and other sites (Domsch *et al.*, 1980). The concentration of AF in the natural environment is low in comparison to other fungal allergens, such as *Cladosporium* and *Alternaria*, but AF is more common when compared with most other fungi capable of being human pathogens. Larsen and Gravesen (1991) reported 0-204 CFU/m^3 for all aspergilli (eight percent of the total fungal airspora) in Denmark. They found peaks of

TABLE 4.
Seasonal counts of viable thermophilic actinomycetes in air in the Washington, D.C. Metropolitan Area during 1979-1980.

Site	Seasonal counts (CFU/m^3) Fall	Winter	Spring	Summer
Lawn				
during mowing	2	2	7	0
with mulch	0	0	0	1
under trees	0	0	5	2
of hospital	0	5	0	1
of park	0	8	2	12
Wooded Area				
arboretum	5	0	5	5
nature trail	0	2	4	5
road side	0	1	4	0
Agricultural				
corn field	2	2	1	5
barn	NS	18	0	5
barnyard	NS	132	1	51
poultry coop	0	29	43	1
mushroom house	204	24,600	35,800	3,470
brush pile	1	4	10	5
Refuse				
municipal dump	1	4	2	6
supermarket dumpster	1	1	0	1
Greenhouse				
potting room	13	0	1	0
low humidity	NS	2	12	2
high humidity	NS	0	4	0
Pool — indoor	11	10	3	1
Library-stacks	0	2	4	7
Attic	NS	0	2	4
Zoo-birdhouse	5	1	4	8
Boiler room	4	0	0	1
Reference Sites				
School playground	3	3	3	3
University parking lot	2	1	2	2
Shopping center	2	2	3	3

total viable fungi in July and August, whereas the peak for Aspergilli was in November. They used a IAP Slit-Sampler with VB agar incubated at 25°C.

Mullins *et al.*, (1976) examined average monthly levels of AF in Cardiff, Wales and reported an average for the year of 33 CFU/m^3 and a peak of 537 CFU/m^3 with the Andersen sampling technique. In contrast to Larsen and Gravesen (1991), Mullins *et al.*, (1976), reported higher levels in winter months with a peak in February. Solomon and Burge (1975) reported that less than eight percent of outdoor air contained AF when there were no sources of self-heating matter in the surrounding area. They found levels of 150 CFU/m^3 and less with peaks in the early spring and fall in the U.S. They emphasized that the U.S. data from Michigan is different in amount and peak times from previous English studies. In subsequent studies outside and within a Michigan clinical center, Solomon et at., (1978) found that AF concentrations were similar to those in Cardiff, Wales (Mullins *et al.*, 1976) except that there was no peak in the win-

ter. Later, Mullins *et al.*, (1984) made a direct comparison of air outside of hospitals in Cardiff, Wales and St. Louis, Missouri, in the U.S. They reported very similar yearly patterns and amounts between 0-50 CFU/m^3 except that the peak month was October for St. Louis and November for Cardiff. They further noted that the "*A. fumigatus* concentrations are no higher than those recorded for *Cladosporium* which suggests that conditions for abundant growth of *A. fumigatus* are rare."

Jones and Cookson (1983) sampled air (with Andersen samplers) in the vicinity of a proposed sewage sludge composting site near Washington, DC. They reported 0-7,220 CFU/m^3 of mesophilic fungi (geometric mean 273); 0-193 CFU/m^3 thermophilic fungi (median 2.1); 0-71 CFU/m^3 of AF (median 1.0); 4.2-1,640 CFU (aerobic bacteria)/m^3 (geometric mean 79); 0-5.7 CFU (fecal streptococci)/m^3 (median 0) and no fecal coliforms. They found a November/December peak.

Millner (1985) reported background concentrations for different urban and suburban situations. Low concentrations of AF, i.e. < 10 CFU/m^3, were reported for lawns during mowing, under trees, in front of a hospital and in a park. When the lawn had a mulched area, higher concentrations of AF were found, i.e., 686 CFU/m^3, in summer and fall. Wooded areas also had relatively low concentrations of AF, i.e., 1-10 CFU/m^3, except for a nature trail in fall with 56 CFU/m^3. A school playground, university parking lot and shopping center, likewise had relatively low concentration of AF, i.e., < 12 CFU/m^3. The study also detected very high concentrations of AF in agricultural, home and greenhouse situations.

Domestic Interiors: AF and Other Fungi

There are several studies on the normal fungal airspora of households (most recently reviewed by Summerbell *et al.*, 1992; Flannigan *et al.*, 1991). Many of these studies examined households for the production of agents capable of eliciting allergic reactions in atopic individuals and therefore are questionable indicators of reference values. Much of the older literature on fungal content of household air uses data obtained by the open petri dish settling plate technique. Settling plate studies provide results that are of limited value in assessing exposure because that sampling technique tends to oversample particles of large aerodynamic diameter and settling velocity (and undersample the respirable size particles). The viable particle settle plate sampling technique simply demonstrates that some airborne microbes are capable of landing, germinating and growing on agar plates; settle plate data cannot be used to quantitatively differentiate between outdoor and indoor spore levels or between different concentrations of microbes present. Hence, the method is inappropriate for use an an indicator of exposure and the potential for respiratory health effects.

Another aspect of sampling technique that strongly influences interpretation and comparison of data is the dependence of the technique on the culturability and viability of the microbes collected in the sampling device. To obtain microbial counts for the air sampled by the Andersen, slit and impinger samplers, the microbes collected must be viable and culturable on the nutrient media used. Because viable microbes constitute only a portion of the total airborne microbial content, the techniques which rely on viability and culturability will tend to underestimate the microbial content. In addition, impactor (air to agar) samplers, are subject to rapid overloading of the agar surfaces in very dusty interiors, and this limits sample time. Under such circumstances, the All-Glass Impinger (AGI-30; Davies 1971) is commonly used since collected microbes can be subsequently dilution plated, subjected to immunoassays or other suitable detection techniques which take advantage of the aqueous matrix.

Outdoor airspora commonly contains fungi associated with degrading plant matter such as *Cladosporium, Alternaria and* other mesophilic saprophytic fungi. Indoor mycoflora consists mainly of *Penicillium, Aspergillus* and other species. While the presence of both groups of fungi fluctuates, indoor species appear to fluctuate less dramatically than outdoor mycoflora and have a population peak in the winter, with Penicillia generally more abundant than Aspergilli (Flannigan *et al.*, 1991; Hunter *et al.*, 1988). However, both appear to survive in greater numbers indoors than outdoors on carpeting, wood and other organic substrates when provided with adequate moisture. As might be expected, the indoor rather than the outdoor mycoflora is more abundant in winter in locations that have killing frosts, extended periods of freezing and snow. Indoor exposure to fungi in winter has increased in recent times primarily because of airtight building construction. It is unclear whether this is true in more moderate or tropical climates, or even if there is a good comparative survey of indoor air prior to the use of energy conservation measures.

Fungi have been implicated in the induction of allergies and asthmatic episodes. These episodes have been correlated with the aerial dispersal of spores by activities such as vacuuming, construction and dust movement. However, when "moldy" and "clean" homes were compared for fungal numbers, it was found that they did not differ significantly in spore numbers recovered (Hunter *et al.*, 1988). This resulted in part from the high variation in the number of spores recovered per sample, but it was also a result of room-to-room variation and sampling time in the same room. Rooms with visible mold growth had higher recovered populations of fungi (median value 2,673 CFU/m^3). Rooms in "moldy" houses, except for those with visible mold, had levels similar to those in apparently clean houses (median values of 360 CFU/m^3 and 236 CFU/m^3, respectively). "Clean" houses occasionally had levels as high as "moldy" houses for no apparent reason (23,070 versus 21,790 CFU/m^3, respectively).

It must be noted that these reports are not specific for AF; routine assays specifically for this fungus in indoor environments are limited in number. The levels of AF in a forced hot air heated house and an office were found to be negligible (<512 CFU/m^3) (ERCO, 1980). Millner (1985) reported that the concentrations of AF usually obtained from indoor air were <175 CFU/m^3. Most frequently, "indoor" sites such as an attic, library or boiler room, had AF concentrations between 0 and 50 CFU/m^3 (Table 3). The occasional high concentrations of AF (approx. 1,100 CFU/m^3) were associated with disturbances that would increase the movement of dust. It is interesting to note that similarly high concentrations of AF were found in potting rooms (Millner, 1985); potted plants have been implicated as the source of AF inoculum in nosocomial, i.e., hospital borne, infections (Flannigan *et al.*, 1991).

The literature shows few studies that have included volumetric indoor measurements of AF. Evaluations to date indicate that the fungus can be present indoors, but is not necessarily the major component of the airborne mycoflora. The fact that 13 percent of tested outpatients reacted positively to AF antigen (Hendrick *et al.*, 1975) and that similar percentages were found in tested samples from blood donors (Belin and Malmberg, 1986) indicates that the AF antigen is widespread. Activities that increase indoor dust movement, such as vacuuming, construction, repair and air ventilation, would also increase exposure.

Solomon (1975) measured fungi in and immediately outside several midwestern homes during two seasons, frost free and subfreezing. *Aspergillus fumigatus* was found in 26 homes at a level of 40 CFU/m^3 during the frost free period and similar levels in 80 homes during subfreezing weather. A subsequent study (Solomon, 1976) reported that *Aspergillus fumigatus* was recovered in 31 percent of 47 homes, with a range of 1-

946 CFU/m^3 and a mean of 24.4 CFU/m^3.

Hirsh and Sosman (1976) conducted a one year survey of mold growth in 12 homes. *Aspergillus fumigatus* was one of the most common molds isolated. It was the most common mold found in basements; the second most common in bathrooms; and fourth and fifth most common in front rooms and bedrooms, respectively. Aspergillus was significantly more frequent in homes with pets in comparison to other molds.

Su *et al.* (1992) sampled 150 households in Topeka, Kansas using Andersen samplers. They found that *Aspergillus fumigatus, Penicillium* spp. and other fungi were present in a significant number of homes. These fungi were primarily associated with homes with gas stoves for cooking and basement crawl spaces. The authors attributed this to increased relative humidity in these places.

Occupants (people and pets) and their level of activity (moving or stationary) significantly influence the indoor air concentration of fungi, however, this parameter has not been considered in many of the studies to date.

Other Microorganisms

A study of bacteria in background air by Jones and Cookson (1983) reported the presence of aerobic bacteria at 4.2-1,640 CFU/ m^3 (geometric mean = 79 CFU/m^3), fecal streptococci at 0-5.7 CFU/m^3 (median: 0 CFU/m^3) and no fecal coliforms. The study was conducted in a suburban area of Washington, D.C. near the proposed site for a biosolids composting facility (now known as WSSC Site II).

Thermotolerant and thermophilic actinomycetes are typically sampled with Andersen samplers and enumerated from colony counts obtained from plates incubated at 44°C. *Streptomyces* spp. are particularly abundant but *Actinobifida, Microbispora, Micropolyspora, Saccharomonospora, Saccharopolyspora, Streptosporongium, Thermoactinomyces and Thermonospora* are all possible components of air (Millner, 1985) although there are no published quantitative measurements of individual species. Millner *et al.* (1980) reported from 0-59 CFU/m^3 thermotolerant/thermophilic actinomycetes in background air samples collected at a Beltsville, Maryland site unaffected by composting.

Exposures: Occupational

Noncompost Sites

Much information is available concerning the presence of biohazards in industrial environments. These data are predominantly related to agriculture and to processing agricultural materials. General pertinent reviews are available and include presentations of such topics as agents, diseases and prevention/control (Rylander *et al.*, 1986); work related respiratory diseases and their occurrence, environmental factors, immunological and lung function studies and prevention/socioeconomic aspects (Terho *et al.*, 1987); and health effects, worker risk and symptomatology related to organic dusts and lung disease (Rylander and Peterson, 1990).

No quantitative surveys of AF specifically in office or manufacturing environments are available. Based on results from other ambient surveys, AF concentrations in air of office and manufacturing centers would be expected to be comparable to that in houses, unless free moisture and warm temperatures conducive to fungal growth were present. Wood processing operations such as those at sawmills and pulp/paper factories are primary candidates for concern with respect to potential industrial exposures (other than waste handling) that could present a risk of exposure to AF.

Agricultural

Agricultural exposures to microbes are extensive and often similar to those expected at composting facilities. Exposure to moldy hay, organic or grain dusts have been associated with decreased lung function, chronic bronchitis and farmer's lung. The development of farmer's lung has been related to a chronic exposure to large amounts of respirable, microbially colonized dusts in enclosed barns. The symptoms become more severe as the winter progresses and a thermotolerant microflora develops in the molding hay. The implicated causal agents are certain thermophilic actinomycetes: *Micropolyspora faeni* and *Thermoactinomyces vulgaris* and *T. viridis*. Other environments in which the exposure to thermophilic microbes is similar to that described for farmer's lung are sugar cane processing areas where bagasse is handled, grain handling and clean out and renovation of mushroom houses. The latter three situations have led to descriptions of bagassosis, grain handler's lung and mushroom picker's lung, in the medical literature.

Although AF specifically has not been shown to be involved in farmer's lung, *Aspergillus umbrosus*, occurred in significantly greater numbers along with the thermophilic actinomycetes in a Finnish study of farmers reporting symptoms of chronic bronchitis or farmer's lung (Kotimaa *et al.*, 1987). One report in this supplement also included a measure of the level of microbes such as AF, *A. umbrosus* and thermophilic actinomycetes in the air of barns. Although mean values for AF in air were slightly higher, the researchers found no significant difference in AF concentrations in the barn air of farmers with chronic bronchitis and in barns of asymptomatic farmers (Kotimaa *et al.*, 1987) suggesting an influence of individual susceptibility.

Other measures of AF levels in hay barns have been recorded as low as <70 CFU/m^3 (ERCO,1980) or significantly higher, i.e., 100 up to 5,500 CFU/m^3 (Millner, 1985). The higher levels reported by Millner (Table 3) were similar to those reported in the Finnish studies (Kotimaa *et al.*, 1987). Air measurements of AF which used radioimmunoassays (RIA) for AF have also been conducted in poultry barns during the bedding chopping operation which generated the highest measurements of AF antigen, 70 ng/m^3 (Pratt *et al.*, 1990). These are not comparable to other types of volumetric measurements for AF (CFU/m^3 values) because RIA measures the presence of the antigen which does not require the viability of the microbe. Lack of corresponding viable count data (CFU/m^3) limits appropriate comparison of this study data to that of other studies in which only volumetric concentrations of viable microbes are reported. Other poultry houses in which airborne AF have been measured showed generally low levels (<100 CFU/m^3), except during the spring when 2,060 CFU/m^3 were found (Table 3).

In summary, the available data on airborne AF levels in agricultural occupations show that measurements are highest during mechanical agitation in enclosed structures such as barns, animal holding pens or mushroom houses, especially where fresh air exchange is limited or nonexistent. It is noteworthy that these data show that human exposure has not significantly increased the occurrence of AF infection in farmers; farmers do, however, show higher levels of antibody to AF than do a cross section of blood donors (Belin and Malmberg, 1986). Also, as might be predicted, the tested farmer population had a greater amount of antibody to common, outdoor fungi that are associated with degrading plant material, e.g., *Botrytis, Alternaria, Paecilomyces* and *Penicillium*, compared to the blood donor control group (Malmberg *et al.*, 1985). This increase in antibody levels is, however, an indication of exposure rather than a marker of disease risk.

Mushroom Production

Several studies have examined the biological agents associated with mushroom production. Suspected causes (fungi, bacteria, other organic materials) of mushroom workers' pneumonitis are described in a review (Lockey, 1974). In a study of various phases of the mushroom industry, all materials, from chicken manure to whole mushrooms, were found to be contaminated with endotoxins (Olenchock *et al.*, 1989). Precipitating antibodies were found in both ill and non-ill workers to extracts of the different bulk materials and IgG antibodies were detected to antigens in a panel of HP agents, including *A. fumigatus, A. niger* and thermophilic actinomycetes by enzyme linked immunosorbent assays (ELISA). Although there was no association with disease, antibodies were evidence of biomarkers for exposure and suggest variation in individual susceptibility. Kleyn *et al.* (1981) reported that the total microbial count in airborne dust of stationary bed mushroom houses was 333 CFU/m^3, with 90 percent or more of the isolates belonging to the genus, *Streptomyces.* Fungal spores constituted five percent or less of the airborne dusts. Precipitating antibodies in workers' sera were found against *Bacillus licheniformis, Micropolyspora faeni, Thermoactinomyces vulgaris, Aspergillus fumigatus and Humicola grisea.* Finally, in a study of a specialty mushroom, it was reported that high levels (>10^6/m^3) of 'Shiitake' mushroom spores were found in the growing rooms (Sastre *et al.*, 1990).

Mushroom production facilities can contain very dense concentrations of fungal aerosols during the cleaning operations after harvest: 67,000 to 740,000 AF CFU/m^3 (Millner, 1985). As mentioned above, mushroom house workers can experience an hypersensitivity pneumonitis after exposure to high concentrations of the thermophilic microflora in aerosolized mushroom compost (See also Table 4).

Timber Processing

Specific studies describe the levels of biological agents in industrial situations, e.g., bacteria, fungi and endotoxins were quantified in air of five large wood processing plants and organisms were described in stored timber logs in Poland (Dutkiewicz, 1989). Airborne bacteria and fungi were found at levels of 10^2-10^4 CFU/m^3 and the concentration of endotoxins were observed at levels in the range of 0.24-40.0 µg/m^3. Predominant bacteria included *Enterobacter* sp., coryneform bacteria, and, although the levels were low, *Thermoactinomyces vulgaris.* Predominant fungi included *Penicillium* sp., *Cladosporium brevicompactum* and *Aspergillus fumigatus.* Although microbes within the air of the sawmill were not quantified, types and quantities of gram-negative and positive bacteria, yeasts and filamentous fungi were exhaustively described from stored timber in the U.S. (Dutkiewicz *et al.*, 1992).

Maple bark handler's disease has resulted from a sporadic exposure to a fungus associated with dead maple trees. Also, wood handlers in sawmills in British Columbia have reported occasional respiratory irritations. Inland workers, handling hemlock and fir, reported more symptoms of irritations than did coastal workers who handled red cedar (Enarson and Chan-Yeung,1990). The differences in microbial concentrations among different tree species and between heartwood, sapwood and bark (Dutkiewicz *et al.*, 1992) indicates the possibility for differing exposures to the type and quantity of microbial dusts. Those workers involved in debarking or processing of sapwood wastes into fiberboard or chipboard are also those exposed to high volumetric quantities of bacteria and fungi including AF (12,700, 52,800 and 65,200 CFU/m^3 for total fungi for heartwood, sapwood and bark respectively, de-

scribed as predominantly *Penicillium* and *A. fumigatus*) (Dutkiewicz, 1989). This is relevant to composted wood chips, dust from which may support a considerable load of fungal spores, e.g., 1.4×10^6 CFU/m^3 fungi predominantly *A. fumigatus, A. niger, Penicillium* spp., *Rhizopus stolonifer, Cladosporium* sp. *and Trichoderma* sp. (Olenchock *et al.*, 1991). Measurements of fungi in the air at wood processing facilities have mostly been made in Scandinavian countries which are using wood as a fuel substitute for oil. Air sample analyses provide data on total viable fungi and on total fungal spore (viable and nonviable) concentrations (10^4 - 10^5 /cu. m.) , but do not numerically single out the AF portion of the airspora (Jäppinen *et al.*, 1987; Kolmodin-Hedman *et al.*, 1987). This reflects the recognition that AF is not the only or even the most important bioaerosol of concern in assessment of risk for ODTS, MMI and extrinsic allergic alveolitis associated with exposure to dust from chipped wood and bark. There is one report that includes a measurement for AF in a paper pulp factory, <12 CFU/m^3 (ERCO, 1980).

In summary, industrial exposures to bioaerosols can be quite intense, especially in the wood processing industry. The most significant levels of exposure in these environments are associated with the dust from handling moldy bark and sapwood. Levels are high and are comparable to those in moldy hay exposures, but do not appear to result in fungal pathogenesis, i.e., tissue infection. Data are insufficient to support a specific role for AF exclusively, but exposure to fungal antigens may be responsible for the respiratory complications cited since the measured levels of thermophilic actinomycetes (farmer's lung antigen) are generally low in wood processing exposures.

Cotton Dust

There are several important studies which show a close relation between the extent of different health effects and the amount of endotoxin in airborne cotton dust. In studies in cotton workers, relationships have been found for chest tightness (Cinkotai *et al.*, 1976) and decreases in FEV1 (a measure of pulmonary function) over the workshift (Rylander *et al.*, 1985; Sigsgaard *et al.*, 1992). Other studies that have been performed in experimental cardrooms with cotton workers (Rylander *et al.*, 1983; Rylander and Haglind, 1986) and with previously unexposed subjects (Castellan *et al.*, 1987) using up to 32 different samples of cotton from widely divergent geographic locations show similar relationships. A linear regression model of these data was used to calculate a threshold of *zero effect* for acute FEV_1 at 9 ng/m^3. A level of 33 ng/m^3 was defined as the threshold of acute effect in another study (Haglind and Rylander, 1984).

Associations between airborne endotoxin concentrations and chronic lung disease also have been reported. In an epidemiologic study of 443 cotton textile mill workers in two textile mills in China, a dose-response trend was observed between current endotoxin levels and chronic bronchitis (Kennedy *et al.*, 1987). Results indicated that exposures from 1-20 ng/m^3 constituted an adverse respiratory health effect in exposed workers.

From a separate epidemiologic study in the Netherlands, 315 animal feed workers related symptoms and lung function changes to present and historic endotoxin exposures more than to gravimetric dust levels (Smid *et al.*, 1992a,b). These investigators reported that lung function changes occurred at levels of airborne endotoxins of as little as 0.2 ng/m^3.

Other Estimations

Systemic and respiratory effects are thought to be the result of inhalation of endotoxin-laden organic dusts. The International Committee on Occupational Health, through its Committee on Organic Dusts reported that endotoxin may provoke different reactions when the exposure occurs at different levels (Rylander *et al.*, 1989). The Committee report states that ODTS is elicited at a level of 1000-2000 ng/m^3, acute bronchoconstriction occurs at 100-200 ng/m^3, and Mucous Membrane Irritation may occur at endotoxin levels of 20-50 ng/m^3. It is not known whether even lower concentrations trigger responses in sensitive individuals.

Compost Site Case Summaries

Large-Scale Composting Facilities

Currently there are over 200 biosolids composting facilities, 17 solid waste composting facilities and over 3,000 yard waste composting facilities in the United States (Goldstein *et al.*, 1994; Steuteville, 1994). Since the inception of sludge composting in 1976, there have been several studies on the effects of composting on bioaerosol production in relation to worker and public health in and around facilities (Millner *et al.*, 1977; Hampton Roads Sanitation District, 1981; Lees and Tockman, 1987; Clayton Environmental Consultants, 1983; ERCO, 1980; Kothary and Chase, 1984). The data have indicated that at distances of 250 to 500 feet from compost facility perimeters the airborne concentrations of *A. fumigatus* were at or below background concentrations. Presently, there are no published or documented studies on bioaerosols at solid waste composting facilities in the United States, however, there have been several studies in Europe (Boutin *et al.*, 1987; Lundholm and Rylander, 1980; Clark *et al.*, 1983). Data is also meager for yard waste composting or agricultural composting sites.

Washington Suburban Sanitary Commission (WSSC)

Several studies have been conducted at the Montgomery County Regional Facility first located at Dickerson, Maryland, and later at Site II, Silver Spring, Maryland, from

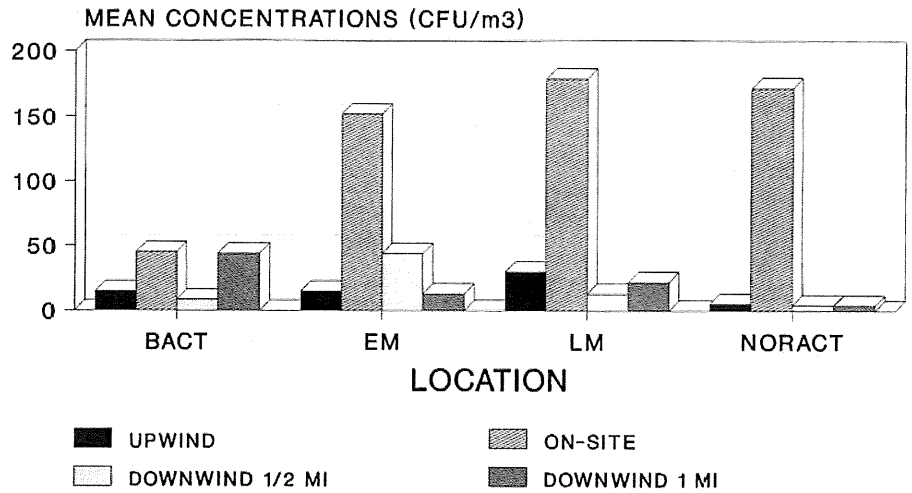

Figure 1. Concentrations of *Aspergillus fumigatus* at a biosolids facility in Dickerson, Maryland. Source: Cookson, 1983
Legend: BACT= before daily activity; EM= early morning w/o screening; LM= late morning w/o screening; NORACT= normal activity

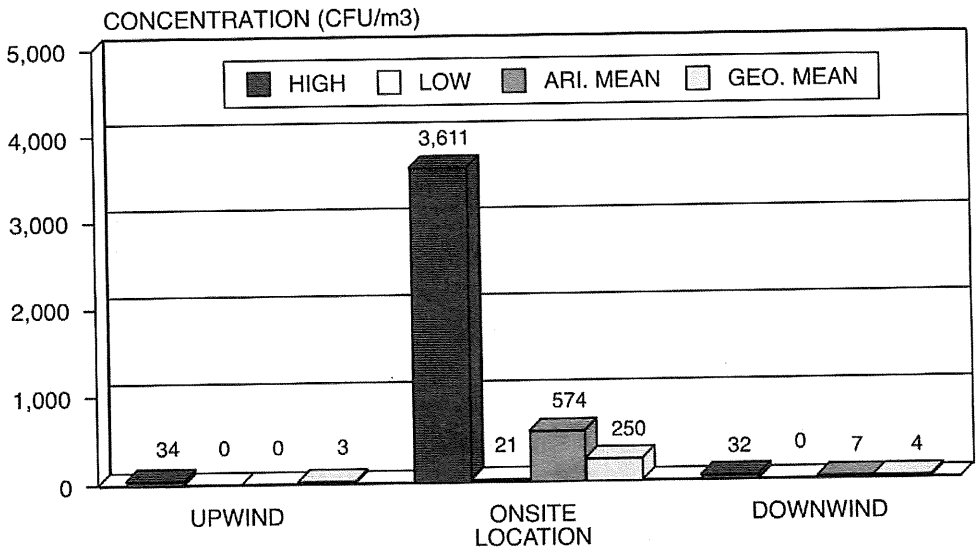

Figure 2. Concentration of *Aspergillus fumigatus* at or near WSSC Site II, 1990-1991 (Data from 12 locations) Source: General Physics Corporation, 1991

1978 to 1991 (Cookson *et al.*, 1983; Lees and Tockman, 1987; General Physics, 1991).

The Dickerson site was totally open with mixing, composting, curing and screening performed outdoors on a paved area (Figure 1). During composting activities the concentration of *A. fumigatus* was the same for both the upwind and downwind locations. These concentrations were comparable to background concentrations reported in the literature.

Studies at Site II began in 1983 (Lees and Tockman, 1987). During the period 1983 to 1986 the site was partially enclosed. Mixing and composting were done under cover, curing was totally in the open and screening was enclosed. The data reported by Lees and Tockman (1987) indicated that since the start of composting operations no increase in the frequency of or in the mean airborne concentration of *A. fumigatus* has occurred at community based monitoring stations. The geometric mean concentration for all 1,427 *A. fumigatus* samples was 3.4 CFU/m^3, with the maximum concentration at 88 CFU/m^3. The geometric mean directly at the compost facility was 22 CFU/m^3, with the maximum concentration being 144 CFU/m^3.

During 1990 and 1991, General Physics Corporation conducted further investigations on levels of *A. fumigatus*, thermophilic fungi, aerobic bacteria and mesophilic fungi. The data for *A. fumigatus* and thermophilic fungi are shown in Figure 2. The downwind sites were located 1,000 feet to 8,600 feet from the Site II perimeter.

An employee health surveillance program was established in 1987 at Site II (Chesapeake Occupational Health Services, 1991). There was no evidence of adverse health effects related to the exposure to *A. fumigatus* during the past five years.

Westbrook, Maine

A comprehensive monitoring of *A. fumigatus* was conducted for the Portland, Maine Water District in 1979 and 1980 at Westbrook (ERCO, 1980). All site activities were performed in the open on a paved surface. The highest concentration was found

at 30 meters from the site center. At 90, 150 and 1,500 meters, levels of *A. fumigatus* were at background levels (Figure 3).

Windsor, Ontario, Canada

A comprehensive air sampling program was conducted in 1983 at the composting site, wastewater treatment plant and surrounding areas (Clayton Environmental Consultants, 1983). At distances greater than 400 feet the airborne concentrations of *A. fumigatus* were below 20 CFU/m^3 (Figure 4).

Solid Waste Composting Studies

Lundholm and Rylander (1980) reported that workers at an experimental refuse compost facility had a higher incidence of subjective symptoms, such as nausea, headaches and diarrhea. Eleven employees were evaluated. Two reported nausea, one reported fever, five had headaches and four had diarrhea. This was attributed to possible exposure to endotoxins.

Rylander *et al.* (1983) studied exposure to aerosols of microorganisms and endotoxins in sewage treatment and composting plants in the United States and Sweden. Data from composting plants showed average airborne dust levels ranging from 0.1 to 12.0 mg/m^3. The highest levels (median 10.6 mg/m^3) were found in the screening areas. The lowest levels were near compost piles. The respirable proportion of gram-negative bacteria was in the range of 50 to 60 percent. They concluded that too little information is available to establish dose-response relationships which might be used to suggest standards. Rylander *et al.* (1983) suggested that a level of up to 1,000 gram-negative bacteria /m^3 and 0.1 ug/m^3 of endotoxins should be considered as safe until additional information is available. More recently, the International Committee on Occupational Health has recorded 0.02-0.05 ug/m^3 as sufficient to elicit an MMI response.

Clark *et al.* (1983) studied the airborne gram-negative bacteria, endotoxins (lipopolysaccharide dust) and *Aspergillus fumigatus* at four Swedish composting

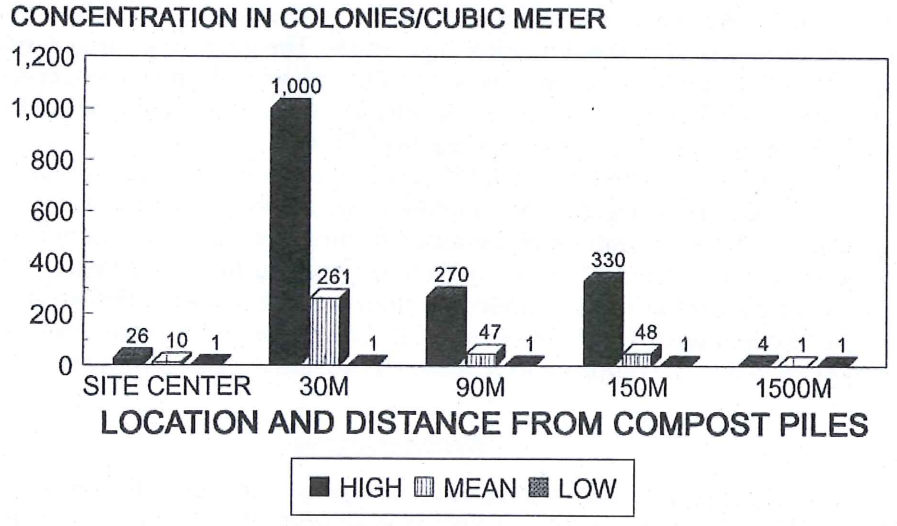

Figure 3. Concentration of *Aspergillus fumigatus* at the Westbrook, Maine Composting Facility
Source: ERCO, 1980

plants. Three plants composted a mixture of solid waste and biosolids. The fourth composted biosolids and wood chips. Both indoor and outdoor sites were sampled at various operational locations. A considerable range of microbial concentrations were found in all plants. Airborne concentrations of gram-negative bacteria ranged from 0 to $3.7x10^5$ CFU/m^3. Refuse hoppers, waste processing areas and screening areas had the highest concentrations, i.e., 0.15×10^5 to 3.7×10^5 CFU/m^3 with medians of 0.43 $\times 10^5$, 0.94×10^5 and 0.96×10^5 for the respective areas. In most cases, more than half of these airborne concentrations were in the respirable size range. Endotoxin values ranged from 0.001 to 0.042 ug/m^3 , well below the suggested safe levels of 0.1 ug/m^3.

Yard Waste Studies

Zwerling and Strom (1991) reported on a study in four communities in New Jersey. They found high airborne concentrations of *A. fumigatus* on-site during activity and concentrations equivalent to low background levels during periods of no activity. During high activity, AF concentrations at the composting sites exceeded 5×10^3 and at some places 7×10^4 CFU/m^3. However, during periods of work activity the concentrations dropped significantly at 100 m (300 ft) and 500 m (1500 ft) downwind. At 100 m downwind, the airborne concentration was at 354 CFU/m^3 and at 500 m it was 86 CFU/m^3. These numbers were within the range typical of background concentrations.

A recent report on AF at a Connecticut yard waste composting site reported concentrations ranging from 0 to 2,648 CFU/m^3 on-site and 0 to 11 CFU/m^3 downwind at distances of 500 feet to 1 mile (Figure 5.). The downwind measurements were similar to levels found at background sites located remotely from the facility (E&A Environmental Consultants, Inc., 1993).

Aerobiology specialists at the New York State Biological Survey analyzed air samples collected at four locations on and around a large yard waste composting facility at Islip, N.Y. (ICF). Continuous samples were collected from August-October, 1992 us-

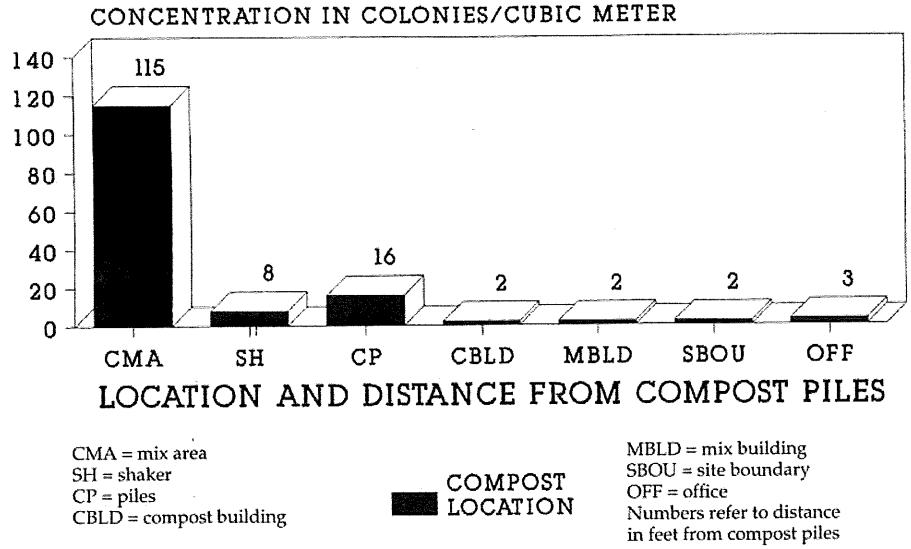

Figure 4. Concentration of *Aspergillus fumigatus* at West Windsor, Canada
Source: Clayton Environmental Consultants, Ltd., 1983

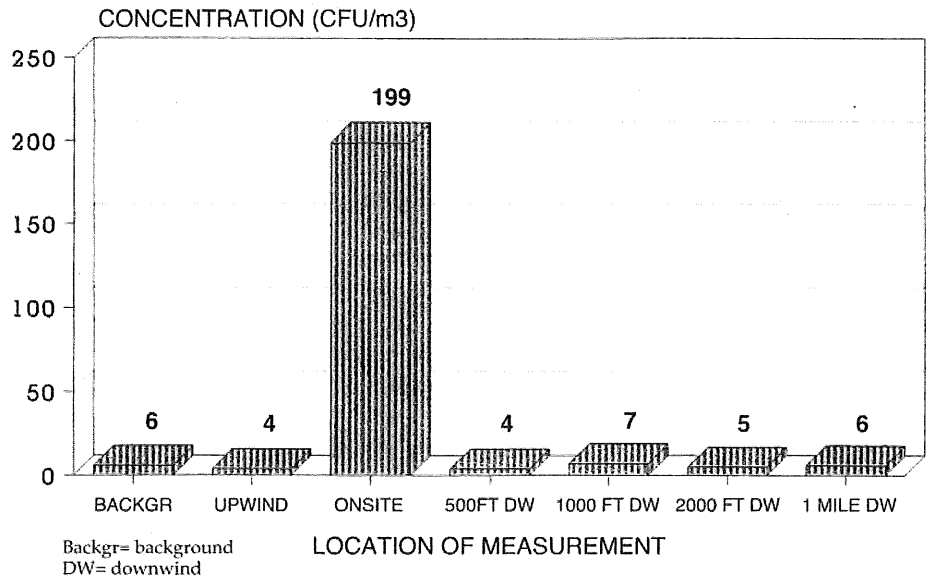

Figure 5. Average concentration of *Aspergillus fumigatus* at or near a Connecticut yard waste facility
Source: E & A Environmental Consultants, Inc., 1993

ing a Burkhard spore trap and were analyzed for total (viable and nonviable) fungal spores and for *Aspergillus fumigatus* (AF). Periodic air samples were also collected concurrently for viable AF and thermophilic actinomycetes with an RCS sampler by Suffolk County Health Department. Volumetric spore counts were obtained for the ICF; Union Ave., a residential neighborhood about 500 m downwind of the ICF (under prevailing summer wind conditions); an airport site about 800 m upwind of the ICF; and at Fisher Ave., Islip Terrace, about six miles (10 km) upwind of the ICF. Total airborne spore concentrations were similar at all four sample sites. The average "background" AF airborne concentrations (at Fisher) were 65 spores/m^3 and mean airborne AF concentrations at ICF and Union were 710 and 300 spores/m^3, respectively (J. Haines and L. Syzdek, pers. communication). Average viable airborne AF were 56 CFU/m^3 at Fisher and 600, 81 and 20 CFU/m^3 for ICF, Union and the airport. Mean viable thermophilic actinomycete concentrations were 36 CFU/m^3 at Fisher, and 480, 110 and 33 CFU/m^3 at ICF, Union, and the airport respectively (J. Haines and L. Syzdek, pers. communication). Allergy and asthma symptoms that were reported by Union and Fisher community participants in a concurrent diary study, were evaluated and analyzed by the New York State Health Department. Analyses showed that symptoms were not significantly associated ($P>0.05$) with airborne concentrations of spores in the two neighborhoods (E. Horn, pers. communication). However, symptoms were significantly associated ($P<0.05$) with an 'environmental factor', which was a derived variable, i.e., a multivariate correlate of temperature, ozone and ragweed pollen.

Ultimately, it appears that a clear resolution to a number of questions about possible health effects associated with siting a large yard waste composting facility within relative close proximity to residential communities will not be achieved using the Islip or other previous study data. The statistical power needed to distinguish significant health effect trends will require a very large number of symptom (diarist) re-

spondents and objective measures of health effect responses wherever possible, along with hourly spore counts, even if only for a relatively short period of time, e.g., 4 weeks. Thus, the existing studies provided explicit site data as well as guidance for improved study designs for the future.

Individual Case Studies

There have been very few reported cases of health effects in workers, nearby residents or other persons associated with composting.

Kramer *et al.* (1989) reported on a case of a young man who was an asthmatic for 16 years and who was treated with immunotherapeutic agents. The individual resided 250 feet from a municipal leaf composting site and developed allergic bronchopulmonary aspergillosis (ABPA) which was related to *A. fumigatus*.

Zuk *et al.* (1989) reported that a young man who worked as a gardener for 14 years contracted a fatal locally invasive pulmonary aspergillosis in the absence of predisposing conditions.

One particular human exposure which received multidisciplinary scientific examination involved a case of severe respiratory illness that developed in a person after shoveling two truckloads of composted wood chips (Olenchock *et al.*, 1991). The lung disease was described as an acute hypersensitivity pneumonitis (HP) or organic dust toxic syndrome (ODTS), (Weber *et al.*, 1993). Bulk materials and airborne dusts (obtained through subsequent reenactment) were tested for the presence of bioaerosol agents. Endotoxins (98.9 to 934.7 endotoxin units (EU)/mg, bulk dust; 636.5 EU/m^3, inspirable dust; 771.89 EU/m^3, respirable dust) were quantified. Viable airborne total bacterial levels were 6.3×10^5 CFU/m^3; gram-negative bacteria, 2.9×10^5 CFU/m^3; thermophilic actinomycetes, 3.2×10^2 CFU/m^3; and total fungi 1.4×10^6 CFU/m^3. In addition, predominant fungi were identified in the bulk materials and airborne dust as *Aspergillus fumigatus, A. niger, Penicillium* spp., *Rhizopus microsporus, R. stolonifer, Absidia* sp., *Cladosporium* sp. and *Trichoderma* sp. Specific antibodies to *A. fumigatus* and *A. niger*, which were biomarkers of that exposure, were detected in the blood serum of the affected individual. Biomarkers for effect, i.e., HP or ODTS, were observed clinically in the patient.

Facility Design and Mitigation of Exposure

Factors Contributing to Exposure

The primary exposure of potential concern is inhalation of bioaerosols and derived products emitted from compost facilities. Health risks might only occur at or around active compost sites where susceptible human receptors are present along with material that contains biologically active agents that may become aerosolized. Factors which may contribute to exposure can be classified as: 1) physical and meteorological characteristics and 2) operational characteristics.

Physical and Meteorological Characteristics

Meteorological characteristics at a site, in conjunction with topography, may affect the exposure of workers and nearby public to bioaerosol emissions from compost facilities. If the design goal is to maximize diffusion and distribute aerosols over a large area (so as to decrease atmospheric concentration), then the facility design should attempt to utilize winds and higher points of release. If the design goal is to keep the

mass of airborne material close to the facility, then composting operations should be shielded from winds and preferably emit aerosols at low heights, or even from elevations below surrounding sensitive areas. Existing atmospheric diffusion models may be used to estimate the impact of facility emissions on sensitive receptors (Millner *et al.*, 1977; Lighthart and Frisch, 1976; Lighthart and Kim, 1989).

Operational Characteristics

Limited data are available to quantitatively evaluate the effectiveness of various operational characteristics on bioaerosol emissions. In the past, facility operations focussed on process efficiency, achievement of pathogen kill and odor control. However, there are data that are relevant to the outdoor static pile composting of sewage sludge utilizing fresh and recycled wood chips as bulking agents. The emission rate for AF spores released from woodchip/sewage compost handled by front end loaders was determined (Millner *et al.*, 1980); mechanical agitation of compost material was a major source of airborne emissions. This suggests that reduced bulk movement of compost and use of dust control measures will minimize bioaerosol releases. The study also suggests that few of the aerosolized microbes originated as wind blown losses from static piles of compost. This was consistent with the situation in the U.K. (Mullins *et al.*, 1976) in which 170,000 CFU/g were recovered from compost, with only an average of 33 CFU/m^3 recovered from the air alongside the compost heap. In tests in the U.S. during mechanical agitation of compost by a front end loader (FEL), downwind concentrations of thermophilic actinomycetes and fungi were 150-200 times greater in the immediate working area.

At least a few other mechanisms are identified that will contribute to increases in airborne bioaerosols losses: mechanical agitation of wheels and tires of equipment; physical handling of the materials, and downdrafts onto dust-laden traffic surfaces. High levels of AF are also associated with FEL movement of stored/stockpiled wood chips, other vehicular movement across dust covered surfaces and screening of compost (21 day) piles. Curing the compost for one month or more can markedly reduce levels of AF found (Millner *et al.*, 1977). It must be stressed that the results from the Beltsville static pile method are very specific for this process method and materials handled. It would be misleading to generally apply these results to other composting processes and materials without evaluating the potentially critical differences, i.e., size of the overall operation and site, the amount and frequency of organic dust generation sources, the feedstocks, the pile configuration and overall process and site management . Other types of composting facilities should be studied to determine the effect of operational characteristics on bioaerosol emissions.

Mitigation Through Facility Siting, Design and Operational Changes

When siting new facilities, critical evaluations should be made of several factors including the proximity to residences and public facilities and meteorological/topographical parameters that contribute to off-site transport of bioaerosols. The proximity to residences and public places should also be a key consideration when upgrading composting facilities. Required buffer areas can be greatly reduced with enclosure, good management practices and increased mechanization of the facility. The layout of composting activities associated with bioaerosolization, particularly material handling processes, should be located downwind or as far as possible from sensitive receptors. From the engineering perspective, the design principle of compost facilities should

closely follow the natural biological process, requiring minimal intervention or handling of composting materials. Dispersion modeling of bioaerosol emissions may be helpful, although such models are predictive in nature and may generate results that are highly uncertain (Millner *et al.*, 1977; Lighthart and Kim, 1989).

Good management practices for the operation of compost facilities are well defined in state and federal regulations. These practices have primarily considered operational efficiency, pathogen kill, production of a good compost quality and dust and odor control. As noted above, data support the contention that an open or an enclosed static pile sewage sludge facility utilizing virgin and recycled wood chips does not contribute to a significant elevation of bioaerosol levels off-site. We believe that additional studies on other types of compost facilities are needed to determine the effectiveness of the following operational methods which should lead to reduced bioaerosol emissions in the event that is prudent to do so with respect to the protection of public and worker health: 1) Use of added moisture in the composting materials and/or area water spraying to control all particulate emissions from the operation (EPA Guidelines for Controlling Fugitive Emissions OAQPS RTP-NC, 1978); 2) Mechanical agitation (handling) of materials with a high potential for creating bioaerosols should be minimized consistent with the need to maintain other controls; 3) Agitation of compost materials should be timed to coincide with the stage of the material when: a) the potential for release of bioaerosols is minimal, b) the potential for off-site dispersal is minimal and/or c) the receptor populations are least; and 4) Temperature and moisture conditions of bulking agents should be managed to minimize formation of bioaerosols.

Site Enclosure

In addition to careful attention to good management practices, the use of enclosures and managed air streams may need to be considered, particularly at sites in close proximity to potentially sensitive populations. Careful attention will need to be paid to worker exposures and protection in such circumstances. Data available from the WSSC Site II studies (Lees and Tockman, 1987; General Physics Corp., 1991) indicates that on-site AF levels increased 11-fold when the site was enclosed. Prior to enclosure (4/1/83 - 5/31/86), AF were 22 CFU/m^3 (geometric mean) on-site. After enclosure (May 1990 - April 1991), AF increased to 250 CFU/m^3 (geometric mean). In October 1991, Chesapeake Occupational Health Services reviewed the health surveillance data maintained for Site II workers from its inception in 1987 and found "no evidence of adverse health findings related to exposure to AF" (Chesapeake Occupational Health Services, 1991).

The use of filters has been evaluated primarily for effectiveness of odor control. The use of biofilters or chemical scrubbers (used primarily to control odors in enclosed facilities) has not been evaluated specifically for their capacity to retain bioaerosols. Considerable data exists relating to the efficacy of electrostatic precipitators and dry bag houses in removing particulates from industrial stack emissions. However, the efficiency of bioaerosol removal with these technologies remains untested. We recommend that biofilters and scrubbers be evaluated for their bioaerosol removal efficiency. The design of such studies should consider the fact that previous investigations (Mullins *et al.*, 1976; Millner *et al.*, 1980) have shown that *static* compost piles exposed to low or moderate levels of atmospheric turbulence do not release a continuous stream of AF spores into the air. Furthermore, AF, unlike, some other fungi, does not have an inherent mechanism for forcible ejection and propulsion of its spores (conidia) into the surrounding atmosphere; this fungus depends on external mechanical movements for dispersal of its spores.

Dispersion Control

Site modifications can be used to enhance or inhibit the dispersion of bioaerosols to minimize off-site effects. Berms may be built and/or trees planted at strategic locations on the site to alter wind dispersion patterns and entrainment of bioaerosols generated by the facility. Tree barriers have an aesthetically pleasant appeal around waste treatment facilities and have often been suggested for visual benefit as well as the benefit of reducing dispersion of airborne microorganisms from the facilities. Experimental evidence for a forest barrier benefit was produced by Raynor *et al.*, 1974 in their very detailed study of particulate dispersion into a 90 percent pine forest having a stem density of 1,474 trees per hectare and a mean tree height of 10.5 to 13 m (over the years of the study) at Brookhaven National Laboratory, Upton, New York. They demonstrated that "the forest edge modifies dispersion primarily by changing local meteorological conditions and flow patterns and secondarily by direct removal of particles," i.e., by impaction and deposition onto foliage. Particulate plumes moving unobstructedly over open terrain are significantly broadened, both horizontally and vertically, when they encounter a forest edge; this in effect dilutes the concentration of particulates in the plume. The amount of broadening is influenced by the foliage density (Raynor *et al.*, 1974). Dense foliage at the edge would have the same effect as a solid object, i.e., pronounced vertical dispersion, whereas in a forest with open trunk space at the edge maximum penetration and minimum broadening of the plume would occur with much channeling of air into the subcanopy level. They also found that "the intensity of turbulence within the forest reaches a maximum at midcanopy level." In addition to this study, there is considerable knowledge and experience showing that vegetative windbreaks are very effective controls for wind erosion, particularly in the Great Plains regions of the U.S. Air dispersion models currently available (Turner, 1974; Gifford, 1961; Pasquill, 1961) should be useful in the evaluation of this issue at local sites.

Buffer Distances

In general, the specification of appropriate buffer distances depends on the site location, design, local micrometeorological conditions and emission controls. No setback may be necessary for totally enclosed facilities, if all activities are enclosed and ventilation control is provided. A number of sewage sludge composting facilities have been empirically evaluated. Studies at the closest residence (0.4 and 0.5 miles) to the WSSC Site II and Dickerson in Maryland suggest that bioaerosols are not elevated above background.

Airborne AF levels were measured at the WSSC Site II prior to operation as 0-42 CFU/m^3. When WSSC Site II was operated as an open facility, airborne AF were measured at or below 43 CFU/m^3 in three years of operation. However, slightly elevated levels of AF were detected at the nearest off-site receptor (about 500 feet from the active composting pad). When the site was enclosed, AF levels were similar to prior background. Zwerling and Strom (1991) have measured airborne levels of AF at varying distances from several yard waste composting facilities at different stages of operation. The facilities represent varying levels of management expertise. Sampling was also conducted at the Islip Composting Facility in Islip, New York which was handling yard waste and a complete report has been compiled by the investigators (Department of Health, 1994). We are not aware of any others.

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Limitations To Determinations Of Exposure

Environmental Sampling Data Needed

In order to evaluate the impact of bioaerosols on populations living and working in areas near composting facilities, more data are needed about the natural, ambient concentrations in air of compost process-associated microbes (of concern as potential pathogens and allergens to humans). Statistically significant and consistent increases in natural concentrations in air are inherently difficult to measure in the outdoors because of the multitude of environmental variables that influence individual sampling events. Time averaged data, from multiple sites in an area would be desirable. Technical limitations to the analysis of spore collections currently limit the number to samples that can be analyzed, primarily because the expertise for the microscopic analyses is in short supply. Data also are needed regarding the prevalence of thermophilous and thermotolerant actinomycetes and fungi for more regions of the U.S.

Lack of suitable sampling technology has limited the study and understanding of bioaerosols. Therefore, if the labor cost of generating large data bases containing information on airborne fungi, actinomycetes and bacteria could be reduced, a much clearer understanding of the atmosphere in and around composting facilities as well as the natural environment would be available. Results obtained with Andersen samplers require considerable interpretive skill and are restricted to a single sample for a set time period. In very dusty situations, the agar collection surface can rapidly become overloaded; thus, sample times in dusty environments may need to be quite short. This requires more frequent sample events. The biotest handheld sampler eliminates the need for large numbers of plates of media but requires interpretation and is restricted to a single time period. The Burkard impaction type sampler runs continuously and makes an ongoing record that can be analyzed for any point in time, but microscopic analysis of the spore collection requires intensive use of a high resolution microscope with difficult and subjective identification. The Burkhard sampler has a 50 percent collection efficiency for particles with an aerodynamic diameter of approximately 2.5 μm; consequently the Burkhard may have a suboptimal collection efficiency for small, individual spores such as those of *Penicillium* and *Aspergillus* spp. Slit samplers using media plates are both continuous over time and readable on the surface of the media, but they suffer from high labor input necessary to maintain media and the inability to detect nonviable spores which may be allergens.

A continuous volumetric sampler which is based on objective detection techniques rather than subjective interpretation is needed. The ideal sampler for monitoring compost facilities and neighborhoods would generate data on specific bioaerosols such as AF on-site, automatically read the data and transmit it to a database. A sampler that continuously collects airborne particles so that daily and hourly fluctuations can be detected with molecular or immulogical probes would be ideal. This would be a monitor which could detect problems as they happen so that they might be corrected. The Burkard or other impaction samplers can generate a plastic strip with all particulates 2 μm and longer. This sample might be treated with immunofluorescent antibodies to specific fungal spores and the results could be read easily. Bacteria and actinomycetes would require sampling technology adjusted to the smaller particle sizes involved.

Most downwind collections of bioaerosols have been very patchy, temporally and spatially. With brief samples taken monthly, weekly or daily, the detailed nature of the bioaerosols cannot be elucidated. It is a hit or miss pattern in the complicated nature of a diffusing, rising, turbulent bioaerosol plume which may miss a target sampler (or person) entirely or which may deposit a heavy concentration of material onto

it. Continuous data over a season or year would be desirable, instead of intermittent samples. Total bioaerosol inhalation is important for allergic response. It is the dispersal over time that is important rather than a few spot samples. More downwind measurements are needed to soundly recommend setback distances of compost facilities from population centers.

Specific aspects of the microbial ecology of composting are incompletely understood. What is the location within the pile that produces fungal spores as opposed to fungal vegetative growth? What is the colony configuration of the compost pile? Are the organisms that are present genetically and immunologically uniform? If so, then fungus or actinomycete spores can be identified as to their site of origin. Are different bioaerosols emitted from different composts? Are unique allergens or pathogens produced by food wastes, feedstocks, fruit pomaces, yard wastes and sewage sludge?

Quantitative data are needed on the effects of compost management techniques on bioaerosols. In order to manage a compost facility with minimum emissions of bioaerosols, the effect of turning a static pile at shorter or longer intervals, the effect of hotter or cooler inner and outer pile temperatures, the effect of air movement, the effect of H_2O content and other parameters need to be known.

Risk Assessment

Risk assessment is traditionally divided into the following components: hazard identification, exposure characterization, exposure response analysis and risk characterization. Elements of the first two components of this process, hazard identification and exposure classification, have been addressed in the previous sections of this report. In particular, the hazards associated with bioaerosol exposures include inflammation, allergy and infections from primary and secondary (= opportunistic) pathogens. The principle remaining question is: At what exposure levels do these health effects occur? Thus, this section focuses on discussion of studies that specifically evaluate the risk of exposure to bioaerosols among workers and the community, and what is known about the relationship between exposure levels and response.

Exposure Assessment of Bioaerosols at Composting Facilities

Exposure pathways are one of the first considerations for a risk assessment evaluation (Goldsmith and Berglund, 1974). The mechanisms for transport from composting operations are both direct and indirect. Direct transport results when composting material is transported off-site by individuals or equipment. Compost is essentially pathogen free and is a lower infectious agent risk to workers then is sewage sludge. Other microbial organisms are considered comparable to typical levels found in wood mulching and other soil conditioning materials. Thus, direct transport has not been considered a significant exposure route.

The indirect transport mechanisms include water runoff and air suspension. Water runoff controls, collection and discharge to sewage treatment facilities are standard practice. Thus, exposure by the water route is unlikely. The only potential exposure transport mechanism that requires detailed consideration is air suspension and transport to populations as a bioaerosol.

The transport of bioaerosols was evaluated by WSSC Montgomery County Regional Composting Facility, Silver Spring, Maryland using computer generated air dispersion models as predictors of downwind distance for maximum bioaerosol concentrations. Field sampling was performed twice monthly at upwind, on-site and the

predicted maximum downwind locations for a full year. The numerous environmental and atmospheric conditions that influence the transport of bioaerosols were factored into the predictions. Many parameters that have a major influence are site specific including micrometeorological conditions, the elevation of the bioaerosol discharge from the compost site, the size of the facility and the bioaerosol release rate. Release rate is a function of the facility design and treatment controls. Thus, generalizations on transport and health significance from one facility may not apply precisely to another. Each requires a specific risk assessment evaluation. However, most data indicate insignificant bioaerosol transport from a well operated and designed compost facility. Viable airborne microorganisms were analyzed by groups at Site II: total aerobic bacteria, mesophilic fungi, thermophilic fungi and AF. Of the four groups, three did not demonstrate elevated downwind concentration at a 95 percent confidence level. The downwind aerobic bacterial concentration was elevated at the 95 percent confidence level. Downwind concentrations had a geometric average of 160 CFU/m^3 and the upwind concentrations averaged 83 CFU/m^3.

However, the data for mesophilic fungi and AF do not demonstrate higher downwind than upwind concentrations at sampling locations that should yield maximum off-site concentration.

