



Biennial Review of 40 CFR Part 503 As Required Under the Clean Water Act Section 405(d)(2)(C)

**Reporting Period
2007 Biennial Review**

Biennial Review of 40 CFR Part 503
As Required Under the Clean Water Act Section 405(d)(2)(C)

Reporting Period Biennial Review 2007

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Health and Ecological Criteria Division
Ecological and Health Processes Branch
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NOTICE

This document has been reviewed in accordance with U.S. EPA policy and approved for publication. This report was prepared with the support of Research Triangle Institute, and its subcontractors, under the direction and review of the Office of Science and Technology.

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EXECUTIVE SUMMARY

In 1993, the Environmental Protection Agency (EPA) promulgated regulations in 40 CFR Part 503 as amended, setting numerical standards for certain metals in sewage sludge, requiring vector attraction reduction (e.g., reducing birds, rodents and insects) for pathogens, and establishing operational standards for emissions from sewage sludge incinerators. Section 405(d)(2)(C) of the Clean Water Act (CWA) states that EPA shall review the sewage sludge regulations not less often than every two years for the purpose of identifying additional toxic pollutants and promulgating regulations for such pollutants consistent with the requirements of section 405(d).

In fulfilling this commitment for the 2007 Biennial Review Cycle, EPA collected and reviewed publicly available information. The Agency searched databases with articles published in English and in refereed journals for information on occurrence, fate and transport in the environment, human health and ecological effects, as well as other relevant information for pollutants that may occur in U.S. sewage sludge. If such data are available for pollutants that may occur in sewage sludge, the agency is able to characterize the potential risk associated with exposure to such pollutants when sewage sludge is applied to land as a fertilizer or soil amendment, placed in a surface disposal site, or incinerated.

The data search identified 47 pollutants for which some data were available. However, the available data are not sufficient at this time to allow the Agency to conduct exposure and hazard assessments or determine what, if any, regulatory action may be needed. Therefore, at this time EPA has not identified additional toxic pollutants for regulation under Section 405(d)(2)(C) of the CWA.

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Introduction

This document summarizes the U.S. Environmental Protection Agency's (EPA) activities related to the 2007 biennial review of pollutants pursuant to the Clean Water Act (CWA) Section 405(d)(2)(C). That section requires EPA to review existing sewage sludge regulations at least every two years to identify additional pollutants for possible regulation. The biennial review covered by this report summary – the 2007 Biennial Review – obtained biosolids-related literature through October 2007. This document summarizes the analysis of that literature (a Technical Memorandum contractor summary is included in Attachment A). In this document, we use the term “biosolids” interchangeably with “sewage sludge,” which is defined in the regulations and used in the statute.

History of the Standards for Use or Disposal of Sewage Sludge

In Section 405 of the CWA, Congress set forth a comprehensive program designed to reduce potential health and environmental risks associated with using or disposing of sewage sludge. Under Section 405(d), EPA establishes numeric limits and management practices that protect public health and the environment from the reasonably anticipated adverse effects of chemical and microbial pollutants in sewage sludge. Section 405(d) prohibits any person from using or disposing of sewage sludge from publicly owned treatment works (POTWs) or other treatment works treating domestic sewage, unless the use or disposal complies with regulations promulgated under section 405(d).

On February 19, 1993, EPA identified pollutants which, on the basis of available information on their toxicity, persistence, concentration, mobility, or potential for exposure, were present in sewage sludge in concentrations which may adversely affect public health or the environment. At that time, the Agency promulgated regulations (58 *FR* 9248) - CFR 40 Part 503 *Standards for the Use or Disposal of Sewage Sludge* - specifying acceptable management practices, numeric standards for ten metals (see Table 1) and operational standards for microbial organisms.

The 1993 rule established requirements for the final use or disposal of sewage sludge when it is: (1) applied to land as a fertilizer or soil amendment; (2) placed in a surface disposal site, including sewage sludge-only landfills; or (3) incinerated. These requirements apply to

publicly and privately owned treatment works that generate or treat domestic sewage sludge and to anyone who uses or disposes of sewage sludge. The rule also requires monitoring, record keeping, and reporting of specific information regarding sewage sludge management.

Table 1: Metals Regulated in 40 CFR 503

Metal	Land Application	Incineration ²	Surface Disposal
Arsenic	X	X	X
Cadmium	X		X
Chromium	X ¹	X	X
Copper	X		
Lead	X		X
Mercury	X		
Molybdenum	X ¹		
Nickel	X	X	X
Selenium	X		
Zinc	X		

^{1/} Minor amendments published in 1994 and 1995 improved clarity and responded to the results of judicial review resulting in changes in land application limits for chromium (deleted all limits) and molybdenum (deleted limits in Tables 2, 3, and 4 of Section 503.13).

^{2/} Mercury emissions are regulated as limits to air emissions either by monitoring the exhaust air from the incinerator or the ambient air around the incinerator. In either case, the concentration in the air must meet the National Emission Standards for Hazardous Air Pollutants (NESHAPs, 40 CFR Part 61). Total hydrocarbons (THC) or carbon monoxide (CO) is monitored to represent all organic compounds in the exhaust gas that are covered by the Part 503 Rule. See Subpart E, Section 503.43 for other incineration requirements.

Section 405(d)(2)(C) of the CWA also requires the Agency to review from time to time, but not less often than every 2 years (i.e., biennial reviews), the regulations for the purpose of identifying additional toxic pollutants and promulgating regulations for such pollutants (the Agency uses the term pollutant as defined in the CWA). The purpose of reviewing information on pollutants, or potential pollutants, is to assess the availability and sufficiency of the data to conduct exposure and hazard assessments. Such exposure and hazard assessments, where sufficient data exist, allow the Agency to determine the potential for harm to public health or the environment following use or disposal of biosolids. To inform the exposure and hazard assessments of pollutants in biosolids, EPA typically collects the following data:

- Toxicity to human and ecological receptors (e.g., toxicity defined in terms of reference dose, reference concentrations, cancer slope factor, lethal dose, lethal concentration, or chronic endpoints related to fecundity).
- Acceptable concentration data in sewage sludge. Both the ability to detect a given pollutant in sewage sludge and the concentrations at which that pollutant is present are highly dependent on the existence of acceptable analytical methods for that pollutant in the sewage sludge matrix. Analytical methods for water, effluent, or soil may not necessarily be appropriate for detecting pollutants in biosolids.
- Fate and transport data for pollutants that may be present in sewage sludge. These data are necessary for assessing exposure. Chemical and physical properties that are developed for a given pollutant in sewage sludge should generally include:

Parameter
Molecular weight
Solubility
Vapor pressure
Henry's law constant
Soil-water partitioning coefficient
Soil adsorption coefficient (K_d and K_{oc})
Degradation rates in various media
Log octanol-water partition coefficient ($\text{Log } K_{ow}$)
Diffusivity in air
Diffusivity in water

Air-to-plant transfer factor
Root uptake factor for above ground vegetation
Root concentration factor
Bioconcentration factors for animal products

The Agency evaluates the sufficiency of such data for pollutants having acceptable analytical methods, source concentration values, human health benchmarks, and other pertinent data for two general purposes:

1. To conduct sewage sludge exposure and hazard assessments for humans and the environment.
2. To support potential rulemaking under 40 CFR Part 503.

EPA did not meet the timetable in section 405(d) for promulgating the first round of regulations, and a citizen's suit was filed (*Gearhardt v. Reilly* (Civ. No. 89-6266-HO (D. Ore.)) to require EPA to fulfill this mandate. A consent decree was entered by the court in that case, establishing schedules for two rounds¹ of sewage sludge rules. To comply with the consent decree, EPA was required to:

- Identify toxic pollutants in sewage sludge (not identified pursuant to 33 U.S.C. Section 1345(d)(2)(A)(i) and (ii)) that may adversely affect public health and the environment. In compliance, on February 19, 1993, EPA promulgated the first rule codified at 40 CFR Part 503 (58 *FR* 9248) ("Round One").
- Sign a notice for publication proposing Round Two¹ regulations no later than December 15, 1999, and to sign a notice taking final action on the proposal no later than December 2001. In compliance, on December 21, 2001 EPA published in the Federal Register (66 *FR* 66228) its determination not to regulate dioxin and

^{1/} The terms "Round One" and "Round Two" were used by the consent decree. EPA uses the term "Biennial Review" to refer to subsequent reviews of Part 503 pursuant to Section 405(d) of the CWA.

dioxin-like compounds [i.e., polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like coplanar polychlorinated biphenyls (PCBs)] in sewage sludge that is incinerated or placed in sludge landfills or containment ponds. EPA based its decision on an evaluation of the risk of exposure for people most likely to be exposed to dioxin from these sources. EPA concluded that existing regulations for incinerators, landfills or containment ponds adequately protect human health and the environment by limiting exposure to pollutants, including dioxins in sewage sludge that is disposed of at a surface disposal site or incinerated in a sewage sludge incinerator.

- The consent decree (as amended) required EPA to take final action on the land application Round Two rulemaking from the original date of December 15, 2001, to a new date of October 17, 2003. In compliance, on October 24, 2003 EPA announced its final decision not to regulate dioxins in land-applied sewage sludge, concluding that dioxins from land-applied sewage sludge do not pose a significant risk to human health or the environment. (68 *FR* 61084).

Biennial Reviews

2003 Biennial Review

Consistent with the consent decree mentioned above, EPA agreed to publish a notice in the Federal Register describing how the Agency intends to respond to the National Research Council (NRC) report (<http://www.epa.gov/waterscience/biosolids/nas/complete.pdf>) recommendations and to seek public comment on its planned response. EPA also agreed to review publicly available information to identify additional toxic pollutants in sewage sludge and to publish a notice and seek public comment on the results of the review. Fulfilling these commitments, EPA published a notice in the Federal Register on December 31, 2003 ([68 FR 75531](#)).

