



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

**Reporting Period
2015 Biosolids Biennial Review**

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As Required Under the Clean Water Act Section
405(d)(2)(C)**

Reporting Period Biosolids Biennial Review 2015

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
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NOTICE

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Executive Summary

In 1993, the U.S. Environmental Protection Agency (EPA) promulgated regulations in 40 CFR Part 503 for sewage sludge¹, as amended, that include general requirements, pollutant limits, management practices, operational standards, and requirements for monitoring, recordkeeping and reporting. Section 405(d)(2)(C) of the Clean Water Act (CWA) states that EPA shall review the biosolids 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 2015 biennial review cycle, EPA collected and reviewed publicly available information on the occurrence, fate and transport in the environment, human health and ecological effects, and other relevant information for toxic pollutants that may occur in U.S. biosolids. After conducting the review, if such data are available for pollutants that may occur in biosolids, the Agency will assess the potential risk to human health or the environment associated with exposure to such pollutants when biosolids are applied to land as a fertilizer or soil amendment, placed in a surface disposal site, or incinerated, and, if appropriate, EPA will set numeric limits for these pollutants.

This review process included information collected for pollutants that (1) have been identified in the Targeted National Sewage Sludge Survey (TNSSS; U.S. EPA, 2009) or in the open literature as having concentration data for biosolids or other evidence of occurrence in biosolids, and (2) have not been previously regulated or evaluated (e.g., as potentially causing harm to humans or the environment) in biosolids. Using this search approach, 46 new articles were identified as providing relevant information for pollutants that may occur in U.S. biosolids. Review of these articles identified 29 new chemicals in biosolids: 2-benzyl-4-chlorophenol (120-32-1); bis(5-chloro-2-hydroxyphenyl)methane (97-23-4); 2-chloro-4-phenylphenol (92-04-6); decamethylcyclopentasiloxane (D5) (541-02-6); seven nitrosamines; 2,4,5-trichlorophenol (95-95-4); seven polybrominated dibenzo-p-dioxins (PBDDs); and ten polybrominated dibenzofurans (PBDFs). Human health toxicity values were found for eight of these new chemicals (2,3,5-trichlorophenol and seven nitrosamines) and one chemical (carbamazepine) identified in a previous biennial review. Ecological toxicity values were found for one chemical newly identified in biosolids (decamethylcyclopentasiloxane), but not for other chemicals previously found in biosolids. New physical-chemical properties (log K_{ow} and half-life) were identified for one new chemical and 10 chemicals previously identified in biosolids, and new bioaccumulation factors for aquatic organisms were identified for one new chemical.

The available data for many of the chemicals identified are not sufficient at this time to evaluate risk using current biosolids modeling tools. EPA will consider the newly identified toxicity data for nitrosamines, PBDDs, PBDFs, and carbamazepine in conducting risk assessments.

EPA has not identified any additional toxic pollutants for potential regulation during the 2015 Biennial Review. The Agency will continue to assess the availability of sufficient information for these and other pollutants identified during the biennial review activities pursuant to section 405(d)(2)(C) of the CWA.

¹ EPA often uses the term “biosolids” interchangeably with “sewage sludge,” which is defined in the regulations and used in the statute. Biosolids refers to treated sewage sludge.

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1. Introduction

In Section 405 of the Clean Water Act (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), the U.S. Environmental Protection Agency (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 several pollutants which, based on 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, 40 CFR Part 503 *Standards for the Use or Disposal of Sewage Sludge*, specifying acceptable management practices, numeric standards for 10 metals (arsenic, cadmium, chromium III, copper, lead, mercury, molybdenum, nickel, selenium, and zinc), and operational standards for microbial organisms (58 FR 9248).

The 1993 rule also 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 manages sewage sludge. The rule also requires monitoring, record keeping, and reporting of specific information regarding sewage sludge management.

Section 405(d)(2)(C) of the CWA requires EPA to review the biosolids 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). Prior to the reports known as “biennial reviews,” in order to fulfill this requirement, the Agency made the following decisions and observations: (1) In 2001, EPA decided that regulation of dioxin and dioxin-like compounds disposed via incineration or land-filling was not needed for adequate protection of public health and the environment (66 FR 66227); (2) In 2003, EPA determined that regulation of dioxin and dioxin-like compounds in land-applied sewage sludge was not needed for adequate protection of public health and the environment (68 FR 61084); and (3) In conducting the biennial review for 2003 (68 FR 75531), EPA identified nine pollutants (barium, beryllium, manganese, silver, fluoranthene, pyrene, 4-chloroaniline, nitrate, and nitrite) for evaluation. Molybdenum was also added for reevaluation in 2003. Summaries of the evaluations and past biennial reviews are available on EPA’s Web site at <http://www.epa.gov/biosolids>.

For the 2015 Biennial Review, EPA searched publicly available information in databases and articles published in English in refereed journals from January 2014 through December 2015. The purpose of reviewing this information is to identify pollutants found in biosolids in this timeframe and to assess the availability and sufficiency of the data for conducting risk assessments. After conducting the review, if such data are available for pollutants that may occur in biosolids, the Agency will assess the potential risk to human health or the environment associated with exposure to such pollutants when biosolids are applied to land as a fertilizer or soil amendment, placed in a surface disposal site, or incinerated to determine whether to regulate pollutants.

To inform the risk assessments of pollutants in biosolids, EPA typically uses models that require data from three major categories:

- **Toxicity to human and ecological receptors.** For human toxicity, this type of data includes values such as a reference dose, reference concentration, cancer slope factor, or inhalation unit risk. For ecological toxicity, it includes values such as lethal dose, lethal concentration, or chronic endpoints related to fecundity.
- **Concentration data for pollutants in biosolids.** Both the ability to detect a given pollutant in biosolids and the determination of the concentration at which that pollutant is present are highly dependent on the existence of analytical methods for that pollutant in the biosolids matrix.
- **Environmental Fate and transport data for pollutants that may be present in biosolids.** These data are necessary for assessing exposure. Examples of chemical and physical properties that may be considered, depending on the nature of a given pollutant in biosolids, include:
 - Molecular weight
 - Solubility
 - Vapor pressure
 - Henry's law constant
 - Soil-water partitioning coefficients
 - Soil adsorption coefficients (K_d and K_{oc})
 - Degradation rates in various media
 - Log octanol-water partition coefficients ($\text{Log } K_{ow}$)
 - Diffusivities in air and water
 - Bioavailability
 - Air-to-plant transfer factors
 - Root uptake factors for above ground vegetation
 - Root concentration factors
 - Bioconcentration factors for animal products (e.g., meat and milk).

2. Literature Search Approach

To determine if data are available to evaluate human health risks or ecological risks, EPA searched databases and the published literature for articles in English in refereed journals from January 2014 through December 2015 to identify data sources published since the previous search performed in support of the 2013 Biosolids Biennial Report (EPA-822-S18-002).

The bibliographic databases searched included PubMed, Science Citation Index Expanded (Web of Science), Toxline, Aquatic Sciences and Fisheries Abstracts, Biological Sciences Database, Environmental Sciences and Pollution Management, and Soil Science journal website. The data search included a combination of the following key words:

Biosolids-related keywords: (sewage sludge OR biosolids OR treated sewage OR sludge treatment OR sewage treatment)

AND

Pollutant- and health-related keywords: (pollutant* OR toxic* [toxicant, toxicology, etc.] OR pathogen* OR concentration* OR propert* OR fate OR transport OR health OR ecolog* OR effect OR effects OR micro* [microbial, etc.] OR *Salmonella*)

AND

Geographic keywords (limiters): (United States OR Canada OR USA OR U.S.A. OR U.S. OR US)

AND

Land Application-related keywords: (land application OR farm OR agriculture OR soil)

AND

Health-related keywords: (occurrence OR concentration OR properties OR fate OR transport OR health effects OR ecological effects).

In addition to the bibliographic databases searched, EPA also employed the search strategies described in **Sections 2.1** and **2.2** for human health toxicity values and ecological toxicity values, respectively.