For this 400 wet ton per day sewage sludge composting facility, the transport mechanism is not sufficient to expose an off-site population to levels of AF that are above those measured in nature and that occur as part of the common airspora. If the transport mechanism is insufficient to expose a population group, then further evaluation is unnecessary and close relationship data is not required.

Dose Response Information

This section presents an overview of the state of knowledge of dose response information available on compost related bioaerosols. Because of the apparent complexity of human and animal immune response to biological agents, clearly defined effect levels are not presently available for the vast majority of compost related bioaerosols. Limited occupational data exist regarding worker exposure to certain bioaerosols. To the extent that these occupational data enable an assessment of acute and chronic health effects, they are incorporated. It should be recognized that, while occupational data are generally more useful than animal dose response data in assessing the relative health effects among the nonworker population, they are not directly applicable without adjusting for a higher exposure frequency to account for 24 versus 8 hours and for sensitivity among individuals in the general population.

Endotoxin

Based on experience from field studies of workers in cotton mills, swine confinement buildings and paper mills, guidelines for endotoxin exposure have been suggested (Rylander,1987) for the various health effects which may appear.

Acute Effects

In 1989, the International Commission on Occupational Health (ICOH) published suggested occupational health criteria for acute effects to endotoxins (Rylander *et al.*, 1989). Three discrete health effects were identified which ICOH felt warranted exposure level criteria. Table 2 shows the different types of responses that can result from inhalation of organic dusts, the symptoms and the suggested threshold levels of en-

dotoxin that would protect against possible acute effects including (1) Organic Dust Toxic Syndrome (ODTS), (Toxic Pneumonitis), (2) Broncho-Constriction (BC) and (3) Mucous Membrane Irritation (MMI).

Chronic Effects

ICOH also provided criteria recommendations on permissible endotoxin levels for workers exposed to endotoxins in the cotton textile manufacturing and animal feed industries (Rylander *et al.*, 1989). Based on adverse respiratory effects (particularly broncho-constriction), ICOH suggested the following exposure levels:

Industry	Permissible Endotoxin Concentration (ng/m^3)
Cotton Mills	1.0-20
Animal Feed	0.2-470

In a recent study, Sigsgaard *et al.* (1990) assessed occupational health effects of workers at a garbage sorting facility in Denmark. A range of threshold limit values (TLVs) were proposed for endotoxins (0.1 to 0.2 $\mu g/m^3$);gram-negative bacteria were not to exceed 1,000 CFU/m^3 on average for an eight hour work day; and total bacteria were suggested to be maintained at a level ranging from 5,000 to 10,000 CFU/m^3 (Sigsgaard *et al.*, 1990).

Aspergillus fumigatus

There is an absence of dose-response information available characterizing either acute or chronic human health effects associated with exposure to AF in compost related bioaerosols. Available data qualitatively associate AF concentrations with the presence or absence of clinical health effects. There is an apparent lack of data correlating specific exposure levels to observed health effects. Nevertheless, until such quantitative studies are completed (if at all possible), the lack of compost worker health effects suggests that the highest exposure group (albeit "healthy") are not at increased risk.

Occupational Studies

There are two studies on workers exposed to bioaerosols at composting facilities and the results from these studies are inconclusive. Lees and Tockman (1987) conducted a formal study of respiratory effects among a group of workers employed at a compost facility (WSSC Site II) in Montgomery County, Maryland. The records used were collected by the Maryland Department of Health and Mental Hygiene and not by the study investigators. A total of 31 people with at least one follow up visit were included in the analysis. The average length of time between the preemployment and follow up examination was approximately 3.7 years. Information from pulmonary function testing, questionnaires and chest x-rays were evaluated. The investigators (Lees and Tockman, l987) did not find any significant differences between the preemployment results and the follow up tests. These results must be viewed in the light of the small number of workers involved and the relatively short period before the follow up examination.

There were some statistically nonsignificant differences in this study that were notable. Workers at the follow up examination reported a higher percentage of allergies, asthma, 'lung trouble' and shortness of breath than at the preemployment examina-

tion. Based on pulmonary function test results, a higher percentage of workers demonstrated obstructive lung function patterns at follow up. However, abnormal pulmonary function results were very high in both the preemployment screening and follow up studies with no consistent pattern of abnormal results which make these results suspect. Finally, the report cited two cases which showed abnormal chest x-rays that could be consistent with *Aspergillus* infection, but the individuals were negative for *Aspergillus* serum antibodies.

The second study (Clark *et al.*, 1984) included 84 workers from four facilities who were directly involved at the composting sites with various stages of the process. The study also included 157 workers who were classified as having an intermediate level of exposure and who were occasionally involved in the compost process or whose job locations were within 100 m of a composting activity and 133 controls who were not involved in the compost operations or whose job locations were greater than 100 m from the site. A cross sectional study was performed on these workers which included pulmonary function testing, chest x-rays, serologic tests and culturing of *A. fumigatus* from the throat and nose. An excess of nasal, ear and skin infections, and of eye and skin irritation was observed in the compost workers relative to the control group. However, the same conditions were present in even higher numbers in the intermediate exposure group than it was in the compost group. The results were contrary to expectations since it was assumed that the compost group had higher exposures to bioaerosols than the intermediate exposure group. The study clearly demonstrated elevations of AF in the nose and throat of compost workers, providing evidence of exposure but no evidence of infection. There were also indications of increased white blood cell count, eosinophils and hemolytic complement among compost workers, which is indicative of a low level inflammatory response.

There was one case report of aspergillosis in a worker exposed to moldy wood chips (Conrad *et al.*, 1992). In this case, a subject with chronic granulomatous disease (CGD) was occupationally exposed to AF spores while shoveling moldy wood chips and subsequently developed a fatal pulmonary infection caused by AF. This case is noteworthy in that the subject's congenital immune defect placed him at risk of infection and the authors conclude that such patients should be advised to avoid occupational environments where exposure to high spore concentrations is possible.

Summary and Recommendations for Further Research

The primary goal of this collaborative workgroup was to evaluate the results from the few available quantitative studies that might answer the question of what levels of bioaerosols are "safe" and what levels of protection are adequate for occupational and public health. A second goal was to clearly identify and to whatever extent possible, prioritize future research needed to fill existing gaps in the data.

There is little doubt that if traditional dose response modeling and quantitative risk assessment could be performed, more robust conclusions could be derived from the data. However, differences in human immune responses preclude the likelihood that specific minimum effect levels will be developed. There are some dose-response data for endotoxin. However, direct experimental resolution of the dose-response issue of microbial bioaerosols may not be feasible, especially in terms of human populations. This information would be valuable in determining the relative risk to the general population, the segments of the population that are at increased risk because of their preexisting medical/immunological/genetic conditions or predispositions.

The following conclusions represent the consensus of this committee after their re-

view of the existing scientific and technical literature/reports that deal with exposures to compost bioaerosols and the health effects associated with them:

There is little reason for concern about the risk of potential infections from exposure to *A. fumigatus* among healthy individuals in either the general population (defined as nonoccupational exposure) or workforce exposed to composting bioaerosols.

There are subpopulations within the general population and workforce that may be at increased risk from exposure to composting bioaerosols. Of particular concern, immunocompromised and/or immunosuppressed individuals (e.g., chemotherapy recipients, organ transplant recipients, AIDS patients, individuals with congenital defects and children with cystic fibrosis who may be at increased risk of infection) may have greater susceptibility to colonization and infection by *A. fumigatus*.

Atopic/asthmatic individuals may be at increased risk for developing allergic reactions to various components of composting bioaerosols. A variety of common components of aerosols (e.g., pollen, fungal spores, house dust) are associated with allergic reactions or can induce asthmatic reactions.

Compost worker exposures to bioaerosols may be sufficiently high in certain circumstances to increase the risk of some adverse health effect. Although convincing evidence for an increase in risk has not been documented in any of the studies performed to date, the designs of these studies have limitations that make it difficult to draw definitive conclusions.

There is currently a total lack of dose-response data with which to quantify the risks for components of compost bioaerosols, with the possible exception of endotoxin.

Although there is dose-response information for endotoxin from gram-negative bacteria, there is currently inadequate exposure data to characterize the risk of exposure. Available data suggests that the risk of exposure to the general population may be minimal.

Available epidemiologic studies of both the workers and the general population are inadequate solely to determine whether or not significant risk exists from exposure to compost bioaerosols.

It appears that nonatopic individuals can become sensitized by constant exposure to bioaerosols. This is an area which warrants further investigation.

Epidemiological investigations to address both issues of hazard identification and dose response to bioaerosols are needed, in the occupational and in the general populations, who are either exposed to leaf, biosolids and/or garbage composting. The feasibility of conducting the following types of studies should be explored:

1) Longitudinal studies of occupational cohorts that include: a) quantitative estimates of levels of exposure to the various pathogens contained in the bioaerosols, b) medical history and questionnaire data and c) measurements of pulmonary function, chest x-rays and d) changes in antibody levels to specific antigens made from a pool of known serotypes from that specific environment. This type of study would hopefully provide the critically needed information on dose-response relationships.

2) Longitudinal cohort study that is community/population based and includes: a) medical history and questionnaire data, b) measurements of pulmonary function, chest x-rays and c) changes in antibody levels to specific antigens made from a pool of known serotypes from that specific environment.

3) Case control study of laboratory confirmed cases using Aspergillus isolates obtained within the vicinity of the composting facility. Controls might be other patients from hospitals or other health care facilities that were the source of the cases. The controls might be matched to the cases on other risk factors (e.g., age and sex). Information on residential exposures including indoor air sampling as well as outdoor ambi-

ent air would be collected for both cases and controls.

4) The development and implementation of a questionnaire to solicit information from residents living near composting facilities, as well as residents in neighborhoods where no composting facilities exist. The questionnaire should be structured as a general environmental health questionnaire and not specifically "targeted" at individuals residing around composting facilities. The questions may include inquiries of noise, odor, vibration, as well as specific health interests to assess specific allergy, history of immunosuppressive drug utilization and a general health history of significant illnesses/medical conditions among residential members. This type of research tool and approach has been successfully utilized in Sweden and in New York State in residential areas surrounding manufacturing facilities.

Appendix I

Composting: Scope and Process

Most urban areas of the U.S. currently face serious problems in safe/effective management of wastes, especially two major municipal wastes, i.e., sewage sludge and municipal solid waste (refuse). The limitations to traditional waste management practices are acute. Many landfills and incinerators have closed and new disposal facilities are increasingly difficult and costly to site and operate. In 1990, nearly 196 million tons, or 4.3 pounds per person per day of municipal solid waste (MSW) were generated. After materials recovery for recycling and composting, discards were 3.6 pounds per person per day. Virtually all of these discards were combusted or sent to a landfill. Without any additional source reduction, the amount of waste generated in 1995 will reach 208 million tons; by 2000, waste will near 222 million tons, or 4.5 lbs. per person per day. The per capita figure for the year 2000 represents a five percent increase over 1990 levels (USEPA, 1992). The current annual production of sewage sludge in the United States is approximately 7.7 dry million metric tons or 64 lbs. of sewage sludge per capita (USEPA, 1990).

Composting is considered an economically and environmentally desirable treatment option for wastes because this controlled biological decomposition process converts solid organic matter into a humus-like mixture. The process depends on the growth and activity of mixed populations of actinomycetes, other bacteria and fungi that are indigenous to the various organic wastes that are composted (Golueke, 1992). Composting can be conducted under either aerobic (with oxygen) or anaerobic (without oxygen) conditions (Finstein *et al.*, 1980); however, the aerobic mode is generally preferred since it proceeds more rapidly, is less apt to generate malodors and provides for greater thermal reduction of primary pathogens. The humus-like product is a stable, organic material which is used in agriculture, horticulture and landscaping.

A properly operated composting system accelerates the natural decomposition and stabilization of organic matter by optimizing conditions for biodegradation. Organic materials containing N and P are microbially mineralized such that the N and P are released in plant-available forms, pathogens are destroyed after three days exposure to 55°C and with proper process control and preventative measures for odor treatment malodors can be abated. Destruction of primary, human pathogens by high temperature composting, particularly with recyclable organic resources such as sewage sludge and municipal solid waste, contrasts with the direct land application of sewage sludge which does not completely eliminate existing pathogens within the material.

Compost Systems

Composting is a time honored practice that recently has been mechanized (e.g., shredding equipment, blowers, specially designed containers) and computerized to improve process control, pathogen destruction, odor reduction and product stabilization and quality. Several methods of composting are available (as described below); they are sometimes combined. The fundamental methods are:

Static pile. A pile of blended proportions of various organic materials is constructed (often over perforated pipe or blocks) and air is introduced by positive or negative flow. The mass and pile geometry contribute to heat retention. Sometimes the "static" piles are broken down, remixed and a new pile constructed to continue the process. The static pile can be used indoors or outdoors and is most commonly applied to biosolids composting.

Turned windrow. In contrast to the static pile, windrows (elongated piles of organic material) are turned at regular intervals with either a front end loader or a windrow turning machine. Water or additional organic materials can be added to the windrows during turning to maintain optimal composting conditions. Turned windrows can be constructed indoors or outdoors, with the outdoor method being the most common for composting leaves, grass and brush.

In-vessel. In contrast to static pile and windrow systems, in-vessel composting systems process wastes in containers equipped to control critical conditions, e.g., aeration and agitation. Rotating drums, similar to cement mixers and horizontal or vertical tanks, are the most common in-vessel systems. More recently, tunnel reactors, a type of horizontal tank which has been used for many years by mushroom growers, are being used to compost solid wastes. Retention times for organic material in a vessel depend on the particular technology, but range from 6 hours to several weeks. Most in-vessel systems also utilize static pile or turned windrow methods to accomplish further degradation and stabilization after the material is removed from the vessel. The vessel may or may not be installed in a building, depending on the climate and type of system. Because of their higher capital costs, vessels are most commonly applied to larger quantities of biosolids and/or solid waste.

Hybrid. It is common to find some combination of the above systems, particularly the in-vessel and one of the other approaches. Many turned windrow systems also utilize static piles for final curing and stabilization of the humus. Many of these systems, particularly those housed indoors, utilize chemical scrubbers or biofilters to minimize potential odor dispersal.

Scope of Current and Future Compost Facilities

In the U.S., there were over 2,200 leaf and yard facilities at the end of 1992. At the current rate, the number is expected to approach 3,300 by 1994. In addition, there are over 200 biosolids facilities and 17 mixed municipal solid waste facilities. According to recent trends, there are growing numbers of composters of single stream organic wastes from paper mills, supermarkets and restaurants. These facilities range in size from 20 tons per day for leaf and yard facilities to 500 tons per day for municipal solid waste facilities. In addition, agricultural wastes are composted at numerous sites either on or around farms.

Regional Distribution of Composting Facilities

The Composting Council estimates that as of April, 1993, there are approximately

2,500 composting facilities in the U.S. The Council has begun a database of facilities; this database currently contains about 1,300 entries, most with partial information. The vast majority of facilities compost yard wastes. About 150 compost biosolids, another 30 compost source separated organics (i.e., food waste, yard waste, soiled paper) and 20 extract compostables from mixed waste. Facilities that compost manures and other primarily agricultural wastes have not yet been characterized. Currently, 47 percent of facilities are in the northeast, 27 percent in the midwest, 16 percent in the west and 10 percent in the south. This corresponds roughly to the population distribution in the U.S.; anecdotal evidence suggests most facilities are in suburban areas. To date, 25 states have enacted legislation to encourage composting by banning part or all of their yard wastes (brush, grass clippings, garden trimmings, leaves, prunings, shrubbery and small wood materials) from landfillls; nine of those states are in the northeast, 10 are in the midwest, seven are in the south and one is in the west.

In addition, USDA is increasing its efforts in composting as directed by the 1990 Farm Bill (FACTA, l990, Section 1456). To encourage on-farm composting, the Soil Conservation Service (SCS) developed a standard for on-farm composting (USDA, 1990) that established minimum requirements for design and operation of facilities that handle livestock and poultry manure, dead animal carcasses and food processing wastes. Also, SCS has developed technical support guidance about on-farm composting.

Since 1990, the National Association of Conservation Districts has consistently urged USDA's Agricultural Soil Conservation and Stabilization (ASCS) to cost share the use of compost and/or livestock manure (the crop nutrient value) and has supported the concept of urban/rural waste cocomposting. Some much needed economic incentives to stimulate more on-farm composting of the rural/urban wastes is needed, i.e., assistance in terms of ASCS cost sharing the equipment needed to start an on-farm composting operation. Cost sharing will stimulate use of composting as an alternate animal manure management practice.

Appendix II

Case Definitions for Diseases Caused by Aspergillus Fumigatus

(Excerpted with permission from the "Final Report of the Aspergillosis and Composting Medical Advisory Panel", April 4, 1994, Santa Clara County, Public Health Department, Division of Disease Control and Prevention, San Jose, California)

I. Invasive Aspergillosis

A. *Aspergillus* sp. isolated by culture from a normally sterile site (blood or tissue); culture of sputum is not acceptable.

B. Mycetoma (aspergilloma) diagnosed by sputum culture and chest or sinus radiograph or computed tomography scan.

II. Allergic Bronchopulmonary Aspergillosis

Definite cases meet ALL of the following criteria.

Possible cases have asthma and five of the six remaining criteria.

A) Asthma, i.e. evidence of reversible air flow obstruction defined by: $FEV_1/FVC < 0.70$

B) Evidence of immediate skin reactivity, i.e., a wheal and flare reaction, to *Aspergillus fumigatus* antigen (intradermal injection or "prick" test).

C) The presence of *Aspergillus* - specific IgE in serum.

D) Quantitative serum IgE>400 IU/ml, or IgE>250 IU/ml plus evidence of fluctuation with disease activity.

E) IgG or precipitating antibody to *Aspergillus*.

F) History of peripheral eosinophilia (absolute eosinophil count> 500 cells/mm^3).

G) History of pulmonary infiltrates documented by chest radiograph.

III. Acute Allergic Alveolitis (Hypersensitivity Pneumonitis)

Criteria A and (B, C or D) must be met.

A) Fever and severe dyspnea four to six hours after exposure to a source of *Aspergillus* OR episodic fever and dyspnea and high serum IgG precipitating antibodies to *Aspergillus*.

B) At the time of symptoms, either diffuse micronodular infiltrates by chest radiograph OR restrictive ventilatory defect documented by pulmonary function tests.

C) A positive response to an inhalation provocation test using *Aspergillus* as the antigen.

D) Lung histopathology with mononuclear inflammatory cell infiltrates in alveoli and interstitial spaces documented by lung biopsy.

IV. Asthma Induced by Aspergillus

Criteria A, B, C and D must be met for a definite case; criteria A, B and E must be met for a possible case.

A) Recurrent or intermittent symptoms consistent with asthma including wheezing, dyspnea, cough or chest tightness.

B) Documentation of reversible or variable airway obstruction (improvement of at least 10 percent in FEV_1 with bronchodilator; 2) at least 20 percent variability in serial peak expiratory flow rate (PEFR) measurements in a 24 hour period; 3) Positive inhalation challenge testing with methacholine or histamine (20 percent fall in FEV_1 produced by five inhalations of 8 mg/ml or less).

C) Temporal association between episode of asthma and known exposure to *Aspergillus*.

D) Positive wheal/flare to *Aspergillus* or positive *Aspergillus* specific IgE RAST in absence of reaction to other allergens.

E) Positive wheal/flare to *Aspergillus* or positive *Aspergillus* specific IgE RAST

NOTE: Allergic asthma due to Aspergilli exposure usually occurs in atopic persons, is rarely solely due to Aspergilli exposure and occurs less often than hay fever.

V. Aspergillus Sinusitis

Criteria A and B must be met:

A) Isolation of *Aspergillus* species from sinus culture

B) Severe sinus CT scan abnormalities

VI. Allergies (A Case Definition Was Not Developed)

While persons with sensitivity to *Aspergillus* may have a skin test reaction or IgE

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MICROBIAL AND ENDOTOXIN IMMISSIONS IN THE NEIGHBORHOOD OF A COMPOSTING PLANT*

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Danneberg G, Grüneklee E, Seitz M, Hartung J, Driesel AJ: Microbial and endotoxin immissions in the neighborhood of a composting plant. *Ann Agric Environ Med* 1997, 4, 169-173.

Abstract: Objective of the study was the evaluation of microbial and endotoxin immissions in an area surrounding a composting plant. Immissions of microorganisms and endotoxin originating from a composting plant working with the Herhof box-system were measured. Samples were collected in the vicinity of the plant with filter samplers using gelatin filters (MD 8 of Sartorius). Total aerobic bacteria, staphylococci, coliforms and total Gram-negative bacteria were recorded. Mesophilic fungi were investigated using two different media: DG 18 agar and malt extract agar with two different incubation temperatures (22°C and 30°C). *Aspergillus fumigatus* was scored using malt extract agar with an incubation temperature of 45°C. Measured airborne microbial concentrations expressed as cfu per m^3 air were used to calculate the emission rate expressed as cfu per h by employing a dispersal model according to the German TA Luft, Annex C or a modified formula for emission from chimneys. Immissions in the area surrounding the plant were recalculated using these models. As a result of these calculations no significant increase of microbial concentration compared to the concentrations described in literature for ambient air could be predicted at a distance of more than 500 m under the conditions employed. This indicates that composting facilities of the investigated type do not initiate heavy immissions in their vicinities. Only very small endotoxin concentrations could be detected outside the composting plant.

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INTRODUCTION

The hazards imposed on employees by biological agents have been discussed for a couple of years. The intention of workers protection has led to the formulation of the EC-guideline 90/679 [1] in this field.

Concerning waste management, the separate collection of biological waste has increased in Germany during the last decade. Now separate collection and biological treatment of biological wastes is official policy in Germany [5]. This fact rises the question how to maintain the personal safety of workers employed in biological waste treatment plants (e.g. composting facilities). The problem of providing adequate protection to the workers is discussed at different institutions ([2, 14], CEN/TC 137). Many basic problems related to biological agents (e.g. standards for sampling and analyzing biological agents in the working place atmosphere) are not solved up

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to now [10]. In addition public discussion has spread to the safety of people living in the vicinity of biological waste treatment plants.

In Germany people living in the neighborhood of projected composting plants are concerned by the question whether the biological emissions of the operating plant might have negative impact on their health.

Therefore the current study was conducted to elucidate the levels of biological agents in the vicinity of a composting plant. Immission concentrations of biological agents were calculated using models which are the approved standard in the calculation of immissions of chemical agents.

MATERIAL AND METHODS

Air samples were collected in the vicinity of the composting plant located at Stapelfeld near Cloppenburg in the northern part of Germany. Reference samples were taken at a control location where a similar composting plant is projected. When samples were taken there was no additional microbial load originating from a composting plant in the air of the control site.

The Stapelfeld plant is working with the Herhof box system illustrated in Figure 1. The Herhof procedure is characterized by an intensive decomposition conducted in closed composting boxes. The exhaust air of the intensive decomposition is purified by a biofilter. Following the intensive decomposition the compost material is subjected to a post-curing on windrows for a period between six and 12 weeks. In the Stapelfeld plant the post-curing is conducted in an open hall. This hall is roofed while walls are missing allowing a free flow of the wind throughout the hall. The material is shifted several times during the post curing. The bulk of microbial emissions into the environment is supposed to originate during the shifting and sieving of compost material in this open hall.

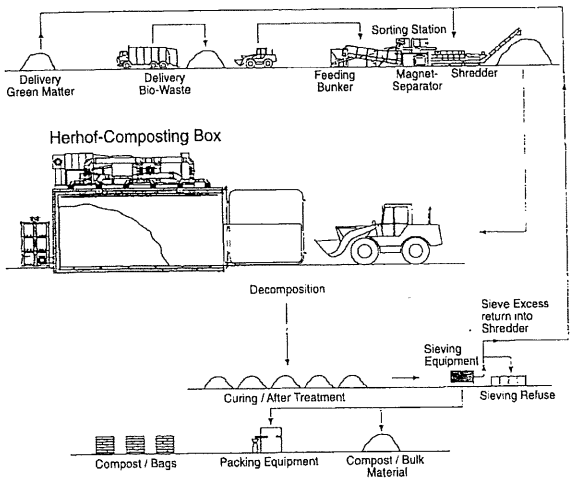

Figure 1. Display of biowaste decomposition with the Herhof box system.

Air samples for the determination of microorganisms and endotoxin content were taken in the neighborhood down-wind from the composting facility. The sampling points were positioned on along four lines down-wind the composting plant. The lines originated in the composting plant and were each separated by an angle of 45°. Controls were performed inside the open hall when the compost material was moved by a rotating sieve and

Table 1. Media and conditions used for the cultivation of airborne microorganisms.

Medium	Selectivity	Incubation temp.	Incubation length	Special remarks
CaSo Nutrient Agar (Merck No. 5458)	no specific selectivity	37°C	2 d	predominantly for bacteria
MacConkey Agar, (Merck No. 5465)	*Enterobacteriaceae*, Gram-negative bacteria	37 °C	2 d	coliforms bacteria produce red colonies, other Gram-negative bacteria like *Salmonella* or *Pseudomonas* can grow as well, growth of most Gram-positive bacteria is inhibited
Malt Extract Agar (Merck No. 5398)	yeasts and molds	22 °C	7 d	only molds were scored
Malt Extract Agar (Merck No. 5398)	yeasts and molds	30 °C	7 d	only molds were scored
Malt Extract Agar (Merck No. 5398)	thermophilic yeasts and molds (e.g. *Aspergillus fumigatus*)	45 °C	2 d	*Aspergillus fumigatus* was identified according to its morphology
DG 18 Agar plus Chloramphenicol (Oxoid CM 729)	xerophilic molds	22 °C	7 d	only molds were scored
DG 18 Agar plus Chloramphenicol (Oxoid CM 729)	xerophilic molds	30 °C	7 d	only molds were scored
Baird Parker Agar (Oxoid CM 275)	staphylococci	37 °C	2 d	suspected isolates were examined microscopically and by coagulation assay

outside the composting plant 75 m directly against the direction of the wind.

Sampling of air was conducted with a Sartorius MD 8 device (Sartorius AG, Göttingen, Germany) using the corresponding gelatin membrane filters. Samples of 1 m^3 volume were taken using a flow rate of 6 m^3/h. Three consecutive samples were taken at a definite sampling point. Filters were transferred into sterile plastic bags directly after sampling. The bags were closed and transferred to the laboratory within 24 hours as described earlier [3]. The gelatin filters were dissolved in 20 ml of 0.9% NaCl solution and shaken on a rotary shaker until a clear homogenous solution was produced. Fractions (volume 0.1 ml) of this solution were plated directly onto the different solid media described in Table 1 (two replicates of each solution). Culture and enumeration of microorganisms were conducted using the conditions listed in Table 1.

Microorganism concentrations were calculated as a mean of the three different filters sampled at each collecting point.

Samples for the investigation of airborne endotoxin were taken using a Stroehlein VC 25 dust sampler and quartz filters (Munktell MK 360, 150 mm, Cryo-Technik, Hamburg, Germany). The samples were collected at the same places as for microorganisms.

The analysis of endotoxin content was conducted using the *Limulus* assay as described earlier [3].

For the calculation of microbial emission per unit of time the mean of the concentrations measured at a distance of 150 m was used. Using these concentrations the emission rate was recalculated with the computer program AUSTAL-PC 3.2 (employing a dispersal model according to the official German TA Luft [4], supplied by Geomet, Berlin, Germany). Based on the calculated microbial emission the immissions in the vicinity of the composting plant were determined. The program AUSTAL-PC 3.2 is routinely used for the calculation of the immission of dust or chemical compounds in the neighborhood of industrial plants.

Alternatively to the AUSTAL-PC program, the means of the concentrations at a distance of 150 m away from the plant were used to calculate the emission of microbes with Giebel's formula. This formula has been originally developed by Giebel [11] empirically based on the measurement of NO_x-emissions in the close neighborhood of industrial plants. The modified formula used in this study is

$$s = \frac{Q}{2 \times E^{1.6} \times u} \times 2 = \frac{Q}{E^{1.6} \times u}$$

with s = immission concentration of microbes in cfu/m^3 air,
Q = emission of microbes from the source in cfu/s,
u = wind velocity in m/s,
E = distance between source and place for which the immission is calculated in m.

In this formula the equation originally published by Giebel is multiplied by a factor of two. This was done since the original Giebel formula was developed for emission from high chimneys. However, the maximum emission from composting plants is comparatively close to the surface. Therefore the factor of 2 was brought into the original Giebel formula to correct for this fact.

RESULTS

Airborne microbial concentrations as determined in the vicinity of the Stapelfeld plant are shown in Table 2.

The emission from the rotating sieve for total bacteria was calculated iteratively to be 1×10^{12} cfu/h corresponding to 2.78×10^8 cfu/s using the TA-Luft dispersal model.

When Giebel's formula was used to calculate the emission of total bacteria based on the concentrations measured at a distance of 150 m from the Stapelfeld composting plant, the emission was calculated to be 4.5×10^{10} cfu/h corresponding to 1.25×10^7 cfu/s.

These values are roughly in the same order of magnitude as published in the literature for the dispersal of *Aspergillus fumigatus* from sewage sludge compost piles [17]. These authors calculated for *Aspergillus fumigatus* an emission of 4.6×10^6 cfu/s corresponding to 1.66×10^{10} cfu/h.

When immission concentrations at defined distances from the source are calculated using the TA-Luft model

Table 2. Microbial concentrations determined in the air near a composting plant at Stapelfeld/Germany (figures are given in cfu/m^3 air for microbes, and in ng/m^3 air for endotoxin).

	Total bacteria on nutrient agar	Gram-negative bacteria on MacConkey Agar	*Aspergillus fumigatus*	Molds at 22°C on malt extract agar	Molds at 30°C on malt extract agar	Molds at 22°C on DG 18 agar	Molds at 30°C on DG 18 agar	Endotoxin[a] (ng/m^3 air)
Stapelfeld: near the rotating sieve	7.67×10^4	9.66×10^2	2.03×10^3	2.73×10^3	2.83×10^3	7.33×10^2	3.63×10^3	20.704
Stapelfeld: 75 m up-wind	4.33×10^2	0.00×10^0	0.00×10^0	2.67×10^2	1.00×10^2	3.30×10^1	1.00×10^2	0.161
Stapelfeld: 150 m down-wind[b]	2.83×10^3	0.00×10^0	2.00×10^2	3.67×10^2	8.16×10^2	8.35×10^1	2.92×10^2	0.236
Exhaust air emitted from the biofilter	3.30×10^1	3.30×10^1	6.00×10^2	1.43×10^3	8.33×10^2	5.67×10^2	9.33×10^2	0.008
Control location[c]	3.11×10^2	0.00×10^0	7.77×10^1	5.22×10^2	7.34×10^2	2.33×10^2	1.14×10^2	0.017

[a]Endotoxin values have been determined from a single sample per sampling point; [b]Mean of four sampling points composed of three single measurements each; [c]Mean of three sampling points composed of three single measurements each.

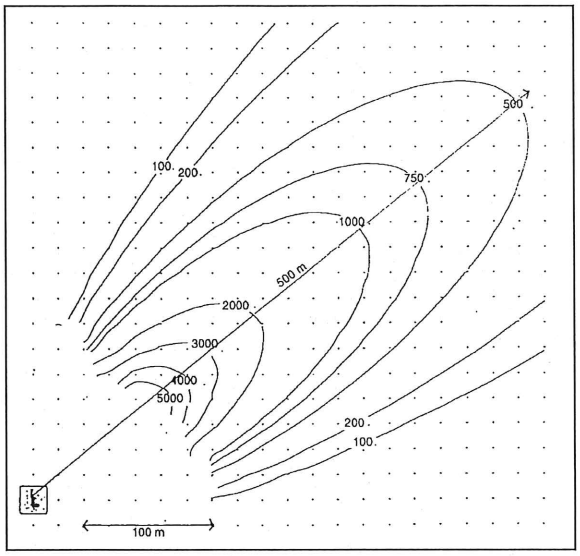

Figure 2. Prediction of microbial immissions in the vicinity of a compost plant (airborne bacteria) according to TA-Luft. L = source of emission. Curves with numbers = calculated concentrations of airborne bacteria (cfu/m^3).

with a maximum assumption (wind blowing constantly into one direction, dispersion class AK 4 (AK 4 means unstable temperature stratification) and wind velocity WG 3 (1.9–2.3 m/s), the calculation yields immission concentrations for total bacteria of less than 500 cfu/m^3 air at a distance of more than 500 m from the source with an orientation with the direction of the wind (Fig. 2).

According to the Giebel equation and assuming a wind velocity of 1.5 m/s, an immission concentration of 400 cfu/m^3 was calculated for a distance of 500 m from the source with the direction of the wind. These recalculations were conducted with approximately identical meteorological conditions as recorded during the sampling period.

DISCUSSION

The endotoxin data of this study clearly demonstrate that much lower endotoxin concentrations are found in the vicinity of the composting plant than in the working place atmosphere when compost material is moved around. Low values were also found at the control location without influence from a nearby composting facility.

Airborne endotoxins are discussed to pose a health risk to humans. However, generally accepted threshold values do not exist. For the working environment in livestock industries and an eight hour shift-length a threshold limit between 50 and 100 ng/m^3 air is discussed [8, 9, 12, 13]. The results of this study show that the endotoxin concentration found at a distance of 150 m downwind of the composting plant is by a factor of 100 lower than close to the rotating sieve in the plant. The endotoxin concentration found outside the plant is also 200 times lower compared to the threshold value of 50 ng/m^3 mentioned above. It seems that people living more than 150 m away from composting facilities of the investigated type do not bear a significant risk due to exposure to endotoxin emitted from the composting plant.

The highest concentrations of airborne microorganisms were determined directly near the rotating sieve where compost material is processed. Since the rotating sieve was located in an open hall microorganisms can be carried by air into the vicinity of the plant. Outside the plant, higher microbial levels were found down-wind compared to the measurements conducted up-wind. This indicates that at least a part of the microbial burden of the air found 150 m down-wind may be due to emissions from the composting plant. However, in some cases the value of ambient air at the control location without any composting plant was higher than the corresponding value determined near the Stapelfeld plant (e.g. 522 cfu/m^3 compared to 367 cfu/m^3 for molds at 22°C on malt extract agar). This fact demonstrates that the natural microbial burden of ambient air may contribute significantly to the microbial concentrations measured down-wind the Stapelfeld plant. Future research should be dedicated to a deeper investigation of the travel distances and the deposition characteristics of particulates from biocompost plants.

In this study the calculation of the emission which is in turn the basis for the dispersal calculation was conducted with the measured data at a distance of 150 m from the plant assuming that the total microbial burden detected originates from the emission of the facility. This leads to an overestimation of immission concentrations. In addition, both the TA-Luft model and the empirical Giebel formula are models for the dispersion of gases. The models do not consider sedimentation or inactivation processes which have a significant impact on bacteria or fungi in an airborne state. These facts also lead to an overestimation of immission concentrations when the above mentioned models are used.

The evaluation of the calculated immission with respect to the sanitary risks is a difficult problem. No generally accepted limiting concentrations for biological agents exist up to now. However, there are some recommendations in the literature, especially for the evaluation of microbial indoor concentrations. Reynolds *et al.* [19] state that airborne fungal concentrations of more than 500 cfu/m^3 indicate an abnormal condition in the indoor environment. Morey *et al.* [18] suggest that a level of viable microorganisms in excess of 1,000 cfu/m^3 indicates that the indoor environment may be in need of investigation and improvement. According to Rüden and Moriske [20] total viable microorganisms of 1,000 cfu/m^3 may occur indoors and do not mean any health risk for the inhabitants. For *Aspergillus fumigatus* Holmberg [15] stated that concentrations of thermotolerant *Aspergillus* of more than 50 cfu/m^3 turned out to be a significant risk factor with regard to eye irritation and respiratory symptoms.

The Niedersächsisches Landesamt für Ökologie, Hannover, Germany, recommends that the number of viable microorganisms in the working place atmosphere should not exceed 10,000 cfu/m^3. This value is under discussion in Germany but has been adopted nationwide

for evaluation of the working place atmosphere in recycling plants [2]. For the protection of people living in the vicinity of possible emission sources this value might be too high since it was stated for workers which are usually healthy whereas people living in the vicinity of plants might be weak, old or immunocompromised.

The published concentrations of airborne microorganisms found in ambient air outdoors vary to a great extent. Bovallius *et al.* [7] published bacterial counts in outdoor air between 100 and 10,000 cfu/m^3. For *Aspergillus* spp. and *Penicillium* spp. 1,000 cfu/m^3 have been published [16]. The same author found 3,000 cfu/m^3 for the mold genus *Cladosporium* and Bagni *et al.* [6] recorded up to 10,000 cfu/m^3 for molds.

The immission concentrations calculated in this study at a distance of about 500 m from the plant indicate that the calculated concentrations probably are within the natural variation range even if a composting plant as an additional source exists with the emissions determined in this study.

The health related properties of the biological emissions of a composting plant should not differ specifically from the properties of the natural ambient airborne microflora. So no specific risks related to biological emissions from composting can be expected.

However, little is known currently about the question whether the dispersion models applied in this study fit for the modeling of microbial emissions. A lot of systematic sampling of airborne microorganisms in the vicinity of significant emitents is necessary to answer this question. Standardization of methods in the field of sampling and laboratory analyses is necessary [8]. In the light of data obtained through systematic measurements, the existing models formulated for the dispersion of chemical compounds might have to be modified.

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Simple emission-reducing measures in an open biological waste treatment plant

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Abstract

In the course of composting biological waste, concentrations of various thermophilic and thermotolerant microorganisms increase. Moving piles of compost results in increased emissions of Actinomycetes and fungi. The present investigation deals with the reduction of airborne microorganism emission and immission in large-scale composting plants with open piles. Simple measures were introduced in order to reduce the release of bioaerosols when turning piles and the release of dust and bioaerols at large. These measures included the sealing of turning machinery with rubber mats, the wetting of piles before and after turning and regular cleaning and wetting driveways during the dry season.

Concentrations of airborne microorganisms during the summer season were determined on 5 days before and after the introduction of emission-reducing measures using the six-stage Andersen cascade sampler. The investigation showed that following the introduction of emission-reducing measures there was, at all locations, a highly significant reduction not only of all culturable indicator organisms (thermophilic actinomycetes and *Aspergillus fumigatus*) but also of total microorganism concentrations ($p < 0{,}001$). The introduction of the simple emission-reducing measures mentioned above, however, reduced the immission in the vicinity of the plant to such a degree that the natural background levels were reached at a distance of 150 m.

1. Introduction

Biological waste treatment plants cause an increase of naturally occurring microorganisms and an increased release of these microorganisms and their metabolic products into the ambient air. Composting plants with open piles cause short-term but characteristic emission peaks, especially when working with turned compost. Depending on the type of plant and local conditions, the impact on the air quality of the neighborhood can be determined at varying distances from the plant (Fanta et al., 1999; Wüst et al., 1999). Unturned piles on the other hand do not release significantly increased volumes of biological agents into the ambient air (Haas et al., 1999b).

Thermophilic and thermotolerant microorganisms are biological agents which are particularly characteristic for these processes. *Saccharopolyspora* sp., *Saccharomonospora* sp. and *Aspergillus fumigatus* are especially significant in this context (Haas et al., 1999a). The representatives of thermophilic actinomycetes mentioned are also used for measuring plant-related immission during the main rot. *A. fumigatus* is thermotolerant and emerges especially in that phase of the composting process when temperature rises (Göttlich et al., 1999). The microorganisms mentioned are potentially pathogenic and can cause extrinsic allergic alveolitis (EAA). In the unpolluted ambient air, their concentration is low. Presently there are no recognized criteria permitting the assessment of

potential health hazards for neighboring residents and employees (Heller and Rabe, 2001). Consequently, there are also no international or national limits or standards. Nevertheless it has been established, that the risk of developing symptoms of diseases rises with increased exposure to infectious, toxic or allergenic substances (Bunger et al., 2000). This is particularly relevant for sensitive individuals living in the vicinity of composting plants. Herr et al. (2003) studied residents living 150–200 m from a large scale composting plant which established for the first time that residents exposed to bioaerosol pollution suffer from respiratory irritations similar to mucous membrane irritations.

Austrian standards ensuring protection from emissions require a distance of at least 1000 m from hospitals, nursing homes or rehabilitation centers and at least 300 m from regular apartment buildings and housing (ÖNORM S 2205, 1999).

Since present-day technology does not guarantee complete prevention of microorganism emissions, especially when turning piles, it is necessary to minimize emissions as much as possible. In closed composting systems, the most important plant-side measures include adequate operation of ventilation systems and biofilters, regular monitoring of biofilters and the avoidance of other diffuse sources, for example by closing plant gates (Gerbl-Rieger et al., 1998). The present investigation served to examine the efficiency of simple technical measures for the reduction of microorganism emission and immission in open composting plants with open-air piles.

2. Material and methods

The present study determined the total numbers of culturable, aerobic, mesophilic and thermophilic microorganisms (mesophilic bacteria and fungi as well as thermophilic actinomycetes and bacillae) and of *A. fumigatus* inside a large-scale composting plant and in the vicinity of the plant (summer 2000). The same numbers were determined in summer 2002 following the introduction of the emission-reducing measures.

- Sealing of the turning machinery with rubber mats for the reduction of microorganism emission in the course of turning piles
- Wetting of piles before and after turning
- Regular cleaning and wetting of driveways in order to keep down dust emission (for this measure a loop line was installed in the plant investigated to wet the driveways during dry weather)

Table 1. Description of the facility investigated.

Input material	wood, sewage sludge, green cut lawn, bush cut (in addition sand and loam for soilification)
Process	mainrot in open table-pile composting windrows
Number of rots and capacity	25–30 windrows (7000–9000 m^3), L 90 m; W 4.5 m; H 1.8 m Capacity: ca. 65.000 t/year

Table 2. Measuring locations (L), positioning towards (P) and distance (D) from the plant.

P	D (ca)	Description
L1	3–10 m	Emission location between rots
L2	150 m	Inside plant (downwind)
L3	300 m	Street (downwind)
L4	300 m	Housing estate (downwind)
L5	500 m	Housing estate (upwind)
L6	800 m	Housing estate (upwind)

Concentrations of airborne microorganisms were determined on five days with approx. 4 weeks between each measurement (April 2000 through September 2000 and, following the introduction of the measures mentioned above, from April through October 2002) inside a large-scale composting plant (Table 1) and in the vicinity of the plant. Measurements took place between 10:30 a.m. and 1:30 p.m. at six different measuring locations (Table 2): two measuring locations (L1, L2) were inside the composting plant, four (L3–L6) were outside, at a distance of 300 m to 800 m from the plant. Locations L2 und L3 were downwind from the plant (prevailing wind direction). In selecting the measuring locations, care was taken to avoid interference from other sources of emission for the microorganisms measured.

Measurements were conducted using four six-stage Andersen samplers for four different culture media which were installed at a sampling altitude of 1.80 m above ground. Measurements at the individual locations was successive from L1 to L6 with 30 min intervals. Measuring time was four minutes at the immission locations (L2–L6) and 30 sec at the emission location at the time of turning piles (sampling volume 28,3l/min). The culture media listed in Table 3 were used as impaction media. Culture media for mesophilic bacteria were incubated at 37 °C

Table 3. Media for impaction.

Media	Incubation	Microorganism
Tryptic-soja-agar (TSA1), add: 1 ml cycloheximid (50 µg/ml) MERCK 5458	50 °C/ 120 h	Thermophilic actinomycetes + thermophilic bacillae
Tryptic-soja-agar (TSA2), add: 2 ml cycloheximid (50 µg/ml) MERCK 5458	37°C/ 48 h	Mesophilic bacteria
Blood-agar-base (BA), add: 5–10% human blood OXOID CM 271	37°C/ 48 h	*A. fumigatus*
Maltextract-agar (MEA), add: 3 ml streptomycin (0,2 g/l) + penicillin (0,1 g/l) MERCK 5398	25 °C/ 120 h	Mesophilic molds

for 48 hours, the culture media for fungi at 25 °C for 120 hours, the culture media for *Aspergillus fumigatus* at 37 °C for 48 hours and the culture media for thermophilic bacteria at 50 °C for 120 hours. Thermophilic actinomycetes and thermophilic bacillae were recorded separately.

Measurement of viable air-borne microorganisms is influenced by turning piles, air humidity, wind speed, temperature and radiation. In order to obtain comparable data, measurements of airborne microorganisms were only conducted during and after turning piles on days with stable, dry weather and at wind speeds of 0–2 m/sec maximum. Moreover, the median values of 5 measurements per location before and 5 measurements per location after the introduction of these measures were used. For all measurements, wind direction, wind speed and temperature were recorded. The results of monthly measurements recorded for the last 10 years using the identical method at unexposed locations in rural and urban regions were used as background levels (Wüst et al., 1999; Köck et al., 1998).

The statistical evaluation of the samples (calculation of median, minimum and maximum values as well as corresponding boxplots) was conducted using SPSS (release 11.0).

3. Results

3.1 *Data prior to the introduction of emission-reducing measures*

In the course of the five measuring days, the emission location (L1) showed the highest individual concentrations and the highest median values (for all microorganism groups and compared to all measuring locations). The median concentrations for mesophilic bacteria were 3.1×10^5 CFU/m^3, for thermophilic actinomycetes 2.4×10^5 CFU/m^3, for thermophilic bacillae 5.9×10^4 CFU/m^3, for fungi 4.7×10^4 CFU/m^3 and for *Aspergillus fumigatus* 1.5×10^3 CFU/m^3. At location L2 (150 m downwind), median concentrations for mesophilic bacteria were 1.9×10^4 CFU/m^3, for thermophilic actinomycetes at 2.9×10^3 CFU/m^3, for *Aspergillus fumigatus* at 1.8×10^2 CFU/m^3, for thermophilic bacillae 6.0×10^3 CFU/m^3 and for fungi 4.9×10^3 CFU/m^3.

At the immission locations, all concentrations were lower by one or two powers of ten compared to the emission location. Median values outside of the plant for mesophilic bacteria were between 3.3×10^3 CFU/m^3 (L5) and 1.3×10^4 CFU/m^3 (L3 downwind) and for fungi between 3.0×10^3 CFU/m^3 (L5) and 4.8×10^3 CFU/m^3 (L6). For *Aspergillus fumigatus*, median concentrations were between 1.4×10^2 CFU/m^3 (L6) and 2.8×10^2 CFU/m^3 (L3 downwind), for thermophilic bacillae between 1.6×10^3 CFU/m^3 (L5) and 6.8×10^3 CFU/m^3 (L3 downwind) and for thermophilic actinomycetes between 4.1×10^2 CFU/m^3 (L5) and 2.0×10^3 CFU/m^3 (L3 downwind).

3.2 *Data following introduction of emission-reducing measures*

Following the introduction of emission-reducing measures the investigations showed a highly significant reduction ($p < 0.001$) for all parameters at all locations. At the locations outside of the plant (L3–L6), significantly lower concentrations were found for all microorganism groups compared to the emission location. Median concentrations for mesophilic bacteria were between 1.4×10^2 CFU/m^3 (L5) and 7.6×10^2 CFU/m^3 (L3), for fungi between 5.3×10^2 CFU/m^3 (L2) and 1.0×10^3 CFU/m^3 (L3). For *Aspergillus fumigatus*, the median concentrations were between 1.7×10^1CFU/m^3 (L4, 5) and 5.3×10^1 CFU/m^3 (L1, 2), for thermophilic bacillae between 5.3×10^1 CFU/m^3 (L4, 5) and $1{,}4 \times 10^2$ CFU/m^3 (L3) and for thermophilic actinomycetes between 1.8×10^1

Table 4. Cfu/m^3 (min, max, med, St.dev. and background values) for all sampling locations.

		2000				2002				background
		Minimum	Maximum	St.dev.	Median	Minimum	Maximum	St.dev.	Median	level
location 1	thermophilic bacillae	4,7E+03	5,1E+05	2,2E+05	5,9E+04	3,5E+01	2,0E+02	7,3E+01	8,8E+01	7,5E+01
	thermophilic actinomycetes	1,1E+02	5,1E+05	2,1E+05	2,4E+05	0,0E+00	2,5E+03	1,1E+03	5,3E+01	2,0E+01
	A. fumigatus	3,9E+02	1,8E+05	7,7E+04	1,5E+03	0,0E+00	1,8E+02	7,9E+01	5,3E+01	1,0E+01
	mesophilic molds	4,8E+03	3,0E+05	1,2E+05	4,7E+04	1,2E+02	1,1E+03	3,9E+02	8,6E+02	1,0E+03
	mesophilic bacteria	1,2E+04	5,1E+05	2,5E+05	3,1E+05	1,1E+02	1,2E+03	5,0E+02	5,4E+02	2,1E+02
location 2	thermophilic bacillae	5,4E+03	7,8E+03	9,2E+02	6,0E+03	8,8E+00	4,1E+02	1,7E+02	6,2E+01	7,5E+01
	thermophilic actinomycetes	2,8E+02	2,9E+04	1,2E+04	2,9E+03	0,0E+00	9,7E+01	4,1E+01	5,3E+01	2,0E+01
	A. fumigatus	1,8E+01	1,7E+03	7,1E+02	1,8E+02	0,0E+00	1,7E+02	7,4E+01	5,3E+01	1,0E+01
	mesophilic molds	2,0E+03	7,8E+03	2,2E+03	4,9E+03	3,7E+02	8,1E+02	1,6E+02	5,3E+02	1,0E+03
	mesophilic bacteria	9,8E+03	2,5E+04	6,1E+03	1,9E+04	2,1E+02	1,4E+03	5,0E+02	3,7E+02	2,1E+02
location 3	thermophilic bacillae	1,9E+03	1,1E+04	3,6E+03	6,8E+03	8,8E+00	2,8E+02	9,9E+01	1,4E+02	7,5E+01
	thermophilic actinomycetes	7,1E+01	1,4E+04	5,6E+03	2,0E+03	8,8E+00	2,6E+01	7,0E+00	1,8E+01	2,0E+01
	A. fumigatus	0,0E+00	3,2E+02	1,3E+02	2,8E+02	0,0E+00	8,8E+01	3,5E+01	1,8E+01	1,0E+01
	mesophilic molds	1,8E+03	7,4E+03	2,1E+03	3,3E+03	1,8E+02	1,1E+03	4,2E+02	1,0E+03	1,0E+03
	mesophilic bacteria	3,9E+03	2,3E+04	8,2E+03	1,3E+04	2,3E+02	1,3E+03	4,1E+02	7,6E+02	2,1E+02
location 4	thermophilic bacillae	6,0E+02	4,7E+03	1,8E+03	2,3E+03	0,0E+00	1,5E+02	6,0E+01	5,3E+01	7,5E+01
	thermophilic actinomycetes	5,3E+01	4,9E+03	2,0E+03	8,7E+02	0,0E+00	7,9E+01	3,2E+01	1,8E+01	2,0E+01
	A. fumigatus	0,0E+00	6,7E+02	2,6E+02	1,8E+02	8,8E+00	4,4E+01	1,4E+01	1,8E+01	1,0E+01
	mesophilic molds	1,8E+03	1,2E+04	4,2E+03	3,5E+03	1,9E+02	6,5E+03	2,6E+03	9,4E+02	1,0E+03
	mesophilic bacteria	3,8E+03	1,2E+04	3,7E+03	5,8E+03	1,5E+02	5,1E+02	1,5E+02	2,6E+02	2,1E+02
location 5	thermophilic bacillae	2,5E+02	4,1E+03	1,6E+03	1,6E+03	2,6E+01	1,6E+02	5,6E+01	5,3E+01	7,5E+01
	thermophilic actinomycetes	1,4E+02	3,7E+03	1,7E+03	4,1E+02	0,0E+00	9,7E+01	3,8E+01	1,8E+01	2,0E+01
	A. fumigatus	1,1E+02	2,8E+02	8,0E+01	1,8E+02	0,0E+00	5,2E+02	2,3E+02	1,8E+01	1,0E+01
	mesophilic molds	1,2E+03	8,4E+03	3,0E+03	3,0E+03	1,5E+02	9,8E+02	3,1E+02	6,0E+02	1,0E+03
	mesophilic bacteria	7,5E+02	1,0E+04	3,5E+03	3,3E+03	1,2E+02	5,9E+02	2,3E+02	1,4E+02	2,1E+02
location 6	thermophilic bacillae	3,6E+02	5,4E+03	2,0E+03	3,8E+03	1,8E+01	1,1E+02	3,8E+01	7,0E+01	7,5E+01
	thermophilic actinomycetes	8,8E+01	7,0E+03	2,9E+03	1,1E+03	0,0E+00	2,6E+01	1,0E+01	1,8E+01	2,0E+01
	A. fumigatus	3,5E+01	2,3E+02	6,9E+01	1,4E+02	8,8E+00	4,4E+01	1,3E+01	2,6E+01	1,0E+01
	mesophilic molds	4,0E+03	5,0E+03	4,0E+02	4,8E+03	2,3E+02	1,2E+03	3,8E+02	8,0E+02	1,0E+03
	mesophilic bacteria	1,1E+03	1,8E+04	6,8E+03	9,2E+03	1,1E+02	4,2E+02	1,2E+02	1,9E+02	2,1E+02

CFU/m^3 (L3, 4, 5, 6) and 5.3×10^1 CFU/m^3 (L1, 2). Results are summarized in Table 4.

The statistical analyses showed a relative decrease of microorganism concentrations following the introduction of emission-reducing measures. The changes (reduction of microorganisms) were not dependent on temperature. For bacteria, there was a correlation with the location ($p < 0{,}05$) whereas for all other microorganisms the changes remained independent of location. If concentrations for the year 2000 are taken as a basis (100%), the introduction of the emission-reducing measures resulted in the following percentage reductions of microorganisms:

Microorganism	reduction to
thermophilic bacillae	1.7%
thermophilic actinomycetes	1.3%
A. fumigatus	16%
mesophilic fungi	0.95%
mesophilic bacteria	L1: 0.5%,
	L2: 3.0%,
	L3: 5.0%,
	L4: 3.8%,
	L5: 6.3%,
	L6: 1.9%

4. Discussion

The results prior to the introduction of emission-reducing measures show that under worst-case conditions (pile turning) significant concentrations of airborne microorganisms develop in composting plants. This, as well as the increased presence of thermophilic microorganisms characteristic of composting processes in the neighborhood of composting plants, has already been established by previous studies (Swan et al., 2003). At the emission location of the composting plant investigated, the concentration of thermophilic actinomycetes measured were even higher than the concentrations found in various other large scale composting facilities (Haas et al., 1999b; Reinthaler et al., 1999). At measuring locations outside of the plant (L3–L6) there were significantly lower concentrations compared to the emission locations. However, median concentrations were higher compared with the natural background levels characteristic of unpolluted rural and urban areas. They were also significantly higher compared to immissions recorded so far at various composting plants in Austria (Wüst et al., 1999; Reinthaler et al., 1999).

The second series of investigations showed that following the introduction of simple emission-reducing measures both the emissions and the immission of microorganisms from composting were significantly reduced. The total microorganism concentration in the immediate vicinity of the plant investigated (L1, L2) was lower than the emissions of large-scale composting plants investigated in the past (Haas et al., 1999a; Reinthaler et al., 1999; Reinthaler et al., 1997). This is especially true for the indicator organisms thermophilic actinomycetes and *Aspergillus fumigatus*. All measurements in 2002 showed significantly lower total concentrations of microorganisms than previously determined in the emission area of composting and other waste treatment plants without such measures. In comparison with the emission location, monitoring locations outside of the plant (L3–L6) showed significantly lower concentrations for all groups of microorganisms. Overall, these concentrations were also significantly lower than so far measured in the vicinity of composting plants (Haas et al., 1999a; Reinthaler et al., 1999; Reinthaler et al., 1997). The median concentrations of indicator organisms thermophilic actinomycetes and *Aspergillus fumigatus* were similar to the concentrations in unpolluted rural and urban areas. Thus, plant-related influences could not be demonstrated any longer. The lesser reduction of total mesophilic bacteria can be explained by additional anthropogenic influences at these locations such as dust caused by street traffic.

Previous studies have shown that, depending on climatic and geographical conditions, natural background levels were determined at distances from 150 m to 500 m (Fanta et al., 1999; Swan et al., 2003; Reinthaler et al., 1999). These differences are due to different plant sizes and types of plant. For a closed system, Fanta et al. (1999) demonstrated no impact on the ambient air starting at a distance of 200 m from the plant, whereas for open plants (6.000 t/a), an impact has been demonstrated for a distance of up to 500 m. Recer et al. (2001) found that bioaerosol emissions (thermophilic actinomycetes and *Aspergillus fumigatus*) from a large yard-waste composting facility (25.000 t/a) can significantly increase exposure levels at least 500 m downwind from the facility. In the present study, the impact of the plant (36.000 t/a) prior to the introduction of the measures was demonstrated as far as 800 m from the plant.

Depending on wind conditions, microorganisms can be transported across even longer distances. This, however, is only shown in individual measurements and is not critical for the assessment of the overall exposure (Bayrisches Staatsministerium, 1999; Mücke and Lemmen, 1997). For the assessment of health hazards for neighboring residents, it must also be taken into consideration that the impact of open plants on the ambient air depends on specific activities like turning of windrows and is thus limited in time. To date, there are only very few investigations of health hazards for residents in the neighborhood of composting plants. In an investigation of residents living in the neighborhood of a grass and leaf composting facility, Browne et al. (2001) established no association between allergy and asthma symptom incidence and *A. fumigatus* spore levels, but they could not asses the risk of unusual, but severe illnesses among very sensitive individuals. In a current study, Herr et al. (2003) were able to demonstrate a health impairment on residents near a large composting plant. In this study, total microorganism counts (fungi, thermophilic actinomycetes, bacteria) of up to 10^5 CFU/m^3 were measured at 200 m from the plant, whereas background levels were reached at a distance of 300 m. Among residents living 150–200 m from the plant complaints such as bronchitis, cough, fatigue were significantly more common when compared to residents at a distance of > 400 m.