For its 2003 Biennial Review, EPA assessed available data on chemical pollutants that had been detected in sewage sludge and that had not been regulated or previously assessed. The

Agency collected and reviewed publicly available information published between 1990 and 2003 on the occurrence of chemicals in sewage sludge; data on environmental properties such as mobility and persistence; and available human health benchmarks (HHBs). Following this review, EPA made preliminary determinations regarding sufficiency of information for conducting an exposure and hazard-based screening assessment. That literature review identified 803 chemicals reported to occur in sewage sludge. Sufficient data for evaluation were available for 40 of these 803 pollutants. EPA conducted a human health and an ecological hazard screening assessment for these 40 pollutants. Of the 40 pollutants evaluated, EPA determined that 15 pollutants presented a potential risk to human health and/or the environment. EPA further reduced the 15 pollutants to nine pollutants based on an updated biosolids exposure and hazard assessment. The results of EPA's review do not mean that EPA has concluded that these nine pollutants in sewage sludge adversely affect human health or the environment. EPA will use the results from the recently completed Targeted National Sewage Sludge Survey (TNSSS) (www.epa.gov/waterscience/biosolids) to complete its risk evaluation for these 9 pollutants. EPA will use that evaluation to inform what action, if any, to take under section 405(d) of the CWA.

2005 Biennial Review

For its 2005 Biennial Review, EPA again collected and reviewed publicly available information. The Agency searched known databases and the published literature designed to capture available information on occurrence, fate and transport in the environment, human health or ecological effects, as well as other relevant information for pollutants that may occur in U.S. sewage sludge. The data search identified 137 pollutants for which some data were available for 118 pollutants (Table 3; EPA-822-R-06-014), the available data were not sufficient to allow the Agency to either conduct exposure and hazard assessments or determine what, if any, regulatory action may be needed.

For the remaining 19 pollutants (Table 2; EPA-822-R-06-014), data are available to enable EPA to evaluate exposure and hazard. This evaluation will use existing data, data collected during the 2005 Biennial Review, and results from the recent Targeted National Sewage Sludge Survey. When EPA evaluates potential risk for these pollutants, EPA will be able to determine what, if any, regulatory action may be needed pursuant to Section 405(d) of the

CWA. However, at this time EPA has not identified additional toxic pollutants for regulation under Section 405(d)(2)(C) of the CWA.

2007 Biennial Review

The remainder of this document presents the 2007 Biennial Review. In conducting the 2007 Biennial Review, EPA again collected and reviewed publicly available information on pollutants to evaluate potential harm to human health or the environment following use or disposal of sewage sludge.

Ecological Assessment

EPA conducted a literature search from 2005 through October 2007. EPA searched databases and the published literature to capture available information necessary for ecological and environmental risk evaluations (e.g., occurrence, fate and transport in the environment, and ecological effects) for pollutants in U.S. sewage sludge. The Agency used articles published in English in peer-reviewed journals, databases such as ECOTOX, Aquatic Sciences and Fisheries Abstracts, Biological Sciences Database, and the Environmental Sciences and Pollution Management Database, as well as secondary sources of data for eco-toxicity benchmarks (e.g., the recent Ecological Soil Screening Level documentation for certain metals). The Agency assessed whether data were sufficient to conduct an ecological exposure and hazard assessment.

Human Health Assessment

To conduct human health risk evaluations, EPA did a literature search from 2005 through October 2007. EPA searched databases and the published literature, such as PubMed, TOXLINE, and the Environmental Sciences and Pollution Management Database for information such as occurrence, fate and transport in the environment, and human health for pollutants in U.S. sewage sludge.

The Agency followed the same methodology as for the 2003 and 2005 Biennial Reviews to determine whether the identified data were sufficient for proceeding with an exposure and hazard screening assessment. This methodology involved identifying the pollutants for which EPA peer-reviewed final human health benchmarks (HHBs) had been developed by the Agency's Office of Pesticide Programs (OPP) Reregistration Eligibility Decisions

(REDs), or EPA's Office of Research and Development for Integrated Risk Information System (IRIS) health assessments. For the biennial reviews, EPA does not include pollutants for which the scientific basis of HHBs is being reassessed at the time of review. For future biennial reviews, the Agency is re-evaluating its process of only relying solely on IRIS or OPP HHBs. The goal is to be able to expand its sources of human health toxicity data and potentially evaluate more pollutants.

Results of the 2007 Biennial Review

During the Agency's search of known databases and the open literature during the 2007 Biennial Review, the Agency collected and reviewed publicly available information for pollutants listed in Tables 2 and 3. The Agency evaluated the availability and acceptability of data addressing toxicity to human and ecological receptors, pollutant concentrations in sewage sludge based on acceptable analytical methods, physical and chemical properties, and fate and transport in the environment in order to be able to conduct an exposure and hazard assessment.

For its 2007 Biennial Review, EPA identified articles published since the 2005 Biennial Review as potential sources of information on pollutants in biosolids. The Agency evaluated the articles as potentially relevant sources containing new information that was not previously available or evaluated for pollutants in a prior biennial review, as well as previously collected information. Two criteria were established for selecting a pollutant for an exposure and hazard evaluation if relevant data were available: 1) the pollutant has either an OPP or IRIS HHB and that the HHB study was not undergoing reevaluation, and (2) the pollutant has nationally representative or otherwise acceptable measured concentrations in U.S. sewage sludge based on acceptable analytical methodology that can be used to detect and quantify such concentrations.

The Agency divided the list of pollutants identified into two major groups:

1. Pollutants that have not previously been evaluated but may have readily available OPP or IRIS human health benchmarks (e.g., toxicity defined in terms of reference dose, reference concentrations, or cancer slope factor). Table 2 lists seven chemicals identified in the 2007 Biennial Review that have health benchmarks (not necessarily limited to IRIS or OPP).

**Table 2. List of Pollutants Evaluated During 2007 Biennial Review
with Human Health Benchmarks**

Constituent Name	CASRN	IRIS or OPP¹	Class
Aluminum	7429-90-5		metal
Bisphenol A	80-05-7	IRIS	plasticizer
Cobalt	7440-48-4		metal
Cresol, p- (4-methylphenol)	106-44-5	IRIS	preservative
Phenanthrene	85-01-8		PAH
Phosphorus	7723-14-0	IRIS	nutrient
Triclosan	3380-34-5	OPP	disinfectant

^{1/} EPA's Integrated Risk Information System (IRIS) or EPA's Office of Pesticide Programs (OPP). If neither IRIS nor OPP, the entry is blank

In spite of the fact that HHBs and other data exist for the seven pollutants listed in Table 2, the available data were not sufficient at this time to allow the Agency to conduct exposure and hazard assessments. Critical information gaps may include source concentration, fate and transport in the environment, and ecological data.

2. Pollutants for which OPP or IRIS human health benchmarks were not available. Table 3 lists pollutants (n=40) in sewage sludge for which the search did not identify IRIS or OPP human health benchmarks. EPA's current process is that in the absence of an IRIS or OPP human health benchmark, EPA will not conduct human health risk evaluations, even if other data on exposure (e.g., fate and transport in the environment) are available, because toxicity data for human receptors are critical pieces of data.

**Table 3. List of Pollutants for which OPP or IRIS Human Health
Benchmark Data Are Lacking**

Constituent Name	CASRN	Class
3-beta-coprostanol	360-68-9	fecal steroid
Acetyl-1,1,3,4,4,6-hexamethyltetrahydronaphthalene, 7- (AHTN)	21145-77-7	fragrance
Amphetamine	300-62-9	pharmaceutical
Azithromycin	83905-01-5	antibiotic
Beta-Sitosterol	19044-06-5	steroid
Calcium	7440-70-2	essential metal
Carbamazepine	298-46-4	pharmaceutical
Cholesterol	57-88-5	steroid
Clarithromycin	81103-11-9	antibiotic
Diphenhydramine	58-73-1	pharmaceutical
d-Limonene	5989-27-5	fragrance
Erythromycin	114-07-8	antibiotic
Fluoxetine	54910-89-3	pharmaceutical
Galaxolide (HHCB)	1222-05-5	fragrance
Indole	120-72-9	fragrance
Magnesium	7439-95-4	essential metal
Methamphetamine	537-46-2	pharmaceutical
Nitrogen	7727-37-9	nutrient
Nonylphenol monoethoxylate	27986-36-3	detergent metabolite
Nonylphenol, 4- (para-)	84852-15-3	detergent metabolite
Nonylphenol, dithoxy-total	Not identified	detergent metabolite
Octylphenol, 4-tert-	140-66-9	detergent metabolite
Organic-nitrogen, NH ₄ -N, NO ₃ -N,	14798-03-9	nutrient
Potassium	7440-09-7	essential metal
Roxithromycin	80214-83-1	antibiotic
Skatole	83-34-1	fecal indicator odor
Stigmastanol	19466-47-8	steroid
Tonalide (AHTN)	21145-77-7	fragrance
Triclocarban	101-20-2	disinfectant
Tylosin	1401-69-0	antibiotic
<i>Clostridium perfringens</i>	Not applicable	microbial agent
Coliphage	Not applicable	microbial indicator
<i>Cryptosporidium parvum</i>	Not applicable	microbial agent
<i>E. Coli spp.</i>	Not applicable	microbial agent
Endotoxin	Not applicable	microbial toxin
Fecal coliform	Not applicable	microbial indicator
Heterotrophic plate count	Not applicable	heterotrophic microorganisms
<i>Salmonella spp.</i>	Not applicable	microbial agent
Sulfite-reducing <i>Clostridia</i>	Not applicable	microbial agent
total bacteria	Not applicable	microbial agent

Consistent with the Agency's current process, the available data were not sufficient at this time to allow the Agency to conduct exposure and hazard assessments. There are significant data gaps for identified pollutants regarding environmental properties, human health and ecotoxicity benchmarks, and acceptable concentration data in sewage sludge. Thus, EPA has not at this time identified additional toxic pollutants during its 2007 Biennial Review for potential regulation.

The Agency will continue to assess the availability of sufficient information for these and other pollutants during subsequent biennial reviews pursuant to Section 405(d)(2)(C) of the CWA. In addition, the Agency is evaluating its process for how future biennial reviews will be conducted. For example, for future biennial reviews, the Agency is re-evaluating its process of only relying solely on IRIS or OPP HHBs.