The Agency applied an abstract screening process to the initial group of articles identified. Articles that included pollutants that fit the following criteria were formally reviewed:

- Identified in the Targeted National Sewage Sludge Survey (TNSSS; U.S. EPA, 2009) or the open literature as having concentration data or other evidence of occurrence in biosolids (see **Attachments A**).
- Not previously regulated or evaluated for biosolids.²

In the formal review process, articles addressing previously identified pollutants that appeared to provide new data on their behavior in the environment or toxicity were included. Articles were excluded from further review for any one of four reasons:

- The study addressed toxicity through a medium other than biosolids (e.g., wastewater effluent).
- The study was conducted in a country other than the United States or Canada.
- The study only described an analytical method.
- An abstract was not available and the title alone did not provide sufficient evidence for inclusion.

International studies that examined the occurrence of pollutants in biosolids were excluded from consideration, because treatment technologies and regulatory requirements in other countries are not necessarily representative of the United States. However, Canadian studies that examined the fate and transport of pollutants from agriculturally applied biosolids in soils were included because of the expected similarities in Canadian and U.S. soil types. Additionally, the Canadian governmental research group, Agriculture and Agri-Food Canada, has conducted numerous studies of interest on the fate and transport of pharmaceuticals and personal care products in agricultural soils.

2.1 Human Health Toxicity Values Data Sources and Selection

To estimate the potential for adverse human health risks from agricultural land application of biosolids, EPA assesses chronic oral and inhalation exposures. EPA uses reference doses (RfDs) and reference concentrations (RfCs) to evaluate non-cancer risk from oral and inhalation exposures, respectively. EPA uses oral cancer slope factors (CSFs) and inhalation unit risks (IURs) to evaluate risk for carcinogens from oral and inhalation exposures.³

The Integrated Risk Information System (IRIS; U.S. EPA, 2016a) is EPA's primary repository for human health toxicity values that have been developed specifically for human health risk

² For more information on pollutants previously regulated or evaluated in biosolids, see the [Statistics Support Documentation for the 40 CFR Part 503 - Volume 1](https://www.epa.gov/sites/production/files/2015-04/documents/statistics_1992_support_document_-_biosolids_vol_i.pdf) (https://www.epa.gov/sites/production/files/2015-04/documents/statistics_1992_support_document_-_biosolids_vol_i.pdf) and EPA's response to the National Research Council of the National Academy of Sciences report on biosolids (https://www.epa.gov/sites/production/files/2015-06/documents/technical_background_document.pdf)

³ For more information about these toxicity values, see <https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system>.

assessment using standardized methods⁴ and have been thoroughly peer reviewed. IRIS is considered the most preferred source for human health toxicity values for EPA risk assessment. However, not all chemicals have a toxicity value in IRIS, and even those that do, do not necessarily have all four toxicity values (RfD, RfC, CSF, IUR). Thus, a variety of other sources were used. To make efficient use of resources, EPA developed a hierarchy (see **Table 1**) that gives higher priority to sources of information that:

- Are developed specifically for use in human health risk assessment using methodologies similar to those used by IRIS;
- Have been peer reviewed to at least some extent and have a transparent basis for the values; and
- Are more recent than published IRIS values.

Table 1. Hierarchy for Human Health Toxicity Value Data

Data Sources Included
<p>Tier 1: Highest Quality EPA Sources Sources in Tier 1 contain values developed by EPA specifically for human health risk assessment according to standard methods and represent the highest quality human health toxicity values available. These toxicity values are frequently used to support EPA risk analyses.</p>
<p>Integrated Risk Information System (IRIS): IRIS is EPA’s primary repository for human health toxicity values that have been developed specifically for human health risk assessment using standardized methods and have been thoroughly peer reviewed. IRIS is considered the most preferred source for human health toxicity values for EPA risk assessment; however, for pesticides, toxicity values are developed by EPA’s Office of Pesticide Programs (U.S. EPA, 2016a).</p>
<p>Human Health Benchmarks for Pesticides (HHBPs): EPA develops chronic <i>oral</i> health benchmarks (RfDs and CSFs) for pesticides for surface and groundwater sources of drinking water using health effects data submitted during the pesticide registration process (U.S. EPA, 2016b).</p>
<p>Provisional Peer Reviewed Toxicity Values (PPRTVs): The Superfund Health Risk Technical Support Center (in the National Center for Environmental Assessment, Office of Research and Development) develops PPRTVs using the same methods as IRIS (U.S. EPA, 2016c).</p>
<p>Office of Water Health Effects Support Documents (HESDs): These documents may provide additional toxicity values not elsewhere available, but developed using the same methodology as IRIS.</p>
<p>Tier 2: Non-EPA Sources Using a Similar Methodology to Tier 1 Sources in Tier 2 contain toxicity values developed specifically for human health risk assessment by another organization using methods similar to IRIS. They represent the highest quality human health toxicity values available and are frequently used to support EPA risk analyses.</p>
<p>ATSDR Minimum Risk Levels (MRLs): The Agency for Toxic Substances and Disease Registry (ATSDR) develops MRLs, which are oral non-cancer toxicity values equivalent to RfDs (ATSDR, 2016).</p>
<p>CalEPA Reference Exposure Levels (RELs) and Cancer Potency Factors (CPFs): The California Environmental Protection Agency (CalEPA) develops RELs, which are non-cancer toxicity values equivalent to RfDs or RfCs (CalEPA, 2016) and CPFs, which are cancer toxicity values equivalent to CSFs or IURs (CalEPA, 2011).</p>
<p>Tier 3: Other Non-EPA Sources Tier 3 sources represent high-quality human health toxicity values that have been developed by other organizations for a use other than human health risk assessment or using methodologies that differ from IRIS.</p>

⁴ For more information about these methods, see <https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance>.

Data Sources Included
JECFA Acceptable Daily Intakes (ADIs): The Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) meets annually and issues ADIs, which are roughly equivalent to an RfD (FAO/WHO, 2014).
NAS Tolerable Upper Intake Levels: The National Academies of Science (specifically the Food and Nutrition Board of the Institutes of Medicine) issues Dietary Reference Intakes every 5 years; in concert with this, although less often, they also issue Tolerable Upper Limits for vitamins and elements. These Tolerable Upper Intake Levels are expressed in mg/day (or µg/day), so have been divided by a body weight of 70 kg to produce a toxicity value comparable to an RfD for use here. Values for non-pregnant, non-lactating adults aged 31–50 were used (male and female are presented separately but are the same values for elements) (NAS, 2010).
RIVM Maximum Permissible Risk Levels (MPRs): RIVM, the Dutch National Institute of Public Health and the Environment, maintains MPRs, which may be tolerable day intakes or tolerable concentrations in air for noncarcinogens (analogous to RfDs and RfCs), or may be a cancer risk oral or inhalation. These latter are not equivalent to a CSF or IUR, in that they are expressed as the dose or concentration in air, respectively, that results in a risk of 1E-4. To obtain a value comparable to a CSF or IUR, divide 1E-4 by the RIVM MPR (Baars et al., 2001). Note that RIVM reviewed a subset of these values in 2009 (Tiesjema and Baars, 2009), but none of the ones used here.
Tier 4: Other EPA Sources <i>This tier consists of outdated or no-longer-maintained EPA sources.</i>
Health Effects Assessment Summary Tables (HEAST): HEAST, once an alternative for chemicals without IRIS toxicity values, has not been updated since 1997 and has largely been superseded by IRIS and other more recent EPA sources described in Tier 1. It is rarely used, and only if no higher tier health toxicity values data are available (U.S. EPA, 1997).
Tier 5: Open Literature <i>These sources include journal articles that contain ADI values similar to RfDs and developed for potential use in assessing human risks but using methods or data (e.g., minimum therapeutic dose) that differ from IRIS.</i>
Tier 6: Other Sources <i>These sources have limited use in human health risk evaluations. For example, the U.S. Food and Drug Administration's (FDA's) tolerances for residues of drugs in food are for animal meat tissue (beef, fish, milk). These values are only used if no other health toxicity values data are available.</i>
FDA Tolerances for Residues of New Animal Drugs in Food. (21CFR556)
FDA Center for Veterinary Medicine. (http://www.fda.gov/AnimalVeterinary/default.htm)
FDA Center for Drug Evaluation and Research. (http://www.fda.gov/Drugs/default.htm)
European Union European Medicines Agency. (http://www.emea.europa.eu/)

For each chemical, the sources presented in Table 1 were searched from most preferred (IRIS) to least preferred. Once a value was found for a particular toxicity value (RfD, RfC, CSF, IUR), no lower ranked sources in the hierarchy were searched for that chemical. The lower tiers (Tiers 4, 5, and 6) were only used if no toxicity value of any kind was found in higher tiers (e.g., if IRIS had a RfD but no CSF, Tiers 2 and 3 would be searched for a CSF, but if none were found, Tiers 4, 5, and 6 would not be searched, as at least one toxicity value was available from a higher tier source).