Generally, the immission for neighboring residents should be kept as low as possible. For the microorganisms released by composting, natural background levels should be reached as quickly as possible. As shown in the present study, they can be achieved starting at a distance of 150 m downwind even in the case of large, open composting plants. The distance of 300 m between composting plants (starting at 50 t/a) and neighboring residents recommended by ÖNORM (1000 m between hospitals and plants) definitely makes sense and is sufficient as protective measures against bioaerosol immissions.

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Sayers, Margery

From: John Tegeris <johntegeris@gmail.com>
Sent: Monday, October 23, 2017 11:12 PM
To: CouncilMail; Kittleman, Allan; Lazdins, Valdis; Gowan, Amy; Peter Jensen; pwood@baltsun.com; mdzwonchyk@baltsun.com; aburnett@wjz.com; Kim Dacey; srorman@sbgtv.com; bzumer@sbgtv.com; ambarnett@sbgtv.com; Ted Mariani; Rick Lober/Annette; Brent Loveless; Stu Kohn; Rob Long; Preserve Dayton; Velculescu Victor; Jeff Harp; Jeff Harp; Luv of My Life; andrew.green@baltsun.com; John Tegeris
Cc: Tim Mayer; Mike Navarre/Lynn; Bethany Hooper; Rob Bundy; Dan O'Leary; Andrew Royle; Lisa Markovitz; Susan Garber; Carol Jane Gray; Ocheltree Janet; Erin Allen; John Allen; Al Risdorfer; Bono Tony V; Paul Morris; Paul Retzbach; Colleen Retzbach; Kristin Robertson; Lora Houck; Trip Kloser; Craig Ostrom; Julius Tunji Akintade; Chelakara Shankar; James Nickel; Banwarth Dave; dave.kromer@tunnellgov.com; Sylvie Leary; Alan Schneider; Paul Shoffeitt; Mike Bucci; Robert Scales; China Williams; Katie Hester; Mike; Patricia Soffen; Joanne Heckman; <darbus37@gmail.com>; Jennifer Bush; sharon.keeny@lnf.com; tilycog@comcast.net; cathydatz@yahoo.com; Richard Valentine; Belkacem Manseur; Alex Xu; Richard Taber; Phil Montag; <bstrickland@wtplaw.com>; ST Balimtas; Michael Burns; Paul Retzbach; <fernandesgj@washpost.com>; Eric Goldberg; <benabili@hotmail.com>; Rob Bovello; Paul Robertson; Michael Price; Doug Lee; Jay and Santa Bhalani; Ajay soodan; jmathew@acidd.us; Om Prakash Gupta; <jthensel61@gmail.com>; Benjamin Lee; <joelhouck66@yahoo.com>; Ty Shrader; sdwerlinich@aol.com; Williams; Z Zhang; Brian Lehman; Lisa Valentine; Denise Howze; Hafida Manseur; Ning Hu; dianawscales@gmail.com; Richard and Susan Taber; Marisa Montag; <estrickland@offitkurman.com>; Robin Balimtas; Kathy Burns; Home; Dahna Goldberg; Michelle Meney; <jmbovello@comcast.net>; Delia Velculescu; Annette Lober; rajput31@yahoo.com; Melissa and Larry Kramer; Jyoti Gupta; <s.hensel@live.com>; Carol Werlinich; Mirra Morris; Sally Ostrom; Karen K; Laurie Lehman
Subject: CB60: Food Waste, Compost and Our Neighborhoods Don't Mix

Below is yet more proof of what a horrible idea County Executive Kittleman and Council Members Sigaty and Fox had when they decided to allow Type 2 feedstock (food waste, animal mortality and manure) to be trucked in for composting and trucked out for commercial sale throughout all farmland in Howard County.

The DEP, Pennsylvania's equivalent of our MDE, suspended the Barnside Farm Composting's permit to collect food waste due to "excessive flies, odor and heavy equipment noises as early as 6:30am." This was less than a year after they received their permit to collect food scraps in the first place. No surprise, this facility also offered mulch and topsoil to its customers.

Please email the County Council (councilmail@howardcountymd.gov) and urge them to introduce amendments that will prohibit Type 2 feedstock materials from being trucked in for industrial composting and trucked out for commercial sale.

Please also email County Executive Kittleman (akittleman@howardcountymd.gov) and tell him that he simply cannot allow this to occur on his watch, and that he needs to make sure these operations stay at county run facilities such as Alpha Ridge.

Thank you for distributing to your network.

John Tegeris, PhD
President, DRPS

https://patch.com/pennsylvania/perkiomenvalley/barnside-farm-requesting-food-compost-reinstatement

Barnside Requests DEP Permit Reinstatement

The DEP is requesting public comment on the facility's permit to compost food, which was suspended after neighbors complained of insects, odors and noise.

Oct 17, 2012 2:15 am ET

The Pennsylvania Department of Environmental Protection (DEP) is requesting public comment on a request from Barnside Farm Composting, of Haldeman Road in Lower Salford Township, to reinstate a food composting permit, according to the DEP.

At the September Perkiomen Township meeting, a resident alerted the township supervisors to Barnside's application, which has since moved to the next step of a DEP review.

"It's a very sticky situation because there are four townships right there," said the Haldeman Road resident. "The people affected by it are in this township, and that is why I wanted to make you aware."

This application for a permit reinstatement comes less than two years after complaints of odors, noise and insects prompted the Department of Environmental Protection to shut down the Lower Salford company's food waste operation.

Since its inception in 1998, the company offered mulch, compost and topsoil to customers. In May of 2010, owners Walt and Nancy Larkin began receiving food waste, after being granted a permit by the DEP.

Less than a year after the Larkins were received the permit to collect food scraps, local residents met with DEP and local officials to testify about their living conditions as a result of the food composting - issues included excessive flies, odor, and heavy equipment noises as early as 6:30 a.m., according to the Souderton Independent.

The DEP then suspended the facility's permit to collect food waste, though it continues to collect yard waste.

Sayers, Margery

From: Pat <pdmilln@aol.com>
Sent: Tuesday, October 24, 2017 11:36 AM
To: CouncilMail
Subject: Mulch and Composting topic reference materials
Attachments: Aspergillus, Aspergillosis & Composting CIWMB 1994.pdf; Exhibit1a_AEM1977AF_Occurrence.pdf; Exhibit1b_AEM1980Dispersal.pdf; BergeJavma2009.pdf; Arikan2009.pdf; Hakk Millner Larsen 2005 JEQ.pdf; Exhibit2b_AEM82ClosedBoll.pdf; Oxytetracycline Sorption to Organic Matter by Metal-Bridging JEQ MacKAY & CANTERBURY.pdf; Interaction of Tetracycline with Aluminum and Iron Hydrous Oxides EST.pdf; Dust and endotoxin from peppermint & chamomile during processing .docx

I tried sending single documents before, but the file size was too large and the message bounced back with that size problem annotated.
I have broken up one of the documents into parts so that there are smaller size files.
I may send you additional files if than those attached herewith if I can figure out how to reduce the size to less than 26MB for the single file.

The attached address issues concerning
1) dispersal of odors and fungi from composting sites
2) degradation of hormones during proper composting
3) degradation of antibiotics during proper thermophilic composting (most antibiotics are thermolabile at composting temperatures)
4) mortality composting
5) information on ODTS (organic dust toxic syndrome) - a respiratory disease afflicting some agricultural workers exposed to organic dusts. Sectors of agriculture affected include cotton and flax mill workers, organic and conventional tea workers, farmers exposed to moldy hay and barn/animal dusts, grain workers, poultry and bird keepers, and related occupations. There is considerable literature available at the national library of medicine documenting agricultural worker exposure relation to this respiratory disease. High, and repeated exposures to organic dust concentrations in the workplace over time can lead about 15-20% of workers to experience varying degrees of this disorder.

Ann Intern Med. 1984 Aug;101(2):157-63.

Acute bronchoconstriction induced by cotton dust: dose-related responses to endotoxin and other dust factors.

Castellan RM, Olenchock SA, Hankinson JL, Millner PD, Cocke JB, Bragg CK, Perkins HH Jr, Jacobs RR.

Abstract

Fifty-four healthy humans, selected for their acute airway responsiveness to cotton dust, had spirometric tests immediately before and after 6 hours of exposure to card-generated cotton dust from seven different cottons (of several grades and growing regions). During exposures, we measured airborne concentrations of viable fungi and bacteria (total and gram negative), vertically elutriated gravimetric dust, and vertically elutriated endotoxin. Correlation between each of these five exposure indices and exposure-related acute changes in forced expiratory volume in 1 s showed a statistically significant relationship between all of the indices except concentration of viable fungi. Of the other four indices, endotoxin was the most highly correlated (r = -0.94; p less than 0.00001), and gravimetric dust was the least correlated (r = -0.34; p less than 0.05). These findings suggest that gram-negative endotoxin may play a major role in the acute pulmonary response to inhaled cotton dust.

PMID:

6742645

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Pat Millner

Aspergillus, Aspergillosis, and Composting Operations in California

Technical Bulletin No. 1

California Integrated Waste Management Board

by

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INTRODUCTION

Californians have been moving rapidly to implement the mandated solid waste diversion goals of the Integrated Waste Management Act (Act) of 1989. The Act allows, and encourages, local governments to use composting to help them attain the diversion goals. Thus, since 1989 many local jurisdictions and the private sector have planned and/or implemented composting operations.

As a part of the process of implementing the Act, the state's Local Enforcement Agencies (LEAs) for solid waste management have been addressing the siting and operation of new and expanded composting facilities, and are continuing their routine compliance investigations and enforcement actions at existing composting facilities. All these facilities may utilize a wide variety of potentially valuable secondary organic materials such as green waste (yard waste), food waste, industrial waste, agricultural waste, sewage sludge, and mixed municipal solid waste, and represent both a facility management challenge for LEAs and an economic opportunity for local governments and entrepreneurs. The presence of *Aspergillus fumigatus*, a ubiquitous fungus which is both a normal and integral part of the composting process and a potential health risk to certain high-risk individuals, compounds that challenge.

The primary audience for this technical bulletin is the officers of the state's local enforcement agencies, but staff hope composting facility operators and local government officials will also find it useful. The bulletin underwent peer review with professional staff of three state agencies: California Department of Health Services, Office of Environmental Health Hazard Assessment, and the Integrated Waste Management Board (see Acknowledgements).

This bulletin was produced as a direct result of the expressed need of several LEAs for information on *Aspergillus fumigatus*, its potential adverse health effects, and its role and management in composting operations. The scientific information in the bulletin, particularly the "best management practices" described in Section 6, are offered to LEAs to help guide them in their interactions with composting facilities and their professional decision-making. This bulletin is not r a Board policy nor a set of regulations or standards.

The bulletin is divided into several parts, in which headings are worded as commonly asked questions about *Aspergillus fumigatus* and composting:

1. What is the *Aspergillus* organism and its life cycle?
2. What are the common sources of exposure to *Aspergillus*?
3. What is the disease called aspergillosis?
4. What are the levels of *Aspergillus* spores in the ambient environment, and in composting operations?
5. What are the potential health hazards of *Aspergillus* in the ambient environment and in composting operations?

6. What "best management practices" can reduce ambient air exposure to *Aspergillus* spores?
7. How do the state minimum standards for composting facilities help reduce exposure to *Aspergillus*?
8. How can one identify (diagnose) an *Aspergillus* problem in a composting operation?
9. How does one monitor and sample for *Aspergillus* spores in the ambient environment?
10. Conclusions

1. WHAT IS THE ASPERGILLUS ORGANISM AND ITS LIFE CYCLE?

Aspergillus fumigatus is a fungus and one of many microorganisms which bring about the everyday decay of leaves, wood and other organic matter in our environment. It may be found virtually everywhere on earth, and, although we are all exposed to it regularly, it does not normally cause disease. Our bodies' immune system normally acts as if it were an innocent visitor, unless it invades tissues. In that event, the immune system responses will protect us from infection, very much as it does from pathogenic bacteria or viruses.

Aspergillus fumigatus, a fungus in the Class Deuteromycetes (also known as Fungi Imperfecti), is, of a multitude of *Aspergillus* species in the world, the one most suited for growth in humans. The fungus is an aerobe, that is, it prefers aerated microhabitats for growth and reproduction, but is capable of surviving at low levels of oxygen (Foster 1949:162, Hawker 1950:104, Lilly and Barnett 1951:88). *Aspergillus fumigatus* normally obtains its nutrients from decaying organic matter (i.e., it is saprophytic), but can obtain nutrients from living cells (i.e., it can be parasitic). The life cycle is normally saprophytic; invasion of tissues is incidental to its normal life cycle (Sinski 1975).

Spores (also called conidia), one of the stages of the fungus' life cycle, are the resistant form of the fungus, and the form responsible for dispersal in the ambient environment. These asexual spores are borne on special structures called conidiophores (see **Figure 1**), from which they are released into the atmosphere. The spores are very light in weight and therefore are easily spread by air currents. Also, the spores' small size -- 2.0 to 3.5 micrometers average -- allows them to gain access to the alveolar space in the lungs (Sinski 1975, Rippon 1982). The spores resist desiccation by means of thick cell walls and cell membranes (Sinski 1975).

Hyphae (singular: hypha; a mass of hyphae is a mycelium) are the vegetative, growing, long and filamentous forms of the fungus. They comprise another stage of the life cycle: the hyphae generate the conidiophores. The hyphae invade decaying organic matter and, in rare cases, living tissues.

Aspergillus fumigatus can germinate at approximately 80% relative humidity (RH), but its growth is more optimal at approximately 98% RH (Panasenko 1967). The moisture

content of the medium upon which the fungus occurs is also important in germination and growth.

Aspergillus fumigatus has been characterized as one of the most frequently found fungal species in airborne spore (airspora) surveys (Composting Council, 1993:9) The organism can readily grow and reproduce in a wide range of temperatures, from about 12° C to 50-55° C. Because it is able to live in temperatures ranging from less than 20° C to more than 50° C, it is classified by Cooney and Emerson (1964) as a thermotolerant fungus, in contrast to thermophilic fungi which by definition do not survive in temperatures below 20° C.

Aspergillus fumigatus is a normal and integral part of the composting process, participating with other microbes in the final breakdown of compostable materials to a finished product, stabilized compost (Boutin and Moline 1987). An enlightening description of microbial population dynamics occurring during composting was offered by Kramer et al. (1989).

> "During composting, organic materials are decomposed by the growth of mesophilic organisms. During the process, heat is generated because of fermentation and results in the elimination of mesophilic organism[s] because of their thermolability. Thus, the substrate becomes ideal for growth of thermotolerant and thermophilic organisms [such as *A. fumigatus*] because of the lack of competition by relatively thermolabile organisms initially colonizing the compost."

See **Figure 2** for a hypothetical diagram of the typical phases of maximum *Aspergillus* concentrations during the composting cycle.

2. WHAT ARE THE COMMON SOURCES OF EXPOSURE TO ASPERGILLUS?

In the ambient environment, *Aspergillus fumigatus* is commonly found in a great range of sites and materials, including soils, moldy grains, straw and hay, bark and woodchips, house dust, and sewage sludge. The spores are very common in bird droppings, and are found in dung of cattle, horses and sheep (Raper and Fennell 1965, Millner et al. 1977, Kwon-Chung and Bennett 1992).

Inhalation of spores is the most common route of human exposure.

A number of everyday activities indoors and outdoors can provide exposure to *A. fumigatus*, including lawn mowing, gardening, home landscaping, potting of household plants in soils, raking leaves (Sporik et al. 1993) and walking through an arboretum or along a nature trail. One author (Kowal et al. 1978) suggested mowing a lawn may be the most common source of exposure to *A. fumigatus* for residential dwellers. Residential or occupational exposure also can occur from contaminated air conditioners, construction dust (Bodey and Vartivarian 1989, Staib 1992, Kwon-Chung and Bennett 1992), and improperly managed compost piles in backyards or commercial

operations. In Kansas, *Aspergillus* spp., *Penicillium* spp. and other fungi were primarily associated with homes with dirt floor crawlspace basements and homes with gas stoves (both offer moist environments; gas combustion generates water vapor and CO_2)(Su et al. 1992).

3. WHAT IS THE DISEASE CALLED ASPERGILLOSIS?

Aspergillus fumigatus is the *Aspergillus* species most pathogenic to humans (Sinski 1975, Mackenzie 1988). Four disease entities (described in **Box 1**) can result from exposure to sufficient quantities of *A. fumigatus* spores (Rosenberg 1993).

Three basic mechanisms of pathogenesis are responsible for illness: immune hypersensitivity of the patient to antigens present in the fungus or its spores; saprophytic colonization of air spaces in sinuses, bronchi or lungs; or invasion of tissues by fungal mycelia (Kwon-Chung and Bennett 1992).

No one has yet demonstrated a clear dose-response curve (Maritato et al. 1992), a threshold spore concentration, or duration of sensitization needed to cause any of the four disease entities described in Box 1[1]. Also noted in Box 1, in the literature reviewed by staff only two cases of illness, one of acute bronchopulmonary aspergillosis (ABPA) in a pre-disposed (asthmatic) individual in the USA and one of hypersensitivity pneumonitis (HP) in a compost worker in Belgium, have been linked to a commercial composting facility. However, in microbiology, like toxicology, "the dose makes the poison". Thus, while we have do not have good data on infective doses of these organisms, it is reasonable to expect that increasing the potential dose increases the likelihood of eliciting a response, even in otherwise normal people[2]. Therefore, in preventing or reducing health risks from composting facilities, it is important to reduce worker exposure to spores by utilizing a set of best management practices (discussed in Section 6, below).

There are individuals who, due to special circumstances, may be at higher risk of one of the four types of aspergillosis. For example, indoor sources of *Aspergillus* spp., including *A. fumigatus,* have been responsible for infecting high risk hospitalized patients, patients who are immunocompromised or suffer certain other serious illnesses discussed in Box 1. The sources include indoor potted plant soil (potting soil),

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[1]The threshold concentrations required to evoke allergic symptoms to two common molds, *Alternaria* and *Cladosporium*, are estimated to be about 100 *Alternaria* or 3000 *Cladosporium* spores/m^3 of air. Holmberg (1987) found that airborne levels greater than 50 Colony Forming Units (CFU) per m^3 of air containing a mixture of thermotolerant *Aspergillus* species were a significant risk factor for eye irritation and respiratory symptoms. (Fifty CFU is equivalent to ~50 viable spores/m^3.)

[2]Source of discussion: Memo from Robert Holtzer, M.D., Office of Environmental Health Hazard Assessment, Cal/EPA, dated 24 November 1993, to Steven Ault, CIWMB.

uncontrolled dust from nearby construction activities, and hospital air ventilation systems (Bodey and Vartivarian 1989, Staib 1992).

BOX 1: Aspergillosis Disease Entities

About 3-5% of the U.S. population suffer from extrinsic (allergic) asthma (Reed 1981). About 20% of the U.S. population is genetically predisposed to react to allergens in the environment (Burge 1988). In the first type of aspergillosis illness, people with this predisposition may develop this form of asthma upon becoming sensitized to *Aspergillus* species. Asthmatics may find their asthmatic condition aggravated upon exposure to *A. fumigatus*.

In the second disease entity seen, some people develop allergic bronchopulmonary aspergillosis (ABPA), a condition in which *Aspergillus* spores germinate and the resultant mycelial growth can potentially block the bronchi (Vaughan 1993). Patients may cough up small, brown plugs of mycelia. There is no invasion of tissue. However, the patient may suffer lung fibrosis and may, over time, become more susceptible to other lung diseases. In one case of ABPA (Kramer et al. 1989), exposure to a nearby
(250 ft. distance) commercial leaf composting operation "...might have contributed to the development of [the patient's] disease", an individual who was already asthmatic (clinically, "atopic").

The third disease entity, related to ABPA only because it is immune-mediated, hypersensitivity pneumonitis (HP) (also called extrinsic allergic alveolitis) is often associated with repeated exposure to an identified -- often occupational -- source of high levels of antigen. Only 5 to 10% of persons regularly exposed to the wide range of allergenic agents which typically produce HP actually develop the disease (Rosenberg 1993). Only one case of HP has been reported in compost plant workers (Vincken and Roels 1984), occurring in Belgium. A case of HP caused by *A. fumigatus* occurred in a patient in Japan who cultivated vegetables in a poorly-constructed home greenhouse (Yoshida et al. 1993).

The fourth disease entity, invasive aspergillosis (IA), is seen in people whose normal immune systems are compromised by other serious diseases such as leukemia, lymphoma, carcinoma, tuberculosis, emphysema, or diabetes; or by use of immunosuppressive drugs (often used with organ or bone marrow transplant operations); or by large doses of corticosteroids. In IA, there is an actual invasion of lung tissue or skin, and often dissemination by means of blood to other parts of the body. The prognosis for IA is grave.

Serious illness or deaths from aspergillosis in patients without any predisposing conditions are quite rare. One reported case of "fatal local invasive pulmonary aspergillosis [IA from *A. fumigatus*]... [occurred] in a previously fit young adult patient who had no predisposing factors other than exposure to fungal spores in his occupation as a gardener." The researchers (Zuk et al. 1989) could find only one similar case in adults in the previous 30 years; in that case the causative organism was *Aspergillus niger*, not *A. fumigatus*. Ten separate cases of IA in children, caused by *A. fumigatus*, have also been reported (Strelling et al. 1966). At least five of the children lived in agricultural environments, where *A. fumigatus* is common.

In summary, in the literature reviewed by staff only two cases of illness (discussed above), one of ABPA in a pre-disposed (asthmatic) individual in the USA and one of HP in a compost worker in Belgium, have been linked to a commercial composting facility.

4. WHAT ARE THE LEVELS OF ASPERGILLUS SPORES IN THE AMBIENT

ENVIRONMENT, AND IN COMPOSTING OPERATIONS?

Ambient Environment.

Figures 3-4, based on preliminary data compiled by the Composting Council (1993: Table 3), show the measured ambient air concentrations of spores (number of spores/m^3 of air sampled) in the ambient environment (neighborhoods, yards), in homes, and in certain workplaces.

Outdoors.

In the outdoors, the *Aspergillus* group of fungi is generally less prevalent than the fungi *Alternaria* and *Cladosporium*, although it is the most common group of outdoor airborne fungi among those that can be pathogenic to people. *Aspergillus fumigatus* concentrations outdoors rarely exceed 150 spores/m^3 (US EPA 1991).

Indoors.

A. fumigatus has been found to be among the most common molds found indoors (Kothary et al. 1984: Tables 2-4). The fungus appears more common in autumn and winter in North America and Europe (Larsen and Gravesen 1991; NRC 1981). In clean houses, indoor airborne concentrations of *A. fumigatus* spores range from 0-200 spores/m^3, typically (NRC 1981). In a study of 68 homes in a cross-section of southern California homes (Kozak et al. 1979), only 2.9% of the homes had *A. fumigatus*, where the airborne spore concentrations ranged from 0-5 spores/m^3, with a mean of 0.2 spores/m^3.

People living in some homes are at higher risk than others. For instance, in one study *Aspergillus spp.* were significantly more frequent in homes with pets, in comparison to homes without pets (Hirsh and Sosman 1976). Homes with neglected potted plants, or faulty air conditioning systems (e.g., dirty ducts and air filters) are also a higher risk environment.

Composting Operations.

A. fumigatus usually occurs in a layer of mycelia found from about 5-40 cm. (2-16 in.) inside a compost pile; concentrations are greatest at about 5-15 cm.(2-6 in.) inside a pile (Gotaas 1956, Boutin and Moline 1987).

Figure 5 provides a list of concentrations of *A. fumigatus* spores at several composting facilities in North America. Concentrations at composting operations are quite variable and often, but not always, higher than concentrations in the ambient air of residential areas (e.g., Kothary et al. 1984). A study of ten commercial compost facilities in the USA (ChemRisk 1991) found airborne concentrations of *A. fumigatus* at the active site of operations to be, on the average, 10-fold higher than background levels, but the concentrations fell off sharply within 500 feet of the operational site. If the nearest human receptor is located beyond the point at which concentrations fall to background

levels, there is no elevated exposure occurring.

The concentrations of airborne *A. fumigatus* spores were measured at four enclosed composting plants in Sweden. Sampling sites in the plants were selected to be representative of worker locations for both waste processing and compost manipulation. Operations included municipal solid waste (MSW) and sludge composting, with and without wood chip bulking agents. At interior sampling sites close to actual composting operations, *A. fumigatus* airborne levels ranged from 1×10^2 to 6×10^6 CFU/m^3, with a median concentration generally less than 1.26×10^5 CFU/m^3 (Clark et al. 1983).[3]

The use of bark or wood chips (e.g., as a bulking agent for sewage sludge composting) typically raises the on-site level of airborne *A. fumigatus* spores (Millner et al. 1977, Millner et al. 1980, Clark et al. 1983). In one study in Maryland, *A. fumigatus* levels in sewage sludge rose from 10^2 or 10^3 CFU/DGW[4] to 2.6-61.0 x 10^6 CFU/DGW when mixed with wood chips which were stockpiled for various lengths of time. The increase appeared to be caused by wood chips stored in moist piles which were allowed to generate heat (Millner et al. 1977).

Increased *A. fumigatus* spore concentrations have also been observed in screened compost; this may be due to re-inoculation by spores as compost passed through contaminated screens multiple times (Olver 1979); others have suggested that multiple screenings may break up spore clusters, releasing more spores.

5. WHAT ARE THE POTENTIAL HEALTH HAZARDS OF ASPERGILLUS IN THE AMBIENT ENVIRONMENT AND IN COMPOSTING OPERATIONS?

Ambient Environment.

Several researchers (Raper and Fennel 1965, Sinski 1975, Olver 1979, Epstein and Epstein 1985, Epstein and Epstein 1989, Maritato et al. 1992, Epstein 1993) have presented persuasive arguments for the lack of health risk from *A. fumigatus* for exposed healthy people, whether they are working in a composting facility or living nearby. Typical of this widespread view, Emmons et al. (1977:289) noted:

> "In routine autopsy examinations of the lungs of individuals dying from other causes, colonies of *Aspergillus* may be found in bronchi which exhibit little or no evidence of inflammatory reaction."

As well, Olver (1979) stated:

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[3]CFU = Colony Forming Unit. One colony-forming unit represents the growth of one viable spore on an appropriate fungal growth medium.

[4]DGW = Dry Gram Weight. That volume of a desiccated material which weighs one gram.

> "The fungus [*A. fumigatus*] is encountered by most people in a wide diversity of environments. The mere presence of the fungus within the human body is very common and is not necessarily indicative of a diseased condition."

Composting Operations.

One should recognize that composting facilities do represent sites where there is a massive culturing of *Aspergillus fumigatus* organisms in relatively small areas compared to most "natural" or background circumstances. Thus, without dust control, there is an elevated risk of exposure to spores for workers at compost facilities. In a worst-case scenario, a respiratory model developed by Boutin et al. (1987) estimated that a completely unprotected worker shovelling mature compost at a highly contaminated site could inhale 25,000 to 30,000 viable spores per hour. However, elevated exposure is not automatically synonymous with an elevated health risk for compost workers (or neighboring communities). Epstein (1993) discusses several composting facilities in the USA in which health monitoring (physical exams) of compost workers has been conducted; the results of the physical exams did not reveal any illnesses directly associated with composting. As discussed in Section 6, dust exposures at composting facilities are readily controllable, and control benefits and protects both facility workers and nearby residences.

However, many public health specialists, scientists, and engineers in North America and Europe believe that properly operated composting and co-composting operations present little health risk to normal compost facility employees, and negligible if any risk for nearby residences (Millner et al. 1977, Clark et al. 1983, Epstein and Epstein 1985, Boutin and Moline 1987, Maritato et al. 1992). Diaz et al. (1992) stated:

> "The existence of hazard from the spores of *A. fumigatus* [at commercial composting facilities] is yet to be demonstrated. The infectivity of the spores is low. Consequently, any danger posed by it would be of significance only to the unusually susceptible individual. Nevertheless, prudence indicates that an open-air compost plant should not be sited in close proximity to human habitations."

Further information on the potential health effects from composting may be obtained from:

Jon Rosenberg, M.D., Public Health Medical Officer III and
Epidemiologist
California Department of Health Services
Division of Communicable Disease Control
Infectious Disease Branch
2151 Berkeley Way, Room 708
Berkeley, CA 94704-1011
(510) 540-3233 or (510) 540-2566

6. WHAT "BEST MANAGEMENT PRACTICES" CAN REDUCE AMBIENT AIR EXPOSURE TO ASPERGILLUS SPORES?

Reducing the dispersal of *A. fumigatus* spores appears to be the best way to reduce exposure and help protect the health of compost workers and the neighboring communities.

Board staff believes that the suggested management practices discussed below, can help reduce the dispersal of spores into the air. Staff believes these practices are suitable for all commercial aerobic composting operations (whether they be windrows, aerated static piles, or the various types of in-vessel reactors: vertical, horizontal, or rotating drum). The best management practices (described below) include:

- Proper composting facility siting, design and construction;
- Facility operational practices;
- Engineering and administrative controls; and
- Use of personal protective equipment.

Board staff believe the reader will recognize these practices are common sense in nature, and focus on a combination of proper composting operations management, dust control, and personal protection to reduce exposure to *A. fumigatus*. The best management practices are drawn in part from Epstein and Epstein (1989), Composting Council (1993), Tchobanoglous et al. (1993), and D. Krause, CIH, personal communication 1993.

Facility Siting, Design and Construction

- **Siting**. Some scientists (Millner et al. 1977, Olver 1979, Kramer et al. 1989, Diaz et al. 1992) have recommended that buffer zones may be considered between certain types of composting facilities and nearby residences, hospitals, or schools to reduce the risk of exposure to odors and air contaminants.

  Millner et al. (1977) noted: "In consideration of off-site health matters related to air dispersal of spores, a buffer distance between a composting operation and health-care facilities or residential areas may be needed." Olver (1979) noted the "...buffer zone that should normally be provided around the composting site for odor control should work equally well to confine the high conidia levels of the fungus to the processing area". Diaz et al. (1992) noted: "... prudence indicates that an open-air compost plant should not be sited in close proximity to human habitations." Kramer et al. (1989) stated: "Consideration should also be given to locating compost sites similar to the present one [a municipal leaf composting

facility] more than 2 miles from residential areas in order to minimize potential microbial contamination of the environment." Only the latter author has recommended a specific buffer zone width.

The Board's current green waste composting regulations (14 CCR Section 17859(a)) require a setback of at least 300 feet of the facility's active compost materials areas from any residence, school or hospital, excluding on-site residences, unless one of three conditions are met. One of these conditions (Section 17859(a)(3)(C)) is that the local enforcement agency (LEA) may approve in writing a variance from this number. Under this subsection the LEA may allow a facility to be sited closer than 300 feet to any residence, school or hospital; alternately, the LEA may require the facility to be sited at a specificed distance greater than 300 feet away from any residence, school or hospital.

In selecting a buffer zone as a mitigation measure for odor, dust or spore exposure, some factors to consider are: variations in wind direction and speeds through the year (windrose data); background (ambient) spore concentrations; spore production, release and transport data; effectiveness of the dust control measures to be used at the site; types of occupied buildings and their distance from the proposed composting facility; types of nearby communities (e.g., retirement or mixed communities) and their health status.

- **Enclosed facilities**. Indoor (enclosed) buildings can be designed and constructed to reduce spore emissions to the atmosphere (see Engineering Controls, below). Also, certain machinery operations (e.g., tub grinders, trommels, hammermills) which generate high volumes of dust outdoors or indoors may need to be enclosed, physically separating the worker from the source of dust.

- **Compacted or paved road surfaces**. Compost leachate and runoff may carry and concentrate spores and other pathogens (Kramer et al. 1989). One may compact or pave earthen surfaces for control of dust, compost leachate, and water runoff. Composting process wastewater may be disposed to sanitary sewers or septic tanks, or reintroduced into the compost material.

- **Berms and windbreaks**. Placement of planted berms and windbreaks (trees) may change ambient wind direction, directing it to flow away from nearby homes.

Facility Operations

- **Proper aeration and mixing**. Regular and uniform (thorough) blending and mixing of windrows will aid proper composting, as will a proper initial blending of wastes in aerated static piles. Bulking agents should be of a quality to minimize the formation of airspora (bioaerosols).

  In a careful study by Millner et al. (1980) of windrow and compost pile operations

where waste was manipulated with and without use of a front-end loader, there was an observed paucity of windspread spores from undisturbed compost piles (range 0-14 spores/m^3; average 2.2 spores/m^3), when compared to the mechanically agitated piles and windrows (range 1390-5020 spores/m^3, from 3-60 m downwind). Spore counts of *A. fumigatus* dropped off very rapidly (as measured 3 m downwind from the pile), from 1390 spores at peak agitation to 39 spores, within 15 minutes after mechanical agitation ceased.

This study, among others (Kothary et al. 1984), indicates that static aerated piles may put fewer spores into the air than a windrow operation with its need for regular mechanical agitation. The study also found that the use of front-end loaders (or other machines which mix and disturb compost) put significant amounts of spores into the air for short periods of time.

- **Moisture control**. Optimal windrow or aerated static pile moisture content is 50 to 60%. Moisture levels of <25% reduce biologic activity in compost (including reproduction of *A. fumigatus*). Moisture levels >60% reduces porosity and increase the likelihood of anaerobic conditions. The latter conditions favor malodor generation and lower windrow or pile temperature. The application of water to control dust during windrow turning will not normally adversely affect moisture levels, since water for dust control is usually applied as a fine spray (little water volume).

- **Temperature control**. Optimal temperatures for thermophilic composting are 55° to 60° C.; or 30° to 38° C. for mesophilic composting. Proper aeration maintains aerobic conditions in compost piles, and helps maintain proper temperature and moisture for composting.

  Under U.S. Federal regulations (40 CFR Part 503), the Process to Significantly Reduce Pathogens (PSRP) in sewage sludge composting operations requires that solid waste be maintained at 40° C. for at least 5 days, and 55° C. or more for 4 hours during this period. In the Process to Further Reduce Pathogens (PFRP) using the aerated static pile and in-vessel processes, the solid waste is maintained at 55° C. or more for 3 days. In the PFRP for windrows, the solid waste is maintained at 55° C. or more for at least 15 days.

  All these processes will help limit *A. fumigatus* populations in compost. Cured, processed sludge compost allowed to achieve uniform temperatures of at least 60° C. is nearly devoid of viable *A. fumigatus* spores (Millner et al. 1977).

- **Dust control**. Use of water sprays, water mists, or approved sprays, as well as the reduced bulk movement of compost, will help control fugitive dust and spores (Millner et al. 1977). Most spores become airborne by the movement or mixing of compost, not by entrainment on the wheels of vehicles on site (Millner et al. 1977), thus dust control of windrows and piles undergoing mixing is important. Windrow and pile turning on windy days is not advisable.

- **Odor control**. Malodor is only a signal of generally poor composting operations, not a specific sign of *A. fumigatus* problems. One may control malodors by aeration, mixing, and moisture control, as discussed above. Use of biofilters may supplement these basic operational methods for odor control, but their ability to control spores is unknown. (Also see pH control.)

- **pH control**. The optimal pH range for composting is 7 to 7.5. The pH varies over the period of composting from an initial reading of pH 5 to 7, dropping to pH 5 or less during the initial days of composting, rising to pH 7 to 8.5 during the thermophilic stage, and returning to pH 7 to 8 during the cooling stage. Anaerobic conditions may allow the pH to become too low (<5), favoring organic acid formation (and accompanying odors). Nearly as objectionable is a high pH (>8.5), which favors release of nitrogen in the form of gaseous ammonia (Tchobanoglous et al. 1993).

- **Facility, vehicle and equipment cleanlines.**: Soap and washdown will result in a reduction of dust and spore levels in the air. Interiors of vehicles may be routinely inspected and cleaned, and cab air filters inspected and replaced as needed.

- **Operator training**. All facility operators and compost workers may be trained in methods of control of dust and *Aspergillus*.

Engineering and Administrative Controls, and Personal Protective Equipment

- **Engineering controls**. Changing the work process (design and use of ventilation systems, machinery, and vehicles) can minimize the employees' potential exposure to *Aspergillus* spores. This may include installation of suitable building ventilation, with filters if needed. One may also use vehicle in-cab ventilation and filtration. Scrubbers, bag houses, and electrostatic precipitators have not been evaluated for spore control (US EPA 1991).

- **Administrative controls**. Changing work assignments (job rotation, reduced task times) can minimize the length of time of the employees' potential exposure to *Aspergillus* spores.

  Baseline physicals (medical exams) may be appropriate for employees (see Clark et al. 1983, Maritato et al. 1992, Epstein 1993). The baseline physical may include a one-second forced expiratory volume (FEV_1) and forced vital capacity (FVC) test. The initial medical history report, taken with the baseline physical, may cover whether or not the employee has any inherited immune defects, immunocompromised states, or other serious health conditions (asthma, carcinoma, diabetes, etc.), in order not to put a high-risk person into an elevated exposure environment in the workplace (Epstein 1993). Annual physicals may be appropriate for highly-exposed employees or employees with health problems (Epstein 1993).

- **Personal protective equipment**. Not every type of compost facility employee may need to use personal protective equipment. For people working where high spore exposure may occur (e.g., individuals who turn compost piles or move compost), correct use of protective clothing (Boutin and Moline 1987), gloves, and respirators (Millner et al. 1977, Kothary et al. 1984) is important. Showers and clothing change facilities may be made available to these workers (Boutin and Moline 1987). Facility operations should, of course, be in full compliance with Occupational Safety & Health Administration (OSHA) standards.

7. HOW DO THE STATE MINIMUM STANDARDS FOR COMPOSTING FACILITIES HELP REDUCE EXPOSURE TO ASPERGILLUS?

The state's current adopted green waste composting regulations are found in 14 CCR Chapter 3.1, commencing with section 17851. The use of setbacks and general dust control measures helps to reduce potential exposure to *A. fumigatus*.

The current green waste composting regulations (14 CCR Section 17859(a)) require a setback of at least 300 feet of the facility's active compost materials areas from any residence, school or hospital, excluding on-site residences, unless one of three conditions are met, as discussed above under Siting issues.
The current green waste composting regulations also include requirements for general dust control, including design requirements (14 CCR 17873); however, no specific operational standards or guidelines are given. General control measures for *A. fumigatus* and *Aspergillus flavus* (another species present in North America) are required for both exempted and non-exempted composting facilities (14 CCR 17875(a)).

8. HOW CAN ONE IDENTIFY (DIAGNOSE) AN ASPERGILLUS PROBLEM IN A COMPOSTING OPERATION?

The presence of very foul odors in a compost operation is an indication of poor composting practice. However, foul odors alone are NOT a necessary or sufficient condition for the presence of high levels of *A. fumigatus* spores, though they may raise the level of suspicion of poor operating practices.

No simple field tests (cultural assays, bioassays, immunological assays) for identification of *Aspergillus* spores and mycelia are available. One must rely on proper sampling methods and devices, which are outlined in Introduction to Indoor Air Quality: A Reference Manual (US EPA 1991), and bring samples into the laboratory for identification and testing. Cultural assays (identifying viable organisms - hyphae and spores - in the laboratory, using fungal cultures and knowledge of fungal morphology and taxonomy) and the direct microscopy of spores are the laboratory methods currently used to identify *Aspergillus fumigatus*. Cultural assays and microscopy should be done by professional mycologists or other specialists. (Also see Section 9 on Monitoring and Sampling, below.)

9. HOW DOES ONE MONITOR AND SAMPLE FOR ASPERGILLUS SPORES IN THE AMBIENT ENVIRONMENT?

As noted above, *A. fumigatus* is a very common fungus found in nearly all environments, and grass clippings generated during lawn mowing may be responsible for most of the *A. fumigatus* spore exposure in residential areas in North America. Bearing this in mind, any routine monitoring or special sampling for *A. fumigatus* spores at compost operation sites should always be compared to "background" levels of *A. fumigatus* and to other allergenic or pathogenic airspora (*Penicillium*, etc.), not as stand-alone data.

The standard sampling devices for bioaerosols and airspora, including *A. fumigatus* include: rotating slit or slit-to-agar impactors, multiple-hole impactors (e.g., Andersen multi-stage samplers), centrifugal samplers, liquid impingers, and filters. Selection of sampling methods and devices depends upon the particular purpose of the sampling, and is always site-specific. The Andersen multi-stage sampler is one of the most frequently used devices to sample air for *A. fumigatus*[5]. The American Council of Governmental Industrial Hygienists (ACGIH) Committee on Bioaerosols recommends the multiple-hole impactor or slit impactor be set to collect particles around the one micrometer size range (US EPA 1991).

Before conducting any bioaerosol (airspora) sampling, Board staff recommends that the investigator contact for advice:

Janet Macher, Sc.D., M.P.H., Air Pollution Research Specialist
Environmental Health Laboratory
California Department of Health Services
2151 Berkeley Way
Berkeley, CA 94704,
phone (510) 540-3130

Assistance with laboratory identification of fungal spores, hyphae and mycelia can be obtained from:

Edward Desmond, Ph.D.
Microbial Diseases Laboratory
California Department of Health Services
2151 Berkeley Way
Berkeley, CA 94704,
phone (510) 540-2074

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[5] Mention of a specific product or device does not constitute an endorsement by the Board or its staff, and shall not be construed as such.

10. CONCLUSIONS

Aspergillus fumigatus spores are very common in our everyday environment in North America. Disease or illness caused by *Aspergillus fumigatus* is a negligible or low-level risk for healthy people. People's everyday activities (e.g., gardening, potting plants, mowing lawns, walking through leaf piles or raking leaves), their sanitation and hygiene practices (e.g., home cleaning, maintenance of air ventilation and air conditioning systems,) and their occupational exposures (e.g., construction workers undertaking digging and earth-moving) account for the great majority of exposures to this fungus.

The vast majority of ordinary exposures result in no asthma or other diseases. Certain groups of people, particularly people who are asthmatic or suffer from certain other serious diseases, immunosuppressed people, or patients taking high doses of corticosteriods, are probably at an elevated risk of developing illness after exposure to large concentrations of spores.

A properly operated compost facility (windrow, aerated static pile, or in-vessel), with proper moisture and pH levels, aeration, and/or turning and mixing, should not normally present an elevated health risk if the best management practices listed above are followed. To keep ambient dust and spore levels low, operators may use all the siting, design and operational measures listed above, including the utilization of water sprays or mists especially while turning compost, and a refrain from turning compost materials outdoors on windy days. To reduce exposure to fugitive dust and spores, compost facility employees may use appropriate personal protective equipment (e.g., OSHA-approved respirators).

It is important for sludge compost facility operators to follow the existing federal standards for proper sludge composting and co-composting, and for green waste composting operators to follow California's green waste composting facility regulations, in order to keep occupational and residential health risks to a negligible level.

Proactive monitoring of the operations at composting facilities and timely enforcement activities by LEAs and other permitting agencies is important; corrective actions should not wait until complaints are generated.

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FIGURE 3. Environmental Exposures to *A. fumigatus* and Other Mycoflora*.

OUTDOOR	INDOOR	OCCUPATIONAL
Suburban Washington D.C. 0-71 CFU/m^3	Forced hot-air heated house, office <512 CFU/m^3	Agricultural:
Denmark (all *Aspergillus* spp.) 0-204 CFU/m^3	Attic, library stack, boiler room 0-50 CFU/m^3	Hay barns <70 CFU/m^3 100 CFU/m^3 5,500 CFU/m^3
Cardiff, Wales 5 CFU/m^3 33 CFU/m^3	Disturbed dust and plant potting rooms ~ 1,100 CFU/m^3	Poultry houses <100 CFU/m^3 2,060 CFU/m^3 (in Spring)
Michigan (no self-heating matter nearby) 150 CFU/m^3	Midwestern homes, 40 CFU/m^3 (frost-free period), 40 CFU/m^3 (sub-freezing period)	Mushroom houses (stationary bed) 333 CFU/m^3 (with 90% being non-mold spores)
St. Louis, MO (outside hospitals) 0-50 CFU/m^3	General indoors <175 CFU/m^3; 0-686 CFU/m^3	Timber processing 10^2-10^4 CFU/m^3, (Includes all airborne micro- organisms.)
Lawn being mowed <10 CFU/m^3 Mulched lawn 686 CFU/m^3		Debarking 12,700 CFU/m^3, heartwood; 52,800 CFU/m^3, sapwood; 65,200 CFU/m^3, bark. (Includes all fungi, *Penicillium* and *A. fumigatus* predominate.)
Nature trail in Autumn 56 CFU/m^3		
School playground University parking Shopping center <12 CFU/m^3		Composted wood chips 1.4 x 10^6 CFU/m^3 (Includes all fungi.)
Compost site, quiescent 0-24 CFU/m^3		Paper pulp factory <12 CFU/m^3

*Source: Composting Council 1993, pp. 5, 18-29. [Secondary source. See the Council's draft report for primary sources]. All data are for *A. fumigatus* unless otherwise noted.

FIGURE 5. *Aspergillus* Spore Concentrations at Large-Scale Composting Facilities (CFU*/m^3).

Site	Minimum	Maximum	Geometric Mean	Notes
WSSC Site II,				Sewage sludge; enclosed
1991				
Upwind	0	34	3.1	2,000 ft. away
On-site	21	3611	250	
Downwind	0	30	4.0	1,000 - 8,600 ft. away
WSSC Site II, 1987				Sewage sludge; open facility
Upwind 0.4 mi.	<1	15	1	
1.0 mi.	<1	15	2.7	
1.1-1.7 mi.	<1	10	1.4	
On-site	<1	133	9	
Downwind 0.4 mi.	<1	35	2	
Downwind 1.0 mi.	<1	37	2.5	
Downwind 1.1-1.7 mi.	<1	34	1.0	
WSSC Dickerson Site, 1981				Sewage sludge; open facility
Upwind	0	100	16	
On-site	0	555	127	
Downwind 0.5 mi.	0	174	20	
Downwind 1.0 mi.	0	228	23	
Islip, NY, 1993				Yard waste; open facility
Mon.-Sat. (operations)	0	32,743 (Spores/m^3)	865	
Sun. (no operations)	0	4473 (Spores/m^3)	354	
Upwind	0	34	3.1	2,000 ft. away
On-site	21	3611	250	

Downwind	0	30	4.0	1,000 - 8,600 ft. away

*CFU = colony forming units (equivalent to viable spores)
Source: Composting Council 1993

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Occurrence of *Aspergillus fumigatus* During Composting of Sewage Sludge

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Aspergillus fumigatus, a medically important fungal opportunist and respiratory allergen, was isolated from woodchips and sewage sludge used in the production of compost at the U.S. Department of Agriculture's composting research facility in Beltsville, Md. It was also regularly isolated as a dominant fungus during forced aeration composting and after 30 days in an unaerated stationary curing pile; in both cases, the fungus was found in pile zones with temperatures less than 60°C. Compost stored outdoors in stationary unaerated piles from 1 to 4 months after screening out of woodchips contained easily detectable amounts of *A. fumigatus* in the exterior pile zones (0- to 25-cm depths). Semiquantitative studies of the airspora at the composting site revealed that *A. fumigatus* constituted 75% of the total viable mycoflora captured. At locations 320 m to 8 km from the compost site, the fungus constituted only 2% of the total viable mycoflora in the air. Of 21 samples of commercially available potting soil, one had levels of *A. fumigatus* nearly equivalent to those of 1-month-old storage compost; 15 others had lower but detectable levels.

Sewage sludge disposal is an immediate and growing problem for many U.S. municipalities. Composting, one of the alternative disposal/utilization options available, has been investigated from engineering, economic, bacteriological, and viral aspects (6, 11, 20, 48). The agronomic characteristics of the compost have also been evaluated (19). One aspect which has not been investigated thoroughly and evaluated with a view toward health safety is the fungal flora which develops during composting.

Aspergillus fumigatus Fres. is one of the relatively few fungi which are known to infect humans. Records of its ecological distribution and pathogenicity, which follow below, suggested that the fungus might proliferate during composting and thereby pose a health problem for certain individuals.

Ecological distribution. *A. fumigatus* has been found frequently in composting vegetative material (7, 17, 21, 39), self-heating woodchip piles (5, 18, 44), municipal refuse compost (30, 32, 43), refuse-sludge compost (32), moldy hay (24), and sewage (12, 14, 15). Unlike truly thermophilic fungi, which cannot grow below 20°C (16), *A. fumigatus* grows over a range of below 20 to about 50°C (16).

In contrast to the frequent occurrence of *A. fumigatus* in the above-mentioned environments, aspergilli in general have been reported only infrequently and in small numbers in outdoor air (23, 28). Austwick (3, 4) reported low concentrations (10 to $10^4/m^3$) of *A. fumigatus* spores in outdoor air and stated, "*Aspergillus*-type spores in the outside air probably rarely exceed $500/m^3$, but within a farm building following the shaking of mouldy hay Lacey and Lacey (35) found up to 21 million/m^3." Airborne spores in pastures consisted largely of *Cladosporium* sp. and a few other forms but included extremely few spores of aspergilli (33).

A. fumigatus does not appear to be predominant in the fungal flora of most soils (25, 41), even though often isolated. Recent data on sun-heated soils revealed that *A. fumigatus* was the second most frequently isolated thermophilic/thermotolerant fungus from that environment (45); no data are provided on population levels of the fungus in terms of colony-forming units (CFU) per gram of soil.

Pathogenicity and hypersensitivity. *A. fumigatus* is a secondary opportunistic invader of the lungs, sometimes spreading to other organs or to the central nervous system in individuals severely debilitated by primary diseases (18, 27). Ajello (1) supports the observation of Emmons et al. (18) that "pulmonary aspergillosis is often superimposed upon tuberculosis, silicosis, an anatomical abnormality or deficient immunologic response related to corticosteroid or antibiotic

therapy." Emmons et al. (18) noted further that even in the absence of other diseases, infections very occasionally result soon after exposure to large doses of conidia.

Spores of *A. fumigatus* can also cause bronchopulmonary hypersensitivity, marked by asthmatic spasm, fever, malaise, and prostration (18). Although many fungi are capable of inducing such responses (8), among atopic individuals, *A. fumigatus* is a strong allergen which incites a hypersensitivity response in a high percentage of exposed individuals (26, 31). It has been widely used in skin prick tests of asthmatic patients and is recommended (38) as one of five standard allergens for this purpose. In studies of respiratory allergies associated with exposure to moldy hay, the levels of *A. fumigatus* have been cited repeatedly (34–36). Although low concentrations of *A. fumigatus* spores in outdoor air (10 to $10^4/m^3$) do not appear to cause infection in healthy individuals, Austwick (3) suggested that such levels probably do incite allergic responses in sensitized individuals. Vithayasai et al. (46) reported that three asthmatic members of a single family almost simultaneously developed allergic aspergillosis. Their use of potting soil containing *A. fumigatus* (quantities not reported) was suggested as the source of infection.

Our investigation was directed toward determining the occurrence of *A. fumigatus* (i) quantitatively, in all stages of the Beltsville composting process, including the starting materials, undigested sewage sludge (containing large amounts of ferric chloride and lime), and woodchips; (ii) quantitatively, in commercially available potting soils, mulches, and manures; and (iii) qualitatively, in the air at the composting site and at several distances from the site. The information would provide some assessment of the magnitude of the potential inoculum.

MATERIALS AND METHODS

Composting. Details of the Beltsville Aerated Pile Composting Method have been provided by Epstein et al. (20). It consists of five operations: mixing, 3-week composting, curing, screening, and storing. This results in finished compost.

The starting materials consist of: (i) woodchips from commercial tree trimming and removal services, and (ii) vacuum-filtered undigested sludge of approximately 75% moisture content obtained from Blue Plains Wastewater Treatment Plant, Washington, D.C. These are mixed (2:1, vol/vol) and formed into piles 25 by 6 by 2.5 m high, oriented in a north-south direction. Piles are covered with a 30- to 45-cm blanket layer of cured compost for insulation and odor control and subjected to forced aeration for 3 weeks, after which time they are disassembled, repiled, and left unaerated for approximately 4 weeks of curing. Cured compost is screened to remove large woodchips and then stored without aeration for varying periods.

Compost sampling. Seven sludge samples from different deliveries were collected randomly prior to mixing with chips. Woodchips were obtained randomly from old stockpiles and from deliveries of fresh chips. Three-week compost was obtained from high (60 to 82°C), intermediate (40 to 60°C), and low (<40°C) temperature zones, estimated from isotherms, in sectioned, aerated piles. At each sample locus, approximately 100 g of compost was obtained from the inner end of a hole made with a sterile trowel; samples were placed into sterile plastic bags and transported immediately to the laboratory refrigerator (5°C, for 3 h or less) until dilutions were prepared. Cured and freshly screened compost samples were collected randomly and handled aseptically.

Storage compost samples were obtained from a pile (9.1 m in diameter by 2.7 m high) initially and at 1 week, 1 month, 4 months, and 6 months after construction. They were removed aseptically from three randomly selected, equivalently sized sectors of the pile. Samples from depths of 10, 25, and 50 cm, respectively, were composted. Subsamples of the initial storage compost were sealed in airtight plastic bags and stored at approximately 21°C; three bags were analyzed simultaneously with the pile samples at each sampling period.

All piles were exposed to prevailing weather conditions at Beltsville, Md. Temperature measurements of sample loci were made immediately after samples were obtained; measurements were made with calibrated YSI thermistors and thermocouples. In some cases, the temperatures recorded at the time of collection may have been less than previously attained maxima.

For comparison with Beltsville compost, samples of an experimental licorice root/sludge compost from Camden, N.J., were analyzed. Sample collection and handling were the same as above. Also, for comparison, samples of potting soils, manures, and mulches commercially available were analyzed by the detection and enumeration procedures described below.

Air sampling. Two plastic petri dishes (90-mm diameter) containing oxgall-antibiotic agar were opened at the Beltsville compost site for 15 s simultaneously, plates perpendicular to the ground, downwind from sites of (i) mixing, (ii) loading (front end loaders moving compost or cured piles), and (iii) screening. The Beltsville composting site is completely surrounded by dense stands of tall trees. Plates were also opened at very short (320-m) and at greater (1- to 8-km) horizontal distances from the composting site. To catch fungal propagules at the greater distances, plates were opened for 1 to 5 min. A plate from each sample set was incubated at 44 and 25°C. A total of 120 pairs of plates were opened at the compost site; 80 pairs of plates were opened at 17 noncompost sites. The latter sites included dairy barns, fields during cultivation and harvesting, high-speed highways, lawns, pine groves, plant nurseries, and orchards.

Detection and enumeration of fungi. The dilution plate method was used because it favors isolation of spores and conidia (41). To assess potential exposure

to the fungus associated with composting, the determination of the conidial population of *A. fumigatus* was emphasized because inhaled conidia are the most likely cause of infection or allergic response.

Compost (50 g) of 40 to 50% moisture content (wet weight basis) or potting soil (50 g) was added to 250 ml of sterile distilled water containing 0.01% Triton X-100; mixtures were shaken on an International radial-head bottle shaker for 10 min. From the resulting suspensions, dilutions of up to 10^{-4} or 10^{-5} were prepared. Six 1-ml samples of these dilutions were plated with oxgall-antibiotic agar; three plates each were incubated at 25 and 44°C.

The agar medium was prepared with Difco components as follows (grams per liter): peptone, 10; dextrose, 10; oxgall, 15; agar, 20. Antibiotics, filter sterilized with a 0.2-μm membrane filter (Millipore Corp.), were added to cooled (45 to 48°C) medium to give final concentrations of 50 μg of streptomycin, 10 μg of penicillin, and 2 μg of aureomycin per ml. This medium, prepared with a different antibacterial agent, 0.001% crystal violet, is recommended for isolation of saprophytic soil fungi and fungi pathogenic to man (2, 29, 37). Radial advance of most fungal colonies is retarded on the oxgall agar, and most colonies produce identifying morphological structures well before colonies become confluent.

For analyses of 3-week compost by direct plating, microsamples (5 to 15 mg) obtained from the interior of aseptically opened clumps of compost were dispersed in separate petri dishes with 5 ml of sterile distilled water containing 0.01% Triton X-100. Oxgall-antibiotic agar was added to each plate; replicates were incubated at 25 and 44°C.

Average number of colonies per milliliter of the primary dilution was calculated from the plate counts and dilution factors. Countable plates contained 20 to 80 colonies. The number of CFU present per gram of dry weight (GDW) of sample was calculated by dividing the average number of colonies per milliliter by the grams of dry compost per milliliter in the primary dilution (computed on a wet weight basis). For some plates, colonies were too numerous to count and growth was recorded simply as positive for the species noted. Identification of *A. fumigatus* was based on morphological and cultural characteristics described by Raper and Fennell (42). Calculations of CFU of *A. fumigatus* per GDW were based on the countable 44°C plates. The percentage of *A. fumigatus* on those plates was calculated and used as the multiplier of the total thermophilic count resulting in the fraction of CFU attributable to *A. fumigatus* per GDW.

RESULTS

Sludge. Sludge samples all contained between 10^2 and 10^3 CFU of *A. fumigatus* per GDW. Total mesophilic fungi, including filamentous and yeast forms, in these samples ranged from 2.3×10^4 to 5.4×10^4 CFU/GDW.