Additional Information

For more information about EPA's Biosolids program, contact Rick Stevens in the Health and Ecological Criteria Division, 1200 Pennsylvania Avenue, N.W., Washington, DC 20460 (telephone: 202-566-1135 or e-mail: stevens.rick@epa.gov).

Attachment A

Technical Memorandum

Report on Pollutants' Database and Suitability

Technical Memorandum
Report on Pollutants' Database and Suitability

U.S. Environmental Protection Agency
Office of Water
1200 Pennsylvania Avenue, NW
Washington, DC 20460

October 2007

Introduction

This technical memorandum constitutes a deliverable under EPA Contract 68-C-04-006, Task 2, Work Assignment 3-04. The purpose of this task is to identify additional chemical and microbial pollutants in U.S. sewage sludge and provide EPA with information on the suitability for modeling and potential rulemaking for these pollutants.

Data Search

The search for new data was primarily based on the strategy developed under previous work assignments (e.g., Contract 68-C-04-006, Work Assignments #B-20 and 1-20); results from bibliographic databases were limited to articles published in English in refereed journals. The bibliographic databases included PubMed, Toxline, Aquatic Sciences and Fisheries Abstracts, Biological Sciences Database, and the Environmental Sciences and Pollution Management Database. Publications from March 2005 to October 2007 were sought. The data search key words included:

Topic/Keyword:	Sewage sludge, biosolids, pollutants, toxicants, pathogens, microbial, Salmonella, treated sewage, sludge treatment, sewage treatment, land application, farm, agriculture, soil.
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Based on previous literature searches (e.g., Biennial Review 2005), EPA did not uncover many new articles or publications with significant new data. Using this search strategy, we identified 97 articles as potential sources of information on chemical and microbial pollutants in biosolids. As this was fewer articles than anticipated, we expanded our search and identified 50 additional potentially useful studies. We also incorporated results from the pharmaceuticals and personal care products (PPCPs) literature search (from Work Assignments #2-43 and 3-43) into the biennial review effort (in summary so as to not duplicate work between these ongoing tasks); however only 1 study was identified to contain data on concentrations in biosolids within the time period sought.

From these articles, we identified the 36 articles shown in Attachment 1 as potential sources of information on chemical and microbial pollutants in biosolids (abstracts are included where available). Many studies were off-topic or addressed pollutants that have been previously modeled; these studies were omitted from consideration and are not included in Attachment 1. Topics of excluded studies included concentration data in other media (e.g., wastewater effluent, surface waters, and soil), non-U.S. data, not municipal waste (e.g., industrial, agricultural), the lack of analytical measurement techniques in biosolids, and the lack of information concerning toxicity in aquatic and invertebrate organisms. The Agency divided the list of pollutants identified in these articles into two major groups:

1. Pollutants that have not previously been modeled but have readily available health benchmarks.
2. Pollutants that have been identified in recent studies on biosolids for which health benchmarks were not identified in a major reference.

Identification of Additional Pollutants in U.S. Sewage Sludge

Pollutants with Health Benchmarks

Table 1 lists the chemicals (n=7) with health benchmarks (not limited to IRIS or OPP) that fit the following criteria: (1) identified in a previous National Sewage Sludge Survey, (2) not currently on EPA's list of potential candidates for addition to the Part 503 standards, and (3) not previously regulated or evaluated for sewage sludge. The chemicals are also identified by analyte groups defined by similarity in structure as well as typical uses when appropriate.

Table 1. List of Pollutants with Health Benchmarks

Constituent Name	CASRN	IRIS/OPP?	Class
Aluminum	7429-90-5		metal
Bisphenol A	80-05-7	IRIS	other
Cobalt	7440-48-4		metal
Cresol, p- (4-methylphenol)	106-44-5		other
Phenanthrene	85-01-8		PAH
Phosphorus	7723-14-0	IRIS	metal
Triclosan	3380-34-5		pharmaceutical

Pollutants without Health Benchmarks

Table 2 lists additional pollutants of concern (e.g., pharmaceuticals) in sewage sludge that do not have human health benchmarks available, according to our search.

Table 2. List of Pollutants without Health Benchmarks

Constituent Name	CASRN	Class
3-beta-coprostanol		sterol
Acetyl-1,1,3,4,4,6-hexamethyltetrahydronaphthalene, 7-(AHTN)	21145-77-7	odor
Amphetamine	300-62-9	pharmaceutical
Azithromycin	83905-01-5	pharmaceutical
Beta-Sitosterol		pharmaceutical
Calcium	7440-70-2	metal
Carbamazepine	298-46-4	pharmaceutical
Cholesterol	57-88-5	pharmaceutical
Clarithromycin	81103-11-9	pharmaceutical
Diphenhydramine	58-73-1	pharmaceutical
d-Limonene	5989-27-5	solvent
Erythromycin	114-07-8	pharmaceutical
Fluoxetine	54910-89-3	pharmaceutical
Galaxolide (HHCB)	1222-05-5	musk
Indole	120-72-9	scent
Magnesium	7439-95-4	metal
Methamphetamine	537-46-2	pharmaceutical
Nitrogen	7727-37-9	nutrient
Nonylphenol monoethoxylate	27986-36-3	NP/AE/APE
Nonylphenol, 4- (para-)	104-40-5	NP/AE/APE
Nonylphenol, dithoxy-total		NP/AE/APE
Octylphenol, 4-tert-	140-66-9	NP/AE/APE
Organic-nitrogen, NH4-N, NO3-N,	14798-03-9	nutrient
Potassium	7440-09-7	metal
Roxithromycin	80214-83-1	pharmaceutical
Skatole	83-34-1	odor
Stigmastanol	19466-47-8	pharmaceutical
Tonalide (AHTN)	21145-77-7	odor
Triclocarban	101-20-2	pharmaceutical
Tylosin	1401-69-0	pharmaceutical
Clostridium perfringens		microbial
Coliphage		microbial
Cryptosporidium parvum		microbial
E. coli		microbial
Endotoxin		microbial
Fecal coliform		microbial
HPC bacteria		microbial
Salmonella		microbial
Sulfite-reducing Clostridia		microbial
total bacteria		microbial

Attachment 1

Potential Sources of Information on Pollutants in Biosolids

Atalay, A; Bronick, C; Pao, S; et al. (2007) Nutrient and microbial dynamics in biosolids amended soils following rainfall simulation. *Soil Sed Contam* 16(2):209–219.

Abstract: Municipal waste treatment plants are mandated by U.S.EPA to treat domestic wastewater prior to releasing it to receiving streams. The dewatering and high temperature drying processes at the plant are considered effective in reducing microbial contaminants in the waste. The resulting solid material (biosolid) is rich in nutrients that may serve as a value-added product for plant growth. In this study, we examined the nutrient value of biosolids, their potential biological and chemical risks that could result from surface application to two Mid-Atlantic soils: Bojac (coarse-loamy, mixed, thermic Typic Hapludult) and Cullen (clayey, mixed, thermic Typic Hapludult). Soils were placed on tilt beds and packed to their respective bulk density. Biosolids were added at a rate of 2.24 Mg/ha equivalent and mixed with the top 5 cm of the soil bed. Simulated rain was applied at a rate of 65 mm h⁻¹ for 45 minutes. Surface runoff and percolation water were collected and analyzed for elemental content, *Escherichia coli* (*E. coli*) and total coliform bacteria. Among the nutrient elements of concern (P, Zn, Mn, and Cu) in biosolids, none were found to be higher than the specified EPA limits. The concentration of P was highest in runoff and percolation water from beds packed with Bojac and biosolids. The combined effects of high clay (35%), Al (1.14%), and Fe (5.11%) in Cullen increased its P-adsorbing capacity. Low levels of *E. coli* and other coliform bacteria were present in samples from biosolids-treated beds packed with Cullen. Microbial counts in runoff and percolation samples varied with soil type; in some instances they were ten-fold higher in Bojac than in Cullen. The results obtained in this study suggest that surface runoff from land applications of biosolids might contribute to microbial contamination of receiving waters near agricultural fields.

Brooks, JP; Tanner, BD; Josephson, KL; et al. (2005) A national study on the residential impact of biological aerosols from the land application of biosolids. *J Appl Microbiol* 99(2):310–322.

Abstract: The purpose of this study was to evaluate the community risk of infection from bioaerosols to residents living near biosolids land application sites. **METHODS AND RESULTS:** Approximately 350 aerosol samples from 10 sites located throughout the USA were collected via the use of six SKC Biosamplers. Downwind aerosol samples from biosolids loading, unloading, land application and background operations were collected from all sites. All samples were analysed for the presence of HPC bacteria, total coliform bacteria, *Escherichia coli*, *Clostridium perfringens*, coliphage, enteroviruses, hepatitis A virus and norovirus. Total coliforms, *E. coli*, *C. perfringens* and coliphage were not detected with great frequency from any sites, however, biosolids loading operations resulted in the largest concentrations of these aerosolized microbial indicators. Microbial risk analyses were conducted on loading and land application operations and their subsequent residential exposures determined. **CONCLUSIONS:** The greatest annual risks of infection occurred during loading operations, and resulted in a 4×10^{-4} chance of infection from inhalation of coxsackievirus A21. Land application of biosolids resulted in risks that were $< 2 \times 10^{-4}$ from inhalation of coxsackievirus A21. Overall bioaerosol exposure from biosolids operations poses little community risk based on this study.

SIGNIFICANCE AND IMPACT OF THE STUDY: This study evaluated the overall incidence of aerosolized micro-organisms from the land application of biosolids and subsequently determined that microbial risks of infection were low for residents close to biosolids application sites.

Brooks, JP; Tanner, BD; Gerba, CP; et al. (2006) The measurement of aerosolized endotoxin from land application of Class B biosolids in Southeast Arizona. *Can J Microbiol* 52(2):150–156.