2.2 Ecological Toxicity Value Data Sources and Selection

To assess the potential for ecological risks from biosolids, EPA assesses direct contact and ingestion pathways. For the direct contact exposure pathway, species assemblages (or communities) are assessed in soil, sediment, and surface water, where they are assumed to be exposed through direct contact with the contaminated medium. For the ingestion pathway,

mammals and birds are assumed to ingest contaminated food and prey from agricultural fields and a modeled farm pond receiving runoff from biosolids-treated fields.

The Agency uses articles published in 1) English in peer-reviewed journals; and 2) databases such as ECOTOX, Aquatic Sciences and Fisheries Abstracts, Biological Sciences Database, and the Environmental Sciences and Pollution Management Database.

The ecological toxicity values are expressed in terms of media concentration (e.g., mg/L for surface water and mg/kg for soil) for the direct contact pathway and in terms of dose (mg/kg-d) for the ingestion pathway. Because there is no single repository for approved ecological toxicity values analogous to IRIS, ecological toxicity values were derived from various EPA and other government reports and data sources (e.g., ECOTOX), and from toxicological studies in the open literature.

Data quality objectives for ecological ingestion toxicity values for use in this analysis included the following:

- Study should include test species, test species body weight, and study duration.
- Route of administration should be oral, not intraperitoneal injection.

Table 2 summarizes the selection criteria for ingestion toxicity values. Note that non-preferred data are used, but only if preferred data are not found. For studies that meet the above two primary criteria, the lowest toxicity values for ingestion exposures for each chemical/receptor combination is selected using a simple hierarchy:

- Endpoints relevant to population-level impacts (e.g., survival, growth, reproduction) are preferred over other endpoints (e.g., neurological effects). Sublethal endpoints are considered but are less preferred.
- Studies with exposure durations that are multigenerational or could be considered chronic or subchronic are preferred over studies conducted with acute exposure durations.

For direct contact toxicity values, environmental quality criteria are identified in existing EPA sources (e.g., national ambient water quality criteria). Other reputable sources of information, such as studies conducted at the Oak Ridge National Laboratories, or published by the Canadian Council of Ministers of the Environment are also used.

Table 2. Summary of Criteria for Selecting Ecological Ingestion Toxicity Data

All Studies
Assessment Endpoint (Effect)
Preferred: Effects related to population or community viability: reproduction, growth
Not Preferred: Mortality as a short-term result is less preferred than long-term or chronic effects
Not used: Effects not related to population or community viability
Study Duration
Preferred: Chronic, longest
Not Preferred: Acute, shorter
Measurement Endpoint
Preferred: Long-term or chronic NOAEL, LOAEL, MATL, or other threshold effects level
Not Preferred: Short-term or acute LC ₅₀ , LD ₅₀ , EC ₅₀
Measured vs. Predicted Values
Preferred: Measured
Not Preferred: Predicted
Mammal and Bird Studies
Type
Preferred: Ingestion (dietary and other) studies
Not used: Injection studies
Reported Data
Preferred: Test species, test duration, and body weight reported
Not Preferred: Test species, test duration, or body weight not reported
Aquatic Studies
Study Design
Preferred: Flow-through for long-term or chronic studies
Not Preferred: Static for short-term or acute studies

3. Results of the 2015 Biosolids Biennial Review

For the 2015 Biosolids Biennial Review, the Agency identified 46 articles that met the eligibility criteria and provided relevant information on chemicals that have been identified in biosolids.

Review of these articles found the following:

- Twenty-nine new chemicals were identified in biosolids in the 2015 Biennial Review (see Section 3.1).
- New human health toxicity data were identified for eight new chemicals (see Section 3.1.1) and one previously identified chemical (see Section 3.2.1).
- New ecological toxicity data were identified for one newly identified chemical (see Section 3.1.2).

- New physical-chemical property data (log K_{ow} and half-life) were identified for 11 chemicals: 10 previously identified chemicals and one newly identified chemical in the 2015 Biennial Review.
- New bioaccumulation factors for aquatic organisms were identified for one newly identified chemical (see Section 3.3).

The abstracts for the articles that provided relevant information are provided in **Attachment B**. Toxicity data for Human Health and Ecological Effects are identified below for new pollutants identified in this 2015 review and new data for pollutants identified in previous biennial reviews.

3.1 Pollutants Newly Identified in the 2015 Biennial Review

Table 3 lists 29 new chemicals identified in the 2015 Biosolids Biennial Review.

Table 3. Chemicals Identified in Biosolids in the 2015 Biennial Review

Chemical Name (CAS)	Class
Benzyl-4-chlorophenol, 2- (120-32-1)	Antimicrobial
Bis(5-chloro-2hydroxyphenyl)methane (97-23-4)	Antimicrobial
Chloro-4-phenylphenol, 2- (92-04-6)	Antimicrobial
Decamethylcyclopentasiloxane (D5) (541-02-6)	Emollient (used in production of cosmetics, lubricants, disinfection products)
Heptabromodibenzofuran, 1,2,3,4,6,7,8- (107555-95-3)	PBDF
Heptabromodibenzofuran, 1,2,3,4,7,8,9- (161880-51-9)	PBDF
Heptabromodibenzo-p-dioxin, 1,2,3,4,6,7,8- (103456-43-5)	PBDD
Hexabromodibenzofuran, 1,2,3,4,7,8- (70648-26-9)	PBDF
Hexabromodibenzofuran, 1,2,3,6,7,8- (107555-94-2)	PBDF
Hexabromodibenzofuran, 1,2,3,7,8,9- (161880-49-5)	PBDF
Hexabromodibenzofuran, 2,3,4,6,7,8- (60851-34-5)	PBDF
Hexabromodibenzo-p-dioxin, 1,2,3,4,7,8,0 (110999-44-5)	PBDD
Hexabromodibenzo-p-dioxin, 1,2,3,6,7,8- (110999-45-6)	PBDD
Hexabromodibenzo-p-dioxin, 1,2,3,7,8,9- (110999-46-7)	PBDD
N-nitrosodibutylamine (NDBA) 924-16-3	Nitrosamines
N-nitrosodiethylamine (NDEA) 55-18-5	Nitrosamines
N-nitrosodimethylamine (NDMA) 62-75-9	Nitrosamines
N-nitroso-di-n-propylamine (NDPA) 621-64-7	Nitrosamines

Chemical Name (CAS)	Class
N-nitrosodiphenylamine (NDPhA) 86-30-6	Nitrosamines
N-nitrosopiperidine (NPIP) 100-75-4	Nitrosamines
N-nitrosopyrrolidine (NPYR) 930-55-2	Nitrosamines
Octabromodibenzofuran, 1,2,3,4,6,7,8,9- (103582-29-2)	
Octabromodibenzo-p-dioxin, 1,2,3,4,6,7,8,9- (2170-45-8)	PBDD
Pentabromodibenzofuran, 1,2,3,7,8- (107555-93-1)	PBDF
Pentabromodibenzofuran, 2,3,4,7,8- (131166-92-2)	PBDF
Pentabromodibenzo-p-dioxin, 1,2,3,7,8- (109333-34-8)	PBDD
Tetrabromodibenzofuran, 2,3,7,8- (67733-57-7)	PBDF
Tetrabromodibenzo-p-dioxin, 2,3,7,8- (50585-41-6)	PBDD
Trichlorophenol, 2,4,5- (95-95-4)	Antimicrobial

3.1.1 Human Health Toxicity Values for Newly Identified Chemicals

Human health toxicity values were found for eight of the new chemicals identified in biosolids in the 2015 Biosolids Biennial Review: 2,3,5-trichlorophenol and the seven nitrosamines (**Table 4**).