Woodchips. Microscopic inspection and culture isolations of scrapings from woodchips obtained randomly from the exterior and a 8-cm depth of old stockpiles revealed an abundance of *A. fumigatus*. The grayish-green coloration of the fungus material on the chips was attributed to the presence of dense masses of dry conidia. Samples of fresh and old woodchips contained 10^3 to 2.3×10^5 CFU and 2.6×10^6 to 6.1×10^7 CFU of *A. fumigatus* per GDW, respectively.

Compost. *A. fumigatus* was detected in compost samples obtained from temperature zones of 63°C or less. Quantities of fungal propagules detected in various temperature zones of a compost pile are shown in Table 1. Fungi were not detected in any of 12 microsamples from pile loci with temperatures at the time of sample collection ranging from 58 to 82°C. Although the temperature history of each sample locus was not recorded directly, a reliable estimate based on isotherms from temperatures recorded throughout the 21-day composting period revealed that loci in the hottest zones (60 to 82°C) had achieved temperatures >60°C since day 5. Before that time, there was a gradual increase in temperature from the ambient levels present at the initiation of pile construction. In general, loci in intermediate (40 to 60°C) and low (<40°C) temperature zones had attained, during the 21-day composting period, temperatures higher than those recorded at the time of sample collection, in a few cases above 60°C, for periods of 1 to 10 days.

Numerous fungi other than *A. fumigatus* were observed in the compost in varying frequency. *Ceratocystis, Doratomyces,* and *Trichoderma,* well known to be associated with wood, were detected and may have entered the compost on the woodchips. The thermophiles *Chaetomium thermophile* La Touche, *Humicola grisea*

TABLE 1. *Occurrence of A. fumigatus in nine compost samples from loci of measured temperature and moisture content*[a]

Conditions at sample locus		Thermophilic fungi	
Temp range (°C)	Moisture (%)	Total CFU/GDW	*A. fumigatus* colonies (%)
62–63	28	2.1×10^3	0
53–55	46	$<10^3$	100
52–57	49	3.0×10^3	100
52–54	45	$<10^3$	100
50–55	38	3.5×10^4	90
46–50	46	3.0×10^3	100
46–50	31	$>5.0 \times 10^5$	100
32–41	49	4.5×10^4	100
26–28	51	3.2×10^3	90

[a] Twelve other samples from areas of generally higher temperature than 60°C yielded no detectable fungal CFU. See text for details.

Traaen var. *thermoidea* Cooney & Emerson, *H. lanuginosa* (Griffon & Maublanc) Bunce, *Talaromyces thermophilus* Stolk, *Thermoascus aurantiacus* Miehe, and *Torula thermophile* Cooney & Emerson were found frequently along with *A. fumigatus*.

Licorice root-sludge compost. Licorice root alone contained approximately 4.7×10^6 CFU of *A. fumigatus* propagules per GDW. When combined with liquid sludge, the mixture contained between 2.0×10^6 and 5.7×10^6 CFU of thermophiles per GDW, of which 47 to 100% or 9.4×10^5 to 5.7×10^6 CFU/GDW were propagules of *A. fumigatus*. After 3 weeks of composting, the blanket (licorice root alone)-compost interface zone contained 1.2×10^4 to 2.9×10^6 CFU of thermophiles per GDW, of which 85 to 99%, or 10^4 to 2.8×10^6 CFU/GDW, were *A. fumigatus*.

Cured compost. Of the 1.3×10^5 CFU/GDW detected in the woodchip-sludge compost at 45°C, 7 to 25%, or 8.4×10^3 to 3.5×10^4 CFU/GDW, were *A. fumigatus*. *H. grisea* var. *thermoidea* was the most abundant fungus in all the samples examined. Freshly screened, cured compost contained 2.4×10^5 to 5.7×10^6 CFU of thermophiles per GDW, of which *A. fumigatus* constituted between 7 to 16%, or 1.7×10^4 to 9.1×10^5 CFU/GDW of the total.

Stored compost. In six random compost samples taken from 2- to 4-month-old storage piles, thermophiles constituted 7.2×10^4 to greater than 1.1×10^5 CFU/GDW, the majority of colonies being *T. thermophile* and *H. grisea* var. *thermoidea; A. fumigatus* comprised 30% (2.1×10^4 CFU/GDW) or less of the total thermophilic population.

Initial samples of compost used to build the 6-month storage test pile contained 4.7×10^6 CFU of thermophiles per GDW, of which 1% or 4.7×10^4 CFU/GDW were *A. fumigatus; Paecilomyces varioti* Bainier was the preponderant fungus. Samples also contained 2.9×10^5 CFU of mesophiles per GDW with *Scopulariopsis brevicaulis* (Sacc.) Bainier as the preponderant fungus.

At 1 week, *A. fumigatus* constituted 1.5% of thermophilic fungi or 2.7×10^4 CFU/GDW at the 10-cm depth. At 25- and 50-cm depths, the fungus constituted less than 1% of thermophiles, or 1.1×10^4 and 5.8×10^3 CFU/GDW, respectively. At 1 and 4 months, *A. fumigatus* was not detected at 25 and 50 cm. At the 10-cm depth, *A. fumigatus* constituted 11.8% of thermophiles, or 3.0×10^5 CFU/GDW, at 1 month, and 1.8% of thermophiles, or 1.3×10^4 CFU/GDW, at 4 months. Negligible to completely undetectable levels of *A. fumigatus* were found in compost from all three depths of the compost pile at 6 months. Similarly, compost bagged and stored 1 week to 4 months exhibited negligible or undetectable levels of *A. fumigatus*. In summary of the above data, the maximum and minimum levels of *A. fumigatus* detected at each stage of the composting process are shown in Fig. 1.

Air spora. Colonies of *A. fumigatus* were frequently detected on agar exposed to air at the compost site (Table 2). Such colonies were detected from air samples obtained during various mechanical activities and machine traffic,

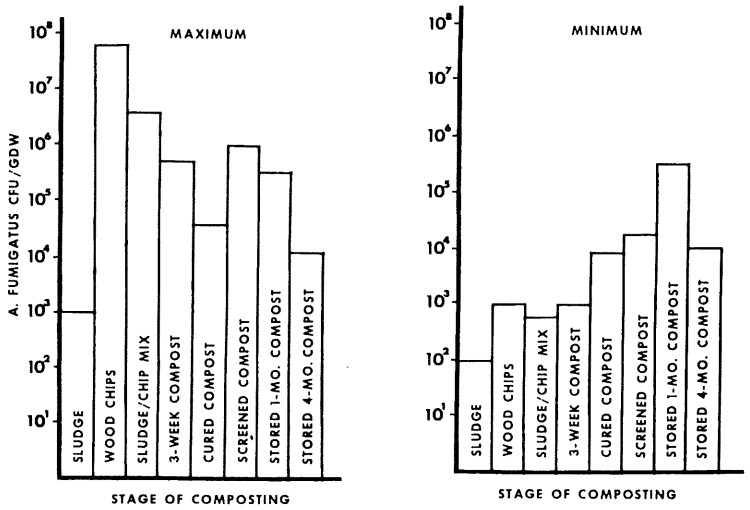

FIG. 1. *Maximum and minimum numbers of CFU of A. fumigatus detected per gram (dry weight) of substrate from each stage of the composting process. Numbers for stored compost represent counts from the 10-cm depth only.*

TABLE 2. *Occurrence of A. fumigatus on oxgall agar plates exposed to air at the Beltsville compost site[a] and at noncompost sites[b]*

Site	Catch frequency[c]		*A. fumigatus* CFU/total CFU at:	
	Plates with *A. fumigatus*/total plates with fungi	Total *A. fumigatus* CFU/total exposure time (min)[d]	25°C	44°C
Compost	167/177	2,991/44.25	1,239/1,969	1,752/2,012
Noncompost	5/50	5/180.75	3/293	2/4

[a] Exposures made during mixing, screening, dumping, loading, and inactivity.
[b] Exposures made at 19 different sites; see Materials and Methods for details.
[c] Total plates exposed: 240 for a total of 60 min at compost site, with 11 plates too numerous to count; 160 for a total of 391 min at noncompost sites.
[d] For plates which contained fungi.

as well as during periods of no machine activity. Of the mesophilic colonies counted, 63% were *A. fumigatus,* and of the thermophilic colonies, 87% were *A. fumigatus.* Plates were exposed for a cumulative total of 60 min to collect a total of 3,981 colonies, of which 2,991 were *A. fumigatus.*

In striking contrast to the above situation, only five colonies of *A. fumigatus* were detected on plates exposed to air at 17 noncompost sites. At these same sites, a cumulative total exposure of 391 min resulted in 50 plates positive for fungal colonies. Of the 297 total fungal colonies detected at 25 and 44°C, only 5, i.e., 2%, were *A. fumigatus.*

Commercial potting soils, manures, and mulches. Levels of *A. fumigatus* in the 21 products analyzed varied greatly; Table 3 shows the levels of mesophiles, thermophiles, and *A. fumigatus* found in each product. Almost all products had at least 10^2 thermophilic CFU/GDW; five products contained no detectable *A. fumigatus.* Of the samples containing the fungus, only one, no. 198, contained levels approximating those found in 1-month-old Beltsville storage compost from a 10-cm depth. Five products, no. 198, 232, 234, 233, and 197, contained *A. fumigatus* levels $>1.3 \times 10^4$ CFU/GDW, the amount found in a 4-month-old Beltsville storage compost from the 10-cm depth. Moisture content and pH of the products varied but were apparently not correlated with the presence or absence of *A. fumigatus.*

DISCUSSION

A. fumigatus occurs at easily detectable levels at each stage of the composting process. Levels varied, reflecting age, temperature, handling, and, to some degree, moisture content of the substrate. Fresh woodchips stored for only a few days contained approximately 10^2 to 10^4 fewer *A. fumigatus* CFU per GDW than chips stored in stockpiles for 1 month or more. The levels of *A. fumigatus* in sludge analyzed in this study were not exceedingly high or variable; however, levels higher or lower than these might occur in sludges from elsewhere. For example, samples of sewage sludge from Ohio (13) collected at different times during a 1-year period were found to contain up to 3.8×10^7 CFU of *A. fumigatus* per GDW.

Occurrence of *A. fumigatus* in a 3-week compost is restricted to zones with temperatures of approximately 60°C or less. One zone with slightly higher recorded temperature, 62 to 63°C, contained 2.1×10^3 CFU of *A. fumigatus* per GDW. Compost from this same zone had a moisture content of 28%, whereas samples from zones with effectively lethal temperatures, ≥60°C, had an average moisture content of 45%. The temperature zones of <60°C were generally confined to the exterior 0.75 m of the piles, including the blanket layer. Thus, these fungal zones constitute approximately 60% of the total volume of a free-standing pile, or 25 to 37% of the total volume of an extended (conjunctive) pile.

High levels of *A. fumigatus* in cured and screened compost (Fig. 1) may be a result of mechanical distribution of conidia and thus reinoculation of previously *A. fumigatus*-free patches. Curing piles still moist and self-heating can easily provide suitable growth conditions for the fungus.

Stationary storage for 1 month or more generally caused a decline in the population of *A. fumigatus* (Fig. 1), especially at depths of 25 and 50 cm. Thus, by the end of the composting process, the total volume of compost contained relatively few propagules of the fungus.

Of the 21 commercial products analyzed, 16 contained *A. fumigatus* and 5 contained levels higher than those found in 4-month-old Beltsville compost from the 10-cm depth. Thus, several commercial products and Beltsville compost are potential sources of the fungus to users of potting soils and mulches; therefore, such products may not be entirely risk free from the standpoint of inciting allergic responses in sensitized individuals, as has occurred with the use

TABLE 3. *Comparison of numbers of mesophilic and thermophilic fungi of A. fumigatus, moisture content, and pH in 21 commercially available potting soils, manures, and mulches*[a]

Product no.	Moisture content (%)	pH	CFU/GDW		
			Mesophiles	Thermophiles	*A. fumigatus*
198	33.2	4.4	1.2×10^6	5.9×10^5	5.7×10^5
232	49.4	7.6	2.3×10^5	1.9×10^5	7.4×10^4
234	27.8	6.8	2.4×10^5	4.6×10^4	2.7×10^4
233[b]	34.3	7.3	4.7×10^5	6.1×10^4	2.1×10^4
197	29.1	5.2	3.0×10^7	1.0×10^6	2.0×10^4
230	55.7	4.8	7.4×10^4	7.7×10^3	7.7×10^3
201	38.7	4.2	3.6×10^5	3.0×10^3	3.0×10^3
196	50.6	3.6	1.2×10^7	7.9×10^3	2.7×10^3
235[b]	14.9	7.5	1.7×10^6	3.3×10^4	2.6×10^3
203	37.3	4.1	3.7×10^5	4.2×10^3	2.1×10^3
213	46.8	6.9	1.6×10^4	1.7×10^3	1.6×10^3
239	67.1	7.0	5.9×10^4	1.9×10^3	1.5×10^3
202	23.5	4.3	4.1×10^5	4.3×10^2	4.3×10^2
195	39.4	6.9	1.0×10^6	8.0×10^2	1.0×10^2
194	31.4	7.2	1.3×10^7	6.4×10^3	<60
229	41.0	7.8	5.8×10^5	3.6×10^2	<3.6
212	61.0	5.2	5.7×10^7	1.0×10^6	0
231	56.1	8.9	3.1×10^6	5.4×10^3	0
237	38.5	7.5	3.9×10^5	7.6×10^2	0
238	35.0	7.1	5.4×10^4	2.1×10^3	0
228[c]	5.2	4.6	$<1.5 \times 10^2$	$<1.5 \times 10^2$	0

[a] Potting soil numbers: 194, 198, 201–203, 213, 233–235, 238; manure numbers: 195, 228, 229, 231, 237; mulch numbers: 196, 197, 212, 230, 232, 239. Products ranked in order of declining occurrence of *A. fumigatus*.
[b] Contain Beltsville compost: 33 and 14%, respectively.
[c] Heat treated.

of potting soil (46). Likewise, gardeners with predisposing medical conditions, as mentioned previously, who use any of these products as mulches could be exposed to large numbers of conidia. Emmons et al. (18) stated that in such situations "the inhaled spores may germinate and the fungus may invade lung parenchyma to produce typical aspergillosis."

In the past, frequency data have been used to indicate relative abundance of fungi, on the basis that members of dense populations have a greater probability of being collected in any one random sample. However, frequency does not adequately describe the preponderance of a species in a sample in terms of propagules per GDW. Soil mycoflora investigations indicate that *A. fumigatus* is found with the following frequencies in different soils (i.e., the percentage of samples analyzed from which the fungus was detectable): forest soils, 7.5 and 14% (10, 22); woodland soils, 1.4 to 4.4%, and cultivated soils, 28.7 to 45.5% (40); open bogs, 1%, and swamps, 0.1% (9); and sun-heated soils, 31% (45). For comparison purposes, *A. fumigatus* had the following frequencies of occurrence: 57% in compost, based on 109 sample analyses; 94% in air at the compost site, based on 177 plate exposures; 10% in air at noncompost sites, based on 50 plate exposures.

If composting of sludge becomes a practiced method of sludge disposal in various municipalities, attempts might be made to compost sludge with numerous bulking agents other than woodchips. Possibilities include: bagasse, carob husks, corncobs and husks, grass seed straw, cereal straws and husks, paper refuse, peanut hulls, urban refuse, and licorice root. All of these are of high cellulose content. Based on evidence that *A. fumigatus* is capable of growing and utilizing cellulose as a carbon source (47), the fungus will very likely be found in such situations.

In conclusion, the on-site problems associated with *A. fumigatus* are worthy of precautionary consideration. Individuals who have a history of asthma, chronic respiratory allergy, or serious lung pathology, or who are being treated on a continuing program of therapy with antibiotics, corticosteroids, or cytotoxins are susceptible to allergic response or infections by the fungus (1, 18, 26, 27). Aerosolized particles of compost which include conidia of *A. fumigatus* may pose a health problem for sensitized or otherwise predisposed individuals. However, such individuals who are, become, or remain employed at composting sites with knowledge of these related facts might achieve some alleviation of exposure if certain practices are initiated, such as (i) wear-

ing of respirators, (ii) water or oil spraying of dry, dusty compost sites periodically as required to reduce dust levels, or (iii) isolating the workers from spore-dispersing parts of the process.

In consideration of off-site health matters related to air dispersal of spores, a buffer distance between a composting operation and health-care facilities or residential areas may be needed. The determination of spore concentrations in air at distances from the composting site is currently under investigation in this laboratory.

Any health hazard associated with the use of finished compost or similar commercial products, insofar as *A. fumigatus* is concerned, is probably closely related to the manner in which the product is handled. Operations which generate dust clouds containing high levels of airborne spores represent a potential exposure problem for hypersensitive or predisposed individuals.

Adequacy of the above cautionary measures and suggestions will require careful evaluation by appropriately informed physicians and epidemiologists.

ACKNOWLEDGMENTS

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Terrasa, Jen

From: Bresani , Alexandra
Sent: Friday, October 27, 2017 12:35 PM
To: Bresani , Alexandra
Subject: Howard County to host information sessions on human service grant application process
Attachments: CRS - CSP Grant Application Process.docx

The following news release was issued to the media this afternoon.

Howard County Government
News Release

Office of Public Information
3430 Courthouse Drive
Ellicott City, Maryland, 21043

410-313-2022 / FAX 410-313-3390 / www.howardcountymd.gov

Mark Miller, Administrator
msmiller@howardcountymd.gov

October 27, 2017

Media Contacts:
Mark Miller, Administrator, Office of Public Information, 410-313-2022
Megan Godfrey Jackson, Community Service Partnership Program Manager, Office of Community Partnerships, Department of Community Resources and Services, 410-313-5996 (voice/relay)

Howard County to host information sessions on human service grant application process

ELLICOTT CITY, MD – The Howard County Department of Community Resources and Services will hold two mandatory Pre-Application Information Sessions on Thursday, November 9, for non-profit organizations interested in applying for a Fiscal Year 2019 Community Service Partnership (CSP) grant. The first session will take place from 10:00 a.m. to 12:00 p.m. and the second session will run from 1:00 to 3:00 p.m. Each session will be held in Room 401 at the Gateway Building, 6751 Columbia Gateway Drive in Columbia.

CSP grants support the delivery of essential human services in Howard County. Funding priority is given to services that meet basic needs and provide opportunities to become self-sufficient. CSP funds may be used for agency operating expenses, program costs, direct client assistance or one-time expenses that directly serve the community. To be eligible, an agency must be a 501(c)(3) non-profit and serve the residents of Howard County.

Grant applications will be completed on-line this grant cycle and will be available November 1, 2017 at https://www.howardcountymd.gov/CSP. Applications are due by Tuesday, January 2, 2018.

For more information, contact CSP Grants Manager Megan Godfrey Jackson at 410-313-5996 (voice/relay) or email mgodfrey@howardcountymd.gov.

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Dispersal of *Aspergillus fumigatus* from Sewage Sludge Compost Piles Subjected to Mechanical Agitation in Open Air

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Aerosolization of the thermophilous fungal opportunist *Aspergillus fumigatus* from mechanically agitated compost piles was examined at a pilot-scale sewage sludge composting facility and two other selected test sites. Aerosols of *A. fumigatus* downwind from stationary compost piles were insignificant in comparison with those downwind from agitated piles. These aerosols were generated by a front-end loader moving and dropping compost. Aerial concentrations of the fungus at distances downwind from the point of emission were used to determine an emission rate for *A. fumigatus* associated with the moving operations. The maximum emission rate, 4.6×10^6 *A. fumigatus* particles per s, was used to calculate predicted concentrations in an unobstructed plume with restrictive, neutral, and dispersive atmospheric mixing conditions up to 1 km downwind from the emission source.

Increasingly stringent regulations governing pollutant discharges into the air and water have resulted in increased sludge production from municipal wastewater treatment plants. Proper disposal of these additional volumes of sewage sludge is rapidly becoming an urban problem and a possible constraint to urban growth. Most disposal techniques, such as incineration, ocean dumping, and land application, have negative environmental impacts. However, composting of sewage sludge recovers and recycles the resource, creating a useful agricultural product. Nevertheless, the composting process can affect the odor and the particulate and microbial burden of the local air environment. Part of the latter phenomenon was investigated qualitatively and quantitatively in the research reported in this paper.

Spores and other viable elements produced by actinomycetes, bacteria, and fungi during the thermogenic self-heating stages of composting may be released into the atmosphere when compost piles are disturbed. Earlier work at a sewage sludge compost site (17) indicated that the thermophilous fungus *Aspergillus fumigatus* Fres. was released into the air during the handling of compost by front-end loaders (FEL). However, those observations were based on nonvolumetric data and were thus only semiquantitative.

To quantitatively characterize the airborne concentrations of *A. fumigatus* at a compost site, an aerometric survey was conducted. Data on the aerial concentrations of thermophilous actinomycetes (those able to grow at 45°C) were also sought during this survey, because no information is available on the occurrence of these organisms in this circumstance and because these microbes can incite allergic reactions in humans when inhaled (11). The results of the survey are presented below and verify the initial indications that high ambient concentrations of *A. fumigatus* are possible when compost is disturbed by mechanical handling. Some of these handling operations (e.g., separation of wood chips from the compost in a mechanical drum screen outdoors and entrainment of compost dust by vehicle wheels) were obvious to the unaided eye as possible sources of *A. fumigatus*. Other operations, such as movement of compost by FEL, and windblown losses from static piles were not obvious sources of *A. fumigatus*. Although various enclosures could reduce dust and *A. fumigatus* release from screening and housekeeping practices could reduce surface dust entrainment, the movement of compost by FEL remains an essential part of the operation and a potential source of *A. fumigatus* emission. Thus, *A. fumigatus* release during FEL movement of compost needed further investigation.

The extent of dispersal of any *A. fumigatus* release requires examination because of possible health consequences associated with this organism. These relate to the *A. fumigatus* proclivity to incite allergic reactions of the immediate or delayed types and to cause infections in individuals affected by some underlying medical difficulty. Additional details on the health-related aspects of *A. fumigatus* are discussed in an annotated review of the current literature on aspergillosis (15).

The small size of *A. fumigatus* spores (diameter, 3.0 μm) and their low terminal settling velocity (0.03 cm/s) (9) suggested that the aerial dispersal of *A. fumigatus* spores would follow the Gaussian form and that the extent of aerial dispersal to locations downwind from an emitting source could be estimated by using models (19, 22). Dispersion models have been used previously for atmospheric pollutants and, with modifications, for microbial aerosols (3, 14, 18). Application of these models requires knowledge of the basic rate of emission of the particles of concern, in this case *A. fumigatus*.

In addition to the survey of *A. fumigatus* concentrations in air after dispersal, a second objective of this study was to determine an emission rate for *A. fumigatus* associated with the mechanical agitation of compost. Comments are provided on the use of the study results in evaluating aerosol dispersal from spore-emitting operations to surrounding environments.

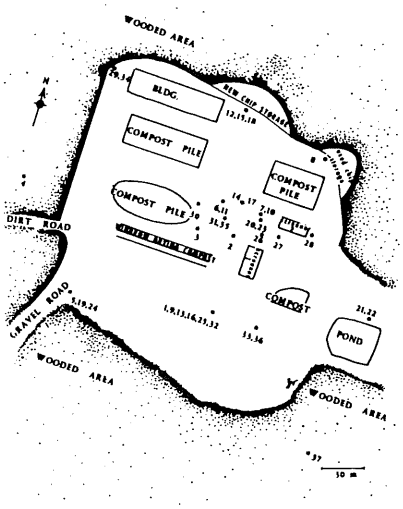

FIG. 1. *BARC compost site: survey tests. Numbers correspond to sample numbers in Table 1.*

MATERIALS AND METHODS

Compost source. The subject composts were self heating and were produced by the Beltsville aerated pile method, which involves 21 days of forced aeration, followed by an additional 30 days of curing (no forced aeration). The composting mixture contained 2.5 parts wood chips and 1 part raw sewage sludge, by volume (or approximately equal parts on a weight basis). Additional details of the composting method are reported elsewhere (6).

Experimental sites. Experiments were conducted at the following three sites: (i) Beltsville Agricultural Research Center (BARC) Compost Site, Beltsville, Md. (site 1), (ii) Blue Plains Wastewater Treatment Plant, Washington, D.C. (site 2), and (iii) BARC Airport (site 3). Measurements at site 1 were made only for the survey portion of the study because of certain site-specific constraints. This site (Fig. 1) was completely surrounded by woods, limiting the distance to which dispersion could be measured to less than 200 m. Also, various dust-generating activities associated with the normal operation of the compost facility, including vehicular traffic, presented confounding influences on emission rate tests.

In contrast, sites 2 and 3 had open areas which allowed dispersal measurements approximately 580 m downwind from the experimental emitting source without the influence of extraneous emitting sources. Site 2 (Fig. 2) extended over concrete surfaces with some metal pipe projections, which were considered relatively minor obstructions to the overall pattern of airflow. In general, the southwest airflow at site 2 was free of turbulence because of its passage over the Potomac River; samples were collected during periods of southwest winds. Site 3 (Fig. 3) extended over fields of soybeans and millet (height, 45 to 60 cm) and a concrete runway (width, 50 m) and was bordered by dense woods. With northwest winds, a stretch of 580 m was available for unobstructed sampling.

The location of each sampler with respect to the emitting source was determined after all tests by using a surveyor's transit, stadia rod, and tape measure.

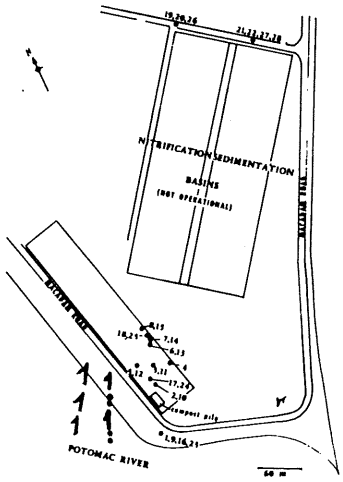

FIG. 2. *Blue Plains Wastewater Treatment Plant site: dispersal tests. Numbers correspond to sample numbers in Table 4. Dashed arrows indicates approximate wind direction (215°) for trials 1 and 3; dotted arrows indicate approximate wind direction (205°) for trials 2 and 4.*

Emission sources. All 37 survey test samples were collected by using typical compost-processing activities as emission sources. These activities included windrow drying of compost, movement of compost by FEL, screening of wood chips from compost in a rotating drum, and various vehicles crossing the composting pad. All activities did not occur simultaneously for every sampling.

The 42 dispersal test samples from six trial runs

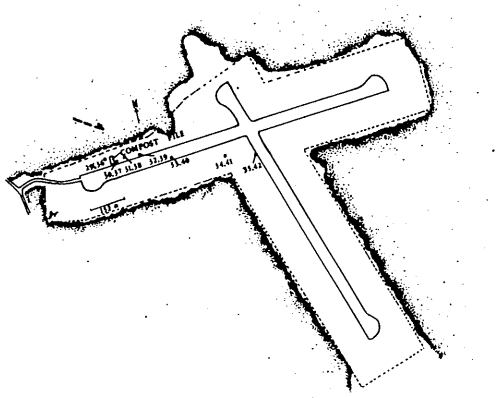

FIG. 3. *BARC airport: dispersal tests. Numbers correspond to sample numbers in Table 4. The dashed arrow indicates the approximate wind direction (295°) for trials 5 and 6.*

were collected by using the movement of compost by an FEL as the emission source. Samples 1 to 15 from trials 1 and 2 were collected during disassembly (i.e., taking the pile apart) and placement in storage of a 21-day compost pile; samples 12 to 22 from trial 3 were collected during movement of this same compost, which had been cured (stored unaerated) for 31 days beyond the initial 21-day composting. Samples 23 to 42 from trials 4 through 6 were collected during disassembly and placement in storage of two other 50-ton (4.54×10^4-kg) piles of 21-day compost. Sample trials were conducted during steady emission conditions, including the FEL working for at least 5 min, cycling bucket loads (capacity, 4.5 cubic yards [3.4 m^3]) at an average rate of five loads per min.

Samples of curing compost pile 1 and aerated pile 2 were analyzed by dilution plating for concentrations of *A. fumigatus*, as described by Millner et al. (17).

Sampler locations. The sampler locations were selected to coincide with the expected plume center line, which was assumed to approximate the mean wind direction during the sampling interval. Since the exact mean wind direction could not be determined in advance, the strip chart trace of wind direction for the interval just before sampling was used to locate the samplers. Generally, the mean wind direction shifted only a few degrees during the trial runs. Thus, the samplers were close to, but not exactly on, the plume center line. Precise location of each sampler with respect to the emitting source was determined after all trials by using a surveyor's transit, stadia rod, and tape measure. The sampler location coordinates were normalized to the mean wind direction for each trial run, with the x and y coordinates corresponding to the downwind and crosswind directions, respectively. The z (height) coordinate was reasonably constant, since the sites were chosen for their flatness. These coordinates were used to calculate the emission rate (Q) in the model described below. The extent of meteorological variation was considered in the determinations of the atmospheric stability classes (see below).

Background samples. For the survey, on each sample date one sampler was located upwind from the emitting area. Events during or immediately before the sample periods sometimes diminished the usefulness of the upwind sites as background checks, so some samples were also collected at a noncompost site at BARC on the dates corresponding to survey samples 13 to 24.

For the dispersal test, a separate sampling during static pile conditions was made upwind and downwind immediately before each of the six trial runs. In addition, 16 samples obtained at site 2 before compost was piled there were included as part of the background check samples available for comparison with dispersal test samples.

Meteorological date. At site 1, wind direction was determined visually and estimates of speed were obtained from local weather reports. At sites 2 and 3 wind speeds were recorded by six sensitive Thornwaite cup anemometers, two of which were mounted at 5- and 10-m heights on a 10-m meterological tower; the other four were mounted 2 m high on sampler poles. Wind speeds during samplings 16 through 28 were recorded with one anemometer mounted on the tower. Temperature and wind direction were measured at 5- and 10 m on the stationary tower. Sky condition was also recorded.

Dispersion model and emission rate. The model of Pasquill (19) for the atmospheric dispersion of inert particles from a point source was used to calculate the emission rate of *A. fumigatus* under the test conditions. The following expression describes the concentration X at points x, y, z from a continuous source at height H:

$$X(x, y, z{:}H) = \frac{Q \exp\left(-0.5 \frac{y^2}{\sigma_y^2}\right)}{2\pi \sigma_y \sigma_z \bar{u}} \cdot \left\{ \exp\left[-0.5 \left(\frac{Z - H}{\sigma_z} \right)^2 \right] + \exp\left[-0.5 \left(\frac{Z + H}{\sigma_z} \right)^2 \right] \right\}$$

where X is the viable spore concentration in spores per cubic meter at coordinate positions, x(downwind), y(crosswind), and z(vertical); Q is the number of spores emitted per second; $\bar{u}$ is the mean wind speed in meters per second; and σ_y and σ_z are the standard deviations of the plume concentration distribution in horizontal and vertical planes, respectively. The values of σ_y and σ_z depend on the downwind distance and atmospheric stability class. The explanation by Turner of the Pasquill stability classification (22) was followed for determining atmospheric stability classes. This classification is based on an integration of several meteorological parameters, such as total cloud cover and ceiling height, solar elevation, and wind speed. Source height (H) was 5.0 m, based on observations of smoke plumes generated from flares attached to the buckets of operating FEL. Receptor height (z) was 2.1 m, estimated as the height of the breathing zone. A decay factor was considered unnecessary for calculations of *A. fumigatus* and actinomycete aerosols since

spores of these microbes may be expected to resist the effects of radiation and desiccation during the dispersal times considered.

Estimations of Q based on the observed concentrations of *A. fumigatus* at the various sampling coordinates were calculated with the aid of a computer programmed for the Pasquill atmospheric dispersion model with the conversion of Gifford (8) for standard deviations of the plume concentration distribution (σ_y and σ_z).

Samplers. Six-stage Andersen viable air samplers were used to collect fungi, actinomycetes, and bacteria from air. The sampler-pump collection train was calibrated to draw 28.3 liters/min (0.028 m^3/min). Before each use, each sampler was cleaned in 10% Wescodyne solution, and the holes in each stage were cleared of any lodged debris; samplers were then rinsed in 70% ethanol and allowed to air dry in a microbiological safety hood. This procedure was adequate, as verified by surface swab tests, to insure against contamination of the samples from residual microbes lost to the inner surfaces of the sampler from previous collections. To prevent contamination, the samplers and plates were assembled and disassembled in a microbiological safety hood.

During collection periods, each sampler was placed on its side in a holder located 2.1 m above ground level. The sampling orifice was directed into the wind, and the intake cone was removed, as recommended by May (16) and P. H. Gregory (personal communication). Samplers were operated simultaneously for designated intervals ranging from 2 to 30 min, depending on sampler location; shorter sample times corresponded to locations close to the emission source.

Culture conditions. Two series of selective media were used in the samplers to obtain data on *A. fumigatus* as well as other thermophilous fungi and actinomycetes. For petri plates placed at stages 1 to 3 of the Andersen assembly, we used oxgall antibiotic agar with the following components (in grams per liter): peptone, 10; dextrose, 10; oxgall, 15; agar, 20. Antibiotics filter-sterilized with a 0.45-μm membrane filter were added to cooled (48°C) medium to give final concentrations of 50 μg of streptomycin per ml, 10 μg of penicillin G per ml, and 2 μg of aureomycin per ml; this medium was designated oxgall-SAP agar. For plates placed at stages 4 to 6 in the sampler, Trypticase soy agar (BBL Microbiology Systems) was used; this agar contained 0.8 μg of aureomycin per ml for plates at stages 4 and 5, but no antibiotic for plates at stage 6. All plates received 27 ml of medium, and they were inverted and incubated at 44°C for 24 and 48 h.

Preliminary tests indicated that *A. fumigatus* grew as well on Trypticase soy agar containing aureomycin as on oxgall-SAP agar. A suspension from a sporulating *A. fumigatus* culture prepared in 0.01% Triton X-100 in sterile distilled water and plated onto both Trypticase soy agar containing aureomycin and oxgall-SAP agar (10 replicates on each medium) produced mean counts of 42 and 40 colonies per plate, respectively, indicating that these media did not differ significantly ($P = 0.05$) in ability to support growth of *A. fumigatus*.

Sample analyses. Colonies were counted at 24 h and again at 48 h. *A. fumigatus* was identified by using the criteria of Raper and Fennell (20); other fungi were identified by the methods of Cooney and Emerson (4), Stolk (21), Fergus (7), and Apinis (2). Actinomycetes and bacteria on plates at stages 5 and 6 were recorded separately where possible; isolations and identifications were made for only a few representative isolates. For the survey portion of the work, calculations of viable microbial particle concentrations were based on the positive hole conversion method (all stages) of Andersen (1). For the dispersion studies, viable microbial particle concentrations were calculated by the following expression: number of colonies/(0.028 m^3/minute × minutes of sample time). A cumulative particle size distribution was tabulated for the dispersion data. Median particle diameter was determined graphically, and the standard deviation was calculated as shown by Dimmick (5).

RESULTS

Survey of BARC compost site. Sample sites are indicated by sample numbers on the map in Fig. 1. The wind speed and direction prevailing during each sampling are shown in Table 1, along with the concentrations of the viable particles of thermophilous microorganisms detected. Data at upwind sites appear as the first sample number for each collection date shown in Table 1. Unanticipated vehicle traffic upwind of the background control sites was a problem during collection of sample 5. Screener 1 (Fig. 1) was in operation immediately before collection of sample 16, confounding the use of sample 16 as a background check sample. Excluding these two occasions, the ranges of aerial concentrations for the different classes of microorganisms at the upwind sites were as follows (in number of particles per cubic meter): total, 13 to 329; actinomycetes, 0 to 317; fungi, 0 to 158; *A. fumigatus*, 9 to 155. For comparison, the concentration ranges of microbes in air collected at a noncompost, background site were as follows (in number of particles per cubic meter): total, 0 to 59; actinomycetes, 0 to 35; fungi and *A. fumigatus*, 0 to 24 (Table 2).

For fungi and *A. fumigatus* most of the upwind concentrations were in the range of concentrations detected at noncompost sites. For actinomycetes, upwind concentrations were frequently higher than the concentrations found at noncompost sites, indicating that upwind sites were not always unbiased background controls. Ideal upwind locations were not always available due to the physical constraints of the sites. Despite this, the marked effect of mechanical agitation of compost on the microbial content of air nearby is obvious from a comparison of the aerial concentrations downwind of the compost site activity with the concentrations upwind (Table 1). The ranges of aerial concentrations at the downwind sites were as follows (in number

TABLE 1. *Aerial concentrations of viable particles of thermophilous microorganisms collected at the Beltsville compost site with Andersen samplers*

Sample[a]	Sample date	Wind speed (m/s)	Wind direction[b]	No. of thermophilous microorganism particles per m^3			
				Total	Actinomycetes	Fungi	*A. fumigatus*
1	13 June 1977	2.7	E	328	317	11	7
2	13 June 1977	2.7	E	16,100	15,300	805	678
3	13 June 1977	2.7	E	12,900	12,600	334	325
4	13 June 1977	2.7	E	86	30[c]	56	11
5	17 June 1977	3.6	SSW	7,220	7,100	115	35
6	17 June 1977	3.6	SSW	13,100	12,500	574	61
7	17 June 1977	3.6	SSW	9,070	8,660	409	191
8	17 June 1977	3.6	SSW	4,570	4,260	314	43
9	20 June 1977	2.7	S	120	104	16	14
10	20 June 1977	2.7	S	1,810	1,450	362	101
11	20 June 1977	2.7	S	2,200	1,610	589	167
12	20 June 1977	2.7	S	1,260	777	480	407
13	24 June 1977	3.2	S	151	132[c]	19	5
14	24 June 1977	3.2	S	1,760	473	1,290	308
15	24 June 1977	3.2	S	1,960	864	1,090	831
16	27 June 1977	2.4	ESE	1,100	744	360	181
17	27 June 1977	2.4	ESE	13,700	9,510	4,170	2,860
18	27 June 1977	2.4	ESE	7,840	4,150	3,690	3,590
19	26 July 1977	4.1	NNW	52	21	31	31
20	26 July 1977	4.1	NNW	367	148	219	219
21	26 July 1977	4.1	NNW	17	10	7	7
22	27 July 1977	4.1	NNE	13	13	0	0
23	27 July 1977	4.1	NNE	1,850	1,690	155	155
24	27 July 1977	4.1	NNE	33	9	24	24
25	19 September 1977	2.5	SSW	13	0	12	0
26	19 September 1977	2.5	SSW	15,000	14,100[c]	896	544
27	19 September 1977	2.5	SSW	10,300	5,070	5,190	1,310
28	19 September 1977	2.5	SSW	7,880	4,970	2,910	2,400
29	28 September 1977	8.2	N	158	0	158	155
30	28 September 1977	8.2	N	26,500	7	26,500	26,500
31	28 September 1977	8.2	N	55,500	42	55,500	55,000
32	28 September 1977	8.2	N	2,880	7	2,870	2,860
33	28 September 1977	8.2	N	4,620	39	4,580	4,460
34	12 October 1977	4.1	NNW	24	0	24	19
35	12 October 1977	4.1	NNW	29,400	35	29,400	28,100
36	12 October 1977	4.1	NNW	20,100	14	20,100	19,000
37	12 October 1977	4.1	NNW	3,300	9	3,290	3,220

[a] For location of sample collection points, see Fig. 1. The first sample in each sample group (i.e., sample date) was an upwind sample; the other samples were downwind samples.

[b] E, East; SSW, south-southwest; S, south; ESE, east-southeast; NNW, north-northwest; NNE, north-northeast; N, north.

[c] Counts include bacteria, but actinomycetes predominated.

of particles per cubic meter): total, 17 to 55,563; actinomycetes, 7 to 15,301; fungi 24 to 55,521; *A. fumigatus*, 7 to 55,021.

The effect of the mechanical agitation of compost on increasing the microbial content of air was also examined by comparing airborne microbe concentrations during and immediately after the piling of compost by FEL. The analysis of this series of samples (Table 3) showed that high concentrations of microbial aerosols were

associated with the piling of compost by FEL. These aerosols rapidly dispersed once the mechanical agitation ceased. Also, these results suggest that few of these aerosolized microbes originated as windblown losses from stationary piles of compost. The total microbial aerosol concentration during compost agitation was approximately 150 to 200 times that present during quiescent periods in the working area. The fungi in the aerosols produced by mechanical agitation were typical of those found in examinations of compost samples (17). Only a few of the many actinomycete isolates obtained have been identified so far; these include *Micropolyspora* sp., *Nocardia* sp. 1, *Nocardia* sp. 2, *Saccharomonospora viridis*, *Streptomyces* spp., and *Thermoactinomyces vulgaris*.

Dispersal tests. Site 2 and 3 sample locations are indicated in Fig. 2 and 3; sample numbers correpond to those given in Table 4. The precise locations of the samplers with respect to the piles and the mean wind direction for the sample period are indicated by the coordinates for each sample (Table 4).

The range of microbial aerosol concentrations obtained in 40 background samples is shown in Table 5, along with several measures of the frequency distribution of each microbe class. Of these 40 background samples, 4 contained 107, 112, 235, and 322 actinomycetes particles per m^3. The sample with 322 actinomycetes per m^3 also had 139 *A. fumigatus* particles per m^2, whereas the previous other 3 had 0, 12, and 14 *A. fumigatus* particles per m^3. These results verify those of the survey; i.e., aerosol concentrations resulting from windblown losses from stationary piles are relatively small in comparison with those generated during mechanical movement of piles.

Data from the dispersal tests were used to calculate emission rates (Q, in viable particles of *A. fumigatus* per second [Table 4]) for each sample by using the equation given above. For these calculations the measured concentrations were corrected for background concentrations of *A. fumigatus* detected at the sample locations immediately before the start of the trials. The log Q was used for analysis of the data; mean emission rates and their 95% confidence limits are shown in Table 6 for the six trials. The overall mean emission rate for 30 determinations was 1.2×10^6 *A. fumigatus* particles per s, and the highest calculated mean emission rate was 4.6×10^6 *A. fumigatus* particles per s.

Because the aerodynamic size of airborne particles influences their penetration and deposition into the respiratory system, the results from the air-sampling tests were evaluated in terms of the aerodynamic sizes of the captured particles. This size determination was based on the fractionation obtained within the Andersen sampler (Table 7 and Fig. 4). For these samples the *A. fumigatus* particle median diameter was 3.8 ± 1.6 μm. The cumulative distribution plot (Fig. 4) shows that approximately 87% of the *A. fumigatus* particles in the aerosol are breathable (≤7 μm), approximately 70% can pass the trachea and primary bronchi, 5% can reach the terminal bronchi, and less than 1% can reach the alveoli (10). The nature of the particles with aerodynamic sizes of ≥4.7 μm is not precisely known at this time. However, the fact that *A.*

TABLE 2. *Concentrations of viable particles of thermophilous microorganisms detected in air at a noncompost site*[a]

Date collected	No. of thermophilous microorganism particles per m^3			
	Total	Actinomycetes	Fungi[b]	*A. fumigatus*
24 June 1977	35	21	14	7
27 June 1977	59	35	24	24
26 July 1977	5	0	5	0
27 July 1977	0	0	0	0

[a] See text for details.
[b] Includes *A. fumigatus* count.

TABLE 3. *Concentrations of viable particles of thermophilous microorganisms in air during and after mechanical agitation of compost by an FEL at BARC compost site*[a]

Sample	No. of thermophilous microorganism particles per m^3			
	Total	Actinomycetes	Fungi[b]	*A. fumigatus*
During compost pile agitation				
Upwind of FEL	2	0	2	2
3 m downwind from FEL	12,000	8,870	3,110	1,390
30 m downwind from FEL	8,920	6,920	2,000	1,820
60 m downwind from FEL	6,310	1,080	5,230	5,020
15 min after compost pile agitation				
3 m downwind from pile	42	3	39	39
30 m downwind from pile	14	7	7	0

[a] Mean wind speed during sampling, 3.1 m/s; mean wind direction during sampling, north-northwest.
[b] Includes *A. fumigatus* count.

TABLE 4. *Results of dispersal tests and calculated emission rates*

Sample	Date	Coordinates		Mean wind speed (m/s)	Atmospheric stability class[a]	Ambient *A. fumigatus* concn (particles per m^3)	Emission rate *Q* (no. of *A. fumigatus* particles per s)
		x Downwind (m)	y Crosswind (m)				
1[b]	12 May	−57.5	−9.1	2.9	B	0	
2	12 May	10.0	4.5	2.9	B	6.2×10^3	7.5×10^6
3	12 May	41.7	7.5	3.1	B	8.6×10^3	8.8×10^6
4	12 May	44.3	−6.5	3.1	B	1.1×10^2	1.1×10^5
5	12 May	30.6	30.4	3.2	B	2.4×10^3	6.7×10^{10}
6	12 May	69.5	11.3	3.2	B	7.2×10^3	1.3×10^7
7	12 May	80.7	17.5	3.3	B	2.7×10^3	8.6×10^6
8	12 May	91.9	24.5	3.3	B	2.1×10^3	1.1×10^7
9[b]	12 May	−58.1	3.0	3.5	B	0	
10	12 May	10.7	2.3	3.14	B	2.3×10^3	7.8×10^5
11	12 May	42.4	−1.3	3.13	B	5.5×10^3	4.0×10^6
12	12 May	40.5	22.4	3.5	B	2.4×10^3	6.7×10^7
13	12 May	70.3	−3.4	3.57	B	2.3×10^3	3.6×10^6
14	12 May	82.6	0.3	3.54	B	4.5×10^2	8.6×10^5
15	12 May	95.0	4.8	3.54	B	1.2×10^3	2.9×10^6
16[b]	16 June	−69.2	21.4	2.6	A	2.14	
17	16 June	10.5	−1.9	2.2	A	4.0×10^3	1.0×10^6
18	16 June	63.8	−11.9	2.2	A	6.4×10^2	1.1×10^6
19	16 June	545.0	−21.9	2.2	A	40	1.9×10^6
20	16 June	475.0	−267.0	2.6	A	21	2.4×10^7
21	16 June	526.0	−128.0	2.2	A	43	5.2×10^6
22	16 June	410.0	−353.0	2.6	A	3.6	
23[b]	16 June	−52.8	4.8	2.7	A	0	
24	16 June	31.9	−4.9	2.2	A	6.3×10^2	4.6×10^5
25	16 June	79.9	−19.9	2.1	A	76.7	1.5×10^5
26	16 June	555.0	−93.9	2.5	A	2.6	4.2×10^5
27	16 June	381.0	−403.0	2.2	A	4.7	1.0×10^6
28	16 June	516.0	−214.0	2.7	A	1.5	
29[b]	7 September	−38.39	21.28	2.9	A	8.3	
30	7 September	33.06	1.24	2.3	A	1.4×10^3	1.0×10^6
31	7 September	78.42	4.67	2.1	A	7.3×10^2	1.4×10^6
32	7 September	139.2	22.3	2.3	A	25.8	1.4×10^5
33	7 September	264.0	46.08	2.4	A	15.8	3.5×10^5
34	7 September	492.45	89.40	2.9	A	2.8	8.0×10^4
35	7 September	619.19	113.64	2.9	A	8.1	3.0×10^5
36[b]	7 September	−43.79	3.06	2.7	A	1.1	
37	7 September	33.05	1.65	2.5	A	375	2.9×10^5
38	7 September	78.54	1.59	2.2	A	77.5	1.8×10^5
39	7 September	140.64	10.08	2.6	A	14.0	2.3×10^4
40	7 September	267.02	22.89	2.7	A	4.6	
41	7 September	498.37	46.14	2.7	A	1.7	
42	7 September	626.75	59.24	2.7	A	5.8	

[a] Atmospheric stability classifications range from A (very unstable) through C (neutral) to F (very stable) according to Pasquill (19).

[b] Upwind checks.

fumigatus spores are exceedingly hydrophobic and have been observed to break from conidiophores in chains suggests that some particles which had aerodynamic sizes of >4.7 μm may have been clumps of *A. fumigatus* spores.

DISCUSSION

The survey at the compost site indicated that the intensity of aerosolization was mainly associated with mechanical movements of the compost piles. After the FEL stopped moving compost, the *A. fumigatus* aerosol at 3 and 30 m downwind of the piles was approximately 33 to 1,800 times less concentrated than that measured during movements and not significantly above background levels. However, because the ground of the site was covered with compost, it was unclear whether aerosolization was due to entrainment of surface dust, etc., by equipment wheels or to mechanical handling of the compost. The survey work suggested that the windblown losses of microbes from stationary piles

TABLE 5. *Range of concentrations of viable particles of thermophilous microbes and measures of frequency distributions obtained from 40 background air samples*

Particle type	Concn (no. of particles/m^3)			
	Range	Mean	Median	Mode
A. fumigatus	0–14	2.2	0.5	0
Fungi	0–19	3.8	1.5	0
Actinomycetes	0–105	8.9	2.5	0

TABLE 6. *Mean emission rates of A. fumigatus based on measured downwind concentrations*

Trial	Samples	Mean emission rate ($\times 10^5$ *A. fumigatus* particles per s)[a]	Pile
1	2 to 8[b]	$210 \geq 46^c \geq 10$	1
2	10 to 15	$140 \geq 35^c \geq 9.4$	1
3	17 to 21	$110 \geq 31^c \geq 8.9$	1
4	24 to 27	$11 \geq 4.3^d \geq 1.7$	2
5	30 to 35	$8.6 \geq 3.5^d \geq 1.4$	3
6	37 to 39	$2.7 \geq 1.1^d \geq 0.49$	3

[a] With the 95% confidence limits.
[b] Excluding sample 5.
[c,d] The values with a superscript *c* differ significantly from those with a superscript *d* ($P < 0.05$).

TABLE 7. *Size fractionation data for A. fumigatus particles collected in Andersen viable samplers (30 dispersion test samples)*

Andersen sampler stage	Size range (μm)	Mean diam (μm)	*A. fumigatus* count	% in size range	Cumulative % less than mean diam
6	0.65–1.1	0.62	43	0.46	0.2
5	1.1–2.1	1.6	886	9.51	5.2
4	2.1–3.3	2.7	3,555	38.18	29.0
3	3.3–4.7	4.0	2,867	30.79	63.5
2	4.7–7.0	5.8	852	9.15	83.5
1	≥7.0		1,107	11.89	94.0

are small in comparison with the losses experienced during active agitation of piles. Airborne transport of these small amounts of windblown losses would result in aerial concentrations which would become rapidly indistinguishable from background levels.

In contrast, microbial aerosols emitted during pile agitation with a certain set of atmospheric conditions produced aerial concentrations significantly above background levels downwind from the piles. Dispersal tests showed that aerosols are generated primarily by mechanical handling of the compost and not from entrainment of surface dust, since tests were conducted on clean concrete surfaces and the *A. fumigatus* concentrations were comparable to those measured during the survey. The atmospheric dispersion model, which was used with some judgment in the selection of the effective source strength (e.g., 4.6×10^6 *A. fumigatus* particles per s), adequately describes the *A. fumigatus* aerosol dispersion at the test sites. Variation in emission rates (Table 6) between piles may be attributed to several factors, including (i) *A. fumigatus* concentration differences in the compost piles, (ii) the uncertainties inherent in estimates of σ_y and σ_z in the model, and (iii) the fact that *A. fumigatus* particles traveling in that portion of the plume which is in contact with the ground may not be completely reflected as the model assumes (also, resuspension of these deposited particles would be possible). The fact that the concentrations of *A. fumigatus* in pile 1 were very nearly the same as those in pile 2 (1.2×10^3 to 3.3×10^3 and 1.8×10^3 to 6.9×10^3 *A. fumigatus* particles per g [dry weight] of compost, respectively) but the emission rates associated with these two piles were significantly different suggests that the efficiency of aerosolization can vary substantially. This is not especially surprising, considering the gross handling method used.

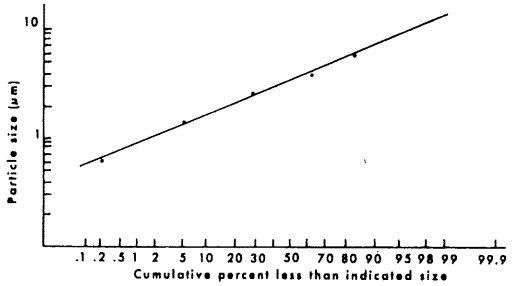

FIG. 4. *Cumulative probit plot of the size distribution of A. fumigatus particles in the Andersen samplers.*

The dispersal tests were made during only a few different meterological conditions, but the results can be extended to a complete matrix of conditions by using the atmospheric dispersion model (19) and statistical data from the National Weather Service. Table 8 lists predicted concentrations of *A. fumigatus* calculated by using the atmospheric dispersion model with 4.6×10^6 *A. fumigatus* particles per s as the emission rate under restrictive (stable), typical (neutral), and dispersive (very unstable) atmospheric conditions. These are predictions for concentrations along the center line of the dispersing plume; predicted concentrations would be decreased at non-center line (crosswind) locations. Furthermore, changes in the meteorological conditions would also change the predicted concentrations.

TABLE 8. *Estimated centerline concentrations of A. fumigatus at unobstructed distances downwind from agitated compost piles for three atmospheric mixing conditions*[a]

Downwind distance (km)	No. of *A. fumigatus* particles per m³ under the following atmospheric conditions		
	Stable[b]	Neutral[c]	Unstable[d]
0.1	1.2×10^4	6.1×10^3	1.1×10^3
0.2	7.8×10^3	2.6×10^3	3.3×10^2
0.3	5.2×10^3	1.3×10^3	1.4×10^2
0.4	3.6×10^3	8.8×10^2	7.2×10^1
0.5	2.7×10^3	6.1×10^2	4.1×10^1
0.6	2.1×10^3	4.2×10^2	2.4×10^1
0.7	1.6×10^3	3.3×10^2	1.5×10^1
0.8	1.3×10^3	2.6×10^2	1.0×10^1
0.9	1.1×10^3	2.2×10^2	7.1×10^0
1.0	9.9×10^2	1.9×10^2	5.2×10^0

[a] Source height, 5.0 m; receptor height, 2.1 m; Q, 4.6×10^6 *A. fumigatus* particles per s.

[b] Class F, $\bar{u} = 3.0$ m/s; based on National Weather Service data for National Airport, Washington, D.C.

[c] Class D, $\bar{u} = 3.58$ m/s; based on National Weather Service data for National Airport, Washington, D.C.

[d] Class A, $\bar{u} = 3.1$ m/s; based on National Weather Service data for National Airport, Washington, D.C.

In the example, with unstable atmospheric mixing the *A. fumigatus* concentration at 0.5 to 0.6 km along the plume center line would approximate the concentrations measured as background (Tables 2 and 3). A survey sampling of actual background concentrations at potential downwind locations would be necessary in case evaluations, however, because of local variations in existing air spora.

In evaluating the dispersal of microbial aerosols to locations around composting sites, the frequency distribution of meteorological factors, such as wind speed, wind direction, and atmospheric stability, which contribute to restrictive (i.e., nondispersive) conditions and which may be expected to occur in the subject locality, should be considered. At a particular downwind location, some meteorological conditions do restrict dispersion, creating a high aerosol concentration; conversely, other conditions enhance dispersion and thus lower aerosol concentration.

The predictions of *A. fumigatus* aerosol concentrations presented here are based on the movement of compost by a single 4.5-cubic yard FEL. It is obvious from these and previous results (17) that fungus growth and release into the air can vary between piles (Table 6). The emission rates reported here are not necessarily typical of those that might occur if the process were modified. Such modifications could include the use of (i) noncellulosic bulking agents, (ii) extended periods of undisturbed storage after peak heating stages of composting, (iii) larger- or smaller-capacity FEL, operating at loading cycles different from those used in these tests, (iv) different methods of moving compost from the piles, including multiple, simultaneous movements of compost by FEL or other equipment, and (v) partial enclosure of compost-moving operations within buildings or other structures.

In addition to the above quantitative aspects of compost-associated aerosols are the qualitative aspects, which formed only a small part of this investigation. The results of the survey indicated that a variety of thermophilous actinomycetes and fungi were aerosolized. Some of these actinomycetes are known to incite hypersensitivity reactions in human respiratory systems when inhaled in large numbers (11). The measured concentrations of thermophilous actinomycetes (maximum, 15,000 actinomycetes particles per m³), however, were not nearly as high as the maximum of 15×10^9 actinomycetes particles per m³ found by previous investigators studying such aerosols in barns during the shaking of moldy hay (12, 13). A thorough characterization of the actinomycete component of the compost-associated aerosols is, however, of potential importance because of the close proximity of compost workers to the aerosols.

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Reference Point

Methods and microbial risks associated with composting of animal carcasses in the United States

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Animal carcass composting for both routine and emergency management of dead production animals is an alternative method of carcass disposal in those situations in which conventional methods are inadequate. Carcass composting differs from composting other materials such as manure and green waste and presents some unique challenges. Carcasses are typically composted whole and do not present uniformly chopped substrate for microbial action, and these compost piles are not turned frequently. Both of these factors contribute to a nonuniform compost composition at the end of the process. Although allowances for this nonuniformity need to be made, well-designed carcass compost systems (with proper maintenance and monitoring) do result in a safe and efficient method of disposing of dead animals with minimal environmental impacts. Importantly, proper composting eliminates many pathogens and may reduce levels of carcass contamination with spore-forming bacteria, prions, and other specific pathogens. When considering options, carcass composting should be evaluated via a risk assessment approach that includes all stages of disposal of dead animals, such as handling, transportation, processing, storage, and disposal; among the various disposal systems under consideration, risk comparisons need to account for the sum of risks from the time of death to sequestration or destruction of potential microbial threats associated with an animal carcass.