Abstract: The purpose of this study was to determine aerosolized endotoxin concentrations downwind of a biosolids land application site. Aerosol samples were collected from biosolids land application sites, tractor operation, and an aeration basin located within an open-air wastewater treatment plant. Aerosolized endotoxin above background concentrations was detected from all sites, at levels ranging from below detection up to 1800 EU m⁻³ of air. Biosolids loading operations resulted in the greatest concentrations of endotoxin (mean 344 EU m⁻³). As downwind (perpendicular to wind vector) distance increased from sources (2-200 m), levels of endotoxin decreased to near background (without biosolids application) concentrations. Overall, the detected levels of aerosolized endotoxin were within past proposed aerosolized endotoxin limits (250-2000 EU m⁻³) by other occupational exposure studies. Occasionally, peak concentrations were found to be above these limits. Sites in which soil was being aerosolized resulted in greater concentrations of endotoxin with or without biosolids, which suggested that the majority of endotoxin may in fact be of soil origin. This study evaluated the presence of aerosolized endotoxin from the land application of biosolids and showed that these levels were within ranges for concern suggested by other studies and that this area of research needs further investigation.

Brooks, J; Gerba, C; Pepper, I. (2007) Diversity of aerosolized bacteria during land application of biosolids. *J Appl Microbiol* 103(5):1779–1790.

Abstract: The purpose of this study was to determine the diversity of bacterial communities associated with bioaerosols generated during land application of biosolids using 16S ribosomal RNA (16S rRNA) PCR. **Methods and Results:** Anaerobically digested Class B biosolids were land applied to an agricultural site located in South Central Arizona. Aerosol samples were collected downwind of the biosolids operations and were collected via the use of SKC Biosamplers and subsequently extracted for the presence of bacterial community DNA. All DNA was amplified using 16S rRNA primers, cloned and sequenced. All sequences were aligned and phylogenetic trees were developed to generate community profiles. The majority of aerosolized bacterial clone sequences belonged to the Actinobacteria and alpha- and beta-proteobacterial taxa. Aerosol samples collected downwind of soil aerosolization produced similar profiles. These profiles differed from upwind and background samples. **Conclusions:** No one clone sequence isolated from the aerosol samples could be solely attributed to biosolids; on the contrary, the majority appeared to have arisen from soil. **Significance and Impact of the Study:** This study demonstrates that in dry, arid climates the majority of aerosols associated with biosolids land application appear to be associated with the onsite soil.

Buyuksonmez, F; Sekeroglu, S. (2005) Presence of pharmaceuticals and personal care products (PPCPs) in biosolids and their degradation during composting. *Journal of Residuals Science & Technology* 2(1):31–40.

Abstract: The presence of pharmaceuticals and personal care product (PPCPs) in biosolids generated during municipal wastewater treatment (WWTP) was monitored weekly for 19 weeks. Biosolids samples obtained from a local WWTP were subjected to extraction with Soxhlet(R) apparatus for 24 hours. The extracts were concentrated and analyzed with a gas chromatograph-mass spectrometer. There were 18 compounds representing a wide range of origins identified in biosolids including phthalate esters, ibuprofen and galaxolide. Phthalate esters were the most frequently detected compounds; and 4-nonyl phenol was the most concentrated chemical at 210 mg/kg-dry weight basis. The efficacy of the composting process to degrade the 10 of the PPCP compounds was also investigated with and without spiking biosolids. Biosolids were amended with straw, and composted for up to 45 days using a laboratory-scale composting system. The lowest degradation rate was observed for 50.11% for octyl-4-methoxy cinnamate (OM) and the highest was 99.73% for butylated hydroxyl toluene (BHT). For all tested compounds except for unspiked OM, degradation efficiencies surpassed 85% at the end of the 45 days of composting study. The results of this study confirm the presence of various organic contaminants originated from personal care products in biosolids, and suggest that composting could be an effective treatment alternative for biosolids.

Campo, NCD; Pepper, IL; Gerba, CP. (2007) Assessment of *Salmonella typhimurium* growth in Class A biosolids and soil/biosolid mixtures. *Journal of Residuals Science & Technology* 4(2):83–88.

Abstract: The potential of *Salmonella typhimurium* regrowth in Class A biosolid pellets and compost after land application was assessed. Mixtures of soil, soil plus biosolids, and biosolids were inoculated with two different concentrations and monitored during a period of 20 days. No *Salmonella* growth occurred in any of the soil/biosolid mixtures regardless of inoculum size or moisture content. No growth occurred in any of the biosolids with a moisture content of 20% except the pellets from Texas when inoculated with 10,000 colony forming units/g. Growth of *Salmonella* did occur in all of the Class A products under saturated conditions. Under all moisture conditions indigenous microflora increased in numbers in the biosolids, soil and biosolid/soil mixtures. In conclusion, these results suggest that while regrowth of *Salmonella* in biosolids may occur under saturated conditions it does not occur after Class A biosolids land application at typical agronomic rates.

Chetochine, AS; Brusseau, ML; Gerba, CP; et al. (2006) Leaching of phage from Class B biosolids and potential transport through soil. *Appl Environ Microbiol* 72(1):665–671.

Abstract: The objective of this study was to investigate leaching and transport of viruses, specifically those of an indigenous coliphage host specific to *Escherichia coli* ATTC 15597 (i.e., MS-2), from a biosolid-soil matrix. Serial extractions of 2% and 7% (solids) class B biosolid matrices were performed to determine the number of phage present in the biosolids and to evaluate their general leaching potential. Significant concentrations of coliphage were removed from the biosolids for each sequential extraction, indicating that many phage remained associated with the solid phase. The fact that phage was associated with or attached to solid particles appeared to influence the potential for release and

subsequent transport of phage under saturated-flow conditions, which was examined in a series of column experiments. The results indicated that less than 8% of the indigenous coliphage initially present in the biosolids leached out of the biosolid-soil matrix. A fraction of this was subsequently transported through the sandy porous medium with minimal retention. The minimal retention observed for the indigenous phage, once released from the biosolids, was consistent with the results of control experiments conducted to examine MS-2 transport through the porous medium.

Choi, CY; Grabau, MR; O'Shaughnessy, SA; et al. (2005) Pathogen reduction in biosolids for land application. *Journal of Residuals Science & Technology* 2(3):159–171.

Abstract: Fecal coliforms or Salmonella criteria have to be met, regardless of which of the six recognized alternatives for achieving Class A pathogen reduction criteria per 40 CFR Part 503 are utilized. Therefore, solar inactivation of fecal coliforms and Salmonella spp. from Class B to Class A levels were evaluated in biosolids drying beds as a function of three treatments: no tilling (control), moderate tilling (twice per week), and intensive tilling (five (5) to six (6) times per week). Experiments were conducted in both summer and winter periods to study seasonal variability. During the 21-day summer drying experiment, total solids in the control bed increased from 30.1% to 41.6%. Total solids in the two tilled beds increased from 30.1% to approximately 90%. Fecal coliforms drastically decreased from 7.7×10^8 MPN/g dry weight to less than 3.0 MPN/g in all beds. Detected Salmonella spp. decreased from 38.6 MPN/4g to less than 3.0 MPN/4g in all beds. Based on fecal coliform criteria, the control, moderately tilled, and intensively tilled drying beds achieved Class A standards within 15, 5, and 7 days, respectively; based on Salmonella spp. criteria, the beds achieved Class A standards within 7, 3, and 5 days, respectively. Fecal coliform inactivation rates increased as a function of tilling intensity during the hot and dry season. However, during the winter experiment (which lasted for 89 days), fecal coliform levels never declined below 1.0×10^3 MPN/g. After 41 days, the levels of Salmonella spp. declined below 3.0 MPN/4g in the control and moderately tilled beds. After 85 days, Salmonella spp. levels in the intensively tilled bed fell below 3.0 MPN/4g. These studies indicate that tilling significantly increases drying bed efficiency and pathogen inactivation during hot, dry periods. However, during cold and wet winter seasons, tilling provides limited measurable benefits. The seasonal studies suggest that the best management practice associated with the drying and disinfection of biosolids in open solar drying beds is to till during hot and dry months and leave biosolids untreated during cold, wet seasons.

Ciparis, S; Hale, RC. (2005) Bioavailability of polybrominated diphenyl ether flame retardants in biosolids and spiked sediment to the aquatic oligochaete, *Lumbriculus variegatus*. *Environ Toxicol Chem* (4):916–925.

Abstract: Polybrominated diphenyl ether (PBDE) flame retardants have become distributed ubiquitously in the environment. High concentrations have been reported in U.S. sewage sludge (biosolids). The burgeoning practice of land-applying biosolids as fertilizer creates an avenue for reintroduction of PBDEs to surface waters and aquatic sediments. Bioavailability of biosolids- and sediment-associated PBDEs was assessed using the freshwater oligochaete, *Lumbriculus variegatus*. Oligochaetes were exposed to composted biosolids (1,600 ng/g total PBDEs) and artificial sediment spiked with penta- and deca-brominated diphenyl ether (BDE) formulations (1,300 ng/g total PBDEs).

Uptake (28-d exposure) and depuration (21 d) of eight congeners were studied. Polybrominated diphenyl ethers in both substrates were bioavailable, but bioaccumulation was 5 to 10 times greater from spiked artificial sediment. The congeners BDE 47 and BDE 99 were the most prevalent congeners in oligochaetes after exposure. Congener BDE 47 was more bioaccumulative, possibly due to the threefold greater depuration rate of BDE 99. Bioaccumulation of penta- and hexa-brominated congeners appeared to be affected more strongly by substitution pattern than degree of bromination. Uptake of BDE 209, the dominant congener in deca-BDE, was minimal. Accumulation of certain PBDE congeners from biosolids and sediments by benthos provides a pathway for transfer to higher trophic levels, and congener discrimination may increase with each trophic transfer.

Cogger, CG; Forge, TA; Neilsen, GH. (2006) Biosolids recycling: Nitrogen management and soil ecology. *Canadian Journal of Soil Science* 86(4):613–620.