Previously, EPA evaluated polychlorinated dioxins⁵ in 2001 for disposal and 2003 for land application and made the decision not to regulate them in biosolids; polybrominated dioxin-like compounds were not evaluated at that time. EPA has published a new methodology for assessing human health risk for dioxins (U.S. EPA 2010), and the WHO-UNEP approach recommends using a similar methodology for brominated and chlorinated congeners for human health risk assessment (van den Berg et al. 2013).

Table 4. Pollutants Identified in 2015 Biosolids Biennial Review for which Human Health Toxicity Values Were Found

Human Health Toxicity Value
Trichlorophenol, 2,4,5- (antimicrobial)
RfD = 0.1 mg/kg-d (human) Source: IRIS: U.S. EPA (2016a); last revised 1/31/1987
N-nitrosodimethylamine (NDMA)
CSF _{oral} = 51 per mg/kg-d (human) Source: IRIS: U.S. EPA (2016a); last revised 12/3/2002
N-nitrosodiethylamine (NDEA)
CSF _{oral} = 150 per mg/kg-d (human) Source: IRIS: U.S. EPA (2016a); last revised 10/28/2003
N-nitroso-di-n-propylamine (NDPA)
CSF _{oral} = 7.0 per mg/kg-d (human) Source: IRIS: U.S. EPA (2016a); last revised 12/3/2002

⁵ Dioxins refer to dioxin-like compounds that consist of 29 specific congeners, including seven 2,3,7,8-substituted congeners of PCDDs, ten 2,3,7,8-substituted congeners of PCDFs, and twelve coplanar PCBs.

Human Health Toxicity Value
N-nitrosodiphenylamine (NDPhA)
CSF _{oral} = 4.9E-3 per mg/kg-d (human) Source: IRIS: U.S. EPA (2016a); last revised 12/3/2002
N-nitrosopyrrolidine (NPYR)
CSF _{oral} = 2.1 per mg/kg-d (human) Source: IRIS: U.S. EPA (2016a); last revised 12/3/2002
N-nitrosodibutylamine (NDBA)
CSF _{oral} = 5.4 per mg/kg-d (human) Source: IRIS: U.S. EPA (2016a); last revised 10/28/2003
N-nitrosopiperidine (NPIP)
CSF _{oral} = 9.4 per mg/kg-d (human) Source: CalEPA CPFs: CalEPA (2011)

CSF_{oral} = oral cancer slope factor

RfD = reference dose

mg/kg-d = milligram/kilogram/day

3.1.2 Ecological Toxicity Values for Newly Identified Chemicals

Ecological toxicity values were found for one new chemical, decamethylcyclopentasiloxane (D5) (Table 5).

Previously, EPA evaluated polychlorinated dioxins in 2001 for disposal and 2003 for land application and made the decision not to regulate them in biosolids; polybrominated dioxin-like compounds were not evaluated at that time. EPA has published new methodology for assessing ecological risk for dioxins (U.S. EPA 2008), and the WHO-UNEP approach recommends using similar methodology for brominated and chlorinated congeners for ecological risk assessment (van den Berg et al. 2013).

Table 5. Ecological Toxicity Values Found for Chemicals Newly Identified in the 2015 Biosolids Biennial Review

Receptor	Medium	Endpoint	Value	Reference
Decamethylcyclopentasiloxane (D5) (541-02-6)				
<i>Lumbriculus variegatus</i> (California blackworm) using spiked sediment	Sediment	28-day LC50	>1272 mg/kg	Dow Corning, 2010a
<i>Lumbriculus variegatus</i> (California blackworm) using spiked sediment	Sediment	LOEC	>1272 mg/kg	Dow Corning, 2010a
<i>Lumbriculus variegatus</i> (California blackworm) using spiked sediment	Sediment	NOEC	>1272 mg/kg	Dow Corning, 2010a
<i>Chironomus riparius</i> (harlequin fly, a non-biting midge) using spiked sediment	Sediment	28-day EC50	257 mg/kg	Dow Corning, 2010a
<i>Chironomus riparius</i> (harlequin fly, a non-biting midge) using spiked sediment	Sediment	LOEC	160 mg/kg	Dow Corning, 2010a
<i>Chironomus riparius</i> (harlequin fly, a non-biting midge) using spiked sediment	Sediment	NOEC	70 mg/kg	Dow Corning, 2010a
<i>Hyalella Azteca</i> (freshwater amphipods)	Sediment	28-day EC50	310 mg/kg	Dow Corning, 2010a
<i>Hyalella Azteca</i> (freshwater amphipods)	Sediment	LOEC	230 mg/kg	Dow Corning, 2010a
<i>Hyalella Azteca</i> (freshwater amphipods)	Sediment	NOEC	130 mg/kg	Dow Corning, 2010a
Barley (<i>Hordeum vulgare</i>) [plant]	Soil	14-day NOEC	77 mg/kg	Fairbrother et al., 2015
Springtail (<i>Folsomia candida</i>) [soil invertebrate]	Soil	28-day NOEC	377 mg/kg	Fairbrother et al., 2015
Earthworm (<i>Eisenia Andrei</i>) [soil invertebrate]	Soil	56-day NOEC	507 mg/kg	Fairbrother et al., 2015
<i>Gromphadorhina portentosa</i> (Madagascar Hissing Cockroach)	Soil	Knockdown at 24 hr EC50 [90% CW ^a] (mg-kg)	1862 mg/kg [range: 788–2708]	Dow Corning, 2011
<i>Gromphadorhina portentosa</i> (Madagascar Hissing Cockroach)	Soil	Immobility at 24 hr EC50 [90% CW ^a] (mg-kg)	1472 mg/kg [range: 1244–1739]	Dow Corning, 2011
<i>Daphnia magna</i> (planktonic crustacean)	Water	48-hour EC50 (with exposure to ZMAT Number 4054113; D5 is a component at 1-5% by weight)	0.73 mg/L (95% confidence limits: 0.61 and 0.87)	Dow Corning, 2010b

3.2 New Information on Pollutants Previously Identified in Biennial Reviews

In each new biennial review EPA searches for new human health and ecological toxicity data, and environmental fate data for pollutants identified in biosolids in the TNSSS, open literature, or previous biosolids reviews. These chemicals are identified in **Attachments A**.

3.2.1 Human Health Toxicity Values for Previously Identified Chemicals

Table 6 presents data for one chemical for which a human health toxicity value was found as a result of the 2015 Biennial Review.

Table 6. Pollutants Identified in Previous Biennial Reviews for which Human Health Toxicity Values Were Found in the 2015 Biosolids Biennial Review

Human Health Toxicity Value
Carbamazepine (298-46-4): Anticonvulsant/mood stabilizer
nHRL _{acute} = 40 µg/L Source: MDH (2013)
RfD = 0.013 mg/kg-d (human) Source: MDH (2013)

RfD = reference dose

mg/kg/day = milligram/kilogram/day

Subsequent to publication of evaluations by EPA of polychlorinated dioxins in 2001 for disposal and 2003 for land application, EPA published new methodologies for assessing human health risk for dioxins (*2010: Recommended Toxicity Equivalence Factors (TEFs) for Human Health Risk Assessments of 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Dioxin-Like Compounds*). EPA will consider these new data and approaches in risk assessments.

3.2.2 Ecological Toxicity Values for Previously Identified Chemicals

No new ecological toxicity values were found in pollutants previously identified in biennial reviews as a result of the 2015 Biennial Review.

Subsequent to publication of evaluations by EPA of polychlorinated dioxins in 2001 for disposal and 2003 for land application, EPA published a new methodology for dioxins for assessing ecological risk (*2008: Framework for Application of the Toxicity Equivalence Methodology for Polychlorinated Dioxins, Furans, and Biphenyls in Ecological Risk Assessment*). EPA will consider these new data and approaches in risk assessments.

3.3 Environmental Fate and Transport Properties for New and Previously Identified Chemicals

Table 7 presents pollutant-specific physical and chemical properties for one newly identified chemical (D5) and 10 previously identified chemicals that could be used to determine the fate and transport of these pollutants. **Table 8** presents bioaccumulation factors for aquatic organisms for one newly identified chemical (D5).