The Current Situation in the United States

Part of the challenge associated with the disposal of animal carcasses includes protection of environmental, animal, and public health against potential microbiological threats. An animal carcass is composed of microbiologically active material that may contain viruses, bacteria, protozoa, parasites, prions, toxins, drug residues, and other chemicals. All of the biologically active materials need to be reduced to safe amounts, eliminated, or sequestered to minimize their potential hazard. Regulations to provide uniform standards for biosecurity, traceability, and environmental protection are necessary. Biosecurity agencies in Australia, New Zealand, the United States, and Canada have recognized the potential benefits of composting for both routine and emergency management of deaths among production animals and have identified it as the preferred method of carcass disposal in certain situations.[1]

Abbreviations

BSE	Bovine spongiform encephalopathy
$\log_{10}$	Logarithm base 10
MRA	Microbial risk assessment
Stx	Shiga toxin

The disposal of dead animals is not federally regulated in the United States and varies between and within states. The principal methods of carcass disposal are rendering, burial, incineration, and composting. Lactic acid fermentation, alkaline hydrolysis, and anaerobic digestion are additional options that currently offer limited capacity for disposal.[2] New technologies continue to enter the market, including microwave sterilization and gasification. All methods have strengths and weaknesses.[2] Several federal and state agencies in the United States have regulations pertaining or relevant to the disposal of animal carcasses. More coordination and harmonization of rules among these regulatory authorities would help eliminate confusing and conflicting information. Although composting as a form of routine or emergency animal carcass disposal has been approved in several states, other states have no rules and some prohibit the practice.[3]

Carcass composting has been referred to as "aboveground burial in a bio-filter with pathogen kill by high temperature".[4] Historically, it is known to be a safe method of disposal of animal manure.[3,5] Compared with carcass composting, the methods for animal manure composting[6–8] and associated risks of disease transmission have been more extensively investigated.

Unusual sudden increases in death rates or other catastrophic losses can exceed the rendering capacity in a local region.[2,9] Such spikes in mortality rates and catastrophic losses may be attributable to epidemic dis-

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ease, severe weather, electric and transportation failure, or other emergency situations including quarantine and market interruption. Even temporary interruptions may have high impacts on intensive animal production operations because those operations have limited storage capacity and production time lines that require regular feed delivery and the entry of replacements and exit of market-ready stock. Effective and safe on-farm disposal of dead animals decreases the potential for environmental contamination and disease spread from the biological hazards associated with animal carcasses.

The US rendering industry collects and disposes of most dead animals and unwanted animal by-products. Economic impacts associated with what is known as the feed rule[10] to protect the United States from the threat of BSE have increased the costs of rendering and resulted in a fee for this service to the producer. Consequently, the amount of animal by-products and carcasses that are disposed of on farms without proper safeguards may have increased since 2000. The approved alternative methods of carcass disposal, including pit burial, individual burial, commercial certified landfills, alkaline digestion, carcass composting, and incineration, vary among states.[2,3] Many of the more traditional methods require specialized equipment and appropriate geologic, hydrologic, and climatic conditions. States that do not allow carcass composting limit their options and may lack the capacity for safe and effective carcass disposal when faced with sudden increases in mortality rates or catastrophic losses among production animals.

The interest in the use of on-farm composting for the disposal of animal by-products and carcasses is growing because the practice is relatively simple, effective, environmentally sound, and economic. It uses materials and equipment that are often available or readily accessible on farms. The finished compost can be applied to the land, if permitted by state and local regulatory agencies, thereby providing an environmentally acceptable means of recycling nutrients and stabilized organic matter into the soil. Proper composting of carcasses requires the same expertise to manage the process and site as that required for proper composting of manure, landscape trimmings, and other materials. Improper composting can result in slower digestion of tissues along with adverse environmental impacts, including odor and leachate production as well as inadequate pathogen reduction. Proper compost system design, maintenance, and monitoring are straightforward. In many states, the poultry industry has successfully used composting for carcass disposal under a variety of environmental conditions for nearly 2 decades.

Several states have guidelines and information on the World Wide Web about composting of animal carcasses. Permits for carcass composting are issued on the basis of the type and extent of composting in some states. Certification for composting is granted in certain states on the basis of livestock producer participation in courses. The National Resource, Agricultural, and Engineering Service has excellent guidance publications on composting, and the National Sustainable Agricultural Information services also provide relevant explanatory documents.[11,12] Composting research has been performed at The Ohio State University,[3,13] Texas Cooperative Extension,[14,15] and the National Agricultural Biosecurity Center at Kansas State University[2] and by the Alberta Provincial government.[11] These groups have provided reviews and databases of resources that describe the economic, environmental, and technical aspects of on-farm composting, including the composting of carcasses. In Canada, a highly biosecure, enclosed system of composting was used by the Canadian Food Inspection Service to deal with thousands of carcasses during an avian influenza outbreak in 2004.[16] The Cornell Waste Management Institute has published an extensive literature review[17] on the expected prevalence and persistence of pathogens in composted New York State roadkill.

Principles and Elements of Composting

Composting is a largely aerobic process in which bacteria, fungi, and other microorganisms convert organic material into stable humus. Composting of animal carcasses requires precautions to minimize the potential spread of diseases, odors, and liquids. The composting process depends on naturally present microorganisms to digest the organic components in the carcass. The carbon-based materials in the composting piles supply energy for the microbes, and the carcass tissues and fluids supply nitrogenous materials for microbial protein synthesis. Heat, water, carbon dioxide, ammonia, and volatile organic compounds are by-products produced in the composting process. Much of the digestion within and at the outer surface of the carcass is anaerobic, but the liquid and gaseous by-products of the anaerobic process diffuse away from the carcass and into progressively more aerobic layers of the composting envelope, where aerobic degradation further reduces them to carbon dioxide and water.

The microbial flora responsible for the decomposition of organic matter are a complex mix of organisms, some of which are able to function and survive at temperatures that are sufficiently high to kill mammalian and avian pathogens.[18–21] These complex microbial decomposer communities occur naturally in the environment, and many of the mesophilic microbes (those that grow best at 20° to 55°C [68° to 131°F]) are responsible for the continuous and normal decay of plant and animal tissues at ambient temperatures. The thermophilic microbes (ie, those that withstand and grow at temperatures > 45°C [113°F]) inhabit naturally self-heating environments such as animal nests, hot springs, and large piles of storm debris. There is no need to add special microbes to the composting matrix.

Composting of all types of organic materials is affected by physical and chemical factors such as the carbon-to-nitrogen ratio, moisture content, oxygen concentration, temperature, and pH.[1,14,22] For optimum carcass composting, recommended conditions include an initial carbon-to-nitrogen ratio in the range of 25:1 to 30:1, an initial moisture content of 50% to 60%, and an initial oxygen concentration > 10%. These conditions facilitate thermophilic composting and supporting temperatures of 43° to 66°C (109.4° to 150.8°F), which are optimal for compost microorganisms.[8,23] Temperatures > 70°C (158°F) or < 40°C (104°F) reduce the thermophilic mi-

crobial population and their accompanying enzymatic activities that are responsible for rapid decomposition and stabilization of the organic feed stocks. At temperatures of 55°C for 3 consecutive days, most pathogenic bacteria and parasites are killed and most viruses are inactivated. Lower temperatures will support carcass decomposition but prolong the duration of the process: as a rule of thumb, general chemical and biochemical reaction rates approximately double with each 10°C (18°F) increase in temperature.[24] The target pH for composting is neutral, although successful composting occurs at pH values of 5.5 to 9.0.[25]

The design of carcass composting systems must address 4 major safety and acceptance issues: protection of ground and surface water, minimization of the risk of spreading disease, prevention of nuisances from scavengers and insects, and maintenance of air quality.[4,15]

Composting systems are divided into open and closed systems.[8] Open systems include windrows, static piles, and bins. The simplest system involves the use of windrows, which have a defined width and variable length as needed. Animal carcasses are added incrementally onto a thick bedding of compostable, absorbent carbonaceous material. As needed, the windrow can be lengthened to accommodate additional large (> 227-kg [500-lb]) carcasses, which are typically composted in a single layer (**Figure 1**). If carcasses are small, windrow height can be increased with additional layers of carcasses separated by sufficient carbonaceous material to absorb and retain liquids. Composting bins are constructed with 3 permanent walls and are sized to accommodate the equipment used to handle the material (eg, a skid-loader). Material is transferred from the primary phase bin to a secondary phase bin at 10 to 21 days (for poultry) or when soft tissue decomposition is complete, pile temperature cools, and fresh aeration is needed to continue the self-heating process. Windrow and bin systems are the most common methods used for on-farm carcass composting.

Closed, in-vessel systems are far less common and typically are used for small species (eg, poultry and nursery pigs). In closed systems, the composting mass is contained within an insulated structure; aeration is provided through a series of vents or during rotation if a horizontal drum configuration is used. Technologically advanced composting systems use reactor vessels with mechanical aeration and mixing of material. Composting processes and facilities range from highly mechanized and intensively managed (frequently turned or mechanically aerated) systems used in production industries to attain maximum throughput and minimum processing time to much simpler naturally ventilated static pile systems that decompose materials more slowly and that require much lower capital and operating costs. In some situations, the process may be started in a closed system (in-vessel system) and eventually transferred to an open system to complete the process.

Most carcass-composting operations employ naturally ventilated, static pile processes. In rainy climates, static pile bins are commonly approximately 5 to 6 feet in height and are covered by a roof to prevent water saturation that can interfere with the process. In dry climates, carcass composting is typically done in unsheltered windrows and may require the addition of moisture to maintain optimal digestion. Open windrows are used during emergencies to enhance biosecurity and reduce environmental pollution risks associated with burial of high numbers of animals deaths. During periods with considerable rainfall, additional precautions are required to minimize leachate contamination of the environment from open windrows. These precautions include use of extra thick layers of absorptive material over and beneath the carcasses or covering the windrows with water-shedding fabrics or plastic sheeting.

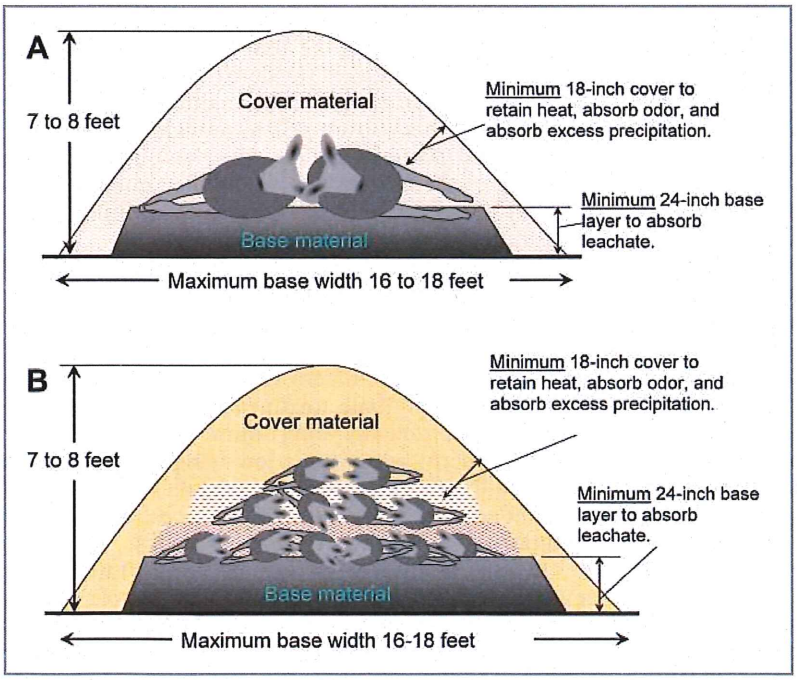

Figure 1—Illustration of the placement of large carcasses (cattle and horses; A) and small carcasses (swine, sheep, calves, or poultry; B) and in a static pile composting system.

Animal carcass composting piles are typically constructed in layers, starting with a thick absorptive layer of carbonaceous plant material. Whole carcasses are laid on top of the base and covered with additional absorptive organic material. Succeeding layers of carcasses are added on a daily basis until the bin is filled or until an appropriate freestanding pile height is reached. Bins containing poultry or similar small carcasses may contain many layers. Composting piles or bins may include 2 or 3 layers of mature sheep and swine carcasses, whereas mature cattle are usually composted in a single layer with 2 carcasses placed back to back (Figure 1). The practice of opening the carcasses of ruminants by lancing the rumen and thorax has been questioned. Although

lancing may prevent the carcass from exploding, which may increase the risk of disease spread, the procedure of lancing could potentially also increase the risk of disease spread. It has been suggested that such lancing does not enhance the overall composting process or outcome.[1] A layer of absorptive carbon material that is approximately the same thickness as the carcasses is used between each carcass layer to retain the heat produced by microbial activity and to absorb excess liquid released from the carcasses during digestion.

The success of naturally ventilated static pile composting processes depends on the characteristics and thickness of the materials used to envelope the carcasses. In reports[26,27] of a 3-year study of emergency cattle carcass–composting procedures, it was concluded that water-holding capacity, biodegradability (for heat production), gas permeability (for oxygen penetration), and mechanical strength (to prevent compaction and loss of gas permeability) are the most important envelope-material factors. Particle size can be used as a practical proxy measure for the all-important gas permeability that permits air to flow into compost. Particle sizes of envelope materials in the 0.6- to 5-cm (0.25- to 2.0-inch) range have been found to work reasonably well on the basis of field observations. Smaller particle sizes restrict gas movement, and larger sizes can lead to excessive airflow and chilling of the composting process during cool weather. Experience indicates that many wood by-products and crop residues are effective in poultry and livestock composting. Some common materials include sawdust, wood chips, ground cornstalks, rice hulls, ground straw, corn silage, straw-manure mixtures, and poultry litter.

Composting times vary depending on the size of the carcasses, ambient temperature, and other physiologic factors. Undisturbed primary composting refers to the first peak–heating phase inclusive of the gradual cooling that ensues. The duration of the primary phase will vary depending on carcass size. The estimated duration of primary composting ranges from 10 days for fowl to 195 days for adult bovids.[4] Primary composting is recommended for all carcasses to minimize the spread of infection and allow for breakdown of soft tissue. Following the primary composting period and cooling to 45° to 48°C (113.0° to 118.4°F), the compost can be turned to stimulate the secondary compost heating phase in which bones will be degraded. Secondary composting is performed for an additional period of 10 to 65 days. The design of an effective composting structure or windrow has been previously described[28] and is applicable for any animal that weighs 2 to 650 kg (4.4 to 1,430 lb). The period required for animal decomposition can be roughly estimated through a function of carcass weight; the required time interval can be calculated by use of the Keener equation as follows: $T = 7.42 \times W^{0.5} \geq 10$, where T is the primary composting time (in days), and W is the weight of the heaviest carcass. This formula is based on a variety of carcass composting demonstration projects, many of which were carried out in warm climates or during warm seasons. However, external temperatures play a highly important role in decomposition time and are not reflected by the Keener equation.

Turning the compost mass infuses it with a supply of oxygen, thereby supporting thermophilic digestion. However, turning piles with large animal carcasses is difficult and unappealing. Often, carcass compost masses are not turned; if the pile is turned, this typically occurs only after the primary composting cycle is complete. As a result, these piles retain a layered structure, which results in considerably higher spatial variation within the pile. Oxygen concentration, for example, which is supplied by natural diffusion from the outer surface, is maximal in the exterior part of the envelope of carbon-based material and low within the core, where areas of dense tissue are located. However, temperatures are often highest near the core because of the extensive insulating effect exerted by the envelope material and lower near the walls or edges of the pile, particularly during cool weather. The carbon-to-nitrogen ratio is usually low at the core near the carcasses because of the high nitrogen content of animal carcasses, and the ratio gradually increases toward the periphery of the compost pile. Similarly, moisture levels are usually greatest in the immediate vicinity of fresh carcasses and at the base of the pile and decline gradually to relatively dry conditions in the outer envelope as a result of the wicking action of the surrounding carbon-based envelope material.

Simple design and construction guidelines greatly improve the potential to achieve important biosecurity and environmental goals. In the final analysis, these goals can be met by sheltering the piles from excess precipitation and high wind and by application of sufficient quantities of acceptable envelope material. Achieving and maintaining temperatures that reduce pathogen survival depend mainly on use of a sufficient thickness of outer envelope materials that have good insulating properties. Similarly, leachate retention depends on use of a sufficient thickness of materials that have high water-holding capacity in the base of the pile. Although oxygen concentrations in the core of unturned piles are likely to be less than the 5% minimum, use of envelope materials cut to a particle size to provide sufficient free airspace and gas permeability can ensure that the outer envelope of the pile sustains an aerobic microbial biofilter layer that decomposes odorous compounds before they are released into the atmosphere.

Livestock carcass–composting facilities should be sited so as to not cause pollution of surface water, groundwater, or soil. Provisions are needed for containment or appropriate diversion and collection of leachate and surface runoff from the pile and, if necessary, subsequent appropriate treatment. The site should be at least 100 m from any water source on public or other private property, but not in a low-lying tract of land. A lime-stabilized clay,[29] asphalt, or concrete surface is recommended to facilitate year-round access and prevent pollution of soil beneath the compost piles.

Regular monitoring of the compost operation is essential. This includes ensuring that all parts of a carcass are properly covered at all times, which often necessitates the addition of envelope material should shift or collapse of the compost pile occur. Temperature monitoring, preferably assessed at the carcass surface, is a key indicator of a properly functioning compost pile,

and daily monitoring is recommended to ensure that the temperature increases to the optimal value; thereafter, at least weekly monitoring should be performed to ensure stable composting conditions. Records of the composting piles temperatures should be maintained. Temperatures should increase from 55° to 66°C and remain in this range for at least 1 week. The time necessary to achieve optimal temperature is dependent on external ambient temperature and on the heat-producing and heat-losing characteristics of the co-compost. Reductions in compost pile temperature from 45° to 48°C indicate a need for more oxygen or substrate. Turning a pile too early can release odor, chill the pile, and increase the risk of pathogen release. Compared with composting procedures for small carcasses, a longer interval should be allowed to elapse before turning a pile containing large carcasses is considered. The frequent turning and aeration practices used in municipal and industrial composting facilities are less important in smaller composting operations with lower carcass-processing loads; compost pile turning may actually disturb and slow down the process if done too frequently or at the wrong time. Turning, in fact, is not necessary at all if the envelope material has good mechanical strength and gas permeability.[26] Odors may indicate a failed composting process. Excessive loss of leachate from the compost may indicate excessive moisture in the pile and insufficient use of absorptive cover beneath and between the carcasses.

Microbial Risks Associated with Composting of Animal Carcasses

A wide variety of potential microbial pathogens are found in manure, food waste, and animal carcasses. Microbial die-off and survival associated with composting animal carcasses and other organic wastes at locations throughout the world have been investigated. Bacterial pathogens, unlike viruses and parasites, can survive outside the host organism if composting temperatures are inadequate for their destruction. An additional concern is the potential for regrowth of organisms that were not completely eliminated if conditions subsequently become favorable. Ova of the parasite *Ascaris lumbricoides* are especially resistant to destruction and have therefore been accepted as a benchmark or proxy for microbial destruction achieved by various treatment systems. Bacterial pathogens potentially found in meat, food scraps, manure, sludge, and other organic residuals will be destroyed by exposure to the time-temperature regimens attained in a well-managed composting environment (**Appendix 1**). The static compost pile coupled with the nonuniform composition of carcass compost presents special conditions that warrant additional research into the potential risks of spore-forming bacteria, materials handling, and the final disposition of the compost product. Laboratory-scale experiments have indicated that enteric pathogens such as *Salmonella* spp and *Escherichia coli* O157:H7 in bovine fecal matter are inactivated at thermophilic composting temperatures.[36–38] Similarly, windrow composting of spent broiler litter resulted in at least 6 $\log_{10}$ reductions in numbers of total coliforms, fecal coliforms, *E coli*, and fecal *Enterococcus* spp.[39] Another study[40] of composting of municipal solid waste has revealed similar trends.

Survival of Stx-encoded bacteriophage in a compost model was measured to evaluate public health risks of compost use as a soil amendment on land used to grow food crops.[41] Results of that study[41] indicated that Stx-encoding phages are quickly eliminated (undetectable after 3 days) in cow manure compost in which peak temperature was 60°C (140°F). In contrast, another study[42] revealed that Stx-encoding phages in the environment were more environmentally persistent and resistant to chlorination and heat treatment than their host *E coli*.

Regrowth of salmonellae and coliform bacteria in mesophilic conditions[43,44] in products that are incompletely stabilized (ie, those containing labile, easily biodegradable, and nonhumified organic fractions[45]) has been reported. In contrast, results of several studies have indicated that *Salmonella* regrowth is strongly suppressed by competing microflora in compost soil[35,46–48] and sludge.[49] However, when compost was tested after being stored for longer than 2 years, the rate of *Salmonella* inactivation was reduced, compared with that observed in composts after 2 to 33 weeks.[50,51] By 117 weeks, the maximum amount of *Salmonella* growth was quite low (90 to 175 most probable number/g), in contrast to the regrowth counts in compost at 2 to 65 weeks (1,400 to 9,800 most probable number/g). Examination of uncovered storage of municipal solid waste windrow compost[52] and dried sewage sludge compost[53] confirmed recovery of certain enteric bacteria and salmonellae. In the latter study, serotyping of the *Salmonella* organisms in the stored, dried biosolids (detected only in samples collected after many weeks) revealed that they were distinct from those in the original biosolids; thus, the conclusion was that wildlife was the source of the pathogen. By use of DNA gene probes to evaluate survival of *Salmonella* ser Typhimurium and *E coli*, differences in pathogen survival between industrial- and laboratory-scale composting operations have been evaluated.[54] In bench-scale trials involving food wastes, both of those species of bacteria survived for 9 days at processing temperatures of 60° to 70°C. However, during industrial trials, both species survived for 59 days at processing temperatures of approximately 60°C. Both species ultimately became undetectable after temperatures were decreased to approximately 40°C during compost curing. The investigators concluded that temperature and time of exposure were difficult to correlate with destruction of pathogens and that the mechanism for removal of these microorganisms during aerobic composting is "complex and not simply the result of a thermal physical environment."[54] The important thing to note from those studies is that the more stable products with low available carbon content and an actively respiring competitive microbial population will decrease the amount of regrowth and hasten the die-off of the regrowth populations. It is also evident that regrowth populations rarely become equivalent to the peak populations present in the untreated materials because of lower concentrations of available nutrients and increased numbers of competitive bacteria.

In a study[55] of rural human sewage sludge composting in France, a straw envelope was placed around the pile and turning was performed monthly; as a result, efficient elimination of nematode eggs, enteroviruses, fecal indicators (*Enterococcus* spp and *E coli*), and pathogenic bacteria (*Salmonella* spp, *Listeria monocytogenes*, and *Clostridium perfringens*) was achieved. Temperatures in the bottoms of the piles were the lowest (< 50°C [122°F]), compared with temperatures in other areas (< 66°C); however, location within the pile had no effect on microorganism survival.[55] The number of *C perfringens* decreased gradually from an initial density of approximately 8×10^4 colony-forming units/g of dry solids to approximately 7.6×10^2 colony-forming units/g of dry solids (the number of *E coli* similarly changed) after 6 months of composting. Biowaste recycling has been implicated as an animal and public health hazard with regard to pathogenic anaerobic spore formers (eg, *Clostridium botulinum*). In 1 study,[56] samples of marketed biocompost in Germany were tested and results indicated that approximately 50% of the tested samples contained *C botulinum*. Also, findings indicated that household biowaste collection in so-called bio-bins was a risk factor for the production of contaminated compost end-products, but that high composting standards and management could minimize the risk.[56]

Because of its potential for toxic effects in humans and other animals, concerns have been expressed about proliferation of *C botulinum* in anaerobic zones during the initial first few days after a carcass compost pile is constructed prior to onset of thermophilic stages. Important facts about the types of this bacterium, their characteristics, and toxin production provide perspective in evaluation of these concerns.

Clostridium botulinum is found worldwide in soils; sediments; intestinal tracts of birds, fish, and mammals; and decaying wildlife carcasses and the insect larvae associated with them as well as on vegetation that contacts contaminated soil.[57–59] This anaerobic bacterium produces an extremely potent proteinaceous neurotoxin, which is released when its heat-resistant endospores germinate. Of the 7 types of toxins (A through G) produced by *C botulinum*, only types A, B, E, and, rarely, F, affect humans, whereas types C, D, and G are toxic to other animals.[59] Some nontoxigenic, proteolytic strains of *C botulinum* (designated *Clostridium sporogenes*) that are present in soil sediments inhibit germination of *C botulinum* spores and destroy the toxin.[60,61] Also, some strains of *C perfringens*, which are present along with *C botulinum* in soil, produce inhibitors that negatively affect germination of spores of *C botulinum* types A and B.[62]

Both *C botulinum* and *C perfringens* are regarded as human pathogens and important food spoilage organisms associated with dairy, meat, and poultry products as well as fresh and canned fruits and vegetables. Spoilage and illness result when products are inadequately heat treated or temperature abused. The source of contamination is thought to be spores in soil residue. *Clostridium botulinum* has been associated with livestock toxicoses following consumption of inadequately fermented (pH > 4.5), contaminated haylage.[63] In addition, wildfowl flock deaths as a result of ingestion of preformed toxin in stagnant water or insect larvae have been reported.[64,65]

In a carcass-composting environment, an anaerobic zone is expected to develop during the early phase of the process, especially with large ruminant carcasses. Until the interior temperature reaches 45° to 47°C (113° to 116.6°F), the anaerobic zone will support vegetative growth of any *C botulinum* that may be present. However, any toxin released from germination of preexisting spores would be inactivated by the ruminal bacterial flora[65] and the developing thermophilic temperatures. As other decomposer bacteria grow throughout the compost pile, the temperature increases and general microbial competition occurs; consequently, *C botulinum* growth slows and the survival response will stimulate sporulation.

Although some spores may germinate and release toxin, others will only partially germinate because of suboptimal conditions, including suboptimal temperatures, inadequate nutrient supply, and presence of inhibitors. Optimal temperatures for germination and toxin release for *C botulinum* types A, C, and E are 38° to 40°C (100.4° to 104.0°F), 40° to 42°C (104.0° to 107.6°F), and 33° to 35°C (91.4° to 95.0°F), respectively.[63] For any toxin released, it will be either immediately or subsequently exposed to thermophilic temperatures because carcass compost piles are constructed so that the animal tissue lies in the core, which heats maximally. Thus, the toxin is inactivated as the temperature and pH increase. Overall, spore germination is followed directly or subsequently with heat inactivation of both toxin and any remaining vegetative cells, which results in breakage of the propagation-sporulation cycle. Rapid spore germination (ie, within 2 hours) at 60°C has been observed for some strains.[66] Because of the lack of suitable conditions for vegetative growth in a thermophilic composting pile, the outcome for continued propagation at these high temperatures with increasingly aerobic conditions as the composting proceeds is problematic. As a result, spore numbers will decline from the peak value that developed during the initial periods. In a recent report[55] on *C perfringens* in sewage sludge compost, viability of anaerobic spore-forming *Clostridium* spp was significantly reduced in thermophilic composting situations.

Some *C botulinum* spores likely survive in micropockets of the final composting mass because of low temperature and anaerobic zones; when the pile is turned and reheating occurs, these spores are faced with the aforementioned survival stressors. Any remaining spore count will be reduced during further exposure to heat, aeration, and microbial competition. The lack of remaining animal tissue in the final compost product reduces the attractiveness of the material to wildlife for scavenging. This limits the likelihood of ingestion and spread of the organism by this means. The final product can also be reserved for use as cover or base material for subsequent compost piles, thereby keeping the material within the immediate composting area.

Remaining spores would become part of the soil site upon which the compost is spread; these spores would increase the population of *C botulinum* spores that is already present in the soil. Following the type of scenarios and risk analyses for land application of catering waste used by Gale,[67,68] the risk of substantially

increasing the environmental and soil populations of *C botulinum*, compared with the existing background populations, appears small.

In a simulation study,[69] the effects of 3 manure-handling methods (thermophilic composting at 55°C, manure packing at 25°C [77°F], and liquid lagoon storage) on *Mycobacterium avium* subsp *paratuberculosis*, *Salmonella* spp, *E coli*, and *Listeria monocytogenes* were investigated. *Mycobacterium avium* subsp *paratuberculosis* DNA was detectable through day 56 in manure samples treated by each method, but no bacteria were cultured after day 0. After 3 days of composting, none of the other zoonotic bacteria were recovered via bacterial culture. Composting was associated with a higher level of pathogen inactivation, compared with the other 2 methods, and was therefore recommended for treatment of manures destined for pathogen-sensitive environments such as rapidly draining fields and areas used for vegetable production or residential gardening. The inactivation of *M avium* subsp *paratuberculosis* in composted manure was also evident in a study[70] on 2 farms in New York State.

Most available data indicate that composting efficiently eliminates viral agents. Parvovirus and enterovirus were effectively eliminated after 28 days during composting of cow manure for land application.[71] There have been concerns about prion agents remaining in compost. A study[72] of the effect of composting on prions revealed that there may be degradation of prions during composting, providing evidence of another safety advantage of composting.

An MRA could quantify the probability of a harmful effect of composting in humans, other animals, and the environment. An MRA can be qualitative or quantitative and can identify areas for further research. Furthermore, an MRA can provide estimates of the magnitude and likelihood of an adverse event, such as the spread of disease through composting.

A quantitative MRA was performed in the United Kingdom to evaluate the risks to farm animals from pathogens in composted catering waste that contained meat.[68] The investigation included assessment of BSE, foot-and-mouth disease, African swine fever, and classical swine fever by use of a quantitative MRA developed for assessment of BSE in sewage sludge.[39,73] It was concluded that the important factors governing risk were sources of composted animal parts, the efficiency of composting, and the decay and dilution in soil when compost was spread on pasture. The net pathogen destruction was heavily influenced by the degree of bypass, which is the compost that does not reach critical temperature because of its location in the pile. Even if an assumption of zero reduction of BSE in compost was applied, composting and compost spread on pasture were deemed safe when a 2-tier (primary and secondary) composting system was used together with a 2-month grazing ban for the treated pasture. The study[67] concluded that CSF constituted the highest risk, but that by use of a 2-tier composting system and a 2-month grazing ban, the risk could be as low as 1 pig/every 190 years in the United Kingdom.

In a situation in which a method of carcass disposal is evaluated for possible approval, it is necessary to estimate and compare the risks of that method with those associated with alternate methods of carcass disposal that are currently approved, such as rendering and incineration. For example, when evaluating risks of on-farm composting, composting has to be evaluated and compared with the rendering process and also against the transport and handling of carcasses prior to rendering.

The transportation of fallen stock (animals that die or are euthanatized other than at slaughter) from the premises of origin to a site of further processing or disposal may be associated with risks for spread of contagious diseases. In a study[74] to evaluate risk factors for the spread of low pathogenicity H7N2 avian influenza virus among commercial poultry farms in Virginia, it was found that transportation of carcasses for rendering increased the risk of spread of avian influenza, and composting of carcasses was recommended. Recently, a renderer in Australia developed anthrax as a result of handling of a *Bacillus anthracis*–infected carcass.[75] During an outbreak of anthrax in Saskatchewan in 2006, more than two thirds of the bovine carcasses were burnt and the remainder were buried. No carcasses were transported off-site for disposal, and the burial and burning occurred on the same pastureland on which a given animal died.[a] Buried *B anthracis*–contaminated carcasses have been the suspected causative factor in several anthrax outbreaks.[76] This indicates that although carcass composting may not eliminate *B anthracis* spores, alternative methods of carcass disposal also present risks to humans and other animals.

Equipment and Methods for Composting Studies

In many instances, evaluation of composting as a means of carcass disposal has been based simply on recovery of microorganisms (with whatever limits of detection are associated with the recovery method) at various stages during composting. Variable times of sample collection, sampling strategies, and microbiological detection methods make comparisons among studies and evaluation of the safety of composting difficult.

Detection of microbial pathogens has become increasingly efficient, and as such, the criteria for zero risk have become increasingly difficult to meet. Therefore, in assessments of composting systems, a relative risk reduction or a risk limit should be defined. The risk reduction estimates obtained from the risk assessment should be compared with standards such as those recommended by the European Food Safety Agency,[77] and acceptable endpoints for microbial burden reduction need to be determined. The European Food Safety Agency Biohazard Panel recommended that a process can be approved if it meets 3 criteria: 5 $\log_{10}$ reductions in the number of non–spore-forming pathogenic bacteria, parasites, and nonthermoresistant viruses; 3 $\log_{10}$ reductions in infectivity titer of thermoresistant viruses; and 3 $\log_{10}$ reductions in the number of parasites (viable stages).

In microbiological studies[78,79] of compost toilets, in situ measurements and indicator bacteria were evaluated in sentinel chambers. In a study[80] of sanitation of human feces, thermophilic composting and ammonia-based treatment were evaluated and compared with storage treatment. Thermal composting of fecal matter and food waste in a 90-L reactor resulted in a treatment

temperature > 65°C (149°F). By use of insulation and turning the compost 3 times during the high-temperature period, it was possible to ensure 5 $\log_{10}$ reductions in numbers of pathogens. A new method to mathematically evaluate and estimate the safety margins of pathogen inactivation during thermal composting has been developed.[80] A laboratory-scale composting reactor has been constructed for systematic studies of the effects of oxygen concentrations and temperature on carbon and nitrogen turnover in household waste compost; this reactor is equipped for independent control of oxygen concentration and temperature.[81] On the basis of the accumulated data, it appears that composting may be inhibited by an excessively rapid increase in temperature, and an improvement of the composting time for household waste during an initial low-pH phase by mesophilic temperature control has been achieved.[82]

Another study[18] investigated the use of a forced-aerated in-vessel system (55-L volume) to compost food waste, cow manure, and bulking materials (wood shavings and mulch hay). A statistical extreme vertices mixture design method was used to design the composting experiments and analyze the collected data.[18] Maximum temperature values of the mixtures were used as a response for both extreme vertices mixture design and statistical analyses. Chemical changes (moisture content, carbon-to-nitrogen ratio, concentration of volatile solids, and pH) and reductions of indicator (fecal coliforms and fecal streptococci) and pathogenic (*Salmonella* spp and *E coli* O157:H7) microorganisms were measured by use of the most probable–number method before and after a 12-day composting period.

Two methods were evaluated for the sanitary process of full-scale industrial composting: spot testing, in which samples are collected directly from the raw material and then periodically throughout the process for evaluation of the numbers of fecal coliforms, *E coli, Enterococcus* spp, and *Salmonella* spp, and direct process evaluation, in which specific organisms (*E coli* and *Enterococcus faecalis*) were inoculated in the raw material and thereafter monitored throughout the process.[83] The direct process evaluation was shown to be a more valuable tool for identifying factors for process optimization in different zones and detecting pathogens that are not typically present in raw material. However, the process is not reliable for evaluation of the overall sanitary process because it is difficult to represent a heterogeneous environment when inoculating a limited number of decomposition zones. The use of indirect process variables (dry matter content, organic matter content, pH, and carbon-to-nitrogen ratio) were found to be unreliable indicators of the sanitation process.

Airborne bacterial risks associated with animal waste handling have been assessed during land application of sewage sludge by use of glass impingers.[84,b] Although airborne salmonellae, fecal coliforms, or coliphages were not detected, data indicated that there were risks for pathogenic *Clostridia* spp and H_2S-producing organisms in locations undergoing high levels of physical agitation. It appears that *Clostridia* spp and H_2S-producing organisms are better indicators of airborne sewage or sludge-derived material than traditionally employed bacterial indicators such as fecal coliforms or *Streptococcus* spp.

Overview

Carcass composting, when done correctly with proper attention to the design, layout, monitoring, maintenance, and environmental impacts of the system used, may be considered an efficient and safe method of disposing of animal carcasses. Composting achieves adequate levels of microbial pathogen reduction, although spore-forming bacteria and prion agents may not be completely eliminated. Further studies are encouraged to determine the effects of composting on spore-forming bacteria and prion agents in carcasses. Federal and state agencies are encouraged to evaluate carcass composting via a risk assessment approach that involves consideration of all stages of the process (including transportation, treatment, and storage of animal carcasses and compost) and to compare the risks associated with composting with those associated with other methods of carcass disposal.

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Appendix

List of D and z values for selected microbial pathogens and indicators derived during evaluation for thermal resistance with regard to thermophilic composting.

Type of matrix	Pathogen	Mean D value* (min)				Mean z value (°C† [95% CI])
		55°C	60°C	65°C	70°C	
Liquid	*Salmonella* spp[30]	3.7	0.4	0.04	0.001	5.2 (5.1–5.3)
	Salmonella Senftenberg 775W[30]	40.8 (293 at 50°C)	5.7	NR	NR	5.8 (5.4–6.4)
	Campylobacter jejuni[30]	0.83	0.13	0.02	0.0016	6.4 (5.8–7.0)
	Escherichia coli[30]	4.43	0.65	0.09	0.006	6.0 (5.9–6.1)
	Enterococcus faecium[30]	63	19	5.8	1.08	9.6 (8.8–10.5)
	Listeria monocytogenes[30]	10.7	1.45	0.2	0.011	5.7 (5.6–5.9)
	Yersinia enterocolica[30]	2.8	0.5	0.09	0.008	6.7 (6.0–7.7)
	Clostridium perfringens—vegetative cells[31]	16.3	NR	0.9	1.3	7.8
	C perfringens—spores[31]	NR	NR	NR	(2.2 at 100°C) (34.2 at 90°C)	8.4
	Clostridium botulinum—spores[32]	NR	NR	NR	72–100	6.8–7.5
	Bacillus cereus— vegetative cells[31]	(33.2 at 50°C)	1.0	NR	0.2	6.6
	B cereus—spores[31]	NR	NR	NR	(2 at 95°C) (32 at 85°C)	8.5
	Cryptosporidium spp—oocysts[33]	NR	1.0	NR	NR	NR
Compost	*Salmonella* spp[34]	30–60	15–20	NR	NR	NR
	Salmonella Senftenberg 775W[35]	89	7.5	NR	NR	NR
	E coli[34]	60	15–20	NR	NR	NR
	Mycobacterium tuberculosis[34]	NR	NR	15–20	20	NR
	Ascaris lumbricoides—ova[35]	NR	1.7	NR	NR	NR
	Entamoeba histolytica—cysts[35]	44	25	NR	NR	NR
	Bacteriophage f2[35]	267	47	NR	NR	NR
	Poliovirus type 1[35]	32	19	NR	NR	NR
	Adenovirus 12 NIAID[35]	11	0.17	NR	NR	NR

*The D value (min) is the amount of time required to cause a 10-fold (1 $\log_{10}$) reduction in the number of organisms (in various matrices). †The mean *z* value is the temperature (°C) change needed and 95% confidence interval (CI) to change the D value by a factor of 10 (ie, the slope of the thermal death-time semilog$_{10}$ plot).
NR = Not reported.

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Minimally managed composting of beef manure at the pilot scale: Effect of manure pile construction on pile temperature profiles and on the fate of oxytetracycline and chlorotetracycline

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ABSTRACT

Oxytetracycline (OTC) and chlorotetracycline (CTC) are broad-spectrum antibiotics used in livestock production. Although laboratory-scale studies have shown that extractable concentrations of these compounds decrease over time within treated and untreated manures and soils, there is relatively little information from farm-scale experiments. The objective of this study was to determine the effect of different levels of management on manure pile temperature profiles and on the fate of OTC and CTC in manure from therapeutically treated calves. Four treatments were designed to span a range of management options – from simply piling up the manure to amending it with straw to increase aeration and adding insulating layers of straw. Replicate samples of antibiotic-containing calf manure were held at ambient temperature or placed in three locations within replicate 3 m^3 piles of beef manure. During the 28-day incubation period, concentrations of buffer-extractable OTC and CTC/ECTC (the summed concentrations of CTC and its epimer 4-epi-chlorotetracycline (ECTC)) in manure samples incubated at ambient temperature (11–24 °C) decreased 75% (from 18 to 4.6 mg kg^{-1} dry weight (DW)) and 90% (from 192 to 16 mg kg^{-1} DW), respectively. Concentrations of the CTC metabolite iso-chlorotetracycline (ICTC) decreased 90% (from 37 to 3 mg kg^{-1} DW). OTC and CTC/ECTC concentrations in samples incubated for 28 days within a non-amended manure pile decreased 91% and >99%, respectively. During that period, the manure pile temperature ranged from 36 °C to 45 °C. Manure piles insulated with a blanket of straw and/or amended with straw (3:1, v/v) attained temperatures up to 70 °C and contained very low levels of OTC, CTC/ECTC, and ICTC (ranging from <0.1 to 0.4 mg kg^{-1} DW) after 28 days.

1. Introduction

Raw manure can be a significant source of pathogens and antibiotics (Sarmah et al., 2006). When animal producers periodically remove manure from pens, housing units, barns or sheds, the manure is often stockpiled until time and circumstances are available to land apply the material. Depending on soil saturation and freeze conditions as well as availability of work crews, stockpiles may remain for several months, for example in winter before being applied to pasture or cropland in spring. Manure stockpiles typically have a tendency to self-heat when piled in sufficient volume (at least 3 m^3) so as to retain the heat of microbial respiration. However, the steep temperature gradient from the self-heated thermophilic core zones of stockpiles to the ambient exterior areas leaves a substantial portion of the stockpiled mass unaffected by the thermophilic stabilization that occurs in the core. Pathogens may persist in the outer or cool pile areas and antibiotic residues from feeding may also persist in these same zones because of low or minimal microbial decomposition. To the extent that manure stockpiles contain pathogens and antibiotics, then a portion of the land-applied material will contribute to pathogen transfer to water, soil, and crops (Nicholson et al., 2005), and antibiotic residues will contribute to potential for antibiotic persistence and development of antibiotic resistance among native microbial communities (Kelley et al., 1998; Rysz and Alvarez, 2004; Sapkota et al., 2007; Witte, 1998). Therefore, it is important to implement appropriate management practices that minimize the risk of disseminating pathogens and antibiotics from the stockpiled manure.

Previous laboratory-scale composting studies have shown that thermophilic temperatures and aerobic conditions increase antibiotic removal rates (Sarmah et al., 2006). However, there is little comparable information on the fate of antibiotic residues from farm-scale experiments using bovine manure. Although on-farm manure composting is a well-described approach for stabilization

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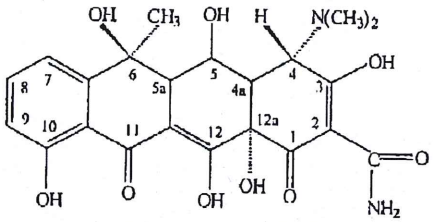
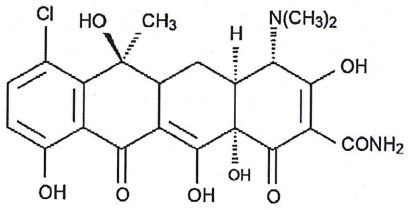
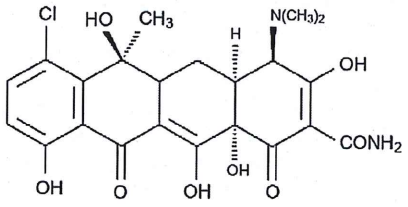
of nutrients and reduction of pathogens and odors (US Composting Council, 2000), few producers have the time, staff or economic incentive to compost their manure according to prescribed protocols for producing products that can be distributed to the public for unrestricted use. However, with a few simple modifications, on-farm manure stockpiles could be manipulated to promote a more extensive zone of destructive temperature, more like that achieved by intensively managed composting practices. By attending to the conformation of the pile and utilizing on-farm resources like old straw, old hay, sawdust, or existing cured compost, an increased zone of aerobic thermophilic decomposition can be achieved. Through a minimal management process configuration it is likely that pathogen and antibiotic concentrations in manure stockpiles can be reduced substantially if not significantly from the starting concentrations. The objective of the study reported here was to evaluate the efficacy of a series of minimal management options for composting manure on-farm to reduce concentrations of the widely used antibiotics oxytetracycline (OTC) and chlorotetracycline (CTC) (Fig. 1). Four treatments were designed to span a range of management options – from simply piling up the manure to amending it with straw (to increase aeration) and adding insulating layers of straw.



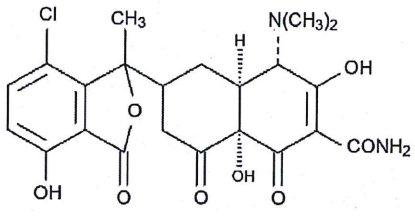
Oxytetracycline (OTC)



Chlortetracycline (CTC)



4-epi-chlortetracycline (ECTC)



Iso-chlortetracycline (ICTC)

Fig. 1. Chemical structures of OTC, CTC, ECTC (an epimer of CTC) and ICTC (a metabolite of CTC).

2. Methods

2.1. Experimental design

2.1.1. Manure collection and treatment design

Beef manure, consisting of feces and urine from beef calves mixed with sawdust bedding was scraped from concrete pens and temporarily stored in a covered area prior to being transported to the USDA's Composting Research Facility at the Beltsville Agricultural Research Center (Beltsville, MD, USA). Water was added to the manure–bedding mixture (hereafter simply termed "manure") and mixed with a front-end loader to achieve approximately 70% moisture content prior to subdividing it into seven separate conically-shaped piles. Each pile had a volume of approximately 3 m^3, dimensions of 2.8 × 1.5 m (diameter × height) and mass of approximately 1500 kg. Four treatments were included in the experiment: treatment 1, one manure pile was placed directly on the concrete floor; treatment 2 (straw base), two replicate manure piles were placed on 6" bases of straw; treatment 3 (straw base, amended with straw), two replicate manure piles were mixed with straw (3:1, v/v) to increase aeration within the piles and the amended piles were placed on 6" bases of straw; treatment 4 (straw base, amended with straw, straw blanket), two replicate manure piles were mixed with straw (3:1, v/v), the amended piles were placed on 6" bases of straw, and covered with a 6" blanket of straw. The piles were constructed in separate concrete bins each with a leachate collection trough embedded in the concrete floor. Bins were located outdoors but under cover in a three-sided pole barn. Table 1 shows characteristics of the manure and straw-amended manure.

2.1.2. Preparation of cassettes containing manure with antibiotics

Collection and characteristics of the OTC and CTC-containing beef calf manures used in this study have been described previously (Arikan et al., 2007; Arikan, 2008). The concentration of buffer-extractable OTC in the fresh manure from OTC-treated calves was 225 ± 15 mg kg^{-1} DW (Arikan et al., 2007). An aliquot of the OTC-containing manure was stored at 4 °C for 25 months until its use in this study. During this storage period, the concentration of extractable OTC in the manure declined to 18 ± 2 mg kg^{-1} DW. The concentrations of extractable CTC/ECTC (the summed concentrations of CTC and its epimer 4-epi-chlorotetracycline (ECTC)) and the CTC metabolite iso-chlorotetracycline (ICTC) in the fresh manure from CTC-treated calves were 208 ± 10 and 33 ± 15 mg kg^{-1} DW, respectively (Arikan, 2008). An aliquot of the CTC-containing manure was stored at −20 °C for 11 months until its use in this study. During this storage period, the concentration of extractable CTC/ECTC in the manure declined (to 192 ± 14 mg kg^{-1} DW), but the concentration ICTC remained constant (37 ± 4 mg kg^{-1} DW).

Table 1
Characteristics of beef manure and straw-amended beef manure[a].

	Manure[b]	Manure–straw mixture (3:1, v/v)[c]
pH	8.7	8.9
EC (mS cm^{-1})	1.75	1.66
Moisture content (%)	69	67
Density (g l^{-1})	550	475
C content (% DW)	12.4	13.9
N content (% DW)	0.54	0.60
P content (% DW)	0.34	0.28
C/N	23	23

[a] Values are from single measurements of composite samples taken on day 0.
[b] Used in treatments 1, 2, and samples incubated at ambient temperature.
[c] Used in treatments 3 and 4.

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Aliquots (30 g wet weight) of the two antibiotic-containing manures were placed in vented plastic cassettes (Tissue Path IV cassettes, Fisher Scientific) prior to being loaded into sections of plastic pipe (hereafter termed BioSentry tubes). BioSentry tubes were constructed of 2" (5 cm) diameter × 3/16" (0.48 cm) (wall thickness) × 10" (25 cm) long polyvinylchloride (PVC) pipe with fiberglass screen covering each end. Each BioSentry tube had forty holes (0.6 cm diameter) equidistantly arranged around the circumference of the tube to allow gas exchange between bulk pile material and the cassette contents. Each BioSentry tube contained a temperature datalogger (WatchDog 100 datalogger, Spectrum Technologies, Plainfield, IL or Tidbit datalogger, Onset Computer Corp., Pocasset, MA). BioSentry tubes were placed approximately 30 cm beneath the surface at three locations in each pile: near the base, in the middle, and near the top (Fig. 2). Two additional BioSentry tubes with manure-containing cassettes and temperature data-loggers were placed on the concrete floor next to the manure piles and were incubated at ambient temperature. On days 7, 14 and 28, tubes were pulled out of the treatment piles and one set of manure-containing cassettes (along with the temperature data-loggers) was removed. After data downloading, the temperature data-loggers were reloaded into the BioSentry tubes and the tubes were reinserted into their respective locations within each manure pile. Manure from the cassettes was removed in the laboratory and immediately processed for analysis of moisture content and antibiotic concentrations.

2.1.3. Analysis of OTC, CTC/ECTC, and ICTC

2.1.3.1. Extraction. Manure subsamples were extracted in duplicate for OTC, CTC/ECTC and ICTC analyses using amethod described previously (Cappone et al., 1996; Arikan et al., 2007). Briefly, 1 g subsamples were extracted three times with 3 ml of 0.1 M Na_2EDTA–McIlvaine buffer by vortexing for 30 s followed by sonication for 3 min in a 100 W sonication bath (Bronson Ultrasonics, Danbury, CT). After each extraction, the extracts were subjected to centrifugation (500g, 5 min, 5 °C), the supernatants were pooled, again subjected to centrifugation (1650g, 20 min, 5 °C), filtered through Whatman glass microfiber (grade GFB) filter paper, and passed through cartridges (Waters Sep-Pak C-18 for OTC and Waters 60 mg HLB Oasis for CTC/ECTC and ICTC) after the cartridges had been prewashed with 5 ml methanol and 10 ml 0.1 M Na_2EDTA–McIlvaine buffer. After the extracts were loaded, the cartridges were flushed with 20 ml distilled water, followed by sample elution using 8 ml of 0.01 M methanolic oxalic acid. The eluents were concentrated under a flow of N_2 to a volume of 0.5 mL. Distilled water (0.5 ml) was added to each tube of concentrated eluent, the tubes were mixed by vortexing (30 s), and the contents were transferred to amber autosampler vials. Demeclocycline (2 μg) was added to each sample as an internal standard prior to analysis by LC–MS/MS.

2.1.3.2. LC–MS/MS analysis. The analyses of OTC, CTC, ECTC and ICTC were performed using LC–MS/MS as previously described (Arikan et al., 2006; Arikan, 2008) using a Waters 2690 XE (Waters Corp., Milford, MA) separations module with an Xterra MS C_{18} column (150 mm × 2.1 mm i.d., 5 μm) (Waters Corp., Milford, MA) at 50 °C for OTC and 45 °C for CTC, ECTC and ICTC. The flow rate was 0.25 ml min^{-1} and the injection volume was 10 μl. A mobile-phase gradient was necessary to separate the compounds. For OTC analysis, the solvent compositions were: (A) methanol–water (5:95, v/v) with formic acid (308 μl/l) added; (B) methanol–water (95:5, v/v) with formic acid (308 μl/l) added. The solvent program was: 0–6 min 89% A, 11% B; 6–11 min, a linear gradient to 50% A, 50% B; 11–21 min, 50% A, 50% B; 21–22 min, linear gradient back to initial conditions (89% A, 11% B). The column was stabilized for 13 min prior to the next analysis. For CTC, ECTC and ICTC analyses, the solvent compositions were: (A) 1% formic acid–methanol (70:30, v/v); (B) water; and (C) methanol. The solvent program was: 0–1 min, 50% A, 50% B, 0% C; 1–12 min, a linear gradient from initial conditions to 70% A, 0% B, 30% C; 12–20 min, 42% A, 0% B, 58% C; 20–22 min, 0% A, 0% B, 100% C; 22–25 min, 0% A, 0% B, 100% C; 25–27 min, a linear gradient back to initial conditions (50% A, 50% B). The column was allowed to stabilize for 10 min prior to the next analysis. Calibration curves were generated using results from 10 μl injections of standards ranging from 0.1 to 10 mg l^{-1}. Atmospheric pressure ionization-tandem mass spectrometry was performed on a benchtop triple quadrupole mass spectrometer (Quattro LC from Micromass Ltd., Manchester, UK) operated in positive electrospray ionization mode. Analyte concentrations were calculated by the internal standard method using demeclocycline as an internal standard (Zhu et al., 2001). Peak integration and quantitation were performed automatically using MassLynx 3.5 software (Waters Corp., Milford, MA). According to European regulations, CTC levels in foodstuffs of animal origins are calculated as the summed levels of the antibiotically-active CTC and ECTC. Therefore, results from CTC and ECTC analyses were summed and are reported as CTC/ECTC values.

2.1.3.3. Recoveries. To determine extraction efficiencies, duplicate samples of unmedicated manure were spiked with stock solutions to obtain samples with 1 and 10 mg kg^{-1} (wet weight) of OTC, CTC, ECTC and ICTC. Samples were thoroughly mixed and then extracted as described above prior to LC–MS/MS analysis. Average recoveries of 1 mg kg^{-1} spikes were slightly higher than recoveries of 10 mg kg^{-1} spikes (Table 2). The moisture content of the manure samples was 69%. Thus, the concentrations in the spiked 1 and 10 mg kg^{-1} (wet weight) samples expressed on a dry weight basis

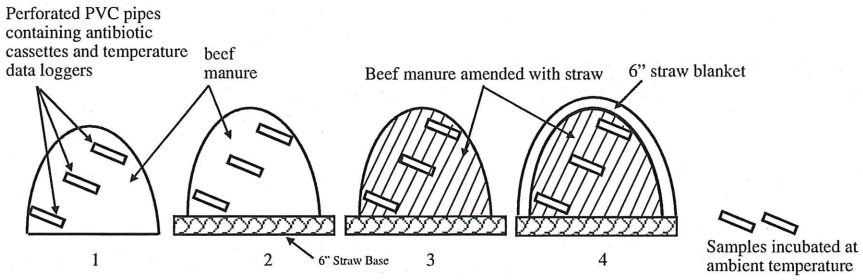

Fig. 2. Schematic drawing of manure treatments and approximate locations of manure-containing cassettes within the manure piles. Treatment 1, manure pile without further treatment; treatment 2, manure pile placed on straw base; treatment 3, manure amended with straw (3:1, v/v) and placed on a straw base; treatment 4, manure amended with straw (3:1, v/v), placed on a straw base, and covered with a layer of straw. In addition, duplicate BioSentry tubes with manure-containing cassettes and temperature data-loggers were incubated at ambient temperature. Each manure pile was approximately 3 m^3 in volume and contained approximately 1500 kg of manure.

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Table 2
Recovery of OTC, CTC, ECTC and ICTC in beef manure.

Compound	% mean recovery (95% confidence limits)[a] Spike level (mg kg^{-1} wet weight)[b]	
	1	10
OTC	71 (63–79)	70 (61–79)
CTC	79 (75–83)	73 (69–77)
ECTC	72 (64–80)	61 (52–69)
ICTC	66 (60–72)	59 (54–64)

[a] Values are means from duplicate samples.
[b] The corresponding concentrations in the spiked samples expressed on a dry weight basis are 3.2 and 32 mg kg^{-1} DW.

were 3.2 and 32 mg kg^{-1} DW, respectively. Average recoveries of OTC, CTC, ECTC and ICTC were 71%, 76%, 66% and 63%, respectively.