Abstract: Biosolids are municipal wastewater treatment solids that meet regulatory standards for land application. Most biosolids are a rich source of N, P, and micronutrients. Although the use of biosolids on food crops remains controversial in the public eye, decades of research have led to the development of regulations for the safe and beneficial use of biosolids in agriculture. Emerging areas of research include biosolids in commercial and home horticulture, the fate of pathogens and organics in biosolids, the use of biosolids in the remediation of contaminated sites, and biosolids effects on soil ecology. Nutrient management remains the most critical day-to-day issue for land application of biosolids. Recent research on plant-available nitrogen (PAN) in biosolids has found that N availability is similar over a range of biosolids processing types, and that growing-season climate is a key factor affecting available N. Regionally based predictions of PAN have been developed for the United States, and could be extended into Canada. Relatively little is known about the effects of biosolids applications on soil ecology, but soil nematodes offer an opportunity to evaluate the structure and function of the soil ecosystem following biosolids applications. We have studied responses of nematode communities to application of municipal biosolids and composts, in forage production systems and orchards. Both types of amendments increased the abundance of enrichment opportunists, for up to 3 yr after single applications. These data on the persistence of increased enrichment opportunists have provided insight into the longevity of amendment-induced enhancement of biological activity and nutrient cycling. Cumulative biosolids applications of 90 Mg ha⁻¹ have caused reductions in abundance of pollutant-sensitive *Dorylaimida*. The extent to which this change is the result of metal or nutrient loading is unclear and deserves more detailed study.

Das KC, XK. (2007) Transformation of 4-nonylphenol isomers during biosolids composting. *Chemosphere* Sept 6 Epub ahead of print.

Abstract: 4-Nonylphenol, a degradation intermediate of commercial surfactant and known endocrine disruptor, has been frequently detected at levels up to several thousand µg l⁻¹ in surface waters and up to several hundred mg kg⁻¹ (dry weight) in soil and sediment samples. Large quantities of 4-NP can be quickly sorbed by the organic rich solid phase during wastewater treatment and are concentrated in biosolids, a possible major source for 4-NP in the environment. Microbial transformation in culture studies

followed different mechanisms for different 4-NP isomers, which have different estrogenic activity. Composting is a process of solid matrix transformation where biological activity is enhanced by process control. This approach has been used successfully in remediation of contaminated soils and sludges. In this study, the transformation kinetics of 4-NP and its isomers were characterized during biosolids composting. Five distinctive 4-NP isomer groups with structures relative to alpha- and beta-carbons of the alkyl chain were identified in biosolids. Composting biosolids mixed with wood shaving at a dry weight percentage ratio of 43:57 (C:N ratio of 65:1) removed 80% of the total 4-NP within two weeks. At this biosolids/wood shaving ratio (B:WS), the transformation of total 4-NP and its isomers followed second-order kinetic. Higher B:WS ratios yielded significantly slower 4-NP transformation which followed first-order kinetic. Isomers with alpha-methyl-alpha-propyl structure transformed significantly slower than those with less branched tertiary alpha-carbon and those with secondary alpha-carbon, suggesting isomer-specific degradation of 4-NP during biosolids composting.

Elliott, HA; Brandt, RC; O'Connor, GA. (2005) Runoff phosphorus losses from surface-applied biosolids. *J Environ Qual* 34(5):1632–1639.

Abstract: Runoff losses of dissolved and particulate phosphorus (P) may occur when rainfall interacts with manures and biosolids spread on the soil surface. This study compared P levels in runoff losses from soils amended with several P sources, including 10 different biosolids and dairy manure (untreated and treated with Fe or Al salts). Simulated rainfall (71 mm h⁻¹) was applied until 30 min of runoff was collected from soil boxes (100 x 20 x 5 cm) to which the P sources were surfaced applied. Materials were applied to achieve a common plant available nitrogen (PAN) rate of 134 kg PAN ha⁻¹, resulting in total P loading rates from 122 (dairy manure) to 555 (Syracuse N-Viro biosolids) kg P ha⁻¹. Two biosolids produced via biological phosphorus removal (BPR) wastewater treatment resulted in the highest total dissolved phosphorus (13-21.5 mg TDP L⁻¹) and total phosphorus (18-27.5 mg TP L⁻¹) concentrations in runoff, followed by untreated dairy manure that had statistically ($p = 0.05$) higher TDP (8.5 mg L⁻¹) and TP (10.9 mg L⁻¹) than seven of the eight other biosolids. The TDP and TP in runoff from six biosolids did not differ significantly from unamended control (0.03 mg TDP L⁻¹; 0.95 mg TP L⁻¹). Highest runoff TDP was associated with P sources low in Al and Fe. Amending dairy manure with Al and Fe salts at 1:1 metal-to-P molar ratio reduced runoff TP to control levels. Runoff TDP and TP were not positively correlated to TP application rate unless modified by a weighting factor reflecting the relative solubility of the P source. This suggests site assessment indices should account for the differential solubility of the applied P source to accurately predict the risk of P loss from the wide variety of biosolids materials routinely land applied.

Harrison, EZ; Oakes, SR; Hysell, M; et al. (2006) Organic chemicals in sewage sludges. *Sci Total Environ* 367(2-3):481–497.

Abstract: Sewage sludges are residues resulting from the treatment of wastewater released from various sources including homes, industries, medical facilities, street runoff and businesses. Sewage sludges contain nutrients and organic matter that can provide soil benefits and are widely used as soil amendments. They also, however, contain contaminants including metals, pathogens, and organic pollutants. Although current

regulations require pathogen reduction and periodic monitoring for some metals prior to land application, there is no requirement to test sewage sludges for the presence of organic chemicals in the U. S. To help fill the gaps in knowledge regarding the presence and concentration of organic chemicals in sewage sludges, the peer-reviewed literature and official governmental reports were examined. Data were found for 516 organic compounds which were grouped into 15 classes. Concentrations were compared to EPA risk-based soil screening limits (SSLs) where available. For 6 of the 15 classes of chemicals identified, there were no SSLs. For the 79 reported chemicals which had SSLs, the maximum reported concentration of 86% exceeded at least one SSL. Eighty-three percent of the 516 chemicals were not on the EPA established list of priority pollutants and 80% were not on the EPA's list of target compounds. Thus analyses targeting these lists will detect only a small fraction of the organic chemicals in sludges. Analysis of the reported data shows that more data has been collected for certain chemical classes such as pesticides, PAHs and PCBs than for others that may pose greater risk such as nitrosamines. The concentration in soil resulting from land application of sludge will be a function of initial concentration in the sludge and soil, the rate of application, management practices and losses. Even for chemicals that degrade readily, if present in high concentrations and applied repeatedly, the soil concentrations may be significantly elevated. The results of this work reinforce the need for a survey of organic chemical contaminants in sewage sludges and for further assessment of the risks they pose.

Heidler JC; Sapkota A; Halden R. (2006) Partitioning, persistence, and accumulation in digested sludge of the topical antiseptic triclocarban during wastewater treatment. *Environ Sci Technol* 40:3634–3639.

Abstract: The topical antiseptic agent triclocarban (TCC) is a common additive in many antimicrobial household consumables, including soaps and other personal care products. Long-term usage of the mass-produced compound and a lack of understanding of its fate during sewage treatment motivated the present mass balance analysis conducted at a typical U.S. activated sludge wastewater treatment plant featuring a design capacity of 680 million liters per day. Using automated samplers and grab sampling, the mass of TCC contained in influent, effluent, and digested sludge was monitored by isotope dilution liquid chromatography (tandem) mass spectrometry. The average mass of TCC (mean (standard deviation) entering and exiting the plant in influent (6.1 (2.0 $\mu\text{g/L}$) and effluent (0.17 (0.03 $\mu\text{g/L}$) was 3737(694 and 127(6 g/d, respectively, indicating an aqueous-phase removal efficiency of 97 (1%. Tertiary treatment by chlorination and sand filtration provided no detectable benefit to the overall removal. Due to strong sorption of TCC to wastewater particulate matter (78 (11% sorbed), the majority of the TCC mass was sequestered into sludge in the primary and secondary clarifiers of the plant. Anaerobic digestion for 19 days did not promote TCC transformation, resulting in an accumulation of the antiseptic compound in dewatered, digested municipal sludge to levels of 51 (15 mg/kg dry weight (2815 (917 g/d). In addition to the biocide mass passing through the plant contained in the effluent (3 (1%), 76 (30% of the TCC input entering the plant underwent no net transformation and instead partitioned into and accumulated in municipal sludge. Based on the rate of beneficial reuse of sludge produced by this facility (95%), which exceeds the national average (63%), study results suggest that approximately three-quarters of the mass of TCC disposed of by consumers in the sewershed of the plant ultimately is released into the environment by application of

municipal sludge (biosolids) on land used in part for agriculture.

Ippolito, JA; Barbarick, KA; Norvell, KL. (2007) Biosolids impact soil phosphorus accountability, fractionation, and potential environmental risk. *J Environ Qual* 36(3):764–772.

Abstract: Biosolids land application rates are typically based on crop N requirements but can lead to soil P accumulation. The Littleton/Englewood, Colorado, wastewater treatment facility has supported biosolids beneficial-use on a dryland wheat-fallow agroecosystem site since 1982, with observable soil P concentration increases as biyearly repeated biosolids applications increased from 0, 6.7, 13, 27, to 40 Mg ha⁻¹. The final study year was 2003, after which P accountability, fractionation, and potential environmental risk were assessed. Between 93 and 128% of biosolids-P added was accounted for when considering conventional tillage soil displacement, grain removal, and soil adsorption. The Fe-P fraction dominated all soil surface P fractions, likely due to an increase in amorphous Fe-oxide because Fe₂(SO₄)₃ was added at the wastewater treatment facility inflow for digester H₂S reduction. The Ca-P phase dominated all soil subsurface P fractions due to calcareous soil conditions. A combination of conventional tillage, drought from 1999 to 2003, and repeated and increasing biosolids application rates may have forced soil surface microorganism dormancy, reduction, or mortality; thus, biomass P reduction was evident. Subsurface biomass P was greater than surface biomass, possibly due to protection against environmental and anthropogenic variables or to increased dissolved organic carbon inputs. Even given years of biosolids application, the soil surface had the ability to sorb additional P as determined by shaking the soil in an excessive P solution. Biosolids-application regulations based on the Colorado Phosphorus Index would not impede current site practices. Proper monitoring, management, and addition of other best management practices are needed for continued assurance that P movement off-site does not become a major issue.