Table 7. Physical-Chemical Properties Identified in the 2015 Biennial Review

Chemical	Half-life (days)	log Kow	Reference
2-Benzyl-4-chlorophenol	ND	3.6–4.2	Holzem et al., 2014
Bis(5-chloro-2-hydroxyphenyl)methane	ND	4.3	Holzem et al., 2014
Bisphenol A*	2.54	ND	Dodgen et al., 2014
2-Chloro-4-phenylphenol	ND	3.92	Holzem et al., 2014
Decamethylcyclopentasiloxane (D5)	12.6 (soil)	8.09	Mackay et al., 2015
Decamethylcyclopentasiloxane (D5)	70.4 (water at pH 7 and 25°C)	8.09	Mackay et al., 2015
Decamethylcyclopentasiloxane (D5)	3100 (sediment)	8.09	Mackay et al., 2015
Diclofenac*	2.93	ND	Dodgen et al., 2014
Naproxen*	4.45	ND	Dodgen et al., 2014
Nonlyphenol-111*	3.45	ND	Dodgen et al., 2014
2,4,5-Trichlorophenol	ND	3.6	Holzem et al., 2014
Triclocarban	ND	2.5–4.2	Holzem et al., 2014
Triclosan	ND	4.8	Holzem et al., 2014

* Laboratory study using agricultural soils from California or Arizona.

Table 8. Bioaccumulation Factors for Aquatic Organisms Exposed to Sediment Identified in the 2015 Biennial Review

Receptor	Endpoint	Value (mg/kg)	Reference
Decamethylcyclopentasiloxane (D5) (541-02-6)			
<i>Lumbriculus variegatus</i> (California blackworm) using spiked sediment	BSAF (biota-sediment accumulation factor)	6.9 (low treatment group) 0.74 (high treatment group)	Dow Corning, 2010a
<i>Lumbriculus variegatus</i> (California blackworm) using spiked sediment	BAF (bioaccumulation factor)	4.3 (low treatment group) 0.46 (high treatment group)	Dow Corning, 2010a
<i>Lumbriculus variegatus</i> (California blackworm) using spiked sediment	BAFK (kinetic bioaccumulation factor)	4.3 (low treatment group) 0.46 (high treatment group)	Dow Corning, 2010a

4. Conclusions

In order to complete a risk assessment using current tools the following data are needed:

- **Human health and ecological toxicity values** (i.e., studies that are adequate for evaluating hazards following acute or chronic exposure).
- **Exposure data and/or physical chemical properties.**
- **Pollutant concentrations in U.S. biosolids.** Pollutant concentration data are considered adequate when details are provided regarding sampling, handling, and analysis based on a suitable analytical methodology for detecting and quantifying pollutant concentrations. An analytical methodology is acceptable when the processes and techniques have been independently replicated and/or validated, and when written standard operating procedures exist.

- **Environmental fate and transport properties.** Data on half-life, mobility, and bioaccumulation are needed to model exposure to humans and wildlife.

Twenty-nine chemical pollutants in biosolids were newly identified in the 2015 Biosolids Biennial Review: 2-benzyl-4-chlorophenol (120-32-1); bis(5-chloro-2 hydroxyphenyl) methane (97-23-4); 2-chloro-4-phenylphenol (92-04-6); decamethylcyclopentasiloxane (D5) (541-02-6); seven nitrosamines; 2,4,5-trichlorophenol (95-95-4); seven polybrominated dibenzo-p-dioxins (PBDDs); and ten polybrominated dibenzofurans (PBDFs).

Data gaps limit the use of EPA's current biosolids modeling and risk assessment tools at this time for all newly identified chemicals. EPA will consider new data and approaches identified in this review in conducting risk assessments for the nitrosamines, three PBDDs, and five PBDFs. In addition, in the 2015 Biosolids Biennial Review, EPA identified new human health toxicity data for carbamazepine, previously found in the TNSSS (U.S. EPA 2009). EPA will consider these new data, along with other existing data, in conducting risk assessments.

Subsequent to publication of evaluations by EPA of polychlorinated dioxins in 2001 for disposal and 2003 for land application, EPA published new methodologies for assessing human and ecological risks of dioxins. EPA will consider these new approaches in risk assessments. In future biennial reviews, EPA intends to search the literature for updated information on toxicity and environmental fate properties for chemicals previously evaluated in addition to chemicals newly identified in biosolids. If new data are available these data will be considered to determine if these updated toxicity data change the prior assessment.

EPA has not identified any additional toxic pollutants for potential regulation during the 2015 Biosolids Biennial Review. The Agency will continue to assess the availability of sufficient information for these and other pollutants identified during future biennial review activities pursuant to section 405(d)(2)(C) of the CWA.

5. Additional Information

For additional information about EPA's Biosolids Program, please visit EPA's website at: <http://epa.gov/biosolids>.

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Attachment A. List of Pollutants Identified in Biosolids

Pollutant	CAS No.	Category	TNSSS Analyte?	When Identified?	Last BR Mention^a
Acetaminophen	103-90-2	Other drugs	X	2005BR	2005
Albuterol/Salbutamol	18559-94-9	Other drugs	X	2005BR	2013
Alprazolam	28981-97-7	Other drugs		2013BR	2013
Aluminum	7429-90-5	Metals	X	2005BR	2007
Amitriptyline	549-18-8	Other drugs		2013BR	2013
Amlodipine	88150-42-9	Other drugs		2013BR	2013
Amphetamine	300-62-9	Other drugs		2007BR	2007
Androstenedione	63-05-8	Hormones	X	2009 TNSSS	2009
Androsterone	53-41-8	Hormones	X	2009 TNSSS	2009
Anhydrochlortetracycline	13803-65-1	Antibiotics	X	2009 TNSSS	2009
Anhydrotetracycline	4496-85-9	Antibiotics	X	2009 TNSSS	2009
Antimony	7440-36-0	Metals	X	2005BR	2005
Aspirin	50-78-2	Other drugs		2005BR	2005
Atenolol	29122-68-7	Other drugs		2013BR	2013
Atorvastatin	134523-00-5	Other drugs		2013BR	2013
Azithromycin	83905-01-5	Antibiotics	X	2007BR	2011
Barium	7440-39-3	Metals	X	2009 TNSSS	2009
BDE-100 (2,2',4,4',6-PeBDE)	97038-97-6	PBDEs	X	2009 TNSSS	2009
BDE-138 (2,2',3,4,4',5'-HxBDE)	67888-98-6	PBDEs	X	2009 TNSSS	2009
BDE-153 (2,2',4,4',5,5'-HxBDE)	68631-49-2	PBDEs	X	2009 TNSSS	2009
BDE-154 (2,2',4,4',5,6'-HxBDE)	207122-15-4	PBDEs	X	2009 TNSSS	2009
BDE-183 (2,2',3,4,4',5',6'-HpBDE)	207122-16-5	PBDEs	X	2009 TNSSS	2009
BDE-209 (2,2',3,3',4,4',5,5',6,6'-DeBDE)	1163-19-5	PBDEs	X	2009BR	2009
BDE-28 (2,4,4'-TrBDE)	6430-90-6	PBDEs	X	2009 TNSSS	2009
BDE-47 (2,2',4,4'-TeBDE)	5436-43-1	PBDEs	X	2009 TNSSS	2009