3. Results

Manure pile temperatures are influenced by factors affecting bacterial metabolism (manure nutrient content, moisture content and bulk density) and by factors affecting heat loss from the pile (size and conformation of pile, presence or absence of insulating layers). The treatments were designed to span a range of management options – from simply piling up the manure to amending it with straw and adding insulating layers of straw. The first level of treatment was to place a non-amended manure pile on a concrete floor within a three-sided pole barn (Fig. 2). Increasing levels of treatment included: placing manure pile on a 6" layer of straw to reduce heat loss from the base of the pile (treatment 2), amending the manure with straw to increase aeration within the pile (treatment 3), and covering the manure piles with a 6" layer of straw to reduce heat loss from the top of the pile (treatment 4). Treatment 1 was not replicated; treatments 2–4 were conducted in duplicate. We predicted that piles of straw-amended beef manure would attain higher temperatures than piles of non-amended manure because improved aeration would lead to improved bacterial metabolism. We also predicted that bottom locations within the manure piles would be significantly cooler than middle or top locations because of heat loss to the cool floor. Adding straw layers below and on top of the manure pile would decrease heat loss and would thereby raise and unify temperatures within the piles. With

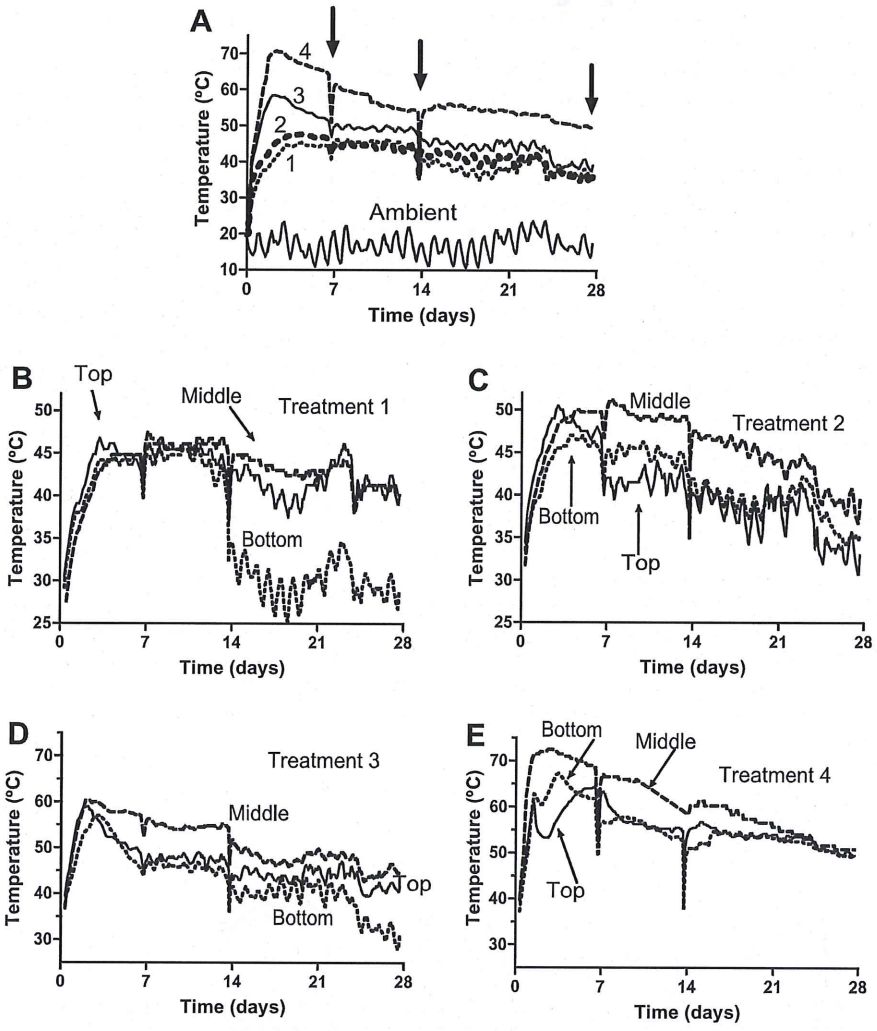

Fig. 3. Mean whole-pile and mean within-pile temperature profiles from manure pile treatments during the 28 day experiment. Panel A, mean whole-pile temperature profiles for each treatment. Values are the means of results from three locations within a single manure pile (treatment 1) or within duplicate manure piles (treatments 2–4). Ambient temperature values are from dataloggers placed within BioSentry tubes located on the floor of the three pole barn. Panels B–E, within-pile temperature profiles for each treatment. Values are from single readings from three locations (top, middle and bottom) within one manure pile (treatment 1) or are the means of readings from three locations in duplicate manure piles (treatments 2–4). Temperature data-loggers were temporarily removed from manure piles on days 7, 14 and 28 (shown by arrows in panel A) in order to download temperature results.

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regard to concentrations of antibiotics within the manure, we predicted that increased temperatures within the manure piles would lead to increased adsorption and lower concentrations of extractable antibiotic residues.

3.1. Effect of manure treatments on pile temperature profiles

Mean manure pile temperatures (averaged values from top, middle and bottom locations from replicate treatments) and within-pile temperature profiles were significantly influenced by treatment. During the incubation period (April 13–May 10, 2006), ambient temperature in the covered building fluctuated from 11–24 °C (with a mean of 17 °C) (Fig. 3, panel A). Mean temperatures within the manure piles rose quickly above ambient temperatures, and reached their maximum values within 2–3 days of incubation (Fig. 3, panel A). The mean temperature of treatment 1 (manure pile placed directly on the concrete floor) increased to a maximum of 45 °C by day 3. The mean temperature of treatment 2 (manure pile placed on a 6" layer of straw) followed a similar profile and increased to a slightly higher maximum of 47 °C by day 3. The mean temperatures of treatments 3 and 4 (manure amended with straw, placed on straw layer, without and with an overlying straw blanket, respectively) increased to maximums of 58 °C and 70 °C, respectively, within two days. In all treatments, mean pile temperatures gradually declined after reaching their maximum values. At the end of the 28 day incubation period, mean pile temperatures of treatments 1–3 were nearly identical (36–38 °C) and were much lower than the mean temperature of treatment 4 (50 °C).

The within-pile temperature profile for treatment 1 showed very similar temperatures from the three within-pile locations until day 14 when the bottom data-logger was removed for data retrieval. After data retrieval, the BioSentry tube was apparently relocated to a much cooler position within the bottom of the pile (presumably closer to the cool floor) (Fig. 3, panel B). In general, the within-pile temperature profiles for treatments 2–4 (Fig. 3, panels C–E) showed bottom and top location temperatures to be very similar and approximately 5 °C lower than middle pile temperatures.

3.2. Effect of pile construction on concentrations of extractable OTC, CTC/ECTC, and ICTC

During the 28 day incubation period concentrations of OTC, CTC/ECTC, and ICTC decreased 75–91% within samples incubated at ambient temperatures and decreased >99% within the four manure treatments (Fig. 4, Table 3). The rates of residue removal for the first 14 days of incubation were similar for treatments 1 and 2 and were lower than the initial removal rates shown in treatments 3 and 4 (Fig. 4). Concentrations of the metabolite ICTC transiently increased in some samples after 7 days of incubation.

Within the samples incubated for 28 days at ambient temperature, extractable concentrations of OTC decreased 75% from 18 to 5 mg kg^{-1} DW, CTC/ECTC concentrations decreased 91% from 192 to 16 mg kg^{-1} DW, and ICTC concentrations decreased 91% from 37 to 3 mg kg^{-1} DW (Fig. 4, Table 3). Within the samples from the non-amended manure piles (treatments 1 and 2), extractable concentrations of OTC, CTC/ECTC, and ICTC (0.3–1.6, 1.2–1.4, and <0.1 mg kg^{-1} DW, respectively) were 3–10 fold lower at the end of the experiment than the samples held at ambient temperature. In comparison, within the samples from the straw-amended manure piles with the highest temperatures (treatments 3 and 4), extractable concentrations of OTC, CTC/ECTC, and ICTC were even lower (0.3–0.4, 0.1–0.3, and <0.1 mg kg^{-1} DW, respectively) at the end of the experiment (Table 3). Although there were within-pile variations in concentrations of extractable residues, we observed no consistent trend with regard to residue concentrations as a function of location within the manure piles (not shown).

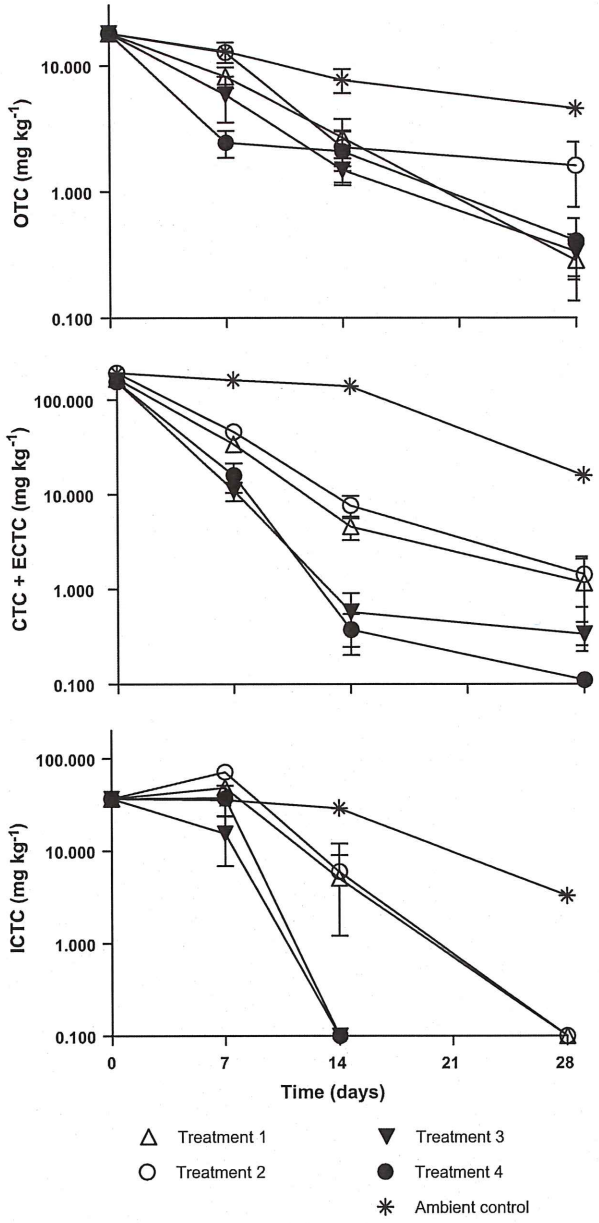

Fig. 4. Extractable concentrations of OTC, CTC/ECTC, and ICTC as a function of treatment during the 28 day experiment.

4. Discussion

Temperature profiles for the manure treatments in this study are comparable to results reported in previous studies. Sommer and coworkers measured the temperatures of a cone-shaped pile (3.1 m diameter, 1.1 m height) of manure from a beef cow feedlot as part of a gas emissions study (Sommer et al., 2004). At 10 cm depth, the manure pile temperature reached 11–49 °C after 2 days and ranged from 50–60 °C from 3–6 days. Chadwick and coworkers measured core pile temperatures in 7–13 m^3 piles of beef manure. Temperatures reached 65 °C within several days, then gradually declined to 55 °C on day 20 and 45 °C on day 40 (Chadwick, 2005). Temperature profiles of large-scale windrows of beef man-

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Table 3
Concentrations (mean ± SE) (in mg kg^{-1} DW) of OTC, CTC/ECTC, and ICTC during the incubation period.

Treatment	1	2	3	4	Ambient temperature
Day					
OTC					
0	18.0 ± 1.9				
7	12.7 ± 0.3	8.2 ± 1.6	5.9 ± 2.3	2.5 ± 0.6	12.9 ± 2.4
14	2.3 ± 0.8	2.7 ± 1.1	1.5 ± 0.4	2.1 ± 0.9	7.7 ± 1.7
28	1.6 ± 0.8	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	4.6 ± 0.3
$T_{½}$ (days)	7.5	4.7	4.7	5.6	14.0
CTC/ECTC					
0	192 ± 14				
7	46.0 ± 2.7	33.8 ± 4.9	10.9 ± 2.4	15.9 ± 5.4	160 ± 7
14	7.6 ± 2.0	4.5 ± 1.2	0.6 ± 0.3	0.4 ± 0.2	139 ± 3
28	7.6 ± 2.0	4.5 ± 1.2	0.6 ± 0.3	0.4 ± 0.2	139 ± 3
$T_{½}$ (days)	4.0	3.8	3.0	2.6	7.8
ICTC					
0	36.8 ± 4.0				
7	71.5 ± 6.8	48.6 ± 6.7	15.4 ± 8.5	37.2 ± 13.4	35.5 ± 1.7
14	6.0 ± 6.0	5.1 ± 3.9	DL[a]	DL	29 ± 0.5
28	DL	DL	DL	DL	3.3 ± 0.4
$T_{½}$ (days)	ND[b]	ND	ND	ND	ND

[a] DL, value below detection limit (0.1 mg kg^{-1} DW).
[b] ND, not determined.

ure show similar peak temperatures but longer time intervals of self-heating and cooling (Hao et al., 2001; Larney et al., 2003; Michel et al., 2004; Storteboom et al., 2007).

The fate of tetracyclines in soil, sediments, and in manure treated by composting or anaerobic digestion at a laboratory-scale has been the subject of numerous studies (reviewed in; Sarmah et al., 2006; Arikan et al., 2007). In general, tetracyclines adsorb strongly to organic matter and, consequently, extractable concentrations of tetracyclines and their metabolites decline over time in organic matrices. In this study, the half-life values of OTC and CTC/ECTC in beef manure incubated at ambient temperature (11–24 °C) were approximately 14 and 8 days, respectively. These ambient temperature half-life value are lower than the 30-day value reported from a study in which manure from OTC-treated calves was stored outdoors in a 3–4 ton pile (De Liguoro et al., 2003) and are much lower than the >40 day values from laboratory scale incubation studies at 25 °C using sterile and non-sterile manure that had been treated with OTC or CTC (Arikan et al., 2007; Arikan et al., in press). At present, we have no explanation for our lower values.

We predicted that increased temperatures within the manure piles would lead to increased adsorption and lower concentrations of extractable antibiotic residues. Our results showed that there was a rapid reduction of extractable concentrations of OTC and CTC/ECTC within the first seven days of all manure treatments. The half-life values of OTC ranged from 5 to 8 days between the different treatments and decreased with increasing mean whole-pile temperatures. These values compare very well with results from a previous laboratory-scale composting experiment using the same OTC-containing calf manure (Arikan, 2008). In that experiment, the temperature of a composted manure–straw–woodchip mixture ranged from 65–70 °C within the first 6 days of treatment and the calculated half-life for OTC was approximately 3 days. In the present experiment, treatment 4 (manure amended with straw and insulated with straw blanket) showed a similar temperature profile over the first 7 days and yielded a calculated half-life for OTC of 4 days. Our values are lower than those recently reported in a pilot-scale treatment study characterizing the fate of CTC, OTC, tylosin and monensin in spiked horse manure, and OTC and CTC in dairy and beef feedlot manure (Storteboom et al., 2007). In that study, the half-life value for OTC was 10–18 days in composted dairy manure and was 15–31 days in composted feedlot manure. It is likely that the different results are due to the differences in the compositions and moisture contents of the different manures used in the these studies.

The half-life values of CTC/ECTC varied very little (ranging from 2–4 days) between the different treatments in our study but decreased with increasing mean whole-pile temperatures. These half-life values are comparable to those reported by Pruden and coworkers (Storteboom et al., 2007) in the pilot-scale manure treatment study described above. In that study, the half-life value for CTC was 5–8 days in composted horse manure, 6–7 days in composted dairy manure and 13 days in composted feedlot manure. The values presented here also compare very well with more recent results from a laboratory-scale composting experiment using CTC-containing calf manure (Arikan et al., in press). In that study, CTC-containing manure from treated calves was collected and an aliquot was sterilized by irradiation. Results showed that concentrations of extractable CTC/ECTC decreased slowly in sterile and non-sterile manure samples during incubation at 25 °C (half-life values of 40–47 days) and decreased rapidly during incubation at 55 °C (half-life values of 4–5 days). Since there were no significant differences in the rate of antibiotic disappearance from sterile and non-sterile manure samples, the decreased concentrations appear to be entirely due to temperature dependent abiotic adsorption (Arikan et al., in press). Although we did not include comparable sterile samples in the current study, it is likely that the decrease in extractable residues shown here is also due to absorption rather than biodegradation.

We do not have any information on the long-term environmental fate of absorbed OTC or CTC residues in composted manure. However, results from two previous composting studies using OTC- and CTC-containing manures suggest that non-extractable antibiotic residues are not bioavailable to the manures' microbial flora (Arikan et al., 2007; Arikan et al., in press). In those studies, total heterotrophic and OTC- or CTC-resistant bacteria in the respective manures were enumerated before and after composting. Results from both studies showed greatly increased numbers of total heterotrophic organisms after composting and decreased numbers of OTC- or CTC-resistant organisms after composting of the respective antibiotic-containing manures. Although these results suggest that non-extractable residues are not bioavailable in the short-term, we have no comparable information on extractability or bioavailabilty of these residues after the composted mixtures are applied to soil. Nonetheless, in the absence of more definitive information, we believe that absorbed antibiotic residues are much less likely to promote the selection or spread of antibiotic resistant bacteria compared to extractable antibiotic residues in untreated manures.

Our results demonstrate that extractable concentrations of OTC and CTC/ECTC decrease >90% and >99%, respectively, in stockpiled manure that self heats to 40–45 °C for 28 days. Although manure piles amended with straw attained higher temperatures and more rapid decreases in antibiotic concentrations, there is, at present, no compelling justification for producers to expend additional resources needed to achieve the more rapid rates of antibiotic removal. However, the ability of manure piles to self-heat is clearly dependent on manure moisture content as well as its composition. In the present experiment, as in most composting studies, water was added to stockpiled manure to achieve a moisture content of roughly 50–80%. Since stockpiled manure can dry very quickly and is not typically monitored for moisture content, additional experiments are needed to establish the range of moisture content needed to consistently achieve this relatively low level of self-heating.

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Decrease in Water-Soluble 17β-Estradiol and Testosterone in Composted Poultry Manure with Time

Heldur Hakk,* Patricia Millner, and Gerald Larsen

ABSTRACT

Little attention has been paid to the environmental fate of the hormones 17β-estradiol and testosterone excreted in animal waste. Land application of manure has a considerable potential to affect the environment with these endocrine disrupting compounds (EDCs). Composting is known to decompose organic matter to a stable, humus-like material. The goal of the present study was to quantitatively assess levels of water-soluble 17β-estradiol and testosterone in composting chicken manure with time. Chicken layer manure was mixed with hay, straw, decomposed leaves, and starter compost, adjusted to approximately 60% moisture, and placed into a windrow. A clay-amended windrow was also prepared. Windrows were turned weekly, and temperature, oxygen, and CO_2 in the composting mass were monitored for either 133 or 139 d. Commercial enzyme immunoassay kits were used to quantitate the levels of 17β-estradiol and testosterone in aqueous sample extracts. Water-soluble quantities of both hormones diminished during composting. The decrease in 17β-estradiol followed first-order kinetics, with a rate constant $k = -0.010$/d. Testosterone levels declined at a slightly higher rate than 17β-estradiol (i.e., $k = -0.015$/d). Both hormones could still be measured in aqueous extracts of compost sampled at the conclusion of composting. The decline in water-soluble 17β-estradiol and testosterone in extracts of clay-amended compost was not statistically different from normal compost. These data suggest that composting may be an environmentally friendly technology suitable for reducing, but not eliminating, the concentrations of these endocrine disrupting hormones at concentrated animal operation facilities.

Current socioeconomic forces have increased the animal numbers per farm operation, but have limited the distance manure or litter can be economically transported. Hence, considerable amounts of manure generated at concentrated animal feeding operations (CAFOs) are stockpiled, lagooned, or composted before being applied to adjoining farmland. Transport of the manure or contaminants in the manure into surface waters can readily occur after heavy rains. Exposure of livestock or wildlife to these compounds during key stages of development may play a role in subsequent developmental and/or reproductive problems.

17β-Estradiol and testosterone are classified as EDCs when found in the environment. Animal manures are a potentially significant source of sex hormones in the environment because they are directly applied to land in relatively high amounts (Callantine et al., 1961; Knight, 1980). Egg-laying chickens excrete high levels of 17β-estradiol and testosterone, 50 and 250 ng/g dry manure/d, respectively (Shemesh and Shore, 1994; Shore et al., 1988, 1995a). In addition, the endogenous concentration of 17β-estradiol in cattle urine averages 13 ng/L (Erb et al., 1977). However, the excreted concentrations can be even higher, because 17β-estradiol, in the benzoate or palmitate ester forms, is frequently administered as a growth hormone to increase muscle mass and decrease fat deposition (Popp et al., 1997).

Several studies have demonstrated that sex hormones appear in soil, surface water, and ground water as a result of manure application (Nichols et al., 1997, 1998; Finlay-Moore et al., 2000; Peterson et al., 2000). Exposure to EDCs in the environment has been associated with widespread physiological and reproductive disorders in a variety of wildlife (Colburn et al., 1993) and in humans (i.e., increased breast cancer; David and Bradlow, 1995). The U.S. Department of Health and Human Services (National Institute of Environmental Health Sciences, 1994) has classified 17β-estradiol as a carcinogen, based on its link to breast cancer (Dickson et al., 1986). Brown trout gonad development and feeding were halted at 50 to 300 μg/L 17β-estradiol (Ashby, 1957). 17β-Estradiol levels of 2000 ng/L in water resulted in 84 to 100% feminization of salmon (Nakamura, 1984), while 250 to 5000 ng/L resulted in fish deaths (Nakamura, 1984; Kramer et al., 1998). 17β-Estradiol in poultry litter fed to heifers caused premature udder development (Shore et al., 1988). 17β-Estradiol has also been shown to have an effect on plant growth. At concentrations of 5.4 to 544 ng/L, 17β-estradiol increased alfalfa growth, while at concentrations of 54.4 to 544 μg/L, decreased alfalfa growth was observed (Shore et al., 1992). Testosterone is excreted into the environment from the same sources and in comparable amounts as 17β-estradiol (Shore et al., 1995b). To the best of our knowledge, there are no published papers on the adverse effects of testosterone in the environment, despite measured levels of up to 200 ng/L (Kolpin et al., 2002).

Little is known about the transport and fate (metabolism and degradation) of these hormones in field settings or in hydrological systems. Studies by individuals involved in this study have shown that 17β-estradiol and testosterone are strongly sorbed to soils (Casey et al., 2003a, 2003b). The lack of data on the transport and fate of these hormones is interesting given the fact that they are vastly more potent in interacting with estrogen and androgen receptors than most anthropogenic EDCs. In vitro studies have reported that 17β-estradiol has a 10^4

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Abbreviations: EDC, endocrine disrupting compound; EIA, enzyme immunoassay.

to 10^6–fold greater affinity for the estrogen receptor than alkylphenol ethoxylates (Jobling and Sumpter, 1993).

Composting is a self-heating, aerobic process that accelerates the degradation of organic materials by the successive action of a diverse group of microorganisms, including mesophiles, thermophiles, bacteria, actinomycetes, and fungi (Kaiser, 1996; Insam et al., 1996). Composting is a beneficial residuals management option that stabilizes organic by-products, reduces their weight, destroys pathogens and weed seeds, and produces a low-odor soil conditioner that also has some fertilizer value (Rynk, 1992; Willson et al., 1983). Active composting generates considerable heat (>60°C) during the 3- to 4-wk peak-heating phase of the process, during and after which the organic matter may ultimately be converted into gaseous CO_2 and water vapor. It can also provide an economic, effective, and practical solution for managing hazardous waste or remediating contaminated soil (Eitzer et al., 1997). For instance, >99% degradation was achieved when 2,4,6-trinitrotoluene-contaminated soil was composted for 160 d (Williams and Keehan, 1993). Depending on the level of degradation achieved, the costs for disposal of remediated soil resulting from composting are far lower than from incineration or landfilling (Goldstein, 1985).

The purpose of the present study was to evaluate the water extractability of the potent endogenous hormones, 17β-estradiol and testosterone, during the course of aerobic composting. Results will be useful in evaluating the efficacy of composting in decreasing the environmental load of these hormones originating from farms, particularly concentrated animal feeding operations.

MATERIALS AND METHODS

Composting

Chicken layer manure was obtained from a commercial layer operation in Pennsylvania and transported to the Composting Research Facility at the Beltsville Agricultural Research Center (Beltsville, MD) where composting was performed. The composting facility handles about 14 500 m^3 per year from residuals generated at the 3000-ha farm and research center. The operational surface of the composting facility consisted of an outdoor 7200-m^2 pad, which was a 21-cm-thick lime-stabilized soil pad that included coal combustion ash, cement kiln dust, quicklime, and existing Christiana clay (fine, kaolinitic, mesic Typic Paleudults) subsoil. An 8-ha orchard grass (*Dactylis glomerata* L.) runoff buffer surrounds the composting pad. The site is located in the Anacostia River watershed, which is part of the Chesapeake Bay system. Manure was analyzed for moisture and carbon (C) and nitrogen (N), from which a C to N ratio was calculated. Water was added to produce moisture content in the range of 50 to 60 g/kg. The initial C to N ratio was adjusted to 30 by addition of hay, straw, leaves, and starter compost. The standard compost composition was old hay (2 parts by weight), 3-wk-old peak heat starter compost made with no poultry, dairy, or swine manure (2 parts), layer manure (3.3 parts), autumn leaves (4 parts), and straw (2 parts). In a separate compost pile, an additional part of crushed Christiana clay was added. Each windrow was approximately 53 m long, 1.7 m wide, and 0.16 m high. After the first week of composting the windrows were turned once a week to aerate and facilitate mixing and exposure of all parts of the mass to the high-temperature core regions of the windrows. Windrows were routinely monitored at three sites for temperature, oxygen, and carbon dioxide using a Compost Pro datalogger (Morgan Scientific, Haverhill, MA) and the data were averaged.

Sampling

Three composite samples were collected on Days 0, 6, and 13 and two were collected on Days 27, 55, 69, 83, 111, and 139 and placed into plastic bags. A composite sample consisted of three subsamples equidistant from a selected site within the windrow, and which were mixed to assure nonpreferential sampling. Samples were frozen at −20°C and shipped overnight in coolers with frozen blue-ice gel inserts to maintain samples until delivery to the Biosciences Research Laboratory (Fargo, ND). They were immediately frozen at −30°C and stored until analyzed. In general, analyses were performed within two weeks of receipt. One replicate windrow each of normal and clay-amended compost was constructed 6 d after the initiation of the first two windrows. Three composite samples were removed from the replicate windrows at Days 0 and 7, and two composites were sampled at Days 21, 49, 63, 77, 105, and 133. The data from the replicate windrows were combined for the analyses.

Sample Preparation

Frozen compost samples were removed from storage, placed into a blender (Waring, Torrington, CT) containing dry ice, and blended until homogeneity was achieved (5 min). Blended samples were placed into a plastic bag and dry ice was allowed to evaporate in a −20°C freezer. An aliquot (1.0–1.2 g wet wt.) was removed, weighed, and dried in a 50°C oven until a constant weight was obtained. This dry weight was used for all calculations of hormone concentration presented in the tables or figures. Two or three determinations of hormone activity were made on composite samples obtained from the first three time points, while one determination was made on samples from later time points.

Hormone Assays

A standard procedure for extracting field samples was adopted. An approximately 200-mg aliquot of the homogenized sample was placed into a 125-mL Erlenmeyer flask with 50 mL of double distilled water. The mixture was shaken horizontally at 25°C for 2 h in a reciprocating water bath. The sample was centrifuged at 1124 × *g* and the aqueous layer was decanted and used for all assays.

Estradiol and testosterone enzyme immunoassay (EIA) kits were purchased from Cayman Chemical (Ann Arbor, MI) and used according to the manufacturer's instructions. The EIA analyses were run in triplicate or quadruplicate with blanks and standards, and both nonspecific and maximum binding were determined. The 17β-estradiol EIA kit cross-reactivities were 17% for 17β-estradiol-3-glucuronide, 4% for estrone, 0.57% for estriol, 0.1% for testosterone, and 5α-dihydrotestosterone, and less than 0.1% for all other steroids. Cross reactivities for the testosterone kit were 21% for 5α-dihydro testosterone, 12.4% for 11-keto testosterone, 10% for 5β-dihydro testosterone, 3.6% for androstenedione, 1.2% for 11-hydroxy testosterone, and less than 1% for all other steroids. Quantitation was performed using a Victor Model 1420 multilabel counter (Wallac, Turku, Finland) by measuring the amount of 5-thio-2-nitrobenzoic acid released from added substrate in an enzymatic reaction at 405 nm. Blank EIA analyses for straw, hay,

leaves, starter compost, and Christiana clay were performed in the same manner.

Recovery Analyses

Recovery data on the extractability of the hormones from the compost matrix were performed in two ways: (i) extraction with water (50 mL × 3) to simulate three successive rainfall events, or (ii) successive 50 mL extractions with water, methanol, and acetone to determine the total recovery. In both cases, three and five aliquots, respectively, of approximately 0.5 g of dried compost were weighed and spiked with [^{14}C] hormones (0.33 μg; 0.66 ppm; [^{14}C]17β-estradiol or [^{14}C]testosterone, 1.7×10^9 Bq/mmol and 1.9×10^9 Bq/mmol, respectively, >98% radiochemical purity; American Radiolabeled Chemicals, St. Louis, MO) in ethanol and allowed to air-dry. The compost was then extracted in each solvent for 2 h at 25°C on a shaker bath, centrifuged, decanted, and assayed immediately for radioactivity. Each extract was spotted onto silica TLC plates and developed with 1:1:2 ethyl acetate to tetrahydrofuran to hexane with standards to quantitate the amount of parent material and any possible metabolites formed.

Statistical Analyses

The combined data from replicate windrows were log-transformed and fitted to a simple linear regression model. A mixed model analysis (PROC MIXED; SAS Institute, 2004) was used to test whether a difference existed between normal and clay-amended compost.

RESULTS

The average temperature of the interior of the normal composting windrow indicated that thermophilic conditions (>40°C) were achieved by Day 3, and remained there until at least Day 41 (Fig. 1A). Mesophilic temperatures (10–40°C) characterized the interior of the normal composting windrow between Days 45 and 139 (temperatures beyond Day 116 not shown in Fig. 1A). Similar results were observed in the clay-amended composting windrow, except that thermophilic temperatures were still measured at Day 45 (Fig. 1B). Replicate windrows displayed similar temperature profiles as their paired windrow.

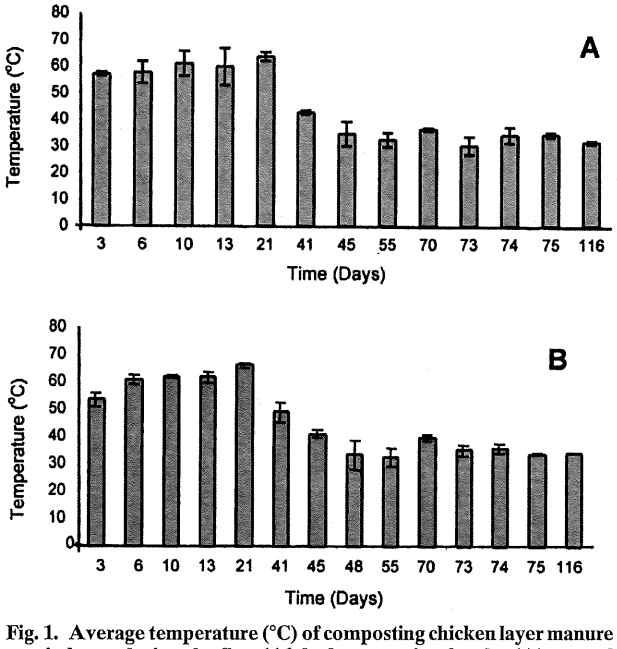

Fig. 1. Average temperature (°C) of composting chicken layer manure windrows during the first 116 d of composting for the (A) normal and (B) clay-amended windrows. Means represent three measurements taken from two windrow core regions and one windrow end region.

The percent concentration of oxygen in the interior of the normal composting windrow was measured and indicated that microbial oxygen consumption was high from Day 0 through Day 10. On Day 0, the percent oxygen was 1.6 g/kg, and recovered steadily to approximately 20 g/kg at Day 41 (Fig. 2), a level that was maintained through Day 139 (percent gas concentrations beyond Day 116 not shown in Fig. 2). The high initial consumption of oxygen was accompanied by a high evolution of carbon dioxide. The observed percent concentration decline of CO_2 over 10 d complemented the increase in the percent concentration of oxygen. The initial concentration of CO_2 was 24 g/kg, but declined to <5 g/kg by Day 21, a concentration that was maintained throughout the remainder of the study (Fig. 2). The gas concentration data were the same for the clay-amended windrow and each of the replicate windrows.

17β-Estradiol and testosterone displayed incomplete recovery from spiked compost following a 2-h water extraction. Mean aqueous recovery of [^{14}C]-spiked 17β-estradiol from compost was 51.9%, and cumulative recovery increased to 67.6 and 75.5% after a second and third 2-h extraction period (Table 1). The recovery of [^{14}C]-spiked testosterone under the same conditions was 61.8, 76.7, and 82.9%, respectively. Quantitative recovery of the spiked dose would have yielded an aqueous hormone concentration of 0.0026 and 0.0027 mg/L for 17β-estradiol and testosterone, respectively. The aqueous solubility of 17β-estradiol and testosterone is 3.6 and 23.4 mg/L (Mansell et al., 2003), respectively. Therefore, the recovery studies were conducted well below the aqueous solubility limits of the two hormones. Recovery of the spiked radiolabeled hormones from compost was also investigated after lengthening the aqueous extraction time to 24 h and decreasing the hormone concentrations; spikes would have yielded maximal aqueous concentrations of 0.00010 mg/L for 17β-estradiol and 0.000068 mg/L for testosterone. Neither variable affected the recovery of spiked hormone from the results presented above: 24-h

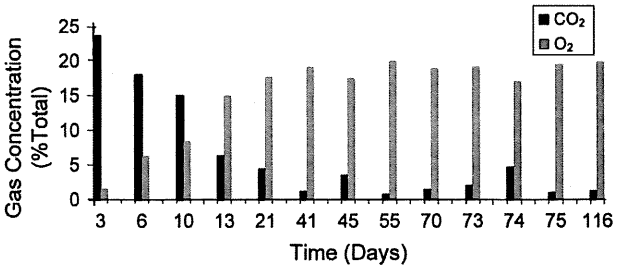

Fig. 2. Average CO_2 and O_2 concentrations (as a percent of total gaseous concentration) from a normal composting chicken layer manure windrow. Means represent three measurements each taken from two windrow core regions and one windrow end region.

Table 1. Percent recovery of spiked [^{14}C]17β-estradiol or [^{14}C]testosterone (0.33 μg) into an aliquot of Day 0 layer manure compost (414–558 mg). Recoveries were measured following successive water extractions (50 mL × 3; n = 3) or successive water, methanol, and acetone extractions (50 mL × 5; n = 5).†

Extraction solvent	[^{14}C]17β-estradiol-spiked compost	[^{14}C]testosterone-spiked compost
	%	
Water #1	51.9 ± 1.9‡	61.8 ± 2.0‡
Water #2	15.7 ± 0.7	14.9 ± 0.3
Water #3	7.9 ± 0.6	6.2 ± 0.8
Cumulative	75.5 ± 0.9	82.9 ± 2.2
Water	43.4 ± 3.9	54.5 ± 1.7
Methanol	54.6 ± 3.1	55.6 ± 2.8
Acetone	1.5 ± 0.4	1.0 ± 0.7
Cumulative	99.5 ± 6.5	111.1 ± 2.5

† Values are means ± SD.
‡ Replicate, 24-h aqueous extraction recoveries (n = 3): 17β-estradiol (48.2 ± 4.8%) and testosterone (59.6 ± 4.2%). Replicate, low hormone concentration aqueous extraction recoveries (n = 3): 17β-estradiol (49.1 ± 4.9%) and testosterone (59.8 ± 11.1%).

extraction recoveries were 49.1 and 59.8% and low concentration recoveries were 48.2 and 59.6% for 17β-estradiol and testosterone, respectively (Table 1).

Recovery of [^{14}C]17β-estradiol and [^{14}C]testosterone from spiked compost following successive 2-h extractions with water, methanol, and acetone yielded incomplete recovery with water for 17β-estradiol and testosterone, 43.4% and 54.5%, respectively (Table 1). Subsequent extraction of each aliquot with methanol then acetone resulted in an additional 54.6 and 55.6% and 1.5 and 1.0% recovery of the two hormones, respectively.

At the initiation of the field composting study (Day 0), after the layer manure had been diluted with hay, starter compost, leaves, and straw, an estimated average of 83 ng of 17β-estradiol was extractable with water per gram of compost (dry weight) using the standard extraction conditions (200 mg compost/50 mL water; ng/g). A general decrease in the concentration of water-soluble 17β-estradiol in the poultry manure compost was observed during the course of the study. The decrease in 17β-estradiol was modeled with a first-order with time expression:

$$dC/dt = kC \quad [1]$$

where C is the concentration of 17β-estradiol (ng/g compost dry wt.) and t is time (d). The rate expression was integrated and written in linear form (Eq. [2]):

$$\ln C = \ln C_0 + kt \quad [2]$$

where C is the EIA-measured concentration of 17β-estradiol at the sampling times, and C_0 is the initial concentration of 17β-estradiol in the compost. The rate constant k for the decrease in water-soluble 17β-estradiol was determined by estimating the slope of the best fit for Eq. [2] (Fig. 3A). After 139 d, the estimated rate constant for the decrease in water-soluble 17β-estradiol in composted chicken manure was −0.010 ± 0.001/d, and the estimate for the 17β-estradiol half-life was 69 d. The amount of removable 17β-estradiol in composted layer manure did not reach 0 ng/g (dry weight) at the conclusion of the 139-d study period. The background levels of removable 17β-estradiol in the hay, starter compost,

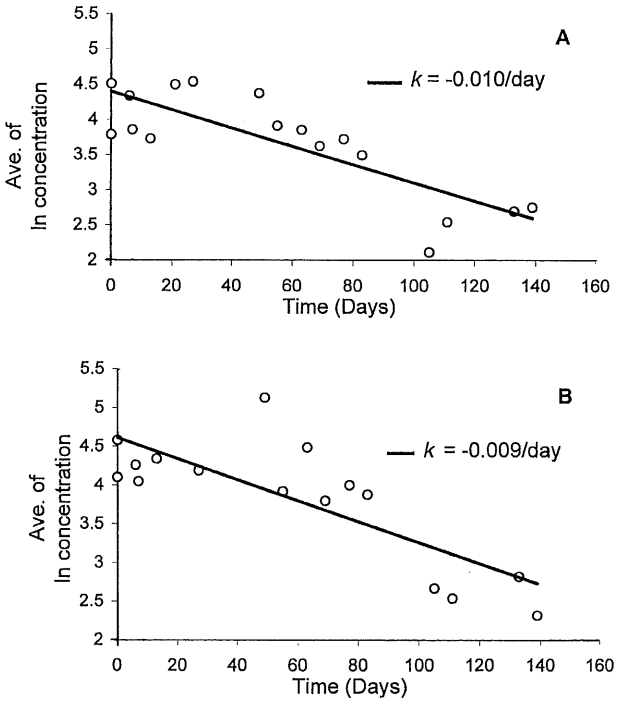

Fig. 3. The average of the natural logarithm (ln) of measured concentration of water-extractable 17β-estradiol in (A) normal and (B) clay-amended windrows with time during chicken layer manure composting. The decrease in 17β-estradiol was modeled with a first-order with time expression (Eq. [2]) to yield the degradation rate constants, k.

leaves, straw, and clay on a dry weight basis were 1.5, 0, 1.0, 4.8, and 0 ng/g, respectively.

Similarly, at the initiation of the field composting study, following dilution of layer manure with hay, starter compost, leaves, and straw, an estimated average of 115 ng of testosterone was extractable with water per gram of compost (dry weight) using the standard extraction conditions. The average water-soluble testosterone concentration displayed a general decrease throughout the study period. The concentration of testosterone in the aqueous extract of compost did not reach zero during active composting by EIA analysis. As with 17β-estradiol, the decrease of testosterone was modeled with a first order with time expression (Fig. 4A). During the 139 d of the study, the estimated rate constant (k) for the decrease in water-soluble testosterone in poultry compost was −0.015 ± 0.001/d, and the estimate for testosterone half-life was 46 d. The background concentration of water-soluble testosterone in the hay, starter compost, leaves, straw, and clay was 1.7, 0.4, 1.0, 4.8, and 0 ng/g dry wt., respectively.

Christiana clay was added to compost prepared in these studies to meet the needs for use in strawberry fields, where a defined topsoil texture is required. The addition of one part clay to the compost mixture resulted in approximately the same estimated rate of decline of water-soluble 17β-estradiol or testosterone during composting. The rate constant k for the decrease in water-soluble 17β-estradiol with time was −0.009 ± 0.002/d (Fig. 3B). The rate constant k for the decrease in water-soluble

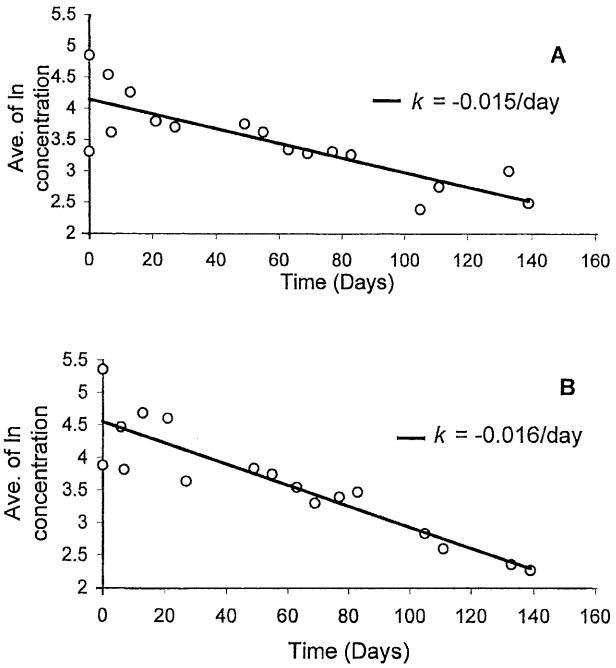

Fig. 4. The average of the natural logarithm (ln) of measured concentration of water-extractable testosterone in (A) normal and (B) clay-amended windrows with time during chicken layer manure compost. The decrease in testosterone was modeled with a first-order with time expression (Eq. [2]) to yield the degradation rate constants, *k*.

testosterone was −0.016 ± 0.001/d for compost containing clay (Fig. 4B). These rate constants were not statistically different from the values obtained from the decrease in water-soluble hormones in normal compost (Fig. 3A and 4A). Half-life estimates for 17β-estradiol and testosterone in clay-amended compost were 77 and 43 d, respectively.

DISCUSSION

The results of the present study demonstrated that aerobic composting reduced the amount of 17β-estradiol and testosterone that could be extracted with water from poultry manure compost. Initial, average concentrations of 17β-estradiol and testosterone in water extracts of compost from replicate windrows were 83 and 115 ng/g compost dry wt., respectively, and diminished to 13 and 11 ng/g by Day 139 (Fig. 3 and 4). This represents an 84 and 90% reduction, respectively, in the water-soluble levels of these potent hormones during aerobic composting. However, composting of poultry manure for 139 d was insufficient to produce compost in which no 17β-estradiol or testosterone could be detected in water-soluble extracts under the standardized conditions selected. The impact of these results is that water-soluble levels of hormones can be reduced during composting, but not completely eliminated.

Many factors can influence the composting process itself, and the methods and feedstock presently used may make the present report unique; however, the same is true of any composting operation. Therefore, the present results have to be understood within the constraints we selected. Fully aerated conditions must be maintained continuously to avoid the onset of anaerobic conditions, which will lead to degradation of organic materials by a different, slower pathway (Rynk, 1992). Moisture content must also be maintained between 40 and 65% to maintain thermophilic degradation. In addition, C to N ratios of 25:1 to 40:1 are needed to provide an adequate feed source for the microorganisms. Porosity and particle size of the composting material can affect the degradation of organic materials by limiting air movement and providing too small a surface area for decomposition.

Very little data has been produced discussing the fate or transport of excreted steroid hormones following their field application in manure. A previous report indicated that soil bacteria are incapable of degrading 17β-estradiol (Zondek and Sulman, 1943). Although not within the scope of the present study, it can be suggested, based on the investigations of others, that the chemical fate of 17β-estradiol and testosterone during aerobic composting was either to extractable or non-extractable products, or the complete mineralization to carbon dioxide. Extractable products could include the parent compound or its metabolites. Non-extractable products may have been the result of covalent bond formation, sequestration, or hydrophobic partitioning with the matrix (Gevao et al., 2000), and would have resulted in the incorporation of the hormones into humic substances.

Many organic compounds have been studied under aerobic composting conditions. Aromatic compounds are seldom used as a sole source of energy by microorganisms found in compost. Therefore, these substances are often partially degraded to water-soluble metabolites rather than fully oxidized to carbon dioxide (Sims and Overcash, 1983). Under composting conditions, the partial degradation of aromatic explosives (Williams and Myler, 1990; Griest et al., 1993; Kaplan and Kaplan, 1982), polyaromatic hydrocarbons (Hogan et al., 1988), Arochlor 1232 (Hogan et al., 1988), chlorophenols (Benoit and Barriusso, 1995; Michel et al., 1995), and persistent pesticides (Lemmon and Pylypiw, 1992) has been demonstrated.

The aromatic steroid hormone 17β-estradiol has on some occasions been reported to be environmentally persistent (Shore et al., 1993), and at other times, readily degraded (Ternes et al., 1999a). Where degradation has been studied, it appears that transformation occurs readily on the D-ring (Fig. 5), and that A-ring conversions are more difficult. Most studies on 17β-estradiol transformation report on the facile formation of estrone (hydroxyl on D-ring oxidized to ketone; Fig. 5). Colucci et al. (2001) has shown that 17β-estradiol can be readily oxidized to estrone in agricultural soil. This conversion was shown to occur in both native and autoclaved soil, suggesting an abiotic cause. Any further metabolism of estrone required microbial action. Lee and Liu (2002) demonstrated that 17β-estradiol could be rapidly oxidized (22 h) to estrone by aerobic degradation with an estradiol-degrading bacterial culture. Lee et al. (2003)

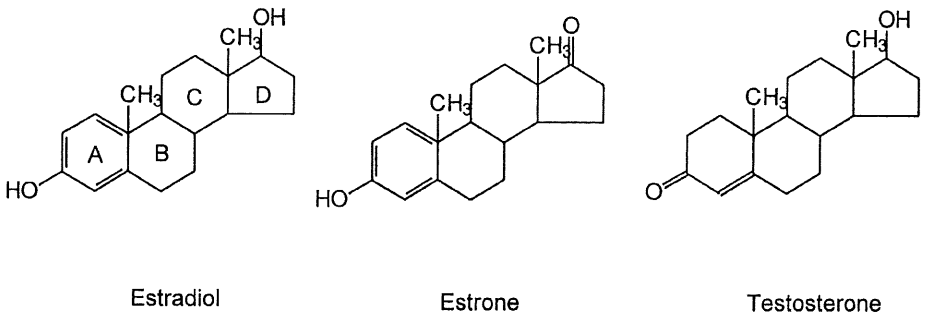

Fig. 5. Chemical structures of 17β-estradiol, estrone, and testosterone. 17β-Estradiol and testosterone are excreted in chicken layer manure, and estrone is a prominent metabolite of 17β-estradiol.

also showed in soil batch equilibrium studies that the major transformation compound of 17β-estradiol was estrone. Other metabolites of the microbial degradation of 17β-estradiol are estriol, 16α-hydroxyestrone, 2-methoxyestradiol, and 2-methyoxyestrone (Lee and Liu, 2002). A-ring catechols of 17β-estradiol were observed in rat hepatocytes (Rathahao et al., 2000), which can undergo further oxidation to quinones, highly reactive electrophilic compounds known to react with DNA to form adducts.

Testosterone (Fig. 5) metabolism has been studied in the estuarine mysid *Neomysis integer* (Verslycke et al., 2002). Eleven metabolites were identified following exposure in the aqueous medium; nine were characterized by liquid chromatography (LC)–mass spectrometry (MS) as monohydroxylated metabolites (2α, 6α, 6β, 7α, 11α, 11β, 15α, 16α, and 16β). The gram-negative bacteria *Comamonas testosteroni* could metabolize testosterone as the sole carbon source (Horinouchi et al., 2001). The bacteria use dehydrogenation, desaturation, hydroxylation, and meta-cleavage reactions to accomplish the degradation of testosterone. Intermediates in the pathway are 4-androstene-3,17-dione, 1,4-androstadiene-3, 17-dione, 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9, 17-dione, and 3,4-dihydroxy-9,10-secoandrosta-1,3,5 (10)-triene-9,17-dione. In soil systems (Lee et al., 2003), the dissipation of testosterone yielded only two compounds: androstenedione (confirmed by gas chromatography [GC]–MS) and androsta-4-ene-3-one-16,17-diol (suspected).

Non-extractable residues of 17β-estradiol and testosterone may also have formed during the process of aerobic composting. This commonly occurs when organic compounds enter soil and form tight complexes with the humic substances of soil (i.e., humic acid, fulvic acid, and humin). Humic acids are complex aromatic macromolecules containing amino acid, amino sugar, peptide, and aliphatic monomeric units and which are insoluble under acid conditions but soluble at higher pH. Fulvic acids are of lower molecular weight than humic acids, water soluble at all pH values, and contain a high proportion of oxygen-bearing polar functional groups. Humin is that portion of soil and/or compost that is not soluble at any pH and has a very high molecular weight (approximately 300 000). It has been demonstrated that metabolism of organic substances to hydroxylated metabolites was followed by covalent coupling to humic and fulvic acids in soil (Richnow et al., 1994; Calderbank, 1989). This led to long-term immobilization, but not the complete destruction of these compounds. Non-extractable organic compounds, when bound to humic substances, tend to lose their inherent biological activity (Calderbank, 1989). The presence of non-extractable residues of 17β-estradiol and testosterone in compost should be investigated and quantitated in future studies by utilizing radiolabeled precursors and determining complete mass balances.

Despite the putative degradation of both hormones via composting, a critical issue requiring additional study is whether a lack of full degradation or the possible formation of non-extractable metabolites is a satisfactory endpoint for these hormones. Colucci et al. (2001) demonstrated a rapid oxidation of 17β-estradiol to estrone in loam, silt loam, and sandy loam soils. Facile conversions of 17β-estradiol to estrone have been observed by other researchers (Ternes et al., 1999a; Raman et al., 2001). Estrone is also estrogenic, although in a yeast estrogenicity assay, it is only one-half as potent as 17β-estradiol (Colucci et al., 2001).

The measured rate of decline in the water extractability of testosterone from normal composted layer manure over 139 d in the present study was approximately 50% faster than that observed with 17β-estradiol (k = −0.015/d and −0.010/d, respectively; Fig. 3A and 4A), although the difference was shown not to be statistically significant. The relative rate of degradation of testosterone has been previously compared with 17β-estradiol and was shown to be more rapid, perhaps due to microbial resistance in degrading the aromatic A-ring of estradiol. The removal and mineralization of 17β-estradiol and testosterone from the aqueous phase of a municipal wastewater treatment plant (WWTP) were measured, and it was reported that testosterone was degraded approximately two times faster than 17β-estradiol (Layton et al., 2000). Adaptation of the bacteria to the hormone was reported to be an important factor in the mineralization of 17β-estradiol and testosterone. Municipal WWTP microbial populations mineralized 17β-estradiol by 84% over 72 h, while bacteria from industrial biosolids only degraded 4% of the 17β-estradiol under identical conditions. Testosterone degradation was not as affected by bacterial adaptation, in that only slight differences were noted when comparing the two systems, that is, 65 vs. 55%, respectively. Differences in the

degradation of these hormones can also arise from different microbe populations present in differing treatment processes. A Brazilian WWTP reported >99% degradation of 17β-estradiol in its waste stream, while only 64% of 17β-estradiol was removed in a German WWTP (Ternes et al., 1999b). Microbe populations may also differ in poultry compost obtained from different regions of the country due to antibiotic administration or other management practices. Additional compost degradation studies with radiolabeled hormones should be performed to assess this potential difference, and to determine the extent to which microbial versus abiotic degradation of 17β-estradiol and testosterone are occurring.

It was theoretically possible that the compost extracts may have contained compounds that cross-reacted in the immunoassay, which would have resulted in the overestimation of 17β-estradiol or testosterone in the aqueous extracts. However, only related steroid hormones would be likely to cross-react with the antibodies contained in the assay kits.

Cumulative recoveries of [^{14}C] hormones spiked into compost were shown to increase with subsequent aqueous extractions (Table 1). The implication of these results for a field setting is that rain would remove only a portion of the residual hormones in compost due to the variable volume of each rainfall event and to mass losses. These data are agreement with work done with simulated rainfall on fields where poultry litter had been applied (Nichols et al., 1997). Second-storm runoff concentrations of 17β-estradiol were 66% less than with the first-storm runoff conducted 7 d earlier. The potential environmental impact of the hormone-bearing runoff into surface or ground waters can be reduced when fields were surrounded with grass filter strips (Nichols et al., 1998).

CONCLUSIONS

In summary, this study demonstrated that the endogenous hormones 17β-estradiol and testosterone, excreted in poultry manure, were extractable into water to a decreasing extent with time while undergoing aerobic, thermophilic composting. This decline was observed to be approximately the same for the two hormones over 139 d. Both hormones could still be detected in aqueous extracts of compost by EIA analyses at the conclusion of the thermophilic and mesophilic periods. The observed decline in the water extractability of the hormones was nearly the same with or without Christiana clay, which is often included as a soil amendment. Composting of layer manure, as an agricultural management tool, may provide an effective and practical means of reducing, but not eliminating, the introduction of these potent hormones into the environment. It was also concluded that, in a field setting, the first rainfall event on fields amended with composted layer manure would not lead to complete removal of residual 17β-estradiol and testosterone. Rather, the hormone levels in runoff would decrease steadily due to mass losses from each previous rainfall event, rainfall water volume, and/or biological degradation.

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Bacteria on Closed-Boll and Commercially Harvested Cotton

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The bacterial content of specially treated cottons used by other investigators to test human pulmonary responses to cotton dust was examined. Cotton from Lubbock, Tex. and Stoneville, Miss. were either (i) harvested by machine and handled as commercial bale cotton, (ii) harvested as closed bolls with bracts intact and opened under special conditions, (iii) harvested as closed bolls, with bracts being removed and opened under special conditions, or (iv) harvested by machine, handled as commercial bale cotton, washed in water, and dried (Stoneville only). *Bacillus* spp. were isolated from all samples and predominated in cotton from Stoneville. *Enterobacter agglomerans* was isolated from all but one sample, the Stoneville closed-boll bract-removed cotton, and predominated in Lubbock samples. Aerogenic and anaerogenic biogroups of *E. agglomerans* were isolated; only aerogenic strain b of *E. agglomerans* was present in samples from both locations. *Klebsiella ozaenae* and *K. pneumoniae* were isolated only from Lubbock samples. Cotton from Lubbock yielded 100 to 1,000 times more bacteria, both total and gram negative, than did comparably treated cotton from Stoneville. Thus, differences in growing and processing conditions at the two locations were associated with large differences in the bacterial content of the cotton, but harvesting green bolls and removing bracts had little effect. The bacterial content of Stoneville unwashed cotton was 10 to 100 times greater than that of the Stoneville washed cotton, and it paralleled the differences reported (Boehlecke et al., Am. Rev. Respir. Dis. **123**:152, 1981) in pulmonary function responses when subjects were exposed to dust (0.6 mg/m^3) from these two cottons. Levels of gram-negative and total bacteria on all samples were comparable to those previously reported for field-weathered cottons from various locations throughout the world.

The possible involvement of microbial agents in byssinosis, the pulmonary disease experienced by some cotton, flax, hemp, and sisal workers (3), has been studied sporadically for nearly 40 years. The earliest evidence which implicated gram-negative bacteria as agents of health disorders in individuals working with cotton was reported by Neal et al. (31) in their account of mattress-maker's fever in 1942. This disorder involved people who had had no previous experience as cotton-textile workers, but who upon using cotton contaminated with bacteria to make mattresses developed respiratory and febrile responses that matched closely those described for mill fever (43), a phenomenon that occurs in cotton-textile workers when they are first exposed to cotton dust. *Aerobacter cloacae* (now known as *Enterobacter cloacae*) was found in concentrations of 10^8/g on samples of mattress cotton (9, 13) and was considered the cause of the illness.

In 1952, Drummond and Hamlin (16) reported on bacterial aerosols in cotton-mill cardrooms in England where American, Egyptian, Indian, and Sudanese cottons were being processed. The majority of the total bacteria in all mills sampled was comprised of *Bacillus* spp. In only a single mill, one that was spinning a mixture of American (58%) and Egyptian (23%) cotton, was the concentration of *Aerobacter* spp. relatively high. Hamlin (23) reported only a few *Pseudomonas* spp. in aerosols of cardrooms; other gram-negative bacteria found were identified as *Achromobacter, Flavobacterium*, and *Escherichia*. The relation of these bacterial aerosols to the incidence of byssinosis was not studied.

For nearly nine years, there were no additional reports relating bacterial agents to byssinosis. In 1961, endotoxin from bacteria was demonstrated in cotton dusts from textile mills (34), and inhalation of purified endotoxins by rabbits and humans resulted in fever and dyspnea. In 1969, Cavagna et al. (10) reported that byssinosis occurred in 32% of cotton cardroom workers

and 47% of hemp cardroom workers exposed to aerosols containing 7.2 and 8.7 μg of endotoxin per m^3, respectively.

However, in a subsequent study, the prevalence of byssinosis was correlated with the concentration of gram-negative bacteria but not with fungal and endotoxin concentrations (12). Later, Cinkotai and Whitaker (12) showed that concentrations of bacterial protease, and 2- to 4-μm particles in the air of 21 cotton-spinning mills in Lancashire, correlated with byssinosis prevalence. Recently, Rylander and Lundholm (39) showed that the spinning of bale cotton containing more than 10^3 gram-negative bacteria per g was highly correlated with decreases in forced expiratory volume (FEV_1) of mill workers. The decreased FEV_1 over the work shift served as the measure of response to the byssinotic agent. Bacteria found in the bulk cotton used in their studies (37, 38) included the following: *Acinetobacter calcoaceticus, Enterobacter agglomerans, E. cloacae, Flavobacterium* spp., *Klebsiella* spp., *Pseudomonas fluorescens, P. maltophilia, P. stutzeri*, and *P. syringae*.

Recent work by Fischer (19, 20) indicates that the concentration of airborne endotoxin is associated with histamine release, the concentration of airborne gram-negative bacteria (genera unspecified), and sometimes a decrease in FEV_1. Also, Fischer et al. (21, 22, 30) indicated that gram-negative bacteria are associated primarily with bract and leaflike and seed trash in raw stock cotton.