Iranpour, R; Cox, HH. (2006) Recurrence of fecal coliforms and Salmonella species in biosolids following thermophilic anaerobic digestion. *Water Environ Res* 78(9):1005–1012.

Abstract: The U.S. Environmental Protection Agency (U.S. EPA) Part 503 Biosolids Rule requires the fecal coliform (indicator) or Salmonella species (pathogen) density requirements for Class A biosolids to be met at the last point of plant control (truck-loading facility and/or farm for land application). The three Southern Californian wastewater treatment plants in this study produced biosolids by thermophilic anaerobic digestion and all met the Class A limits for both fecal coliforms and Salmonella sp. in the digester outflow biosolids. At two plants, however, a recurrence of fecal coliforms was observed in postdigestion biosolids, which caused exceedance of the Class A limit for fecal coliforms at the truck-loading facility and farm for land application. Comparison of observations at the three plants and further laboratory tests indicated that the recurrence of fecal coliforms can possibly be related to the following combination of factors: (1) incomplete destruction of fecal coliforms during thermophilic anaerobic digestion, (2) contamination of Class A biosolids with fecal coliforms from external sources during postdigestion, (3) a large drop of the postdigestion biosolids temperature to below the maximum for fecal coliform growth, (4) an unknown effect of biosolids dewatering in centrifuges. At Hyperion Treatment Plant (City of Los Angeles, California), fecal coliform recurrence could be prevented by the following: (1) complete conversion to thermophilic operation to exclude contamination by mesophilically digested biosolids

and (2) insulation and electrical heat-tracing of postdigestion train for maintaining a high biosolids temperature in postdigestion.

Jolis, D. (2006) Regrowth of fecal coliforms in class A biosolids. *Water Environ Res* 78(4):442–445.

Abstract: Eight types of Class A biosolids were tested for fecal coliform (FC) reactivation and/or regrowth at 20, 35, and 50 degree C for 21 days. Growth of FC did not occur at 20 or 50 degree C, but it was observed in two samples incubated at 35 degree C after a lag period of 48 hours. In undigested biosolids, final FC concentration exceeded 10 super(4) MPN/g, whereas in thermophilically digested biosolids, the final FC concentration remained below 10 super(3) MPN/g, as FC regrowth may have been affected by the presence of the anaerobic bacterial consortium responsible for the digestion process. Fecal-coliform reactivation and regrowth within treatment plant operations seem unlikely but can occur in land application of biosolids.

Jones-Lepp, TL; Stevens, R. (2007) Pharmaceuticals and personal care products in biosolids/sewage sludge: the interface between analytical chemistry and regulation. *Anal Bioanal Chem* 387(4):1173–1183.

Abstract: Modern sanitary practices result in large volumes of human waste, as well as domestic and industrial sewage, being collected and treated at common collection points, wastewater treatment plants (WWTPs). In recognition of the growing use of sewage sludge as fertilizers and soil amendments, and the scarcity of current data regarding the chemical constituents in sewage sludge, the US National Research Council (NRC) in 2002 produced a report on sewage sludge. Among the NRC's recommendations was the need for investigating the occurrence of pharmaceuticals and personal care products (PPCPs) in sewage sludge. PPCPs are a diverse array of non-regulated contaminants that had not been studied in previous sewage sludge surveys but which are likely to be present. The focus of this paper will be to review the current analytical methodologies available for investigating whether pharmaceuticals are present in WWTP-produced sewage sludge, to summarize current regulatory practices regarding sewage sludge, and to report on the presence of pharmaceuticals in sewage sludge.

Kaleta A; Ferdig M; Buchberger W. (2006) Semiquantitative determination of residues of amphetamine in sewage sludge samples. *Journal of Separation Science* 29(11):1662–1666.

Abstract: A procedure based on HPLC and mass spectrometric detection has been developed for screening of residues of the illicit drug amphetamine in sewage sludge. Sample pretreatment consisted in extraction by 50 mM formic acid and methanol (80 : 20 v/v), followed by adjustment of the pH to 10 and preconcentration by SPE at poly(divinylbenzene)-N-vinylpyrrolidone. HPLC separation of the extract was done on a C18 RP with a mixture of 50 mM formic acid and methanol (80 : 20 v/v) as mobile phase. The mass spectrometer was operated in the MS2 and MS3 mode using the transition from m/z 136 to 119 and from m/z 119 to 91. Due to the complex matrix, ionization suppression effects as well as shifts in the sensitivity of the detector within a series of runs could not be fully excluded. Therefore, quantitation was done by standard addition together with external standards, so that semiquantitative results could be obtained down to concentrations of 2 g/kg sewage sludge. Samples taken from various municipal sewage treatment plants indicate that amphetamine residues are ubiquitous in

urban areas.

Kelly, JJ; Favila, E; Hundal, LS; et al. (2007) Assessment of soil microbial communities in surface applied mixtures of Illinois River sediments and biosolids. *Applied Soil Ecology* 36(2-3):176–183.

Abstract: Restoration of the Illinois River and its backwater lakes involves the dredging of millions of cubic meters of sediment, and the relocation of this dredged sediment is a significant challenge. Beneficial use of sediment as landscaping soil on brownfields, strip mines, highway borders and other areas is a potential use for large quantities of this material, as sediments have desirable soil characteristics, including a favorable texture for plant growth and good water holding capacity. The addition of biosolids to Illinois River sediments has the potential to increase the organic and nutrient content of the sediments and thus to make these sediments more useful for the reclamation of damaged soils. The goal of the current study was to assess the impacts of biosolids additions on the physical/chemical characteristics and microbial communities of surface applied river sediments. Field plots containing various sediment/biosolids mixture were established and examined 1 year after application. Results indicated that biosolids addition had significant positive effects on soil organic carbon, total Kjeldahl nitrogen, total phosphorous, and microbial biomass and activity. In addition, the sediment/biosolids mixtures showed lower salinity and lower concentrations of copper, lead and zinc than the pure biosolids. PLFA analysis revealed that biosolids addition resulted in shifts in microbial community composition, with relative increases in Gram negative bacteria and relative decreases in Gram positive bacteria, fungi, and actinomycetes. These data suggest that a mixture of sediment and biosolids is preferable to either sediment or biosolids alone. (c) 2007 Elsevier B.V. All rights reserved

Kinney, CA; Furlong, ET; Zaugg, SD; et al. (2006) Survey of organic wastewater contaminants in biosolids destined for land application. *Environ Sci Technol* 40(23):7207–7215.

Abstract: In this study, the presence, composition, and concentrations of organic wastewater contaminants (OWCs) were determined in solid materials produced during wastewater treatment. This study was undertaken to evaluate the potential of these solids, collectively referred to as biosolids, as a source of OWCs to soil and water in contact with soil. Nine different biosolids products, produced by municipal wastewater treatment plants in seven different states, were analyzed for 87 different OWCs. Fifty-five of the OWCs were detected in at least one biosolids product. The 87 different OWCs represent a diverse cross section of emerging organic contaminants that enter wastewater treatment plants and may be discharged without being completely metabolized or degraded. A minimum of 30 and a maximum of 45 OWCs were detected in any one biosolid. The biosolids used in this study are produced by several production methods, and the plants they originate from have differing population demographics, yet the percent composition of total OWC content, and of the most common OWCs, typically did not vary greatly between the biosolids tested. The summed OWC content ranged from 64 to 1811 mg/kg dry weight. Six biosolids were collected twice, 3-18 months apart, and the total OWC content of each biosolids varied by less than a factor of 2. These results indicate that the biosolids investigated in this study have OWC compositions and concentrations that are more similar than different and that biosolids are highly enriched in OWCs (as mass-normalized concentrations) when compared to effluents or effluent-impacted water.

These results demonstrate the need to better describe the composition and fate of OWCs in biosolids since about 50% of biosolids are land applied and thus become a potentially ubiquitous nonpoint source of OWCs into the environment.

Lee, LS; Carmosini, N; Sassman, SA; et al. (2007) Agricultural contributions of antimicrobials and hormones on soil and water quality. *Advances in Agronomy*, Vol 93 93:1–68.

Abstract: Detection of many emerging chemicals of concern, including antimicrobials and steroid hormones, in the environment has increased in the past decade with the advancement of analytical techniques. There are several potential sources of these inputs, including municipal wastewater discharge, municipal biosolids, pharmaceutical production, and agriculture-related activities. However, the heavy use of antibiotics in the livestock industry and the dramatic shift in recent years toward more highly concentrated animal feeding operations (CAFOs), thus a concomitant increase in the volume of animal wastes per unit of land, has drawn attention to the role of animal waste-borne antimicrobials, antibiotic-resistant bacteria, and steroid hormones on ecosystem and human health. Antimicrobials, although frequently detected, are typically present in water at concentrations in orders of magnitude below what would be considered inhibitory to most biota. Most antibiotics have a high affinity for soil and sediment, thus residual soil concentrations are usually much higher than noted in water but still often below concentrations of concern. The focal point with antibiotic use in animal production is the development of antibiotic-resistant bacteria. Although there is a growing body of evidence of the presence of numerous antibiotic-resistant genes in animal wastes, in soils where wastes are land applied, and in water bodies receiving runoff from manure-amended fields or discharges from aquacultures, conclusive evidence of animal-derived antibiotic-resistant pathogens compromising human health is lacking. In contrast to antibiotics, hormones and related chemicals can cause significant biological responses at very low concentrations. CAFO discharges will include a variety of estrogens, natural and synthetic androgens and progesterones, and phytoestrogens associated with animal feed. Measurable concentrations of many of these hormones have been detected in soil, and ground and surface waters receiving runoff from fields fertilized with animal manure and downstream from farm animal operations. Overall, hormones appear to be moderately to highly sorbed and to dissipate quickly in an aerobic soil environment, but quantitative information on hormone persistence in manure-applied fields and subsequent effects of hormone loads from CAFOs to the aquatic environment is lacking. Research directed toward evaluating the facilitated transport processes with regards to antimicrobial and hormone inputs from manure-amended fields is in its infancy. With the advances in analytical techniques and what has already been learned with regards to transport of nutrients (nitrogen, phosphorus, and carbon) and pesticides from agricultural fields, a reasonable evaluation of CAFOs and associated activities (land application of animal wastes) should be forthcoming in the next decade. Meanwhile, implementation of management practices that optimize reduction in already regulated nutrient releases from CAFOs should also help to minimize the release of antimicrobials and hormones.