Pollutant	CAS No.	Category	TNSSL Analyte?	When Identified?	Last BR Mention ^a
BDE-66 (2,3',4,4'-TeBDE)	84303-45-7	PBDEs	X	2009 TNSSS	2009
BDE-85 (2,2',3,4,4'-PeBDE)	32534-81-9	PBDEs	X	2009BR	2009
BDE-99 (2,2',4,4',5-PeBDE)	60348-60-9	PBDEs	X	2009 TNSSS	2009
Benz(a)anthracene	56-55-3	PAHs		2005BR	2005
Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-amino]	42615-29-2	Other drugs		2005BR	2005
Benzo(a)pyrene	50-32-8	PAHs	X	2005BR	2005
Benzo(b)fluoranthene	205-99-2	PAHs		2005BR	2005
Benzo(k)fluoranthene	207-08-9	PAHs		2005BR	2005
Benzoylecgonine	519-09-5	Other drugs		2013BR	2013
Benzotropine	86-13-5	Other drugs		2013BR	2013
Benzyl-4-chlorophenol, 2-	120-32-1	Antimicrobial		2015BR	2015
Beryllium	7440-41-7	Metals	X	2009 TNSSS	2009
Bezafibrate	41859-67-0	Other drugs		2005BR	2005
Bis (2-ethylhexyl) phthalate	117-81-7	SVOCs	X	2005BR	2005
Bis(5-chloro-2hydroxyphenyl)methane	97-23-4	Antimicrobial		2015BR	2015
Bisphenol A	80-05-7	Plastics		2007BR	2015
Boron	7440-42-8	Metals	X	2005BR	2005
Butylated hydroxy toluene	128-37-0	Other drugs		2005BR	2005
Caffeine	58-08-2	Other drugs	X	2005BR	2011
Calcium	7440-70-2	Inorganics	X	2007BR	2007
Campesterol	474-62-4	Steroids	X	2009 TNSSS	2009
Carbadox	6804-07-5	Antibiotics	X	2005BR	2005
Carbamazepine	298-46-4	Other drugs	X	2005BR	2013
Carbon tetrachloride	56-23-5	Organics		2005BR	2005
Cefotaxime	63527-52-6	Antibiotics	X	2009 TNSSS	2009
Cerium	7440-45-1	Metals		2005BR	2005
Chloro-4-phenylphenol, 2-	92-04-6	Antimicrobial		2015BR	2015
Chloroaniline, 4-	106-47-8	SVOCs	X	2009 TNSSS	2009
Chloroform	67-66-3	Organics		2005BR	2005
Chloronaphthalene, 2-	91-58-7	Organics		2005BR	2005
Chlortetracycline	57-62-5	Antibiotics	X	2009BR	2009
Cholestanol	80-97-7	Steroids	X	2009 TNSSS	2009

Pollutant	CAS No.	Category	TNSSS Analyte?	When Identified?	Last BR Mention ^a
Cholesterol	57-88-5	Steroids	X	2005BR	2007
Chrysene	218-01-9	PAHs		2005BR	2005
Cimetidine	51481-61-9	Other drugs	X	2005BR	2005
Ciprofloxacin	85721-33-1	Antibiotics	X	2005BR	2011
Clarithromycin	81103-11-9	Antibiotics	X	2007BR	2009
Clinafloxacin	105956-97-6	Antibiotics	X	2009 TNSSS	2009
Clindamycin	18323-44-9	Antibiotics		2011BR	2011
Clofibric acid	882-09-7	Other drugs		2005BR	2005
Clotrimazole	23593-75-1	Antibiotics		2011BR	2011
Cloxacillin	61-72-3	Antibiotics	X	2009 TNSSS	2009
Cobalt	7440-48-4	Metals	X	2005BR	2007
Cocaine	50-36-2	Other drugs		2013BR	2013
Codeine	76-57-3	Other drugs	X	2005BR	2005
Coprostanol (3-beta)	360-68-9	Steroids	X	2007BR	2007
Cotinine	486-56-6	Other drugs	X	2005BR	2005
Cresol, p- (4-methylphenol)	106-44-5	Preservative		2005BR	2007
Cyanide	57-12-5	Organics		2005BR	2005
Cyclophosphamide	50-18-0	Other drugs		2005BR	2005
Decamethylcyclopentasiloxane (D5)	541-02-6	Emollients		2015BR	2015
DEET (N,N-diethyltoluamide)	134-62-3	Pesticides		2005BR	2013
Dehydronifedipine	67035-22-7	Other drugs	X	2009 TNSSS	2009
Demeclocycline	127-33-3	Antibiotics	X	2009 TNSSS	2009
Desmethylidiltiazem	130606-60-9	Other drugs		2013BR	2013
Desmosterol	313-04-2	Steroids	X	2009 TNSSS	2009
Diazepam	439-14-5	Other drugs		2005BR	2005
Dichlorobenzene, 1,3-	541-73-1	Pesticides		2005BR	2005
Dichlorobenzene, 1,4-	106-46-7	Pesticides		2005BR	2005
Dichlorocarbaniilide	1219-99-4	Antibiotics		2011BR	2011
Diclofenac	15307-86-5	Antibiotics/ Pesticides		2011BR	2015
Diclofenac sodium	15307-79-6	Other drugs		2005BR	2005
Digoxigenin	1672-46-4	Other drugs	X	2009 TNSSS	2009
Digoxin	20830-75-5	Other drugs	X	2005BR	2005

Pollutant	CAS No.	Category	TNSSS Analyte?	When Identified?	Last BR Mention ^a
Dihydroequilin, 17 α -	651-55-8	Hormones	X	2009 TNSSS	2009
Diltiazem	42399-41-7	Other drugs	X	2005BR	2011
Dimethoate	60-51-5	Pesticides		2005BR	2005
Dimethyl phthalate	131-11-3	Organics		2005BR	2005
Dimethyl-3,5,-dinitro-4-tert-butylacetophenone, 2,6-	81-14-1	Odorants		2005BR	2005
Dimethylaminophenazone	58-15-1	Other drugs		2005BR	2005
Dimethylxanthine, 1,7-	611-59-6	Other drugs	X	2005BR	2005
Di-n-butyl phthalate (Butoxyphosphate ethanol, 2-)	84-74-2	Plasticizers		2005BR	2005
Di-n-octyl phthalate	117-84-0	Organics		2005BR	2005
Diphenhydramine	58-73-1	Other drugs	X	2007BR	2013
Di-tert-butylphenol, 2,6-	128-39-2	Other drugs		2005BR	2005
Doxycycline	564-25-0	Antibiotics	X	2005BR	2009
Endosulfan, α	959-98-8	Pesticides		2005BR	2005
Endosulfan, β	33213-65-9	Pesticides		2005BR	2005
Enrofloxacin	93106-60-6	Antibiotics	X	2009 TNSSS	2009
Epianhydrochlortetracycline, 4-	158018-53-2	Antibiotics	X	2009 TNSSS	2009
Epianhydrotetracycline, 4-	4465-65-0	Antibiotics	X	2009 TNSSS	2009
Epichlortetracycline, 4-	14297-93-9	Antibiotics	X	2009 TNSSS	2009
Epicoprostanol	516-92-7	Steroids	X	2009 TNSSS	2009
Epioxytetracycline, 4-	14206-58-7	Antibiotics	X	2009 TNSSS	2009
Epitetracycline, 4-	23313-80-6	Antibiotics	X	2009 TNSSS	2009
Equilenin	517-09-9	Hormones	X	2009 TNSSS	2009
Equilin	474-86-2	Hormones	X	2005BR	2005
Ergosterol	57-87-4	Steroids	X	2009 TNSSS	2009
Erythromycin	114-07-8	Antibiotics	X	2005BR	2009
Estradiol, 17 α -	57-91-0	Hormones	X	2005BR	2005
Estradiol, 17 β -	50-28-2	Hormones	X	2005BR	2009
Estradiol-3-benzoate, β -	50-50-0	Hormones	X	2009 TNSSS	2009