In addition to harboring bacteria and endotoxin, plant trash in raw cotton is also a source of various pharmacoactive substances considered as possible byssinogenic agents found in cotton dust (5, 17, 41). The presence of these substances has complicated the identification of the causative agent of the disease.

In an effort to determine whether the byssinogenic substance originates with the weathering of cotton in the field, the byssinogenic effects of specially harvested cottons were examined (6). The special cottons were from green, unopened bolls that were allowed to open under conditions in which they were not exposed to field weathering. Bracts from half of the green bolls were removed before the opening of the bolls to eliminate possible pieces of shattered dry bract from the final lot of fiber. Bractless fiber was needed for comparative tests because bracts have been implicated as the source of byssinogenic material. The cotton sample that caused the largest decrease in FEV_1 in individuals exposed to dust generated during carding of the cotton had the highest concentration of endotoxin in comparison with six other samples similarly treated (6). In 1980, the experiment was repeated, using 1979 crop cotton commercially and specially harvested from Stoneville, Miss. and Lubbock, Tex. In addition, part of the Stoneville standard cotton was washed in water and dried before carding to test whether washing might remove the causative agent(s) (29).

The purposes of our study were to determine whether the bacterial content of the test cottons used in the 1980 human exposure tests (i) differed qualitatively or quantitatively from one another, (ii) differed from levels previously reported by other investigators for field-weathered, commercial cottons from various locations throughout the world, and (iii) showed any trend that corresponded to the response trend reported for humans exposed to dust from the samples (Boehlecke et al., Am. Rev. Respir. Dis. **123**:152, 1981).

With regard to the latter objective, ideally the bacterial content of size-fractionated airborne dusts from the exposure tests should be determined to provide data about the actual exposure concentrations and likely inhalations. However, air sampling for total airborne bacteria with subsequent specific identifications was not possible during the exposure tests. Also, we recalled that at least the gram-negative bacterial content of bulk cotton was shown to be highly predictive of decreases in FEV_1 of mill workers (39). In addition, our background studies (unpublished data) showed that the relative bacterial content of airborne cotton dust was reliably predicted by the relative bacterial content of bulk cotton. Thus, it seemed reasonable to us that data on the bacterial content of the bulk cottons would be useful in understanding the relative exposures.

MATERIALS AND METHODS

Samples. Bulk cotton samples used in all tests were subsamples from the 1979 crop harvested during September and October at Stoneville, Miss. and Lubbock, Tex. The samples were treated as previously described (6, 14) and used in tests of respiratory response to cotton carding dust. Three groups of samples from each location were used: (i) bolls, harvested while still green and closed, with bracts and stems removed (closed boll, bract removed) before bolls were allowed to open; (ii) bolls harvested as for (i), but bracts and stems were left intact (closed boll, bract intact); and (iii) bolls harvested after they opened and were exposed to field conditions, as is done commercially (standard). Before being dried and opened, the closed bolls were soaked for 10 min in a solution of NaOCl with 0.05% available chlorine in an effort to eliminate surface saprophytes that might contaminate the fiber during opening.

Drying and opening conditions at the two locations differed. At Lubbock, bolls were dried in sealed bins by pulling filtered (99.99% high efficiency particulate air filter) air through a batch of bolls 1.2 to 1.5 m deep (14). At Stoneville, bolls were dried on shallow screen trays in a controlled atmosphere (30% relative humid-

ity, 95°F [35°C]) (C. K. Bragg, personal communication). Also, a subsample of the Stoneville standard cotton was washed in water (standard, washed) and dried before carding. In 1980, human responses to dust generated during carding of each cotton type in the U.S. Department of Agriculture model cardroom at Clemson, S.C. were measured (Boehlecke et al., Am. Rev. Respir. Dis. **123**:152, 1981). We received samples under code, so that the origin of a particular sample was unknown to us in September, 1980 when our analyses were made.

Microscopic examination and direct plating of fibers. Small samples of fibers from the cottons were examined with a dissecting microscope, and the yellowish, matted areas of fiber were removed, mounted in water on clean microscope slides, and examined at 100 and 400× in a Leitz Ortholux microscope. Small pieces of the yellow, matted fibers were placed directly onto deoxycholate agar (Difco Laboratories) and incubated at 35°C. Colonies were purified and identified as described below.

Enumeration of bacteria. Samples were analyzed for total bacteria by the spread plate method and for gram-negative and coliform bacteria by the most-probable-number (MPN) method. Primary dilutions of samples of the seven cottons were prepared in duplicate by tamping 1.0-g subsamples of bulk cotton in 100 ml of 0.5 M phosphate-buffered water (pH 7.2) containing 0.5% Tween 80 (vol/vol) to dislodge entrapped air and then were shaken at 250 rpm for 15 min at 25°C. Dilutions used for MPNs of gram-negative bacteria were shaken for 1 h at 4°C.

In the plate count method, 10-fold dilutions prepared in phosphate-buffered water were plated onto Trypticase soy agar (TSA) (BBL Microbiology Systems) containing 50 μg of cycloheximide per ml. Representative colonies from TSA plates were purified by successive streaking and subcultured onto TSA slants. Observations on the presence, absence, and relative abundance of the colony types found on the plates were recorded.

In the MPN method, gram-negative selection broth (GNSB) and lauryl tryptone broth were used. The GNSB contained the following components (g/liter of distilled water): peptone, 5; dextrose, 1; K_2HPO_4, 0.03; sodium lauryl sulfate, 1; bromthymol blue, 0.0004. A microdilution technique (36) with GNSB was used to enumerate gram-negative bacteria. Before its use in the microtechnique, the GNSB was tested for its capacity to support growth of bacteria found on the cotton. Bacterial isolates obtained from the TSA plates were inoculated into 10 ml of GNSB, and turbidity after 48 h of incubation at 35°C was considered a positive indicator of growth. For the enumeration of coliforms, five tubes of lauryl tryptone broth for each of five successive 10-fold dilutions were used

TABLE 1. Numbers and types of bacteria associated with cotton samples

Sample source and treatment	No. of bacteria[a]			Identified bacteria[b]
	Total (no./g)	Gram-negative (MPN/g)	Coliform (MPN/g)	
Stoneville, Miss.				
Closed boll, bract removed	1.7×10^5 b	6.3×10^2 a	4.9×10^2 b	*Acinetobacter anitratum, A. lwoffii, *Bacillus* sp., *Pseudomonas stutzeri*
Closed boll, bract intact	6.4×10^5 b	2.8×10^3 a	1.1×10^3 b	**Bacillus* spp., *E. agglomerans, Pseudomonas* sp.
Standard	1.8×10^5 b	2.3×10^4 a	2.3×10^3 b	*A. anitratum, *Bacillus* spp., *E. agglomerans, P. putida*
Standard, washed	5.2×10^3 a	1.0×10^3 a	1.1×10^1 a	**Bacillus* spp., *Enterobacter agglomerans*
Lubbock, Tex.				
Closed boll, bract removed	1.7×10^7 c	4.4×10^6 b	9.2×10^4 c	*Bacillus* spp., **E. agglomerans, Klebsiella ozaenae, P. stutzeri*
Closed boll, bract intact	2.4×10^7 c	4.0×10^6 b	2.7×10^4 c	*Bacillus* sp., **E. agglomerans, Pseudomonas* sp.
Standard	4.2×10^7 c	4.4×10^6 b	6.6×10^4 c	*Bacillus* sp., **E. agglomerans, K. pneumoniae, K. ozaenae, E. sakazakii*

[a] Means of two replications; means within a column followed by the same letter are not significantly different from each other according to Duncan's multiple-range test ($P = 0.05$). Total, gram-negative, and coliform bacteria were grown on (in) TSA, GNSB, and lauryl tryptone broth, respectively (see the text for details).

[b] *, Most abundant colony on TSA plates.

TABLE 2. Characteristics of bacteria isolated from cottons

Strain	Characteristic[a]										
	No. of strains	Motility	Yellow pigmentation	Levan production	Carbon source						
					Anaerobic glucose		Lactose	Arabinose	Dulcitol	Sorbitol	Adonitol
					Acid production	Gas production					
Enterobacter agglomerans											
a	1	+	+	−	+	+	+	+	+	+	−
b	11	+	+	±	+	+	±	+	±	+	−
c	1	+	+	+	+	−	−	+	−	−	−
d	1	+	+	−	+	−	−	+	−	−	−
e	1	+	+	−	+	−	−	+	−	−	−
Enterobacter sakazakii	2	+	−	−	+	+	+	+	−	−	−
Klebsiella pneumoniae	2	−	−	−	+	+	+	+	−	+	+
Klebsiella ozaenae	2	−	−	−	+	+	+	+	−	+	+
Acinetobacter anitratum	1	±	+	−	−	−	−	+	−	−	+
Acinetobacter lwoffii	3	±	−	−	−	−	−	−	−	−	+
Pseudomonas putida	3	+	+	±	−	−	−	−	−	−	−
Pseudomonas stutzeri	1	+	−	−	−	−	−	+	−	−	−

(1). All plates and tubes were incubated at 35°C.

Characterization of isolates. The pure strains were examined for Gram reaction (Hucker's method), cell morphology, and motility at 24 to 48 h and tested for the presence of oxidase (oxidase differentiation disks, Difco). Cultures were further characterized by results from the 15-test Enterotube II (Roche Diagnostics, Div. Hoffmann-La Roche, Inc.) and 9-test Oxi/Ferm tube (Roche) kits (where applicable). There is a high correlation between results with these kits and those with conventional tests (32, 33, 42). Nitrate reduction was recorded at 2 days, using the medium and reagents described by Lennette et al. (27). Levan production was tested on glucose-yeast extract agar supplemented with 5% (wt/vol) sucrose (26). Isolates identified as *Klebsiella* spp. were also treated for their ability to grow at 10°C and to produce gas from lactose at 44.5°C (4).

RESULTS

The selection broth supported the growth of isolates of all gram-negative bacteria listed in Table 1, although *Acinetobacter* spp. and *Pseudomonas* spp. grew slowly. Thus, the medium was accepted for use in enumerating gram-negative bacteria from cotton samples.

Microscopic examination of small pieces of yellow, matted fibers revealed that such yellow spots consisted of dried microcolonies of bacteria, which upon rehydration in water were held in tight clusters in a slime-like matrix. Pure cultures of *E. agglomerans* developed from several pieces of the yellow fibers plated directly onto deoxycholate agar.

The values of total and MPN gram-negative and coliform bacteria given in Table 1 for the seven samples indicated that large populations of viable bacteria persisted on dry cotton stored for up to nearly 12 months. Furthermore, the location of origin and treatment of cotton affected the bacterial populations on these samples. All Lubbock samples showed significantly greater numbers of total, gram-negative, and coliform bacteria than did the Stoneville samples. The differences in numbers of total bacteria among the seven samples paralleled those for the coliforms. Although the MPN of coliforms per gram represented only a select portion of the gram-negative population present, it provided an additional index useful for comparison of the relative bacterial contents of the samples.

Contrary to expectations, the green-boll cottons were not bacteriologically sterile. Thus, differences in harvesting treatments, e.g., closed-boll versus standard, at both locations had no effect on the number of bacteria associated with the cottons. The closed-boll, bract-removed cotton had nearly the same amount of total, gram-negative, and coliform bacteria as did the closed-boll, bract-intact and standard cottons.

Qualitatively, *Bacillus* spp., *E. agglomerans*, and *Pseudomonas* spp. were found on the closed-boll, bract-intact cotton from both locations. Only *Bacillus* spp. and *P. stutzeri* were found on both the Lubbock and Stoneville closed-boll, bract-removed cotton. Also, a few *Acinetobacter anitratum* and *A. lwoffii* isolates were found on the Stoneville closed-boll, bract-removed cotton. Additional qualitative differences among the samples included the relative predominance on TSA plates of *Bacillus* spp. in Stoneville but not Lubbock samples. In contrast, *Enterobacter* spp. were generally more predominant in all Lubbock samples and in the Stoneville standard, unwashed and washed sam-

TABLE 2—*Continued*

Characteristic[a]											
Biochemical test											
H_2S production	Nitrate reduction	Arginine dihydrolase	Lysine decarboxylase	Ornithine decarboxylase	Phenylalanine deaminase	Gelatin liquefaction	Voges-Proskauer	Indole	Urease	Citrate	Oxidase
−	+	NT	−	−	−	NT	+	−	−	+	−
−	±	NT	−	−	−	NT	−	−	−	+	−
−	+	NT	−	−	−	NT	−	−	−	+	−
−	−	NT	−	−	−	NT	−	−	−	−	−
−	+	NT	−	−	−	NT	+	−	−	+	−
−	+	NT	−	+	−	−	+	−	−	+	−
−	+	NT	+	−	−	−	−	+	+	+	−
−	+	NT	−	+	−	−	−	−	−	+	−
−	+	−	−	−	+	−	−	−	−	+	−
−	−	−	−	−	+	+	−	−	+	−	−
−	+	+	−	−	+	−	−	−	−	+	+
−	−	−	−	−	+	−	−	−	−	+	+

[a] NT, Not tested; +, positive or present; −, negative or not present; ±, both positive and negative present, the predominant reaction above.

ples than in Stoneville closed-boll samples. *Klebsiella* spp. were found only in Lubbock standard and Lubbock closed-boll, bract-removed cotton, and the isolates did not grow at 10°C or produce gas from lactose at 44.5°C. Thus, they were not identified as *K. planticola* (4).

Characterization of isolates. Characteristics of the gram-negative strains isolated from all samples are shown in Table 2. Aerogenic and anaerogenic strains of *E. agglomerans* (sensu Ewing and Fife [18]) were found. Lubbock samples contained only aerogenic strains a and b, which corresponded to Ewing and Fife biogroups G1 and G2. Anaerogenic strains c, d, and e, corresponding to Ewing and Fife biogroups 2, 3, and 6, were from the Stoneville standard and washed cottons. Aerogenic strain b of *E. agglomerans* also was isolated from both the standard and the closed-boll, bract-intact cotton from Stoneville.

DISCUSSION

Based on these results, it is evident that the surface treatment with chlorine of closed-boll non-field-weathered cotton did not provide microbially uncontaminated cotton fiber, as had been expected, for human response testing. However, these seven cottons did provide for the comparison of human responses to samples with significantly different numbers of total, gram-negative, and coliform bacteria as well as with different types of bacteria. Lubbock cottons (standard and closed boll) were heavily populated with *E. agglomerans* strain b. Stoneville standard and closed-boll, bract-intact cottons also contained strain b of *E. agglomerans*. In addition, strains c and d of *E. agglomerans* were found from Stoneville standard cotton, and strain e was found from standard, washed cotton. Stoneville closed-boll, bract-removed cotton was the only cotton from which *E. agglomerans* was not isolated.

The occurrence of *Klebsiella ozaenae* and *K. pneumoniae* is in accord with records of their known distribution on soil, plants, and vegetables (8). Undoubtedly, some viable particles are aerosolized during cotton processing. However, at present, the aerosolized concentrations of these klebsiellae and other potential pulmonary pathogens, *Acinetobacter* spp. and *Pseudomonas* spp. (35), found on cotton fiber are unknown.

The human reactions to dust from only the Stoneville standard and washed cottons were reported by Boehlecke et al. (Am. Rev. Respir. Dis. **123**:152, 1981). Pulmonary function responses as measured by FEV_1 decrements over a 6-h exposure to dust from the Stoneville standard unwashed cotton increased from −116 to −237 cm^3, with dust concentrations increasing from 0.6 to 0.9 mg/m^3. Responses to comparable dust levels from washed cotton and to 0.2 mg of dust per m^3 from the unwashed cotton were significantly smaller and comparable with the response to 0.03 mg of dust per m^3 from a clean room (control). The differences in the concentrations reported here for total, gram-negative, and coliform bacteria on the Stoneville standard and washed cottons parallel the differences in the

pulmonary function responses to the high dust levels reported by Boehlecke et al. This parallelism, together with previous reports showing correlations between bacteria and byssinotic response (11, 12, 34), further supports a role for dustborne bacteria in the etiology of acute byssinotic episodes. In contrast, the Lubbock standard cotton, which had significantly more bacteria than Stoneville standard cotton, produced a smaller decrease in FEV_1 than did the Stoneville standard cotton at comparable dust levels (B. Boehlecke, personal communication). We recognize that many substances, including bacteria, can be removed from cotton by washing and that such substances also must be considered in any evaluation of the etiology of byssinosis.

Because of the stated predominance of viable gram-negative bacteria in the dusts studied, previous investigators have emphasized the possible role or activity of endotoxic lipopolysaccharides from gram-negative bacteria in the byssinotic and other occupationally related pulmonary responses (10, 15, 19, 30, 34, 39). However, the endotoxic activity of lipopolysaccharides from different bacteria is known to vary (24). Our data on the two standard cottons indicate that gram-negative bacteria comprise less than half of the total viable bacteria on the bulk fiber. Thus, a relatively large proportion of the viable bacteria were gram positive, as found by Hamlin (23). In view of current information (40) on the adjuvant and pyrogenic activities of, and development of tolerance to, peptidoglycan, this bacterial cell wall component should be assessed in future cotton dust exposures and bacterial inhalation studies in animals. Since viable bacteria probably account for only a fraction of the total microbial biomass present on fiber and in dust, and because viability is not required for immunogenicity, the total bacterial biomass should be assessed.

Detailed comparisons between the present results and those from previous studies are not possible because the techniques and media used to enumerate bacteria on cotton directly affect the results. In general, concentrations of gram-negative bacteria in the Lubbock samples are greater than those reported for bale cotton from Australia, Egypt, Greece, Peru, Russia, Turkey, and the United States (38) and for samples of unwashed, bale cotton, i.e., 1.5×10^6 and 9.4×10^5 colony-forming units per g (19); they are comparable to those in samples of cotton lint and leaf from west Texas and cotton bract and stem from Alabama, North Carolina, west Texas, and Australia (38) and in samples of low-grade stained cotton used for mattress making (31).

The MPN value for gram-negative bacteria on Stoneville closed-boll, bract-removed cotton, 6.3×10^2, was comparable to the values reported for bale cotton which had been precleaned once (20) but less than concentrations of gram-negative bacteria found on cotton lint, bracts, stems, and leaves from Alabama, North Carolina, west Texas, and Australia (38). Stoneville standard and closed-boll, bract-intact cotton had concentrations of gram-negative bacteria comparable to those found in several bales of U.S. cotton (38).

In the present tests, washing of Stoneville standard cotton was effective in reducing total bacterial and coliform contamination but generally ineffective in removing of *E. agglomerans* (Table 1). The latter ineffectiveness may relate to the occurrence of aggregated *E. agglomerans* microcolonies observed microscopically on the fiber surfaces.

Commercial cotton usually contains pieces of bract and other plant trash, and these have been implicated as the major source of byssinogenic material (7) and bacteria (21, 30). However, our results show that bulk cotton with only trace pieces of bract can have relatively large numbers of bacteria. The closed-boll, bract-removed cottons had bacterial levels similar to those of the corresponding standard machine-harvested cottons (Table 1).

With regard to the abundance of bacteria on the closed-boll cottons, the following explanations are possible. During the time (2 to 4 h) between picking and surface sterilization of the closed bolls, some splitting of carpels, even though only slight, along the sutures could have allowed ample space for bacteria to enter the bolls and contaminate the fiber. The fact that bolls were picked and placed into bags so that they were contacting each other may provide for the necessary mechanical transfer of substantial numbers of bacteria among bolls. With the availability of readily utilizable sugar present on the immature fibers during the several days of opening (28), the bacteria could then grow, especially with ample humidity in the immediate region of the fibers. At Lubbock, closed-boll cotton was exposed to relative humidities of 90% or greater for 6 to 8 h during each of the first 2 days of closed-boll opening in bins (25). At Stoneville, the air was dehumidified and heated. Endospore formation in *Bacillus* spp. may account for their predominance on Stoneville closed-boll cottons. Such spores could easily survive the conditioning system used and be carried in the air of the opening facility.

There is a remote possibility that bacteria infected cotton before boll opening. Very occasionally, severe infections of this type are associated with the development of supernumerary carpels which split the placentae and cause an opening to the outside of the boll (2). Internal

boll infection also may be attributed to insect transmission of bacteria or simply to incidental air-, water-, or vector-borne inoculation of 1- and 2-day-old flowers (2). The latter can result in a limited infection of the placental column. *Erwinia herbicola* (= *E. agglomerans* pro parte sensu Ewing and Fife [18]) has been identified as a causal agent of such infections, which may or may not exhibit external symptoms. Due to the reduced amounts of pectin, soluble sugars, and nitrogenous substances in nearly mature bolls, these would be infected much less severely than young bolls (2). The Lubbock cotton used in the present study was grown without pesticides, and a late-season bollworm infestation occurred. Although most bollworm-damaged bolls were eliminated before drying, a few were missed and put into drying bins with the undamaged bolls (Laird, personal communication). Thus, it was possible that contamination from a few bolls spread to other bolls opening in the same bin.

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Terrasa, Jen

From: Feldmark, Jessica
Sent: Friday, October 27, 2017 5:20 PM
To: Terrasa, Jen
Subject: RE: Citizen Concern: inhumane animal conditions

I don't believe I received anything from her on this. Is she looking to do legislation similar to Montgomery County's?

Jess

From: Terrasa, Jen
Sent: Friday, October 27, 2017 4:54 PM
To: Feldmark, Jessica <jfeldmark@howardcountymd.gov>
Subject: FW: Citizen Concern: inhumane animal conditions

Hi Jess,

I know this is an old issue, but we're trying to get caught up here on things that have not been responded to. Jen vaguely recalls sending something to you about this but isn't sure. Do you happen to know whether we can do something as a county about this issue at all?

Thank you!

Colette Gelwicks
District Aide
District 3, Councilwoman Jen Terrasa
Howard County Council
3430 Court House Drive, Ellicott City, MD 21043
cgelwicks@howardcountymd.gov
Phone: (410) 313-3108 Fax: (410) 313-3297

Like Councilwoman Terrasa's page on Facebook and follow her on Twitter!

From: Nathan Eschbach [mailto:enathan1@umbc.edu]
Sent: Monday, July 10, 2017 9:16 AM
To: CouncilMail <CouncilMail@howardcountymd.gov>
Subject: Citizen Concern: inhumane animal conditions

Hello, my name is Nathan Eschbach and I am 20 years old, currently attending UMBC for MechE, and residing in HC. I am emailing today in regard to inhumane conditions and puppy stores.

I am not sure how much you know about animals, their needs, or their distribution chains. I am also unsure if a relevant legislation has been proposed or how that process would work. However, I urge you to do something about the cruel prospect of puppy stores.

There are several puppy stores in this county, each of which is telling consumers their product comes from a "breeder that is USDA certified". This may seem reassuring however the stated certification DOES NOT insure humane treatment of animals and the word "breeder" can be used to describe a puppy mill. In fact, almost all (if not all), animals at puppy retail stores come from puppy mills.

Again, not sure if you are familiar with the practice, but puppy mills are cruel, inhumane breeders which leave their product (puppys) in cages all day and night, over breed and then euthanize the parents, inbreed dogs, and many other cruel practices. This results in genetic problems, aggressive dogs, untrained/poor-mannered dogs, and it takes away from every dog in line for lethal injection in pounds around the country as well as TRULY reputable breeders. The practices puppy mills use are inhumane and disgusting. When I started to educate myself on the issue I was appalled that in a country and county as advanced and knowledgeable as ours, that this was allowed to happen.

Please Please Please contact me. I would love to talk more about this. I have never contacted any member of government representing me before but I am reaching out now and do not want to be disappointing. This is an issue that many people in this county are unaware of, but it is something that 99% of your citizens would not approve of (the other 1% or less are people profiting from the practice).

If it would help I guarantee I could get **thousands** of signatures on a petition.

Additionally, laws banning pet retail stores have been passed all around the country. I will attach the article signed right here in Montgomery County.

Please get back to me, I would love to talk about this and help but I need government interest.

Thank you for your time and interest in the lives of suffering animals,
Nathan Eschbach

Oxytetracycline Sorption to Organic Matter by Metal-Bridging

Allison A. MacKay* and Brian Canterbury

ABSTRACT

The sorption of oxytetracycline to metal-loaded ion exchange resin and to natural organic matter by the formation of ternary complexes between polyvalent metal cations and sorbent- and sorbate ligand groups was investigated. Oxytetracycline (OTC) sorption to Ca- and Cu-loaded Chelex-100 resin increased with increasing metal/sorbate ratio at pH 7.6 (OTC speciation: 55% zwitterion, 45% anion). Greater sorption to Cu- than Ca-loaded resin was observed, consistent with the greater stability constants of Cu with both the resin sites and with OTC. Oxytetracycline sorption to organic matter was measured at pH 5.5 (OTC speciation: 1% cation, 98% zwitterion, 1% anion). No detectable sorption was measured for cellulose or lignin sorbents that contain few metal-complexing ligand groups. Sorption to Aldrich humic acid increased from "clean" < "dirty" (no cation exchange pretreatment) < Al-amended < Fe(III)-amended clean humic acid with K_d values of 5500, 32 000, 48 000, and 250 000 L kg^{-1} C, respectively. Calcium amendments of clean humic acid suggested that a portion of the sorbed OTC was interacting by cation exchange. Oxytetracycline sorption coefficients for all humic acid sorbents were well-correlated with the total sorbed Al-plus-Fe(III) concentrations (r^2 = 0.87, log-log plot), suggesting that sorption by ternary complex formation with humic acid is important. Results of this research indicate that organic matter may be an important sorbent phase in soils and sediments for pharmaceutical compounds that can complex metals by the formation of ternary complexes between organic matter ligand groups and pharmaceutical ligand groups.

The importance of natural organic matter as a sorbent for polar environmental contaminants, such as pharmaceutical compounds, has been overlooked somewhat in the effort to understand the mechanisms governing solid-water exchange of these compounds. The classic hydrophobic partition model, in which the compound octanol–water distribution coefficient is used as a proxy for partitioning between organic matter and water (Chiou et al., 1979; Karickhoff et al., 1979), fails to account for the full extent of polar pharmaceutical compound sorption to soil and sediment samples (Tolls, 2001). To explain the order of magnitude differences between observed sorption coefficients and those predicted using octanol–water distribution coefficients, researchers have investigated pharmaceutical sorption to other soil/sediment components, including various clay minerals (Martin and Gottlieb, 1952; Pinck et al., 1961; Porubcan et al., 1978; Figueroa et al., 2004; Kulshrestha et al., 2004) and oxide solids (Figueroa and MacKay, 2005; Gu and Karthekiyan, 2005) in isolated systems. Pharmaceutical interactions with clay minerals and oxide particles occur through electrostatic ion exchange and ligand complexation interactions. Such interactions may be possible mechanisms for pharmaceutical sorption to organic matter phases that contain cation exchange groups and complexed metals. Given the high organic matter content of manure lagoons and wastewaters that are the sources of many pharmaceutical compounds introduced to the environment, there is a need to understand the mechanisms by which such polar compounds may sorb to natural organic matter phases.

A review of literature studies suggests that metal-bridging may be the most important mechanism of interaction for pharmaceutical compounds with organic matter. Several pharmaceutical sorption studies conducted using isolated organic matter phases from soils or manure have found measured organic carbon (OC)–normalized sorption coefficients (K_{oc}^{obs}) that were up to hundreds or thousands of times greater than estimates (K_{oc}^{est}) using octanol–water distribution coefficients, even after accounting for pH effects on octanol–water partitioning (Sithole and Guy, 1987; Schmitt-Kopplin et al., 1999; Holten Lutzhøft et al., 2000; Loke et al., 2002). For example, norfloxacin sorption to soil humic acid at pH 9.2 had a K_{oc}^{obs} of 1050 L kg^{-1} C (Schmitt-Kopplin et al., 1999), whereas the predicted K_{oc}^{est} was only 0.11 L kg^{-1} C (log K_{oc}^{est} = 0.84 log K_{ow} + 0.41; Karickhoff, 1981) using an octanol–water distribution coefficient of 0.02 at this pH (Takács-Novák et al., 1992). Similarly, OTC sorption to manure particles at pH 7.8 occurred with a K_{oc}^{obs} of 200 L kg^{-1} C, which was much greater than the K_{oc}^{est} of 0.3 L kg^{-1} C calculated using an octanol–water distribution coefficient of 0.08 (Herbert and Dorsey, 1995). Most of these studies applied no specific treatments to remove strongly bound cations from the organic matter sorbents, and most of the sorbates are known to complex metals in solution (Abd El Wahed et al., 1984; Machado et al., 1995; Turel and Bukovec, 1996; Djurdjevic et al., 2000; Schneider, 2001). Thus, it is possible that formation of ternary complexes between the pharmaceutical sorbate and strongly bound cations in the organic matter could explain the high amounts of sorption observed, relative to estimates using only hydrophobic partitioning.

Cation cross-linking is known to alter the hydrophobic nature of natural organic matter (Yuan and Xing, 2001; Lu and Pignatello, 2004); however, this effect does not appear to be of sufficient magnitude to account for differences between observed and estimated sorption coefficients of pharmaceutical compounds for isolated organic matter phases. Lu and Pignatello (2004) found that sorption of nonpolar organic compounds to soil humic acid shifted from partitioning into rubbery regions to hole-filling of glassy regions with Al^{3+}–satura-

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Abbreviations: MWCO, molecular weight cut-off; OC, organic carbon; OTC, oxytetracycline; TOC, total organic carbon.

tion of humic acid by the same Al treatment method used in our study. Overall OC-normalized sorption coefficients for naphthalene and 1,2,4-trichlorobenzene decreased as much as 30% for Al^{3+}–saturated humic acid compared with H^+-saturated humic acid. Similar trends were suggested in the work of Yuan and Xing (2001). Schlautman and Morgan (1993) reported no change in polycyclic aromatic hydrocarbon sorption to Suwanee River humic acid with 1 m*M* Ca^{2+} at pH 4 and a slight increase with 1 m*M* Ca^{2+} at pH 7. Although these studies do not indicate clearly which sorbates will have decreased or enhanced hydrophobic interactions with cross-linked organic matter, they do suggest that cation cross-linking produces relatively small changes to hydrophobic interactions of sorbates with organic matter. Thus, the order of magnitude greater K_{oc}^{obs} than K_{oc}^{est} for pharmaceutical compounds must result from interactions different than from strict hydrophobic partitioning with organic matter.

Additional evidence in support of a metal-bridging mechanism for pharmaceutical sorption to organic matter is provided by pH studies of organic matter sorption and by drug pharmacokinetics. Holten Lutzhøft et al. (2000) showed increased flumequine and oxolinic acid sorption to humic acid with increasing pH, despite the unfavorable electrostatic repulsion expected from the increased deprotonation of both the sorbate and the sorbent. Such a trend of increased sorption to organic matter with increasing pH has been observed for metal complexation to deprotonated ligand groups (Dzombak and Morel, 1990) and thus, formation of a cation bridge between the sorbate and sorbent could be favored at high pH values. Furthermore, ternary complex formation between pharmaceuticals, metal cations, and proteins or nucleic acids are known to be important for antibiotic activity (Schneider, 2001; Turel, 2002) and thus, it is reasonable to hypothesize that similar complexes could form with soil, sediment, and manure organic matter.

The purpose of this research was to investigate whether OTC sorption to organic matter includes a metal-bridging mechanism. Several authors have alluded to possible ternary complex formation between pharmaceutical compounds, metal cations, and organic matter in their studies (Sithole and Guy, 1987; Tolls, 2001); however, no metal concentrations were reported for the sorbents or systems in any of the reviewed studies. Metal-bridging can only occur for pharmaceutical compounds that have functional groups that can complex metal ions in solution. Thus, we chose OTC (Fig. 1), a high-use veterinary antibiotic, as our test sorbate because tetracyclines are known to complex with divalent and trivalent cations (Abd El Wahed et al., 1984; Machado et al., 1995). Our test sorbents were metal-loaded cation exchange resin, cellulose, lignin, and humic acids with varying degrees of metal loading. If metal-bridging is an important sorption mechanism for pharmaceutical compounds that can complex metals in solution, we expect to observe increased sorption to cation exchange resin as: (i) the metal concentration was increased, and (ii) as the stability of the metal–cation complex increased. We also expect higher sorption coefficients for OTC using organic matter with bound metals than for organic matter that could not complex metals.

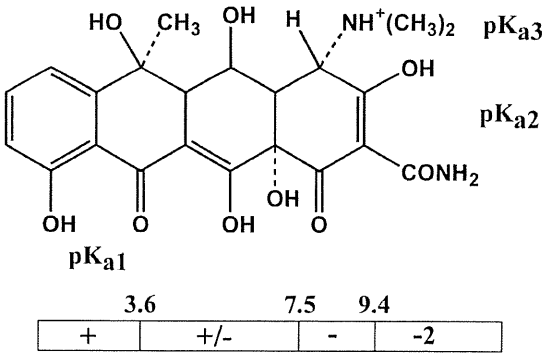

Fig. 1. Structure and chemistry of oxytetracycline (OTC). Bar shows dominant species charge as a function of pH where vertical bars represent pK_a values (Tavares and McGuffin, 1994).

METHODS AND MATERIALS

Materials

Oxytetracycline hydrochloride was used as received from USB Corporation (Cleveland, OH). Chelex-100 resin (Na^+ form, 300–1180 μm) was obtained from Bio-Rad (Hercules, CA). Cellulose (microcrystalline, colloidal), lignin (organosolv), and humic acid were all from Aldrich (Milwaukee, WI). Chloride salts of Ca and Fe(III) and sulfate salts of Al and Cu were from Fisher Scientific (Fair Lawn, NJ). pH adjustments were made with hydrochloric acid, sodium hydroxide, and sodium acetate from Fisher and MOPS (4-morpholinepropane sulfonic acid, sodium salt) from Aldrich. High purity water (18 *M*Ω-cm) was prepared on-site with a Barnstead NANOpure Diamond low-TOC purification system.

Analytical

Oxytetracycline concentrations were quantified by HPLC using a LiChrospher 100 RP-18 endcapped column (5 μm, 4.6 mm i.d., 150 mm) and HP1050 with diode array detector. Isocratic elutions were performed using 80% phosphate buffer (20 m*M*, pH 2.5)/20% acetonitrile at a flowrate of 1 mL min^{-1}. Compound concentrations were quantified by absorbance at 360 nm wavelength. Peak identities were confirmed by comparison of peak spectra with metal-free and metal-containing spectra obtained with a CARY 50 UV-Vis spectrophotometer (Varian) operated in scan mode (200–600 nm).

Quantities of organic matter in solution were determined by high-temperature combustion and CO_2 detection (Shimadzu TOC-5000A with high sensitivity catalyst).

Metal concentrations in aqueous solutions were quantified by inductively coupled plasma–mass spectrometry (ICP–MS) (PE SCIEX, Elan 6000, Environmental Research Institute, Storrs, CT) following USEPA methods 3010A (Acid Digestion of Aqueous Samples) and 6020A (Inductively Coupled Plasma–Mass Spectrometry).

Sorbent Preparation

Humic acid suspensions were created by adding dry powder to 0.01 *M* NaOH and mixing for 24 h. The suspension was centrifuged at 8000 × *g* for 30 min to remove mineral particles. An aliquot of the supernatant was saved as "dirty" humic acid. The remaining supernatant was further treated by acidifying to

pH 2 with HCl for 24 h and centrifuging at 8000 × *g* for 30 min to precipitate the humic acid. The precipitated humic acid was resuspended in 0.01 *M* NaOH and the acid–base treatment sequence was repeated twice more. The precipitated humic acid was resuspended in pH 7 water and dialyzed (1000 MWCO) against high purity water containing strong cation-exchange resin (Dowex 50W8–100, H-form, Aldrich) until the external solution TOC concentration was <0.5 mg L^{-1} C. The dialyzed suspension was designated as "clean" humic acid. The dirty humic acid, cellulose, and lignin were also dialyzed against high purity water until the external solution had TOC < 0.5 mg L^{-1} C, but no cation exchange resin was added. No other treatments were applied to the cellulose and lignin. Dialyzed organic matter suspensions were stored at 4°C and used within 2 d.

Sorption Experiments

Chelex-100 sorption experiments were prepared by adding 0.36 g (dry wt.) of resin to 40 mL of 10 m*M* MOPS buffer containing different concentrations of $CaCl_2$ (up to 4 m*M*) or $CuCl_2$ (up to 0.8 m*M*). pH was adjusted to 7.6 and tubes were mixed for 1 wk. Oxytetracycline was added from a freshly prepared stock solution to an initial concentration of 0.086 m*M* (40 mg L^{-1}). Several tubes were used for kinetic monitoring of equilibrium sorption conditions. Two types of controls tubes were included: (i) metal-free controls to account for nonspecific interactions of OTC with Chelex-100 resin, and (ii) resin-free controls to account for nonsorptive compound losses. Resin-free controls were prepared without metals and also with metal-containing supernatant that had been pre-equilibrated with resin for 1 wk. All experiments were conducted at 26°C. The strong buffer capacity of the resin resulted in some initial trials with final pH values of 9 or 10. These data were used to evaluate pH effects.

Organic matter sorption experiments were conducted using the dialysis tube method (Carter and Suffet, 1982). Dialysis tubing (1000 MWCO, Spectra/Por 6, Spectrum Laboratories, Rancho Dominguez, CA) was pretreated according to the manufacturer's instructions by soaking in high purity water for 2 h, then heating to 60°C in 1 m*M* EDTA–2% $NaHCO_3$ solution for 3 h. This process was repeated a second time and followed by extensive high purity water rinsing. The treated dialysis tubing was stored in high purity water at 4°C until use. Samples were assembled in triplicate by first preparing 200 mL of organic matter suspension (pH 5.5, 10 m*M* acetate buffer) in brown light-block polyethylene plastic bottles to give final concentrations of 53 mg C L^{-1} cellulose, 108 mg C L^{-1} lignin, 19.5 mg C L^{-1} clean humic acid, or 21 mg C L^{-1} dirty humic acid. Additional clean humic acid samples were prepared to contain organic matter complexes with individual cations and minimal formation of cation hydrolysis species. Following the method of Mason et al. (2000) and Vilgé-Ritter et al. (1999a), metal salts were added to clean humic acid to give 0.146 or 1.46 m*M* total Ca, Al (1.46 m*M* only), or Fe(III). The organic matter suspensions were mixed rapidly (140 rpm) for 3 min followed by slow mixing for 30 min. Formation of only mono-, di-, or trimer metal polymer complexes with organic matter functional groups after following this experimental protocol has been verified with spectroscopic observations in previous studies (Vilgé-Ritter et al., 1999b; Mason et al., 2000). Oxytetracycline was then added to the bottles to give an equivalent initial concentration of 0.084 m*M*. A 20-mL aliquot of freshly prepared OTC stock solution at pH 5.5 in 10 m*M* acetate buffer was placed in a knotted length of dialysis tubing and transferred to the bottles containing the organic matter suspensions. The sample bottles were mixed end-over-end gently for 5 d at 26°C, as determined in preliminary equilibration studies. An aliquot of the dialysis bag contents was analyzed for OTC concentration by HPLC. The use of an organic buffer and a chromophore-containing sorbate prohibited TOC or UV-Vis spectroscopy analyses from being used to quantify sorbate transfer across the dialysis bag. Consequently, dialysis pretreatment of the sorbents (1000 MWCO, see Sorbent Preparation) was conducted to minimize the amount of organic matter (<0.5 mg C L^{-1}) that would pass into the bag during the sorption equilibration. No organic matter transfer into the bags was observed during the sorption experiments. Several control tubes were assembled by the same procedure, but contained only pH 5.5 acetate buffer to account for nonsorptive OTC losses. Sorption losses to the polypropylene sample vessels were negligible (Figueroa et al., 2004). An aliquot of the dialysis bag contents was also analyzed for total metal concentrations by ICP–MS. These measurements were assumed to represent the total dissolved metal concentrations. The external organic matter suspension was analyzed for total metal concentrations so that sorbed metal concentrations in the organic matter could be calculated from the difference between the metal concentrations in the external solution and the dialysis bag contents.

Assuming that the dissolved OTC concentration inside the dialysis bag (no organic matter) was equal to the dissolved OTC concentration outside of the bag (with organic matter) at the end of the experiment, sorbed OTC concentrations, C_s (mol kg^{-1} C) were calculated as follows:

$$C_s = (C_{ctl} - C_w)V_w/M_s \quad [1]$$

where C_{ctl} (mol L^{-1}) is the OTC concentration in a sorbent-free control prepared by the same procedure; C_w (mol L^{-1}) is the total aqueous phase concentration of dissolved OTC species inside the bag at the end of the experiment; V_w (L) is the total volume of water in the bottle and dialysis bag, and M_s (kg C) is the mass of organic matter in the bottle. Desorption steps were not performed because the total sorption-plus-desorption time would have resulted in unacceptable (>40%) mass loss of OTC. Sorbed OTC concentrations were used to calculate sorption coefficients as follows:

$$K_d = C_s/C_w \quad [2]$$

where K_d has units of L kg^{-1} C because our measure of sorbent mass was by total organic C analysis. Note that the K_d values calculated from Eq. [2] are effective OTC distribution coefficients because the total dissolved OTC concentrations were used in all calculations, without accounting for aqueous phase speciation. Thus, K_d values are specific to the total metal, sorbate, and sorbent masses used in these experiments.

RESULTS AND DISCUSSION

Sorption to Metal-Chelating Resin

Ternary complex formation by metal-bridging between OTC and sorbent ligand groups was demonstrated using Chelex-100 resin. Experiments were performed at pH 7.6 ± 0.1 to minimize electrostatic attraction between OTC (Fig. 1) and negatively charged resin sites. In the absence of complexing metal cations, OTC concentrations in resin tubes were 6 to 10% lower than concentrations in resin- and metal-free controls, indicating that cation exchange still accounted for a small amount of OTC sorption to the beads at pH 7.6 (Fig. 2a, Na data). The addition of Cu or Ca to the resin resulted in >10% decreases in OTC concentrations, relative to controls, with more OTC sorption at increasing metal/

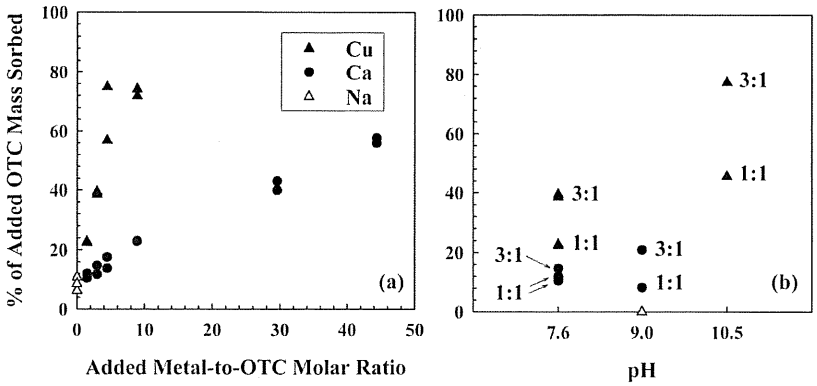

Fig. 2. Sorption of oxytetracycline (OTC) to Chelex-100 resin with presorbed Cu, Ca, or Na cations: (a) at pH 7.6, and (b) variation with pH where numbers denote metal/OTC ratio. Note that metal/sorbate ratios were computed from the initial masses added to the tubes.

sorbate molar ratios (Fig. 2a). For each metal/sorbate ratio, OTC sorption was greater for the Cu systems than for the Ca systems. Greater OTC sorption to Cu-containing resin than to Ca-containing resin is consistent with a metal-bridging mechanism of sorption: Cu forms stronger complexes both with the resin sites and with OTC than does Ca. Distribution coefficients for Cu and Ca with Chelex-100 resin have been reported to be 10^5 L kg^{-1} and 10^3 L kg^{-1} (Leyden and Underwood, 1964), respectively, above pH 6 for systems with metal/resin molar ratios similar to our system. Stability constants for 1:1 complexes of Cu and Ca with OTC are $10^{12.4}$ (Jezowska-Bojczuk et al., 1993) and $10^{4.5}$ (Lambs et al., 1988), respectively. Consequently, the trends of OTC sorption with Chelex-100 suggest that OTC sorption to ligand-rich sorbents will occur in systems that have metal cations complexed to sorbent ligand sites.

pH trends in OTC sorption to Chelex-100 resin were also consistent with ternary complex formation between OTC, metal ions, and resin sites, rather than OTC interactions by cation exchange (Fig. 2b). No significant difference in OTC concentration was observed between Na-resin tubes and resin-free controls at pH 9, indicating that cation exchange was not an important contributor to OTC sorption at high pH (Fig. 1). Oxytetracycline mass sorbed to Cu- and Ca-containing resin increased when the pH was raised. At pH 10.5, the mass fraction of OTC sorbed at a Cu/sorbate ratio of 1 was 45% and at a ratio of 3 was 77%, compared with 22 and 38%, respectively, at pH 7.6. Similar increases in OTC sorption were observed for Ca-containing resin at pH 9 (Fig. 2b). Such trends of increased sorption with pH would not be observed for cation exchange interactions of OTC with the Chelex-100 resin, especially at pH values >9.4 (Tavares and McGuffin, 1994) when the majority of OTC molecules are deprotonated at the dimethyl amine group (Fig. 1). Increased metal complexation, and hence ternary complex formation, is expected at high pH because of decreased competition from H^+ both for OTC acid groups and for resin sites. In contrast, at the lower pH conditions of 5 to 8 that are typical of environmental systems, ternary complex formation between OTC sorbate and sorbent ligand groups would be attenuated by competition from protons, resulting in a decrease of sorption by metal bridging to some extent.

Sorption to Organic Matter

Oxytetracycline sorption was measured for a variety of organic matter types characterized by differing abilities to complex metals and different concentrations of metals complexed to organic matter ligand groups. Although ternary complex formation between OTC, metal cations, and organic matter ligand groups is expected to be greatest around pH 8 (lowest competition from protons or hydroxide ions), these experiments were conducted at pH 5.5, which is more representative of typical soil or sediment conditions and where the dominant aqueous phase species was the zwitterion (Fig. 1). Previous experiments with clays have shown that cation exchange interactions may still be significant at pH 5.5, despite the low abundance of cation species (Figueroa et al., 2004). The use of pH 5.5 enabled these observations to complement the expanding study of OTC interactions with other sorbents that have been conducted at pH 5.5 (Figueroa et al., 2004; Figueroa and MacKay, 2005; Jones et al., 2005).

The lowest OTC sorption coefficients for organic matter samples were measured for those sorbents with the poorest abilities to complex metals (Table 1). Cellulose structure has repeating cellobiose saccharide units with functional groups that are poor ligands, and thus little sorption to this sorbent would be expected if metal-bridging were an important interaction mechanism. Sorbed metal concentrations were below detection limits for this sorbent (Table 1). There was no significant difference (95% CI) between the dissolved OTC concentrations in cellulose-containing samples (64 μM) and sorbent-free controls (64 μM, Table 1, footnote †); therefore, no sorption coefficient K_d was calculated for cellulose. Lignin also has a poor ability to complex metals due to the lack of good chelating groups on the cross-linked phenylpropanoid monomers that make up this polymer. Since lignin could be chemically altered to contain metal-complexing thiol groups during the Kraft extraction process (Lin and Lebo, 1996), solvent-extracted "organo-solv" lignin was chosen for this study. Concentra-

Table 1. Oxytetracycline sorption coefficients and measured metal concentrations for systems with different organic matter types.

Sorbent	pH	C_w†	K_d‡	Dissolved metal conc.§				Sorbed metal conc.§			
				Ca	Mg	Al	Fe	Ca	Mg	Al	Fe
		μM	L kg^{-1} C	μM				mmol kg^{-1} C			
Cellulose	5.7	64	no sorption: 95% CI	11	–¶	8	–	<0.1	<0.4	<0.2	<0.1
Lignin	5.6	64	no sorption: 95% CI	6	–	6	–	<0.1	<0.4	<0.1	<0.1
				Humic acid							
Dirty	5.5	40	32 000 ± 190	23	5	4	–	3 040	390	600	1 480
Clean	5.4	66	5 500 ± 600	4	–	–	–	<50	<20	35	140
				Clean humic acid with metal addition							
Low Ca^{2+}	4.9	66	5 300	94	–	–	–	2 710	<20	35	140
High Ca^{2+}	5.4	68	2 980 ± 240	1 060	–	–	–	20 000	<20	35	140
High Al^{3+}	5.2	45	48 300 ± 15 500	6	–	80	–	<50	<20	71 000	140
High Fe^{3+}	5.2	15	250 000 ± 40 000	8	–	–	10	<50	<20	35	74 400

† Final control concentrations were 64 μM for cellulose, lignin, and dirty humic acid; and 74 μM for all other cases.
‡ N = 3 replicates, except single measure for low Ca^{2+}.
§ Triplicate analyses varied by <10%, except dirty humic acid which varied up to 20%.
¶ Denotes value below detection limit of 4 μM for Mg and Al or 2 μM for Fe.

tions of metals sorbed to lignin were below the detection limits at the end of the equilibration time. No significant difference (95% CI) in dissolved OTC concentrations was observed between the lignin-containing samples and sorbent-free controls (Table 1). Note that a K_{oc}^{est} of 0.4 L kg^{-1} C was estimated for OTC at pH 5.5 (98% as neutral zwitterion) using the relationship log K_{oc}^{est} = 0.84 log K_{ow} + 0.41 (Karickhoff, 1981) with a log octanol–water distribution coefficient of −0.96 previously measured at this pH by the shake-flask method (Krach, 2002). Actual hydrophobic partitioning of OTC to cellulose is likely somewhat less given that cellulose is a poor sorbent phase, even for nonpolar sorbate compounds (Rutherford et al., 1992; Xing et al., 1994). Possible interactions of OTC with cellulose or lignin by hydrophobic partitioning were below the levels of quantitation at the sorbent concentrations used (53 mg C L^{-1} for cellulose, 108 mg C L^{-1} for lignin).

Detectable OTC sorption was observed for humic acid (19.5–21 mg C L^{-1}), a sorbent with functional groups that are known to complex metals (Table 2). Although humic acid chemical structures are less well-defined than for cellulose and lignin, it is generally believed that a distribution of metal-complexing phenol and carboxylic acid ligand groups are present at an abundance of about 1 functional group per 10 C atoms (Morel and Hering, 1993). There is debate in the literature as to whether or not this model is appropriate for Aldrich humic acid, since complexation constants and K_{oc} values for this sorbent differ widely from those of organic matter isolated from well-classified soils or aquatic sources. Aldrich humic acid was used for this study instead of organic matter from a well-classified source because several-gram quantities of humic acid were required and the Aldrich material was available in semi-purified form to demonstrate the concept of OTC sorption by metal-bridging without labor intensive extraction steps. Additional treatments, including with strong cation-exchange resin, gave clean humic acid that had significantly reduced, but still measurable, metal concentrations of Al and Fe (Table 1). Clean humic acid sorbed between 8 and 10% of the added OTC mass giving a K_d of 5500 ± 600 L kg^{-1} C. The OTC sorption coefficient for clean humic acid was significantly less than the K_d of 32 000 ± 190 L kg^{-1} C measured for dirty humic acid that had no acidification or cation-exchange resin treatments. Clearly, manipulating the metal content in the humic acid had large effects on the sorption of OTC; thus, OTC sorption to organic matter was consistent with a metal-bridging mechanism of interaction.

Table 2. Logarithms of the formation constants for the 1:1 metal–ligand complexes with organic ligand analogues of humic acid functional groups and oxytetracycline (25°C, zero ionic strength).

Ligand	Ca^{2+}	Al^{3+}	Fe^{3+}	Reference
Malonate (fulvic acid)	2.4	–†	9.3	Morel and Hering, 1993
Salicylate	0.4	14.2	17.6	Morel and Hering, 1993
Phthalate	2.4	5.0	–	Morel and Hering, 1993
Oxytetracycline	4.46	–	–	I = 0.15 M; Lambs et al., 1988

† Dash denotes values not reported in literature.

Sorption to Metal-Amended Humic Acid

Clean humic acid was amended with common soil cations (Ca, Al, or Fe) to investigate how OTC sorption to organic matter would be affected by metals with differing organic ligand formation constants. By analogy to the Chelex-100 resin, Al and Fe(III) exhibit much greater formation constants with organic matter ligand analogs, than does Ca (Table 2), and thus might be expected to control OTC sorption to humic acid through ternary complex formation. Aluminum and Fe(III) formation constants have not been reported for OTC; however, the strong complexes formed between OTC and these cations have been exploited in analytical methods (Monastero et al., 1951; McCracken et al., 1995). Aluminum and Fe(III) formation constants for anhydrotetracycline reported for 1:1 metal/ligand complexes with various protonated ligand species suggest several orders of magnitude stronger interactions between Al and Fe(III) than between Ca and Mg (Machado et al., 1995; Schneider, 2001). Thus, it was expected that OTC would also have a more favorable interaction in ternary complexes with Al and Fe(III) than with Ca.

Low and high metal amendments to clean humic acid were tested using initial metal salt concentrations of 0.146 and 1.46 mM, respectively. These concentrations were chosen to maintain the organic matter/metal ratio

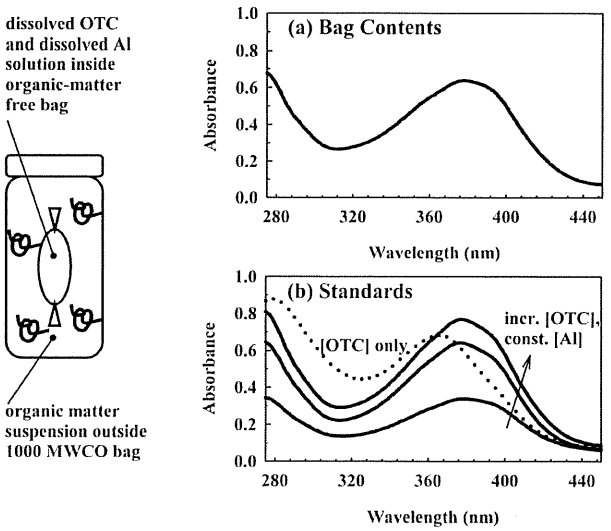

Fig. 3. Schematic of oxytetracycline (OTC) concentration measurements for metal-amended humic acid at pH 5.2: (a) sample spectrum of bag contents with dissolved OTC and 80 μM dissolved Al, and (b) comparison spectra of OTC standards prepared with 80 μM total Al. The dotted line in (b) shows the OTC spectrum in the absence of Al.

that was employed by Mason et al. (2000) and Vilgé-Ritter et al. (1999a) in their careful study of Al and Fe complex formation with organic matter. These researchers demonstrated that the experimental protocol yielded mono-, di-, and trimer metal polymer complexes with organic matter functional groups. We assume that by following the same protocol, similar complexes between the added metal cations and organic matter functional groups in our systems were achieved. Iron- and Al-amended clean humic acid suspensions were slightly cloudy compared with unamended clean humic acid; however, no flocculation was observed during our experiments. Possible analytical interferences from humic acid colloids were minimized by the dialysis pretreatment of clean humic acid that removed the <1000 MW fraction before metal amendment.

Several checks were conducted to evaluate whether dissolved metal–OTC complexes in any of our test systems could cause overestimation of the fraction of compound sorbed to metal-amended organic matter. Early eluting complexes of OTC with metals could not be quantified reliably by HPLC using external standards. Furthermore, co-elution of metal complexes with uncomplexed OTC could introduce quantification errors by causing shifts in the peak spectra to lower absorbances at the wavelengths monitored for uncomplexed OTC. Thus, peak area response factors in solutions with OTC and Ca (up to 1 M) were compared with those of OTC in Ca-free solutions and showed <5% variation, indicating that our HPLC method yielded accurate measures of total dissolved OTC in systems with Ca. Such agreement between metal-containing and metal-free systems was not observed for the Al and Fe systems and so a UV-Vis technique was used to quantify the total dissolved OTC (Fig. 3). In these cases, sorbent-free standards were prepared at the same total dissolved metal concentration as measured in the dialysis bag contents, but containing differing amounts of OTC up to the initial sorbate concentration of 84 μM. The absorbance spectra of these Al- or Fe-containing standards were used to develop OTC-metal complex response factors for each test system. Figure 3b shows example standards spectra for the Al case (solid lines) with the characteristic shift of absorbances to longer wavelengths (Machado et al., 1995), relative to the no-Al case (dotted line). The spectra of OTC in the metal-containing controls showed no shifts when normalized to the total OTC concentration, indicating that the same OTC metal complex(es) was dominant over the full range of OTC concentrations. Total OTC concentrations in the samples from the sorbent-containing tubes were then obtained from adsorption measurements (e.g., Fig. 3a) using the system-specific response factors (e.g., solid lines in Fig. 3b). Iron-containing controls showed the expected shift in absorbance to shorter wavelengths (Machado et al., 1995) from 365 to 354 nm and were used to calculate dissolved concentrations of OTC in dialysis bags of the high Fe samples. The solution phase spectra for the low Fe case showed a shift to 317 nm such that sample spectra did not match that of Fe-containing controls; therefore, K_d was not calculated. Aluminum was omitted from the low concentration tests because of limited quantities of clean humic acid prepared from the same batch.