McBride, MB; Barrett, KA; Kim, B; et al. (2006) Cadmium sorption in soils 25 years after amendment with sewage sludge. *Soil Science* 171(1):21–28.

Abstract: Long-term changes in the solubility and bioavailability of heavy metals in soils, accumulated as the result of waste application, cannot be predicted without knowledge of

the nature of metal retention in these soils. To test the theory that Fe- and Al-rich mineral phases in sewage sludge-amended soils can act as long-term sinks for heavy metals, soils were sampled from two field sites, each with a similar history of multi-year application of either high-Fe, high-Al, or high-Ca sludge (similar to 25 years earlier) but with different textural characteristics. These soils were amended with Cd in the form of CdCl₂ in the laboratory to determine Cd solubility as a function of total added Cd over the range of 0 to 20 mg/kg. The slopes of these linear solubility functions, used to determine the strength of Cd sorption, revealed that the high-Fe and high-Al sludge amendments did not improve the soils' affinity for Cd at either site. The high-Al sludge treatment decreased the affinity for Cd in the more coarse-textured soil. The high-Ca sludge amendment, conversely, increased the affinity, for Cd, probably a result of the higher soil pH that has persisted since the sludge application. The results suggest that sludge Fe and Al may not be effective in binding Cd in all soils of humid temperate climates.

Mohillo, S; Montgomery, A; Fuman, D; et al. (2006) Detection of *Cryptosporidium parvum* oocysts in sediment and biosolids by immunomagnetic separation. *Water Environ Res* 78(9):1013–1016.

Abstract: A method for the detection of *Cryptosporidium parvum* oocysts in sediment and wastewater biosolids has been developed using immunomagnetic separation kits that were designed for use with water. This method requires no pretreatment of the sediment or biosolids samples before the commercial kit application. Oocyst recovery efficiencies from sediment and biosolids using the modified Dynal (Lake Success, New York) and Crypto-Scan commercial methods (Immucell Corporation, Portland, Maine) ranged from 20 to 60%. While the sensitivity of the method is dependent on the amount of sediment processed and the equivalent volume examined under the microscope, it was able to detect 0.48 oocysts per gram dry weight sediment. Using this method, *Cryptosporidium parvum* oocysts were found at levels as high as 97 oocysts/g of primary biosolids and at levels up to 4 oocysts/g in polluted sediment.

Montgomery, MB; Ohno, T; Griffin, TS; et al. (2005) Phosphorus mineralization and availability in soil amended with biosolids and animal manures. *Biological Agriculture & Horticulture* 22(4):321–334.

Abstract: Land spreading of biosolids (sewage sludge) and animal manures on agricultural soils is a means of disposal that is both environmentally and economically attractive. As with any soil amendment, there is potential for adverse impact from the land application of these materials. Applying biosolid or manure to meet crop N requirements can often result in excessive P application. A greenhouse bioassay study was conducted to evaluate bioavailable P from biosolid and manure amendments and to determine the effectiveness of the modified Morgan soil test (pH 4.8, 1.25 M ammonium acetate) and an in situ anion exchange membrane resin P soil test (P (InSitu)) to estimate plant-available P in the amended soil. The effects of biosolid stabilization processes on P availability were investigated by using lime stabilized (LSB), composted (CB), anaerobically digested (ADB) and unstabilized (UB) biosolids. The three animal manures studied were: dairy (DM), poultry (PM), and swine (SM). Triple super phosphate (TSP) served as an inorganic reference. Cumulative net P uptake by ryegrass (*Lolium perenne* L.) was highest for DM and SM, intermediate for PM, LSB, UB and TSP, and lowest for CB and ADB. Soil test P levels measured by the Morgan and P-AEM Methods were

highly correlated ($r = 0.88$, $p = 0.01$). The P uptake by ryegrass (*Lolium perenne* L.) agreed well with predicted P availability, indicating that both the modified Morgan soil test and in situ exchange resin methods are appropriate for determining P availability in biosolid- and manure-amended agricultural soils.

Osemwengie, LI. (2006) Determination of synthetic musk compounds in sewage biosolids by gas chromatography/mass spectrometry. *J Environ Monit* (9):897–903.

Abstract: A review of sewage sludge regulations and land application practices by the United States National Research Council (2002) recommended development of improved analytical techniques to adequately identify and quantify new chemical contaminants, such as synthetic musk compounds in Class A sewage sludge (i.e., biosolids). This prompted the development of a rugged analytical method using gas chromatography coupled to mass spectrometry to detect this group of organic pollutants in biosolids. In this paper, the term "biosolids" is used interchangeably with "sewage sludge", which is defined in the regulations and used in the statute (Clean Water Act). Samples of Class A biosolids obtained from sewage treatment plants in Los Angeles, California, the City of Las Vegas, Nevada, and also in the form of a commercial fertilizer, were extracted using pressurized liquid extraction technique, subjected to gel permeation chromatography cleanup, and analyzed by GC/MS using the selected ion monitoring mode. The method developed has the potential to detect synthetic musk compounds in complex matrices, may provide accurate data useful in human health and environmental risk assessment, and may be useful in determining the efficacy of municipal sewage treatment plants for removing synthetic musk compounds.

Paez-Rubio, T; Ramarui, A; Sommer, J; et al. (2007) Emission rates and characterization of aerosols produced during the spreading of dewatered class B biosolids. *Environ Sci Technol* 41(10):3537–3544.

Abstract: This study measured aerosol emission rates produced during the spreading of dewatered class B biosolids onto agricultural land. Rates were determined in multiple independent experimental runs by characterizing both the source aerosol plume geometry and aerosol concentrations of PM₁₀, total bacteria, heterotrophic plate count bacteria (HPC), two types of biosolids indicator bacteria, endotoxin, and airborne biosolids regulated metals. These components were also measured in the bulk biosolids to allow for correlating bulk biosolids concentrations with aerosol emission rates and to produce reconstructed aerosol concentrations. The average emission rates and associated standard deviation for biosolids PM₁₀, total bacteria, HPC, total coliforms, sulfite-reducing Clostridia, endotoxin, and total biosolids regulated metals were 10.1 +/- 8.0 (mg/s), 1.98 +/- 1.41 x 10⁹ (no./s), 9.0 +/- 11.2 x 10⁷ (CFU/s), 4.9 +/- 2.2 x 10³ (CFU/s), 6.8 +/- 3.8 x 10³ (CFU/s), 2.1 +/- 1.8 x 10⁴ (EU/s), and 36.9 +/- 31.8 (mu g/s) respectively. Based on the land application rates of spreaders used in this study, an estimated 7.6 +/- 6.3 mg of biosolids were aerosolized for every 1 kg (dry weight) applied to land. Scanning electron microscopy particle size distribution analysis of the aerosols revealed that greater than 99% of the emitted particles were less than 10 mu m and particle size distributions had geometric mean diameters and standard deviations near 1.1 +/- 0.97 mu m. The demonstrated correlations of bulk biosolids concentrations with aerosol emission rates, and the reconstruction of aerosol concentration based on PM₁₀ and bulk biosolids concentration provide a more fundamental, bulk biosolids-based approach for extending

biosolids aerosol exposure assessment to different land application scenarios and a broader range of toxins and pathogens.

Paez-Rubio, T; Xin, H; Anderson, J; et al. (2006) Particulate matter composition and emission rates from the disk incorporation of class B biosolids into soil. *Atmos Environ* 40:7034–7045.

Abstract: Biosolids contain metal, synthetic organic compound, endotoxin, and pathogen concentrations that are greater than concentrations in the agricultural soils to which they are applied. Once applied, biosolids are incorporated into soils by disking and the aerosols produced during this process may pose an airborne toxicological and infectious health hazard to biosolids workers and nearby residents. Field studies at a Central Arizona biosolids land application site were conducted to characterize the physical, chemical, and biological content of the aerosols produced during biosolids disking and the content of bulk biosolids and soils from which the aerosols emanate. Arrayed samplers were used to estimate the vertical source aerosol concentration profile to enable plume height and associated source emission rate calculations. Source aerosol concentrations and calculated emission rates reveal that disking is a substantial source of biosolids-derived aerosols. The biosolids emission rate during disking ranged from 9.91 to 27.25 mg s⁻¹ and was greater than previously measured emission rates produced during the spreading of dewatered biosolids or the spraying of liquid biosolids. Adding biosolids to dry soils increased the moisture content and reduced the total PM_{sub(10)} emissions produced during disking by at least three times. The combination of bulk biosolids and aerosol measurements along with PM_{sub(10)} concentrations provides a framework for estimating aerosol concentrations and emission rates by reconstruction. This framework serves to eliminate the difficulty and inherent limitations associated with monitoring low aerosol concentrations of toxic compounds and pathogens, and can promote an increased understanding of the associated biosolids aerosol health risks to workers and nearby residents.

Pepper, LL; Brooks, JP; Gerba, CP. (2006) Pathogens in biosolids. *Advances in Agronomy* 90:1–41.

Abstract: The world population of 6.8 billion people produces sewage. In the developed world most of this is treated by the activated sludge process, which results in large volumes of sludge or biosolids being produced (NRC, 2002). These result in millions of tons of biosolids produced each year in the United States, which must either be disposed of or recycled in some manner. Land application has been seen as the most economical and beneficial way of handling biosolids. Biosolids that result from municipal wastewater treatment processes contain organic matter and nutrients that, when properly treated and applied to farmland, can improve the productivity of soils or enhance re-vegetation of disturbed ecosystems. However, besides the documented benefits of land application, there are also potential hazards, which have caused the public response to the practice to be mixed. Here we review one of the potential hazards associated with biosolids and its land application, namely human pathogens associated with biosolids. (c) 2006, Elsevier Inc

Pillai, SD. (2007) Bioaerosols from land-applied biosolids: Issues and needs. *Water Environ Res* 79(3):270–278.