Pollutant	CAS No.	Category	TNSSS Analyte?	When Identified?	Last BR Mention ^a
Estriol (estradiol)	50-27-1	Hormones	X	2005BR	2005
Estrone	53-16-7	Hormones	X	2005BR	2011
Ethanol, 2-butoxy-phosphate	78-51-3	Organics		2005BR	2005
Ethylbenzene	100-41-4	Organics		2005BR	2005
Ethynyl estradiol, 17 α -	57-63-6	Hormones	X	2005BR	2005
Fenofibric acid	26129-32-8	Other drugs		2005BR	2005
Fenthion	55-38-9	Pesticides		2005BR	2005
Fipronil	120068-37-3	Antibiotics		2011BR	2011
Floxacillin	5250-39-5	Antibiotics		2005BR	2005
Flumequine	42835-25-6	Antibiotics	X	2009 TNSSS	2009
Fluoranthene	206-44-0	PAHs	X	2009 TNSSS	2009
Fluoride	16984-48-8	Inorganics	X	2005BR	2005
Fluoxetine	54910-89-3	Other drugs	X	2005BR	2007
Furosemide	54-31-9	Other drugs		2013BR	2013
Galaxolide	1222-05-5	Fragrance		2005BR	2011
Gemfibrozil	25812-30-0	Other drugs	X	2005BR	2011
Glyburide	10238-21-8	Other drugs		2013BR	2013
Heptabromodibenzofuran, 1,2,3,4,6,7,8-	107555-95-3	PBDF		2015BR	2015
Heptabromodibenzofuran, 1,2,3,4,7,8,9-	161880-51-9	PBDF		2015BR	2015
Heptabromodibenzo-p-dioxin, 1,2,3,4,6,7,8-	103456-43-5	PBDD		2015BR	2015
Heptachlor epoxide	1024-57-3	Pesticides		2005BR	2005
Hexabromobiphenyl, 2,2',4,4',5,5'-	59080-40-9	PBBs		2005BR	2005
Hexabromodibenzofuran, 1,2,3,4,7,8-	70648-26-9	PBDF		2015BR	2015
Hexabromodibenzofuran, 1,2,3,6,7,8-	107555-94-2	PBDF		2015BR	2015
Hexabromodibenzofuran, 1,2,3,7,8,9-	161880-49-5	PBDF		2015BR	2015
Hexabromodibenzofuran, 2,3,4,6,7,8-	60851-34-5	PBDF		2015BR	2015
Hexabromodibenzo-p-dioxin, 1,2,3,4,7,8-	110999-44-5	PBDD		2015BR	2015
Hexabromodibenzo-p-dioxin, 1,2,3,6,7,8-	110999-45-6	PBDD		2015BR	2015
Hexabromodibenzo-p-dioxin, 1,2,3,7,8,9-	110999-46-7	PBDD		2015BR	2015
Hydrocodone	125-29-1	Other drugs		2013BR	2013
Hydroxyamitriptyline, 10-	1246833-15-7	Other drugs		2013BR	2013
Ibuprofen	15687-27-1	Other drugs	X	2005BR	2005

Pollutant	CAS No.	Category	TNSSS Analyte?	When Identified?	Last BR Mention ^a
Indole	120-72-9	Fragrance		2007BR	2007
Indometacine	53-86-1	Other drugs		2005BR	2005
Iron	7439-89-6	Metals	X	2005BR	2005
Isochlortetracycline	514-53-4	Antibiotics	X	2009 TNSSS	2009
Ketoprofen	22071-15-4	Other drugs		2005BR	2005
Limonene, d-	5989-27-5	Fragrance		2007BR	2007
Lincomycin	154-21-2	Antibiotics	X	2009BR	2009
Lomefloxacin	98079-51-7	Antibiotics	X	2009 TNSSS	2009
Magnesium	7439-95-4	Metals	X	2007BR	2007
Manganese	7439-96-5	Metals	X	2009 TNSSS	2009
Mefenamic acid	61-68-7	Other drugs		2005BR	2005
Mesalazine	89-57-6	Other drugs		2005BR	2005
Mestranol	72-33-3	Other drugs		2005BR	2005
Metformin	657-24-9	Other drugs	X	2009 TNSSS	2009
Methamphetamine	537-46-2	Other drugs		2007BR	2009
Methylenedioxymethamphetamine, 3,4-	42542-10-9	Other drugs		2009BR	2009
Methylnaphthalene, 2-	91-57-6	PAHs	X	2005BR	2005
Metoprolol	37350-58-6	Other drugs		2005BR	2013
Miconazole	22916-47-8	Other drugs	X	2009 TNSSS	2009
Minocycline	10118-90-8	Antibiotics	X	2009 TNSSS	2009
Molybdenum	7439-98-7	Metals	X	2009 TNSSS	2009
Monuron	150-68-5	Pesticides		2005BR	2005
Nadolol	42200-33-9	Other drugs		2005BR	2005
Naproxen	22204-53-1	Other drugs	X	2005BR	2015
Napthalene	91-20-3	PAHs		2005BR	2005
Nitrate	14797-55-8	Inorganics	X	2009 TNSSS	2009
Nitrite	14797-65-0	Inorganics	X	2009 TNSSS	2009
Nitrofen	1836-75-5	Pesticides		2005BR	2005
Nitrogen	7727-37-9	Inorganics		2007BR	2007
Nitrogen, organic	14798-03-9	Organics		2007BR	2007
Nitrophenol, p-	100-02-7	Organics		2005BR	2005

Pollutant	CAS No.	Category	TNSSS Analyte?	When Identified?	Last BR Mention ^a
N-nitrosodibutylamine (NDBA) 924-16-3	924-16-3	Nitrosamines		2015BR	2015
N-nitrosodiethylamine (NDEA) 55-18-5	55-18-5	Nitrosamines		2015BR	2015
N-nitrosodimethylamine (NDMA) 62-75-9	62-75-9	Nitrosamines		2015BR	2015
N-nitroso-di-n-propylamine (NDPA) 621-64-7	621-64-7	Nitrosamines		2015BR	2015
N-nitrosodiphenylamine (NDPhA) 86-30-6	86-30-6	Nitrosamines		2015BR	2015
N-nitrosopiperidine (NPIP) 100-75-4	100-75-4	Nitrosamines		2015BR	2015
N-nitrosopyrrolidine (NPYR) 930-55-2	930-55-2	Nitrosamines		2015BR	2015
Nonylphenol	25154-52-3	Surfactants		2005BR	2011
Nonylphenol (branched), 4-	84852-15-3	Surfactants		2005BR	2005
Nonylphenol monoethoxylate	27986-36-3	Surfactants		2007BR	2007
Nonylphenol, 4-	104-40-5	Surfactants		2005BR	2007
Nonylphenol, diethoxy- (total)	NA	Surfactants		2007BR	2007
Norethindrone (norethisterone)	68-22-4	Hormones	X	2005BR	2005
Norfloxacin	70458-96-7	Antibiotics	X	2005BR	2011
Norfluoxetine	57226-68-3	Antibiotics		2011BR	2013
Norgestimate	35189-28-7	Other drugs	X	2009 TNSSS	2009
Norgestrel (levonorgestrel)	797-63-7	Hormones	X	2005BR	2005
Norverapamil	67812-42-4	Other drugs		2013BR	2013
Octabromodibenzofuran, 1,2,3,4,6,7,8,9-	103582-29-2	PBDF		2015BR	2015
Octabromodibenzo-p-dioxin, 1,2,3,4,6,7,8,9-	2170-45-8	PBDD		2015BR	2015
Octylphenol	67554-50-1	Organics		2005BR	2005
Octylphenol, 4-	1806-26-4	Organics		2007BR	2007
Ofloxacin	82419-36-1	Antibiotics	X	2009 TNSSS	2009
Ormetoprim	6981-18-6	Antibiotics	X	2009 TNSSS	2009
Oxacillin	66-79-5	Antibiotics	X	2009 TNSSS	2009
Oxolinic acid	14698-29-4	Antibiotics	X	2009 TNSSS	2009
Oxycodone	76-42-6	Other drugs		2013BR	2013
Oxytetracycline	79-57-2	Antibiotics	X	2005BR	2009
Paroxetine	61869-08-7	Other drugs		2013BR	2013
Penicillin G	61-33-6	Antibiotics	X	2009 TNSSS	2009