The Ca amendments of the clean humic acid suggested that a portion of the sorbed OTC was interacting with the humic acid by cation exchange at the pH 5.5 used in the organic matter studies. The factors of sorbed Ca concentration and pH must both be considered to evaluate the significance of OTC cation interactions with organic matter. For cation exchange, lower sorbed OTC concentrations would be expected for organic matter samples with higher sorbed Ca concentrations, while lower pH favors high sorption by OTC. Therefore, according to the sorbed Ca concentration (Table 1), sorption coefficients for OTC should follow the order clean > "low Ca^{2+}" > "high Ca^{2+}" humic acid. High Ca^{2+} humic acid had the lowest OTC sorption coefficient by a factor of about 2; however, low Ca^{2+} humic acid and the clean humic systems had similar sorption coefficients (Table 1). The pH of the low Ca^{2+} humic acid system was lower than clean humic acid, which may have offset some of the competition between Ca^{2+} and OTC resulting in coincidently similar sorption coefficients. At pH 4.9 (low Ca^{2+} system) and pH 5.4 (clean) approximately 4.8 and 1.5%, respectively, of the dissolved OTC exists as a cation. Note that solution phase complexes between OTC and 1060 μM Ca^{2-} in the case of high Ca^{2+} humic acid were insignificant at pH 5.5 (Table 2).

Although sorption observations for the low Ca^{2+} vs. high Ca^{2+} cases were consistent with a cation exchange interaction of OTC with humic acid, an additional interaction was required to resolve the dirty humic acid with the clean and low Ca^{2+} systems. The dirty humic acid sorption coefficient was six times greater than that of

the clean humic acid, but the dirty humic acid had 2 orders of magnitude greater Ca concentration (plus other sorbed cations) (Table 1). The latter would have resulted in lower sorption coefficients in the dirty humic acid system, if cation exchange was the only sorption mechanism. Similarly, the dirty humic acid had a six times greater K_d than that of the low Ca^{2+} humic acid, even though the system pH was greater for the dirty humic acid, which is also inconsistent with a cation exchange mechanism. However, the trends in dirty vs. clean or low Ca^{2+} humic acid are consistent with the higher sorbed Al and Fe concentrations of the dirty humic acid; Al and Fe can enhance sorption by serving as metal bridges.

Addition of Al or Fe(III) salts to clean humic acid to increase the bound concentration of these potential bridging cations increased OTC sorption to this sorbent. Amendment of the clean humic acid with 1.46 m*M* of Al resulted in an increase in sorbed Al concentration by a factor of 2000 compared with a factor of 6 increase in sorbed OTC concentration, or a factor of 9 increase in K_d over the unamended clean humic acid case (Table 1). Amendment with Fe(III) increased sorbed Fe concentrations by a factor of 530 while the sorbed OTC concentration was 10 times greater and K_d was 45 times greater than for unamended clean humic acid. Such increases in sorbed OTC were not consistent with alterations of the hydrophobic nature of the organic matter by cation cross-linking since Al-saturation of humic acid actually decreased C-normalized nonpolar organic compound sorption, but only by 30% (Lu and Pignatello, 2004). The trends in OTC sorption to the Al- or Fe(III)-amended humic acid were consistent, however, with observations for the dirty humic acid. Log-log plots of OTC K_d vs. total metal concentration sorbed to the humic acid sample showed good correlation between the total sorbed Al-plus-Fe(III) concentrations (r^2 = 0.87) (Fig. 4), whereas inclusion of Ca and Mg in the calculation of total sorbed metal gave a much poorer correlation between log K_d and log sorbed metal concentration (r^2 = 0.39). Similar trends are observed of the log distribution coefficient of complexed/dissolved ligand varying linearly with log total metal concentration for aqueous systems with varied metal/ligand ratios. The correlation between OTC log K_d and log of total sorbed Al-plus-Fe(III) for all humic acid systems suggests that manipulation of the metal content in humic acid by the addition of cations with high stability constants (Al and Fe vs. Ca) increases the extent of OTC sorption to natural organic matter by complexation at typical soil pH values (e.g., 5.5). These findings, when considered together with the tendency for OTC to sorb to metal-loaded cation exchange resin, indicate that an important mechanism for OTC interaction with natural organic matter is by ternary complex formation with tightly bound polyvalent metal ions.

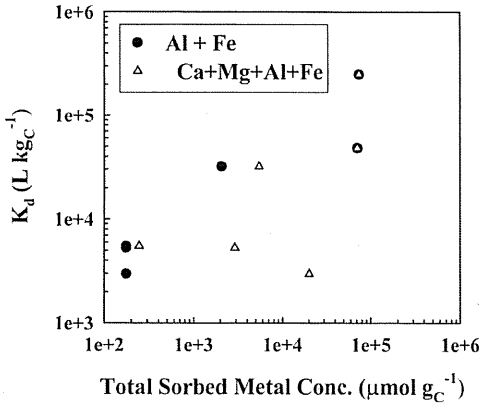

Fig. 4. Correlation between K_d and sorbed metal concentration for all humic acid sorbents. Solid dots are for total sorbed Al plus Fe concentrations (r^2 = 0.87). Open triangles are for total sorbed Ca plus Mg plus Al and Fe concentrations (r^2 = 0.39). Note that the high concentration points (105 mmol kg^{-1} C) are coincident for both data sets because sorbed Ca and Mg concentrations were negligible for these cases (high Al, high Fe; Table 1).

Environmental Significance

The results of our research demonstrate that organic matter phases in soils and sediments may be important sorbents for pharmaceuticals with metal-complexation chemistry. More research is required to quantify thermodynamic constants (K) for ternary organic matter–metal–sorbate complexes so that accurate a priori predictions of pharmaceutical distributions between sorbent and aqueous phases can be made. The introduction of polyvalent metal cations to soil or sediment pore waters could have two possible impacts on pharmaceutical sorption: If the ternary complex is more stable than dissolved metal–pharmaceutical complexes, sorption of the pharmaceutical compound would result, thereby limiting the compound mobility and bioavailability. Our results suggest that that this would be the case for polyvalent cations, such as Al and Fe(III), that are strongly complexed by both organic matter and sorbate ligand groups. Competition between sorbed and dissolved complexes with the pharmaceutical compound could occur for high concentrations of pore water cations, such as Ca, that are only weakly held by organic matter ligand groups. The exact distribution of pharmaceutical compounds between ternary- and dissolved complexes in each case will depend on the total concentrations of organic matter, sorbent ligand groups, and sorbate compound.

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Interaction of Tetracycline with Aluminum and Iron Hydrous Oxides

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The effect of solution chemistry (pH, sorbate-to-sorbent ratio, ionic strength (*I*)) and reaction time on the sorption of tetracycline to the hydrous oxides of Al (HAO) and Fe (HFO) was examined. Sorption to HAO increased with increasing pH up to pH 7 (no such trend for HFO) above which it decreased at higher pH values for both the hydrous oxides. Experimental results indicate that ligand-promoted dissolution is occurring during tetracycline sorption to these hydrous oxides. Ligand-promoted dissolution was more significant for HAO than HFO attributable to the difference in labile surface sites between these two sorbents. The ability of tetracycline to form strong complexes with Al and Fe will increase the solubility of these minerals. Sorption of tetracycline was quite rapid and equilibrium was achieved after 8 h. However, soluble metal (Me: Al or Fe) concentrations attained equilibrium only after 24 h. Ligand-promoted dissolution appears to be a two-step process; initially, 1:1 Me-tetracycline soluble complexes are formed and as the reaction progresses 2:1 complexes existed. Increasing *I* (from 0.01 to 0.5 M) decreased the sorption extent only at higher sorbate-to-sorbent ratios suggesting the dominance of inner-sphere type complexes at low equilibrium tetracycline concentrations. Spectroscopic evidence indicates that tricarbonylamide and carbonyl functional groups of tetracycline could be responsible for sorption to mineral surfaces. Our research findings will increase understanding of the environmental occurrence, fate, and transport characteristics of antibiotics, which are considered as emerging organic contaminants.

Introduction

Antibiotics are used extensively as human infection medicine, veterinary medicine, and husbandry growth promoters. In the United States (U.S.) alone, annual antibiotic production exceeded 50 million lbs by the late 1990s (*1*). Currently, over 70% of all the antibiotics manufactured are for agricultural use (*2*). Most of the antibiotics are poorly absorbed by human and animals after intake, with about 25–75% of added compounds leaving the organisms unaltered via feces or urine (*3*). Because of the dispersion of manure and sewage sludge in fields as fertilizers, antibiotics have the potential to reach soil and aquatic environments. In the first national reconnaissance survey on emerging organic contaminants conducted by the U.S. Geological Survey, antibiotics were detected in half of the sites from a network of 139 streams across 30 states (*4*). A total of six antibiotic compounds were detected including two sulfonamides, one tetracycline, fluoroquinolone, macrolide, and trimethoprim in our Wisconsin (WI) statewide survey of wastewater treatment plants.

Compared to other well-known xenobiotics such as pesticides, PAHs, and PCBs, there is very little information available on the fate and transformation of antibiotics in soil/water environments. A striking difference between the antibiotics and the above xenobiotics is in their hydrophobicity (log K_{ow} of well-known xenobiotics = 3–7) and aqueous solubility (10^{-5} to 0.1 mM for these xenobiotics). The high polarity (e.g., log K_{ow} = −1.97 to −0.47* for tetracycline, *pH-dependent (*5*)) and aqueous solubility (0.52–117* mM for tetracycline, also *pH-dependent (*6*)) of antibiotics can enhance their environmental mobility. Sorption processes, in particular, are important, since association of antibiotics with mineral particles and organic matter will determine their transportability in surface runoff, leachability through soils, and mobility in aquifers. Bioassay studies also revealed that the antibiotics would lose their antibacterial activity when they are adsorbed to soils (*7*, *8*).

Tetracyclines constitute one of the most important antibiotic families, ranking second in production and usage worldwide (*9*). Tetracyclines have been detected in soils (*5*, *10*, *11*), surface waters (*12*), groundwater samples collected near waste and wastewater lagoons (*13*), and hog lagoon samples (*14*). In our statewide survey of wastewater treatment plants in WI, the compound tetracycline was the most frequently detected antibiotic (among 25 antibiotics), being present in 80% of the wastewater influent and effluent samples. As shown in Figure 1a, tetracycline possesses tricarbonylamide (C-1:C-2:C-3), phenolic diketone (C-10:C-11:C-12), and dimethylamine (C-4) groups that confer a marked pH dependent behavior on solubility and lipophilicity. Tetracycline has three pK_a's (3.3, 7.68, and 9.69) causing it to exist as a cationic, zwitterionic, and anionic species under acidic, moderately acidic to neutral, and alkaline conditions (Figure 1b), respectively. The ionization behavior can be expected to significantly influence tetracycline sorption to soil components.

Hydrous oxides of aluminum (Al; HAO) and iron (Fe; HFO) are important mineral components of environmental particles. In highly weathered soils, HAO/HFO can account for as much as 50% of the total soil mass (*15*). Poorly crystalline or amorphous Al and Fe oxides are short-range-ordered minerals and exist as a discrete phase or in association with clay minerals and organic matter. Although they may not be found in large quantities in soils, these minerals are considered as major "sinks" for many inorganic and organic contaminants because of their high surface area and reactivity (*16–18*). Although there are a few studies on the interaction of tetracycline with clay minerals (e.g., *5*, *10*, *19*, *20*), investigations related to tetracycline-hydrous oxide systems are rare.

It is fairly well-known that sorption of chelating compounds, such as ethylenediaminetetraacetic acid (EDTA) and salicylic acid, possessing carboxylic and phenolic functional groups capable of forming stable complexes with metals (Me), will significantly increase the solubility of oxide minerals through ligand-promoted dissolution (*21–25*). Although there is a lack of agreement on the coordination sites, many studies have shown that tetracycline forms strong complexes with various Me cations in solution (*26–32*). Since tetracycline

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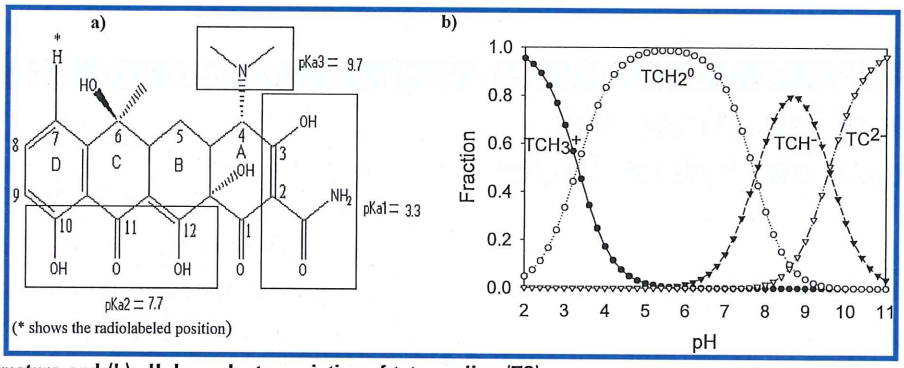

FIGURE 1. (a) Structure and (b) pH-dependent speciation of tetracycline (TC).

possesses similar functional groups and can form strong Me complexes, ligand-promoted dissolution can be expected to occur during its interaction with Al and Fe hydrous oxides.

The major goal of this study, therefore, was to understand the role of hydrous oxide minerals (HAO and HFO) in influencing the environmental fate and transformation of tetracycline. Specific focus was on the effect of important solution chemistry variables (pH, ionic strength (I), sorbate-to-sorbent ratio) and reaction time on the sorption of tetracycline to HAO and HFO. Spectroscopic methods were used to elucidate the mechanisms of interaction.

Materials and Methods

Materials. Tetracycline hydrochloride was obtained from Sigma-Aldrich Chemical (St. Louis, MO) and was used without any purification. Important physicochemical properties of tetracycline are (*5, 6*) MW: 444.43; aqueous solubility: 0.52–117 mM; log K_{ow}: −1.97 to −0.47; and pK_a: 3.3, 7.7, and 9.7. The tetracycline solution was thoroughly mixed with radiolabeled 7-^{3}H-tetracycline (specific activity = 5 Ci mmol^{-1}, American Radiolabeled Chemicals Inc.) to provide a stock with <1% of the total tetracycline ^{3}H labeled. Reagent ACS-grade ($AlCl_3$, $Fe(NO)_3$, 2-(4-morpholino)ethanesulfonic acid (MES), NaCl, and NaOH) and trace-metal grade HCl were purchased from Fisher Scientific (Fair Lawn, NJ). Fresh stock solutions were prepared for each experiment. 4-Epitetracycline hydrochloride (ca. 100% pure) was obtained from Fisher Scientific and was used to prepare analytical standards for high-performance liquid chromatography (HPLC) analysis.

Preparation and Characterization of Hydrous Oxides. HAO was synthesized by gradual neutralization of a 0.5 M $AlCl_3$ solution to pH 7 using 0.5 M NaOH (*16*). HFO was precipitated by dissolving $Fe(NO)_3$ salt using 0.01 M HCl and then rapidly increasing the solution pH to 7.0 using 0.1 M NaOH (*33*). The suspensions (both HAO and HFO) were then aged for 48 h at room temperature and centrifuged in 250 mL polypropylene bottles at 10300 relative centrifugal force (RCF) for 20 min. Supernatant solutions were discarded and the precipitate was dialyzed to remove Na^+ and Cl^- and then was freeze-dried. The hydrous oxides were characterized with BET surface area measurement, zeta potential for determination of zero point of charge (ZPC), and X-ray diffraction (XRD) analysis. The BET surface area and pH_{ZPC} for the hydrous oxides are, respectively, HAO: 386 ± 2 m^2 g^{-1} and 9.5; HFO: 322 ± 1 m^2 g^{-1} and 8.7. The surface area and pH_{ZPC} were in good agreement with values reported elsewhere (*17, 33, 34*). Since only broad peaks appeared in the XRD spectra, it confirmed that both the hydrous oxides are noncrystalline minerals.

Batch Sorption Experiments. The sorption experiments were conducted as a function of pH (4–10). For each batch system, 0.03 g (oven-dried mass) of hydrous oxide was added to tared 15-mL glass centrifuge tubes. Varying proportions of 0.01 M HCl and NaOH were used for pH adjustment. Stock tetracycline solution was added to obtain an initial concentration of 0.1 mM in a total suspension mass of 15 g. The centrifuge tubes were covered with Al foil to prevent exposure to light. Suspensions were equilibrated at 25 °C by end-over-end rotation at 7 rpm for 24 h. At the end of reaction period, suspensions were centrifuged at 5083 RCF (8000 rpm) for 20 min and the supernatant was analyzed for pH, tetracycline (HPLC), ^{3}H radioactivity (liquid scintillation counting, LSC), and Al/Fe (atomic absorption spectrometer, AAS). All experiments were conducted in duplicates. Control experiments (no HAO/HFO) were also conducted, using a similar preparatory scheme as indicated above, to account for losses as sorption to glass tubes and other reactions in solution.

Adsorption Isotherms. Adsorption isotherms were generated by reacting different amounts of tetracycline (5×10^{-4} to 1 mM) with 0.03 g of HAO/HFO (solid-to-solution ratio of 1:500). A constant pH of 5.3 was maintained using 0.04 M MES buffer, which has been shown to have minimal effect on the sorption of organic compounds to minerals (*35*). The isotherm experiments were conducted at three different I values (0.01, 0.1, and 0.5 M NaCl).

Dissolution Kinetic Experiments. To study the ligand-promoted dissolution of tetracycline with HAO/HFO, kinetic experiments were conducted at 25 °C for 7 days. The initial tetracycline concentration was 0.1 and 1 mM and suspension was kept at a constant pH of 5.3 using 0.04 M MES buffer. Sample aliquots were taken at specified time intervals (0.25, 0.5, 1, 2, 4, 8, 24, 72, and 168 h) and centrifuged immediately. Tetracycline, Al/Fe concentrations, and radioactivity in the supernatant were measured. The initial dissolution rates (D_i) were calculated following the method of Liang et al. (*36*); data for the first 8 h were fitted to a polynomial; the first derivative was then taken and the constant at time 0 was defined as D_i.

Analytical Methods. Tetracycline analysis followed the method of Sokol and Matisova (*37*). The concentration in supernatant solution was measured by reverse-phase HPLC (Gilson, WI) with a 4.0 × 250 mm Waters Spherisorb ODS-2 column followed by UV detection at 360 nm. The mobile phase was a mixture of 0.01 M oxalic acid–acetonitrile–methanol (45:35:20, v/v) in an isocratic system at a flow rate of 1 mL min^{-1}. The limits of detection and quantification are 0.5 and 1 mg L^{-1}, respectively. The correlation coefficient of the standard curve (n = 6) was greater than 0.995. Solution radioactivity was determined by LSC (Packard Tri-Carb 1600, MA). Metal (Al/Fe) concentrations in solution were measured by atomic absorption spectrometer (AAS) (GBC Inc., Australia); the detection limits for Al^{3+} and Fe^{3+} are 0.1 and 0.03 mg L^{-1}, respectively. Surface charge measurements were preformed using a Zeta Sizer 3000HS (Malvern, United Kingdom).

FTIR Analysis. Samples were obtained following the procedure used above for the batch sorption experiments but at a higher sorbate-to-sorbent ratio (2.25×10^{-3} M

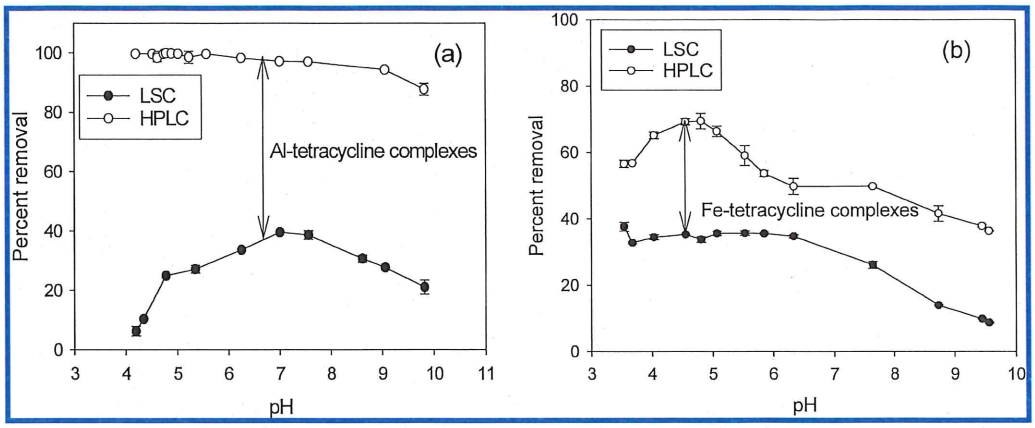

FIGURE 2. Sorption of tetracycline (TC) onto (a) HAO and (b) HFO as a function of pH ($[TC]_{initial}$ = 0.1 mM; ionic strength = 0.01 M NaCl; equilibration time = 24 h). Error bars (±1 standard deviation) if not shown are within the symbols.

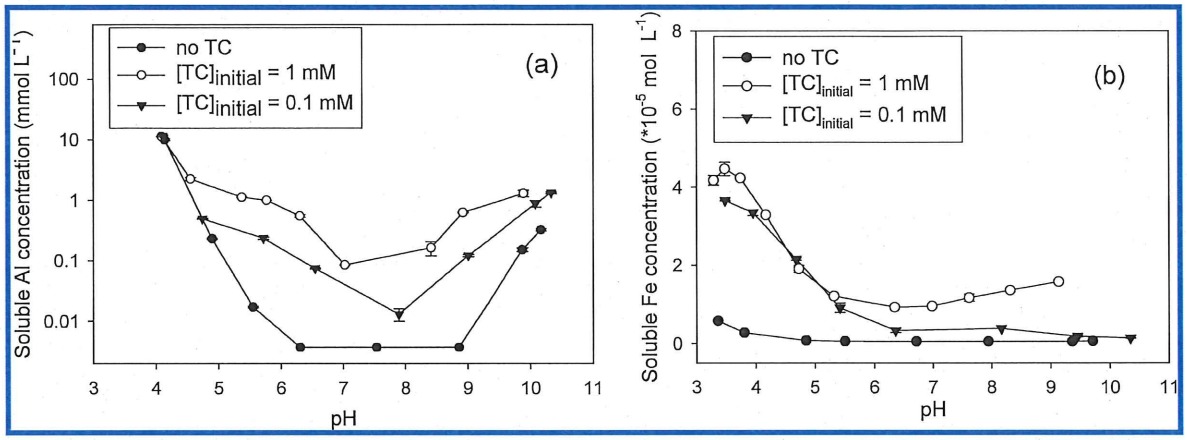

FIGURE 3. Solubility of (a) HAO and (b) HFO as a function of pH in the presence and absence of tetracycline (TC) ($[TC]_{initial}$ = 0.1 mM; 1 mM; ionic strength = 0.01 M NaCl; equilibration time = 24 h). Error bars (±1 standard deviation) if not shown are within the symbols.

tetracycline reacted with 0.05 g of HAO/HFO). After initial centrifugation, the slurry was resuspended in 15 mL of MilliQ-grade deionized water by gentle stirring and was centrifuged again. The slurry was then freeze-dried and stored at 35 °C for 2 days for further drying before analysis. Samples were analyzed by FTS7000 spectrometer (Digilab, MA) equipped with a photoacoustic detector. Reference spectra of tetracycline and hydrous oxide minerals were also acquired.

Results and Discussion

Effect of pH. Results from control experiments (no HAO/HFO) showed that there were no significant losses of tetracycline (recovery always greater than 95%) because of sorption to glassware and other reactions in solution under our experimental conditions (Figure SI-1 in Supporting Information). Figure 2 shows the pH-dependent sorption of tetracycline to HAO and HFO as determined by two independent measurements, HPLC (compound-specific) and LSC. Sorption of 7-^{3}H-tetracycline (measured as loss from solution of ^{3}H label) to HAO increased with increasing pH between pH 4 (almost no sorption) and 7, above which the trend was reversed (Figure 2a). No pH effect was noticed for tetracycline sorption to HFO up to pH 6 above which the sorption extent decreased sharply with increasing pH (Figure 2b). The observed sorption behavior can be attributed to a combination of pH-dependent speciation of tetracycline (Figure 1b), surface charge characteristics of HAO/HFO, and the occurrence of ligand-promoted dissolution (discussed below) of these hydrous oxides in the presence of tetracycline. Both the sorbate (tetracycline) and the sorbents (HAO/HFO) become progressively negatively charged with increasing pH. Differences in sorption levels between these hydrous oxides below circum-neutral pH could be attributed to the higher solubility of HAO compared with HFO (Figure 3a and 3b), especially in the presence of tetracycline below pH 6.

For both the hydrous oxides, there was a significant difference in the extent of tetracycline loss from solution as determined using the independent HPLC and LSC measurements (Figure 2). For HAO, almost no tetracycline could be detected in solution by HPLC within our experimental pH range. However, on the basis of radioactivity measurements, maximum sorption onto HAO was around 40% at pH 7. For HFO, the difference between HPLC and LSC measurements (20−30%) was smaller than those observed for HAO. While LSC provides a measure of the total substrate concentration, including the parent compound (tetracycline) and its products from other reactions in solution, HPLC is compound-specific and accounts for only the free form of tetracycline. Therefore, the difference between these two independent measurements could correspond to the fraction of tetracycline subjected to other reactions in solution (*38*).

The difference could be caused by photodegradation, epimerization of tetracycline, or by the existence of Me−tetracycline (Me: Al or Fe) complexes in solution. The above reactions can affect the retention time (RT) in the HPLC chromatogram and the UV absorption spectrum. As mentioned earlier, tetracycline recoveries in controls (no hydrous oxides), determined by both HPLC and LSC, were >95% at pH between 4.2 and 9.3 (Figure SI-1 in Supporting Information) indicating its stability against degradation. However, it is important to confirm whether epimerization is occurring in the presence of hydrous oxides. Using analytical standards,

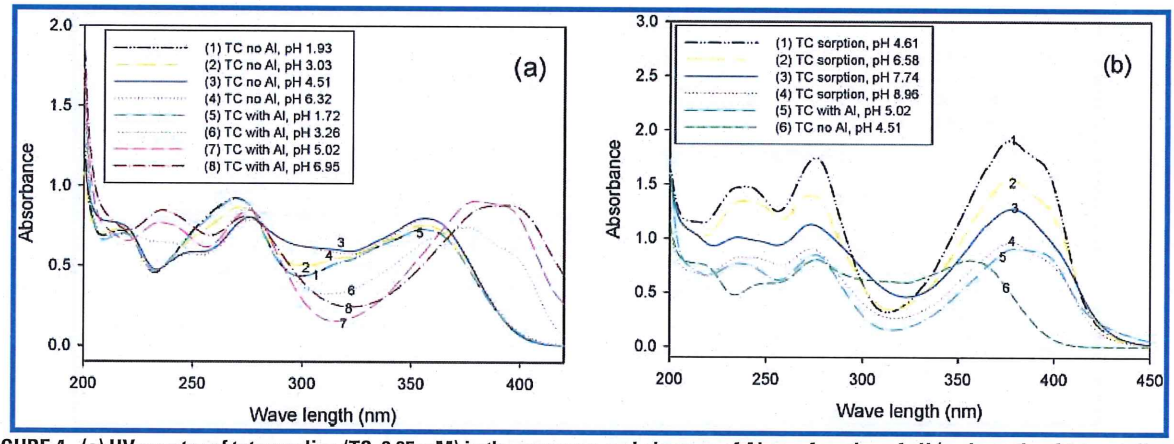

FIGURE 4. (a) UV spectra of tetracycline (TC, 0.05 mM) in the presence and absence of Al as a function of pH (molar ratio of tetracycline:Al = 1:10); (b) UV spectra of supernatant remaining after interaction of tetracycline with HAO as a function of pH ($[TC]_{initial}$ = 0.1 mM; ionic strength = 0.01 M NaCl; equilibration time = 24 h). The UV spectra for tetracycline–Al complexes formed in solution ([TC] = 0.05 mM; molar ratio of tetracycline:Al = 1:10 at pH 5.02) and for tetracycline ([TC] = 0.05 mM; pH = 4.51) are also included for comparison.

we determined the RT in the HPLC chromatogram for 4-epitetracycline and tetracycline to be 8.91 and 9.46 min, respectively. The HPLC chromatograms of the supernatant remaining after reaction with HAO and HFO (Figures SI-2 and SI-3 in Supporting Information, respectively) clearly showed that the major peak present is due to free tetracycline and that contributions from 4-epitetracycline are insignificant. Also, the tetracycline peak height in the supernatant was inversely related, not to the extent of sorption (as determined by LSC), but to the sum of sorption amount and the magnitude of difference between HPLC and LSC measurements. As shown in Figure SI-4 (Supporting Information), the UV spectrum of 4-epitetracycline is similar to that of tetracycline (especially between 300 and 400 nm), and those for the supernatant from reaction with HAO resembled the UV spectra of Al–tetracycline complexes (Figure 4b). Therefore, epimerization of tetracycline cannot account for the observed differences between HPLC and LSC measurements.

A concomitant increase in soluble Al and Fe concentration was observed as the initial tetracycline level was increased (Figure 3a and 3b), which also correlated well with the difference in sorption amounts determined using HPLC and LSC. Therefore, we formulated a hypothesis that ligand-promoted dissolution of HAO and HFO is occurring during tetracycline sorption, and the difference between independent measurements is due to the formation of tetracycline–Me (Me: Al or Fe) complexes. Chromatographic separation and direct quantification of Al/Fe–tetracycline complexes proved to be extremely difficult. We hypothesize that the Al–tetracycline complex is multivalent with a high polarity causing difficulties in chromatographic separation/quantification.

The tetracycline-promoted dissolution was more pronounced for HAO than HFO. The solubility of HAO increased significantly in the presence of tetracycline with an increase of up to 2 orders of magnitude noticeable at 10^{-3} M. Also, increasing levels of tetracycline produced a proportional increase in HAO dissolution. In comparison, the effect on HFO was limited (note: difference in *y*-axis scale for Figure 3a and 3b). Tetracycline forms strong complexes with Me such as Al and Fe as supported by the formation constants (log *K*) of 12.5 and 13.4, respectively (*39*). These values compare favorably with other well-known chelating agents (log *K* for Fe complexation with nitrilotriacetic acid, citric acid, and EDTA is 15.9, 11.4, and 25, respectively (*40*); log *K* for Al-EDTA complexation is 19.07 (*41*)), except EDTA which has higher log *K* values.

Figure 4a shows the UV spectra of tetracycline at different pH in the presence and absence of Al. In the absence of Al, the UV spectra of tetracycline were similar in the pH range of 1.93~6.32, with the characteristic absorption peak at a wavelength of 360 nm (used in HPLC analysis as well). On the other hand, the UV spectra of Al–tetracycline complexes were pH dependent. At pH 1.72, significantly lower than the pK_{a1} of tetracycline (pH = 3.3), the UV spectrum was unaffected by the presence of Al. However, with increasing pH, a significant shift in the absorption peak (to a wavelength of 390 nm) occurred (Figure 4a). It, therefore, appears that the tricarbonylamide group (pK_{a1}) in ring A might be involved in complexation reaction with Al. As shown in Figure 4b, the UV spectra of supernatant after interaction with HAO matches very well with that of Al–tetracycline complex and, importantly, are different from that of tetracycline and 4-epitetracycline (Figure SI-4 in Supporting Information contains UV spectra for 4-epitetracycline at various pH values). With Fe absorbing significant UV radiation between 300 and 400 nm, it was not possible to distinguish the band for Fe–tetracycline complexes.

Effect of Sorbate-to-Sorbent Ratio and Ionic Strength. Isotherms for tetracycline sorption onto HAO and HFO (Figure 5) indicate that *I* effect on tetracycline sorption is dependent on surface coverage. An increase in *I* (from 0.01 to 0.5 M NaCl) lowered the extent of sorption only at surface coverages exceeding 0.073 and 0.036 mol kg^{-1} for HAO and HFO, respectively, and the corresponding equilibrium tetracycline concentrations are 0.347 and 0.182 mM. The isotherms were generated at a pH of 5.3 when the zwitterionic form is the predominant tetracycline species. Therefore, increased competition from both Na^+ and Cl^- with increasing *I* can be expected to lower sorption levels. However, a lack of competitive effect at low surface coverages is suggestive of initial complex formation via the inner-sphere (I. S.) mechanism (*17*, *42*). At higher surface coverages, the isotherm data indicates the likelihood of the presence of both I. S. and weak outer-sphere (O.S.) type complexes.

The isotherm data were all adequately described using the Freundlich isotherm equation, and the parameters are listed in Table 1. The *n* values were always less than 1 (Table 1), suggesting that the sorption sites on hydrous oxide minerals are not homogeneous (*34*). The sorption capacity (K_f) decreased with increasing *I* attributable to the effect of competing ions on tetracycline sorbed as O.S. complexes. The isotherms resembled the L-type curves that are normally observed when the adsorbate has a high affinity for the sorbent at low surface coverage and a decreasing affinity with increasing surface coverage (*34*). The isotherm for HAO at 0.01 M *I* displayed a significant increase in sorption capacity

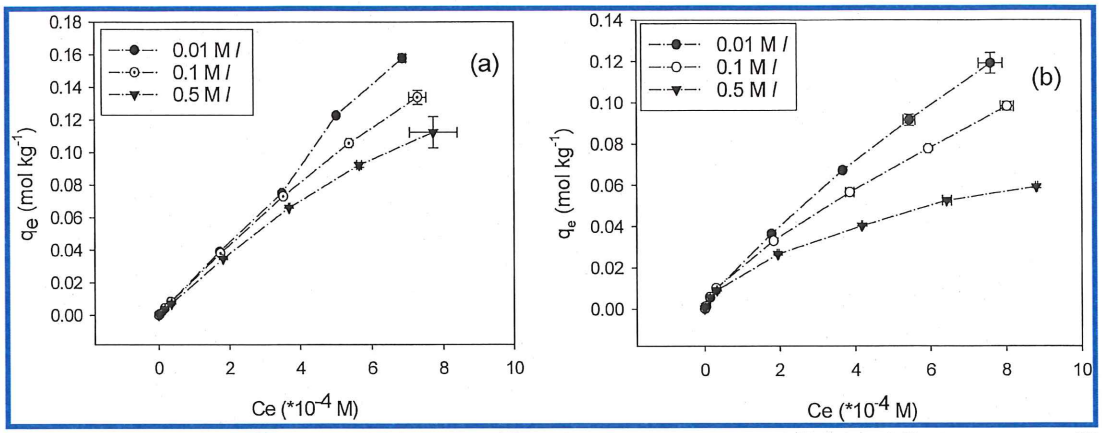

FIGURE 5. Isotherms for tetracycline sorption to (a) HAO and (b) HFO at different ionic strength (*I*) values (pH = 5.3 ± 0.05; equilibration time = 24 h). C_e is the equilibrium tetracycline concentration and q_e is the amount of tetracycline sorbed onto the hydrous oxides. Error bars (±1 standard deviation) if not shown are within the symbols.

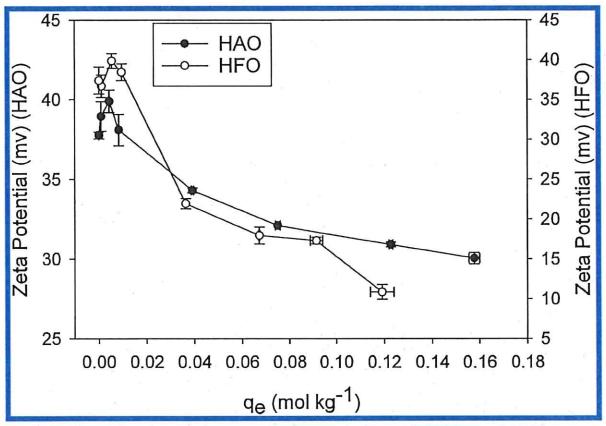

FIGURE 6. Surface charge characteristics of HAO and HFO as a function of increasing tetracycline surface coverage (pH = 5.3 ± 0.05; ionic strength = 0.01 M NaCl; equilibration time = 24 h). q_e is the amount of tetracycline sorbed onto the hydrous oxides. Error bars (±1 standard deviation) if not shown are within the symbols.

TABLE 1. Freundlich Isotherm[a] Parameters for Tetracycline Sorption to HAO and HFO

sorbent	K_f	n	r^2
Hydrous Al Oxide (HAO), pH = 5.3 ± 0.05			
ionic strength = 0.01 M	150	0.95	0.99
ionic strength = 0.1 M	118	0.93	0.99
ionic strength = 0.5 M	83.0	0.91	0.99
Hydrous Fe Oxide (HFO), pH = 5.3 ± 0.05			
ionic strength = 0.01 M	59.1	0.85	0.99
ionic strength = 0.1 M	35.1	0.81	0.99
ionic strength = 0.5 M	14.6	0.75	0.98

[a] Freundlich isotherm: $q_e = K_f \times C_e^n$; where q_e is the amount of tetracycline sorbed onto the hydrous oxides in mol kg^{-1}; C_e is the equilibrium tetracycline concentration in M; and K_f and n are dimensionless Freundlich isotherm constants.

at high surface coverage. As proposed by Molis et al. (*21*) and Poirier and Cases (*43*), during sorption at high tetracycline concentrations the adsorbed molecules would orient themselves parallel to each other to have the lowest Gibbs free energy. The adsorbed layer can accommodate additional tetracycline molecules via hydrophobic interactions.

A progressive decrease in the surface charge of both the hydrous oxides (experiments at pH 5.3 ± 0.05, below the pH_{ZPC}) with increasing tetracycline sorption levels (Figure 6) can be considered to provide evidence for I.S. complex formation (*17, 21*). A sharp decrease in zeta potential at low sorption densities (<0.04 mol kg^{-1}) was followed by a declining rate at increasing surface coverage (Figure 6). Importantly, the sorption densities at which significant slope change occurred, 0.073 and 0.036 mol kg^{-1} for HAO and HFO, respectively, correspond to those above which competitive effects with increasing *I* were observed (Figure 5). The predominance of I. S. type complexes at low sorption densities could have caused the sharp decrease in surface charge for both the hydrous oxides.

FTIR Analysis. FTIR spectra of tetracycline that reacted with HAO and HFO at pH 5.3 are shown in Figure 7. Since the most characteristic region of the tetracycline spectrum occurred between 1200 and 1800 cm^{-1}, only this region is interpreted in detail. In this range, the IR absorption of both the hydrous oxides was negligible (spectra not shown). Peak assignments for tetracycline (Figure 7a) followed the study of Lacher et al. (*44*) and Myers et al. (*45*). The 1673 and 1537 cm^{-1} bands were assigned to the carbonyl and amino groups of the amide in ring A, respectively. The frequencies at 1619 and 1586 cm^{-1} correspond to the carbonyl groups in A and C rings, respectively, and the 1458 cm^{-1} band was assigned to the skeletal vibration. There was only one frequency observed at 1619 cm^{-1} for the oxygens on C-1 and C-3, which suggests that they are equivalent (*45*).

The spectra of tetracycline equilibrated with HAO/HFO were similar (Figure 7b and 7c), indicating that similar type of surface complexes could exist. Although it was difficult to assign the band shift in the spectra of tetracycline equilibrated with the hydrous oxides, they clearly showed the band changes of amide carbonyl and amino groups in ring A and the carbonyl group in ring C (Figure 7b and 7c). There was no band shift for the carbonyl in ring A, but a decrease in relative peak intensity was noticed. From the FTIR results, it was not possible to determine the preferred carbonyl (C-1 vs C-3) binding site. The results indicate that tetracycline complexation with HAO and HFO could be occurring at the tricarbonylamide (C-1:C-2:C-3 in ring A) and carbonyl (C-11 in ring C) functional groups, consistent with the interpretation provided in Myers et al. (*45*). Other studies also provide evidence for the involvement of the tricarbonylamide group of ring A (*26*) and the O11:O-12 oxygen (*29, 31*) to form complexes with soluble Al.

Dissolution Kinetics. Sorption of tetracycline to both HAO and HFO was a fast process, as indicated by the loss of radioactivity from solution (Figure 8a, 8d). Equilibrium sorption levels were attained after 8 h. In comparison, it took longer than 24 h for the soluble Me concentration to approach equilibrium (Figure 8b, 8e). On the basis of these results, a two-step mechanism of tetracycline-promoted dissolution of HAO and HFO is proposed. Initially, tetracycline is

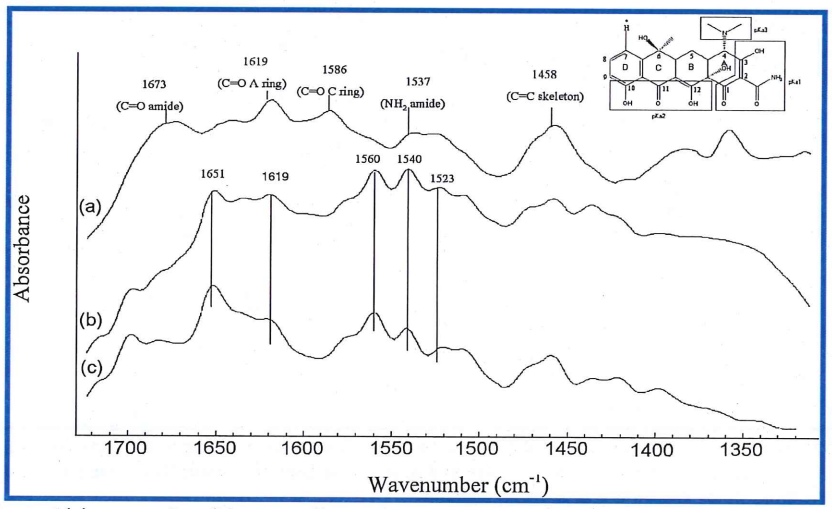

FIGURE 7. FTIR spectrum of (a) tetracycline, (b) tetracycline equilibrated with HFO at pH 5.3, and (c) tetracycline equilibrated with HAO at pH 5.3.

TABLE 2. Initial Dissolution Rate (D_i) and Dissolution Plateau for HAO and HFO after Reaction with Tetracycline[a]

	HAO		HFO	
initial tetracycline concentration (mM)	D_i ($\times 10^{-8}$ mol m^{-2} h^{-1})	dissolution plateau (mM)	D_i ($\times 10^{-8}$ mol m^{-2} h^{-1})	dissolution plateau (mM)
0.1	7.22	0.24	0.148	0.0060
1	23.3	1.56	0.241	0.0092

[a] 2 g L^{-1} HAO and HFO; pH = 5.3 ± 0.05; ionic strength = 0.01 M.

reversibly sorbed onto the mineral surfaces weakening the Me–O bond by destabilizing the metal center in the hydrous oxides. The second step is the detachment of a Me ion from the surface. Soluble Al levels were much higher compared with soluble Fe after tetracycline sorption, which is consistent with the results from sorption experiments described earlier. The D_i values (up to 8 h) and the dissolution plateau for HAO and HFO are compared in Table 2. Dissolution of HAO and HFO is significantly influenced by the surface coverage of the ligand (i.e., tetracycline), consistent with the results obtained for other chelating agents (*22*–*25*).

The difference between LSC and HPLC measurements was used to calculate the concentration of Al–tetracycline complexes. The ratio of levels of soluble Al to Al–tetracycline complexes was plotted as a function of reaction time in Figure 8c. After about 1.25 h (beginning of sorption), when a relatively small amount of Al was solubilized, this ratio was close to 1. As the reaction progressed, increased tetracycline sorption led to greater HAO dissolution, causing the ratio of soluble Al to Al–tetracycline complexes to attain an equilibrium value of 2.4 ± 0.1 after 24 h. It therefore appears that 1:1 Al–tetracycline complexes were initially formed transitioning to 2:1 complexes once equilibrium was attained. From FTIR analysis, it appeared that complexation with HAO could be occurring at two distinct functional groups in tetracycline, namely, the tricarbonylamide (C-1:C-2:C-3 in ring A) and carbonyl (C-11 in ring C) groups. Other researchers (*30*, *32*) have suggested that Ca^{2+} and Mg^{2+} could form 2:1 Me: tetracycline complexes when excess Me was available. Since the soluble Fe concentration was fairly low, the stoichiometry of Fe–tetracycline complexes could not be determined.

Surface Complexation Mechanism. On the basis of the above results, the proposed mechanism for tetracycline sorption to HAO/HFO minerals would involve the formation of complexes between tetracycline and hydrous oxides. This mechanism has been proposed for the interaction between tetracycline and clay minerals as well (*10*). From the FTIR analysis, it appears that the tricarbonylamide group in ring A and carbonyl group in ring C can react either independently or in concert with the mineral surfaces. Kinetic studies showed that a two-step ligand-promoted dissolution could be occurring with the initial formation of 1:1 Me–tetracycline complexes transitioning to 2:1 type complexes at equilibrium. The formation of a binuclear complex, as described below, is hypothesized to occur through a reaction involving charged sites and their noncharged neighbors (*21*).

$$\equiv Al(Fe)-OH_2^{+0.5} + \equiv Al(Fe)-OH_2 + [\text{tetracycline}]^- \rightarrow [\equiv Al_2(Fe_2)-\text{tetracycline}]^{-0.5} + 2H_2O \quad (1)$$

where ≡ represents the bonds of the Me atoms at the solid surface. The proposed complexation reaction can help explain the change in zeta potential of HAO and HFO with increasing tetracycline sorption as shown in Figure 6. Because of the singly coordinated surface hydroxyl groups, the edge Al/Fe atoms in HAO/HFO minerals are more reactive, possessing high Gibbs free energy, and are considered as the sites for surface reactions (*21*, *34*). At the pH value of 5.3 (below pH_{ZPC} of both the hydrous oxides) used in the isotherm and kinetic experiments, both HAO and HFO would be positively charged. As mentioned by Wessels et al. (*30*) and Dos Santos et al. (*31*), surface complexation could drive the ionization of tetracycline to occur at even lower pH (<pK_a), so at pH 5.3 both the $K_{a,1}$ and $K_{a,2}$ groups of tetracycline could be deprotonated.

Compared with HAO, HFO has a much lower solubility (Figure 3), even in the presence of tetracycline indicating that HFO has a more stable structure. It could be difficult for the protons or tetracycline to weaken the Fe–O bonds (compared to Al–O bonds). While almost all the free tetracycline was in the Al-complexed form in the presence of HAO, a significant portion exists in an uncomplexed form during interaction with HFO. As shown in Figure 9, the concentration of dissolved Al was linearly related to the amount of tetracycline sorbed, indicating that ligand-

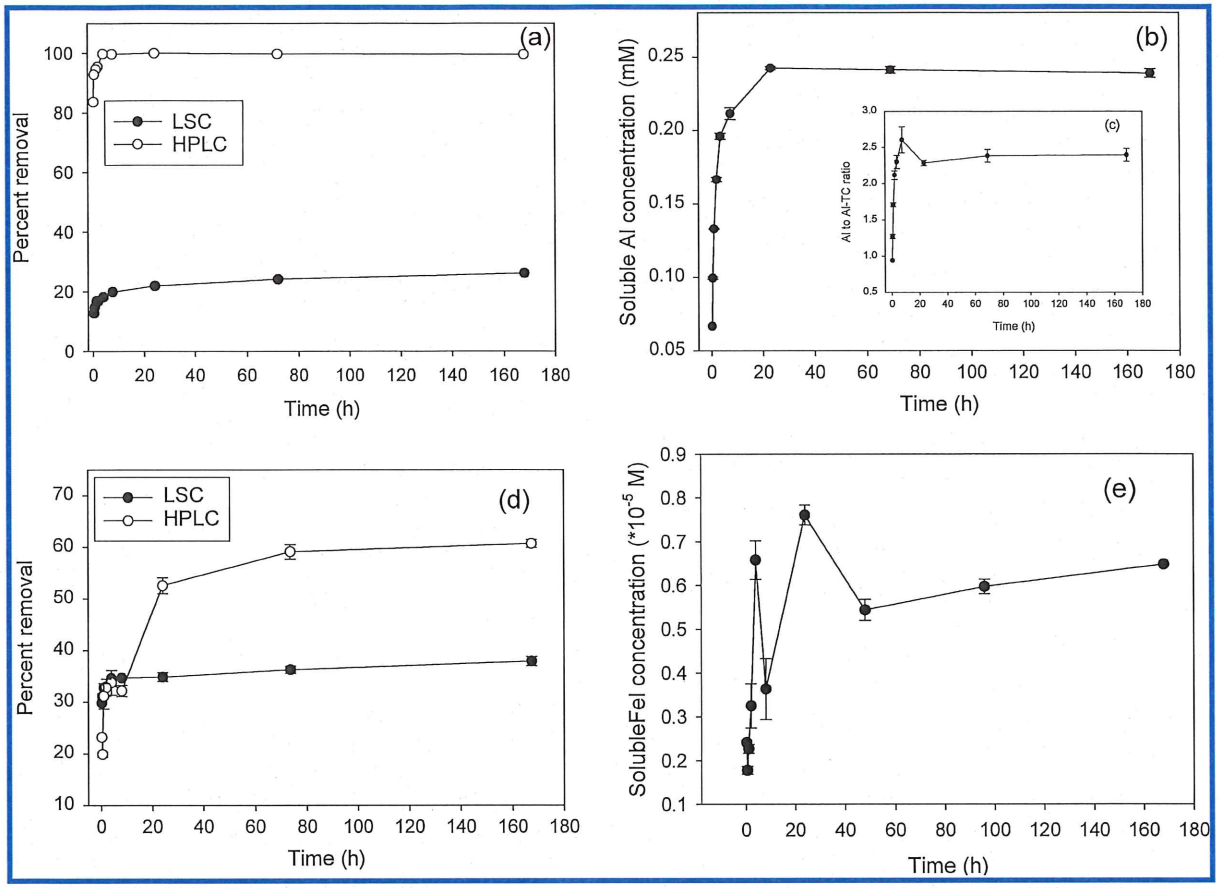

FIGURE 8. Time-dependent sorption of antibiotics and soluble metal release during the interaction of tetracycline (TC) with the hydrous oxides: (a) tetracycline sorption to HAO, (b) soluble Al release, (c) ratio of soluble Al to Al–tetracycline complexes, (d) tetracycline sorption to HFO, and (e) soluble Fe release ($[TC]_{initial}$ = 0.1 mM; pH = 5.3 ± 0.05; ionic strength = 0.01 M NaCl). Error bars (±1 standard deviation) if not shown are within the symbols.

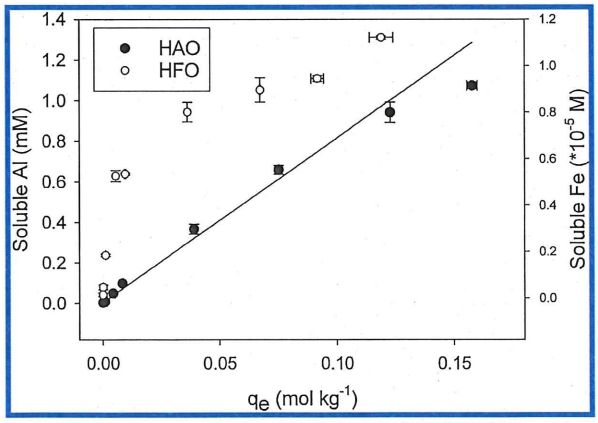

FIGURE 9. Release of soluble Al and Fe as a function of increasing tetracycline sorption onto HAO and HFO, respectively (pH = 5.3 ± 0.05; ionic strength = 0.01 M NaCl; equilibration time = 24 h). q_e is the amount of tetracycline sorbed onto the hydrous oxides. Error bars (±1 standard deviation) if not shown are within the symbols.

promoted dissolution takes place only at the sites where sorption has occurred (*21*). Such a trend was not evident for HFO (Figure 9). Rea et al. (*46*) using iron isotopic exchange and Mössbauer spectroscopy reported that ferrihydrite contains both labile and nonlabile sites for surface interactions. It is, therefore, possible that only the more reactive labile sites on HFO surface could be weakened by tetracycline and the proportion of labile sites might be the limiting factor for the ligand-promoted dissolution process.

Our results indicate that Al and Fe hydrous oxides, important components of environmental particles, can influence the mobility and environmental reactivity of tetracycline. We have sufficient evidence to support the occurrence of tetracycline-promoted dissolution (after initial formation of surface complexes) during its sorption to Al and Fe hydrous oxides. For other pharmaceutical compounds, such as fluoroquinolone and sulfonamide antibiotics, capable of forming strong complexes with metal cations, a similar reaction could occur in the presence of hydrous oxide minerals. To extend the study findings to complex environmental matrixes and soil types, the role of other factors such as the presence of competing cations (Ca^{2+}, Mg^{2+}), dissolved humic substances, and other organic chelating agents needs to be considered.

Acknowledgments

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Supporting Information Available

Four figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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ES048603O

Exposure to airborne microorganisms, dust and endotoxin during processing of peppermint and chamomile herbs on farms.

Ann Agric Environ Med. 2005;12(2):281-8. Skórska C[1], Sitkowska J, Krysińska-Traczyk E, Cholewa G, Dutkiewicz J, Department of Occupational Biohazards, Institute of Agricultural Medicine, Jaczewskiego 2, 20-090 Lublin, Poland. skorska@op.pl

Abstract

The aim of this study was to determine the levels of microorganisms, dust and endotoxin in the air during processing of peppermint (Mentha piperita) and chamomile (Matricaria recutita) by herb farmers, and to examine the species composition of airborne microflora. Air samples were collected on glass fibre filters by use of personal samplers on 13 farms owned by herb cultivating farmers, located in Lublin province (eastern Poland). The concentrations of total viable microorganisms (bacteria + fungi) in the farm air during processing of peppermint herb were large, within a range from 895.1-6,015.8 x 10(3) cfu/m(3) (median 1,055.3 x 10(3) cfu/m(3)). During processing of chamomile herb they were much lower and varied within a range from 0.88-295.6 x 10(3) cfu/m(3) (median 27.3 x 10(3) cfu/m(3)). Gram-negative bacteria distinctly prevailed during processing of peppermint leaves, forming 46.4-88.5 % of the total airborne microflora. During processing of chamomile herb, Gram-negative bacteria were dominant at 3 out of 6 sampling sites forming 54.7-75.3 % of total microflora, whereas at the remaining 3 sites the most common were fungi forming 46.2-99.9 % of the total count. The species Pantoea agglomerans (synonyms: Erwinia herbicola, Enterobacter agglomerans), having strong allergenic and endotoxic properties, distinctly prevailed among Gram-negative isolates. Among fungi, the most common species was Alternaria alternata. The concentrations of airborne dust and endotoxin determined on the examined herb farms were large. The concentrations of airborne dust during peppermint and chamomile processing ranged from 86.7-958.9 mg/m(3), and from 1.1-499.2 mg/m(3), respectively (medians 552.3 mg/m(3) and 12.3 mg/m(3)). The concentrations of airborne endotoxin determined during peppermint and chamomile processing were within a wide range 1.53-208.33 microg/m(3) and 0.005-2604.19 microg/m(3) respectively (medians 57.3 microg/m(3) and 0.96 microg/m(3)). In conclusion, farmers cultivating peppermint are exposed during processing of this herb to large concentrations of airborne microorganisms, dust and endotoxin posing a risk of work-related respiratory disease. The exposure to bioaerosols during processing of chamomile is lower; nevertheless, peak values create a respiratory risk for exposed farmers. PMID: 16457486

Terrasa, Jen

From: Feldmark, Jessica
Sent: Friday, October 27, 2017 5:24 PM
To: Terrasa, Jen
Subject: RE: Long Gate Overlook - Housing Project

Categories: Leg CB 61-62-2017 APFO

See the second highlighted portion below...looks like they waited their time. She may want to speak to her amendments to try to extend the waiting period...

Let me know if you have additional questions.

Thanks,
Jess

From: Terrasa, Jen
Sent: Friday, October 27, 2017 4:56 PM
To: Feldmark, Jessica <jfeldmark@howardcountymd.gov>
Subject: FW: Long Gate Overlook - Housing Project

Hi Jess,

Here's another one that Jen asked me to touch base with you about. I've highlighted the main question below. Do you happen to know what the law is in regards to this? We'd love to respond to him before APFO gets voted on, of course.

Thank you!

Colette Gelwicks
District Aide
District 3, Councilwoman Jen Terrasa
Howard County Council
3430 Court House Drive, Ellicott City, MD 21043
cgelwicks@howardcountymd.gov
Phone: (410) 313-3108 Fax: (410) 313-3297

Like Councilwoman Terrasa's page on Facebook and follow her on Twitter!

From: Kurt Schwarz [mailto:krschwa1@verizon.net]
Sent: Thursday, October 05, 2017 2:47 PM
To: CouncilMail <CouncilMail@howardcountymd.gov>
Subject: Long Gate Overlook - Housing Project

Dear Howard County Council Members,

I am mystified how or why the Zoning Board in the person of Howard County Council could have approved this housing project after it had failed the schools test under AFPO FIVE times. I would like some explanation as to why this happened, and you felt it to continue overburdening local schools, and fit to inject likely over 100 more cars onto Rt. 103 per day. Rt 103 approaches impassability every week day about 5:00 p.m. Again, am flabbergasted that you would consider approving the project as it currently stands. Kindly explain to me how this serves the public interest. Below as received from Dr. Ball's email several days ago.

Long Gate Overlook - Housing Project: A Housing project has been approved to construct 79 townhomes with a max density of 111 townhomes. The entrance (ingress/egress) is being reconfigured in consultation with the Church property to align with the entrance to the Long Gate Shopping Center. The Site Development Plan (SDP) was submitted to the County and is technically complete. The County is awaiting originals. By January 2018, the original mylars must be submitted to the County and then the developer can begin pulling construction permits. In January 2014, the County approved the housing allocations; however, they were placed on hold because they failed the schools test as required by APFO. As of July 3, 2017, they failed their 5th schools test - per APFO, they will now be permitted to proceed with construction although EMMS remains closed.

Kurt Schwarz
9045 Dunloggin Ct.
Ellicott City, MD 21042
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