Abstract: Bioaerosols are a vehicle for the dissemination of human and animal pathogens. Because of land-filling costs and the ban on ocean dumping of municipal biosolids, land application of biosolids and animal manure is increasing all over the globe. There is no doubt that the creation, generation, and disposal of human and animal wastes increases the aerosolization potential of a wide variety of microbial pathogens and related pollutants. In an attempt to address public health issues associated with the land application of municipal biosolids, the U.S. National Research Council (Washington, D.C.) published a report on this issue in 2002. This paper focuses on the current information and technology gaps related to estimating the public health risks associated with bioaerosols during the land application of biosolids.

Schwab, AP; Lewis, K; Banks, MK. (2006) Biosolids-amended soils: Part II. Chemical lability as a measure of contaminant bioaccessability. *Water Environ Res* 78(11):2231–2243.

Abstract: Biosolids recycling by amending agricultural soils has increased significantly over the last few decades. The presence of contaminants in small, bioavailable quantities has generated concerns about health threats resulting from accumulation of potential toxins in the food chain. In this study, land application of biosolids was evaluated for environmental risk. Chemical lability tests for metals were used for the test soils and included analyses for water soluble, exchangeable, and metals extractable by the physiologically based extraction test. Chemical extractions detected slight increases in labile metal concentrations for many of the treated soils, particularly those receiving long-term applications of 5 years or more. Significantly higher metal concentrations were observed in the soils that had been exposed to biosolids before the U.S. Environmental Protection Agency (Washington, D.C.) 503 Rule (U.S. EPA, 2004) was implemented.

Shober, AL; Hesterberg, DL; Sims, JT; et al. (2006) Characterization of phosphorus species in biosolids and manures using XANES spectroscopy. *J Environ Qual* 35(6):1983–1993.

Abstract: Identification of the chemical P species in biosolids or manures will improve our understanding of the long-term potential for P loss when these materials are land applied. The objectives of this study were to determine the P species in dairy manures, poultry litters, and biosolids using X-ray absorption near-edge structure (XANES) spectroscopy and to determine if chemical fractionation techniques can provide useful information when interpreted based on the results of more definitive P speciation studies. Our XANES fitting results indicated that the predominant forms of P in organic P sources included hydroxylapatite, PO(4) sorbed to Al hydroxides, and phytic acid in lime-stabilized biosolids and manures; hydroxylapatite, PO(4) sorbed on ferrihydrite, and phytic acid in lime- and Fe-treated biosolids; and PO(4) sorbed on ferrihydrite, hydroxylapatite, beta-tricalcium phosphate (beta-TCP), and often PO(4) sorbed to Al hydroxides in Fe-treated and digested biosolids. Strong relationships existed between the proportions of XANES PO(4) sorbed to Al hydroxides and NH(4)Cl- + NH(4)F-extractable P, XANES PO(4) sorbed to ferrihydrite + phytic acid and NaOH-extractable P, and XANES hydroxylapatite + beta-TCP and dithionite-citrate-bicarbonate (DCB)- + H(2)SO(4)-extractable P ($r^2 = 0.67$ [P = 0.01], 0.78 [P = 0.01], and 0.89 [P = 0.001], respectively). Our XANES fitting results can be used to make predictions about long-term solubility of P when biosolids and manures are land applied. Fractionation

techniques indicate that there are differences in the forms of P in these materials but should be interpreted based on P speciation data obtained using more advanced analytical tools.

Sullivan, TS; Stromberger, ME; Paschke, MW. (2006) Parallel shifts in plant and soil microbial communities in response to biosolids in a semi-arid grassland. *Soil Biology & Biochemistry* 38(3):449–459.

Abstract: Approximately 70,150 dry Mg of biosolids from over 450 wastewater treatment facilities are applied to the semi-arid rangelands of Colorado every year. Research on semi-arid grassland responses to biosolids has become vital to better understand ecosystem dynamics and develop effective biosolids management strategies. The objectives of this study were to determine the long-term (similar to 12 years) effects of a single biosolids application, and the short-term (similar to 2 years) effects of a repeated application, on plant and microbial community structure in a semi-arid grassland soil. Specific attention was paid to arbuscular mycorrhizal fungi (AMF) and linkages between shifts in plant and soil microbial community structures. Biosolids were surface applied to experimental plots once in 1991 (long-term plots) and again to short-term plots in 2002 at rates of 0, 2.5, 5, 10, 21, or 30 Mg ha⁻¹. Vegetation (species richness and above-ground biomass), soil chemistry (pH, EC, total C, total N, and extractable P, NO₃-N, and NH₄-N), and soil microbial community structure [ester-linked fatty acid methyl esters (EL-FAMES)], were characterized to assess impacts of biosolids on the ecosystem. Soil chemistry was significantly affected and shifts in both soil microbial and plant community structure were observed with treatment. In both years, the EL-FAME biomarker for AMF decreased with increasing application rate of biosolids; principal components analysis of EL-FAME data yielded shifts in the structure of the microbial communities with treatment primarily related to the relative abundance of the AMF specific biomarker. Significant ($p \leq 0.05$) correlations existed among biomarkers for Gram-negative and Gram-positive bacteria, AMF and specific soil chemical parameters and individual plant species' biomass. The AMF biomarker was positively correlated with biomass of the dominant native grass species blue grama (*Bouteloua gracilis* [Willd. ex Kunth] Lagasca. ex Griffiths) and was negatively correlated with western wheatgrass (*Agropyron smithii* Rydb.) biomass. This study demonstrated that applications of biosolids at relatively low rates can have significant long-term effects on soil chemistry, soil microbial community structure, and plant community species richness and structure in the semi-arid grasslands of northern Colorado. Reduced AMF and parallel shifts in the soil microbial community structure and the plant community structure require further investigation to determine precisely the sequence of influence and resulting ecosystem dynamics. (c) 2005 Elsevier Ltd. All rights reserved

Sullivan, TS; Stromberger, ME; Paschke, MW; et al. (2006) Long-term impacts of infrequent biosolids applications on chemical and microbial properties of a semi-arid rangeland soil. *Biology and Fertility of Soils* 42(3):258–266.

Abstract: A plot study was conducted to quantify long-term (> 12 years) impacts of a single biosolids application, and short-term impacts (< 2 years) of a repeated application, on semi-arid rangeland soil chemical and microbial parameters. In 2003 and 2004, plots which had received 0, 2.5, 5, 10, 21, or 30 Mg biosolids ha⁻¹ once in 1991 (long-term plots), or again in 2002 (short-term plots), were sampled and analyzed for soil chemical

parameters, microbial biovolumes, C and N mineralization activities, Biolog EcoPlate substrate utilization potential, and plant productivity and tissue quality. Repeated applications temporarily exacerbated differences in soil chemical properties among treatments, but after 2 years, soil chemistry trends were similar between short-term and long-term plots. Soils which received a repeated application of 21 or 30 Mg biosolids ha⁻¹ had greater bacterial biovolumes and C and N mineralization activities. Biosolids-amended soil communities also utilized Biolog substrates more quickly compared to communities from control plots. Plant biomass increased, whereas plant diversity and plant C/N ratio decreased with increasing application rate for both short-and long-term plots. Infrequent biosolids application had positive ecosystem effects in terms of site management objectives, with relatively low extractable metal levels in soil and greater plant biomass and tissue quality despite reduced species richness.

Zaleski, KJ; Josephson, KL; Gerba, CP; et al. (2005) Potential regrowth and recolonization of salmonellae and indicators in biosolids and biosolid-amended soil. *Appl Environ Microbiol* 71(7):3701–3708.

Abstract: This study evaluated the potential for conversion of Class B to Class A biosolids with respect to salmonellae and fecal coliforms during solar drying in concrete lined drying beds. Anaerobically (8% solids) and aerobically (2% solids) digested Class B biosolids were pumped into field-scale drying beds, and microbial populations and environmental conditions were monitored. Numbers of fecal coliforms and salmonellae decreased as temperature and rate of desiccation increased. After 3 to 4 weeks, Class A requirements were achieved in both biosolids for the pathogens and the indicators. However, following rainfall events, significant increase in numbers was observed for both fecal coliforms and salmonellae. In laboratory studies, regrowth of fecal coliforms was observed in both biosolids and biosolid-amended soil, but the regrowth of salmonellae observed in the concrete-lined drying beds did not occur. These laboratory studies demonstrated that pathogens decreased in numbers when soil was amended with biosolids. Based on serotyping, the increased numbers of salmonellae seen in the concrete lined drying beds following rainfall events was most likely due to recolonization due to contamination from fecal matter introduced by animals and not from regrowth of salmonellae indigenous to biosolids. Overall, we conclude that the use of concrete-lined beds created a situation in which moisture added as rainfall accumulated in the beds, promoting the growth of fecal coliforms and salmonellae added from external sources.

Zaleski, KJ; Josephson, KL; Gerba, CP; et al. (2005) Survival, growth, and regrowth of enteric indicator and pathogenic bacteria in biosolids, compost, soil, and land applied biosolids. *Journal of Residuals Science & Technology* 2(1):49–63.

Abstract: In the U.S. approximately 60% of all biosolids are currently land applied. Although it is known that bacteria in biosolids normally decrease to low or non-detectable levels following treatment, a major concern is that regrowth of pathogens may occur. Specifically the question arises: "Does regrowth occur following reintroduction or recolonization of pathogens after land application or during storage under favorable conditions?" The following paper reviews available information on survival and potential regrowth of pathogenic and indicator bacteria in biosolids, compost, soil, and land applied biosolids. Based on the literature, a conceptual framework is provided to explain the phenomenon of "regrowth."