Pollutant	CAS No.	Category	TNSSS Analyte?	When Identified?	Last BR Mention ^a
Penicillin V (phenoxymethylpenicillin)	87-08-1	Antibiotics	X	2005BR	2005
Pentabromodibenzofuran, 1,2,3,7,8-	107555-93-1	PBDF		2015BR	2015
Pentabromodibenzofuran, 2,3,4,7,8-	131166-92-2	PBDF		2015BR	2015
Pentabromodibenzo-p-dioxin, 1,2,3,7,8-	109333-34-8	PBDD		2015BR	2015
Pentachloronitrobenzene	82-68-8	Pesticides		2005BR	2005
Perfluorheptanoate (PFHpA)	375-85-9	PFASs		2013BR	2013
Perfluorobutanesulfonate (PFBS)	45187-15-3	PFASs		2013BR	2013
Perfluorobutanoate (PFBA)	375-22-4	PFASs		2013BR	2013
Perfluorodecanoate (PFDA)	335-76-2	PFASs		2013BR	2013
Perfluorododecanoate (PFDoDA)	307-55-1	PFASs		2013BR	2013
Perfluorohexanesulfonate (PFHxS)	108427-53-8	PFASs		2013BR	2013
Perfluorohexanoate (PFHxA)	307-24-4	PFASs		2013BR	2013
Perfluoronoanoate (PFNA)	375-95-1	PFASs		2013BR	2013
Perfluorooctane sulfonamide (PFOSA)	754-91-6	PFASs		2013BR	2013
Perfluorooctanesulfonate (PFOS)	45298-90-6	PFASs		2013BR	2013
Perfluorooctanoate (PFOA)	335-67-1	PFASs		2013BR	2013
Perfluoropentanoate (PFPeA)	2706-90-3	PFASs		2013BR	2013
Perfluoroundecanoate (PFUnDA)	2058-94-8	PFASs		2013BR	2013
Phenanthrene	85-01-8	PAHs		2007BR	2007
Phenazone	60-80-0	Other drugs		2005BR	2005
Phosphate (total)	14265-44-2	Inorganics		2005BR	2005
Phosphorus	7723-14-0	Inorganics	X	2007BR	2007
Polyethylene glycol	25322-68-3	Organics		2005BR	2005
Potassium	7440-09-7	Metals		2007BR	2007
Progesterone	57-83-0	Hormones	X	2005BR	2009
Promethazine	60-87-7	Other drugs		2013BR	2013
Propoxyphene	469-62-5	Other drugs		2013BR	2013
Propranolol	525-66-6	Other drugs		2005BR	2013
Pyrene	129-00-0	PAHs	X	2009 TNSSS	2009
Quinine sulfate	7778-93-0	Other drugs		2005BR	2005
Ranitidine	66357-35-5	Other drugs	X	2005BR	2005
Roxithromycin	80214-83-1	Antibiotics	X	2007BR	2007
Rubidium	7440-17-7	Metals		2005BR	2005
Salicylic acid	69-72-7	Other drugs		2005BR	2005
Sarafloxacin	98105-99-8	Antibiotics	X	2009 TNSSS	2009
Sertraline	79617-96-2	Other drugs		2013BR	2013

Pollutant	CAS No.	Category	TNSSS Analyte?	When Identified?	Last BR Mention ^a
Silver	7440-22-4	Metals	X	2009 TNSSS	2009
Sitosterol, β -	83-46-5	Steroids	X	2007BR	2007
Skatole	83-34-1	NA		2007BR	2007
Sodium	7440-23-5	Metals	X	2009 TNSSS	2009
Sodium valproate	1069-66-5	Other drugs		2005BR	2005
Stigmastanol, β -	19466-47-8	Steroids	X	2007BR	2007
Stigmasterol	83-45-4	Steroids	X	2009 TNSSS	2009
Styrene	100-42-5	Organics		2005BR	2005
Sulfachloropyridazine	80-32-0	Antibiotics	X	2009 TNSSS	2009
Sulfadiazine	68-35-9	Antibiotics	X	2009 TNSSS	2009
Sulfadimethoxine	122-11-2	Antibiotics	X	2009BR	2009
Sulfamerazine	127-79-7	Antibiotics	X	2005BR	2005
Sulfamethazine	57-68-1	Antibiotics	X	2005BR	2009
Sulfamethizole	144-82-1	Antibiotics	X	2009 TNSSS	2009
Sulfamethoxazole	723-46-6	Antibiotics	X	2009 TNSSS	2009
Sulfanilamide	63-74-1	Antibiotics	X	2009 TNSSS	2009
Sulfasalazine	599-79-1	Other drugs		2005BR	2005
Sulfathiazole	72-14-0	Antibiotics	X	2009 TNSSS	2009
tert-Butyl-4-hydroxy anisole, 3-	25013-16-5	Other drugs		2005BR	2005
Testosterone	58-22-0	Hormones	X	2009BR	2009
Tetrabromobisphenol A	79-94-7	Organics		2005BR	2005
Tetrabromodibenzofuran, 2,3,7,8-	67733-57-7	PBDF		2015BR	2015
Tetrabromodibenzo-p-dioxin, 2,3,7,8-	50585-41-6	PBDD		2015BR	2015
Tetrachloroethylene	127-18-4	Solvents		2005BR	2005
Tetracycline	60-54-8	Antibiotics	X	2009BR	2009
Thallium	7440-28-0	Metals	X	2005BR	2005
Thiabendazole	148-79-8	Other drugs	X	2009 TNSSS	2009
Tin	7440-31-5	Metals	X	2005BR	2005
Titanium	7440-32-6	Metals	X	2009 TNSSS	2009
Toluene	108-88-3	Solvents		2005BR	2005

Pollutant	CAS No.	Category	TNSSS Analyte?	When Identified?	Last BR Mention ^a
Tonalide (AHTN)	21145-77-7	Fragrance		2007BR	2011
Triamterene	396-01-0	Other drugs		2013BR	2013
Trichlorobenzene, 1,3,5-	108-70-3	Organics		2005BR	2005
Trichlorofon	52-68-6	Pesticides		2005BR	2005
Trichlorophenol, 2,4,5-	95-95-4	Antimicrobial		2015BR	2015
Triclocarban	101-20-2	Antibiotics	X	2007BR	2015
Triclosan	3380-34-5	Antibiotics	X	2005BR	2015
Trimethoprim	738-70-5	Antibiotics	X	2005BR	2009
Triphenyl phosphate	115-86-6	Pesticides		2005BR	2005
Tris(2-chloroethyl) phosphate	115-96-8	Organics		2005BR	2005
Tylosin	1401-69-0	Antibiotics	X	2005BR	2007
Valsartan	137862-53-4	Other drugs		2013BR	2013
Vanadium	7440-62-2	Metals	X	2005BR	2005
Verapamil	52-53-9	Other drugs		2013BR	2013
Virginiamycin	11006-76-1	Antibiotics	X	2005BR	2009
Warfarin	81-81-2	Other drugs	X	2009 TNSSS	2009
Xylene, m-	108-38-3	Solvents		2005BR	2005
Xylene, musk	81-15-2	Odorants		2005BR	2005
Xylene, o-	95-47-6	Solvents		2005BR	2005
Xylene, p	106-42-3	Solvents		2005BR	2005
Yttrium	7440-65-5	Metals	X	2005BR	2005
Microbial Pollutants					
Aerobic endospores	Not applicable	Bacteria		2013BR	2013
<i>Aeromonas</i> spp.	Not applicable	Bacteria		2009BR	2009
Antibiotic-resistant bacteria (ARB) or Antibiotic-resistant genes (ARG)	Not applicable	Bacteria		2013BR	2013
<i>Clostridia</i> spp.	Not applicable	Bacteria		2007BR	2011
Coronavirus HKU1	Not applicable	Virus		2013BR	2013
Cosavirus	Not applicable	Virus		2013BR	2013
<i>Cryptosporidium parvum</i>	Not applicable	Protozoan parasite		2007BR	2007
Enterovirus	Not applicable	Virus		2009BR	2013
<i>Escherichia coli</i> (<i>E. coli</i>)	Not applicable	Bacteria		2009BR	2013
Endotoxin	Not applicable	Microbial toxin		2007BR	2007
<i>Giardia</i> spp.	Not applicable	Protozoan parasite		2009BR	2011
Human Adenoviruses	Not applicable	Virus		2009BR	2013

Pollutant	CAS No.	Category	TNSS Analyte?	When Identified?	Last BR Mention ^a
Human polyomaviruses	Not applicable	Virus		2011BR	2011
Klassevirus	Not applicable	Virus		2013BR	2013
<i>Listeria</i> spp.	Not applicable	Bacteria		2009BR	2011
Human norovirus	Not applicable	Virus		2013BR	2013
<i>Salmonella</i> spp.	Not applicable	Bacteria		2007BR	2013

^a This is the date of the most recent biennial report that mentions this pollutant. That does not necessarily mean there was new data found, just that it came up in the literature search that year.

Attachment B. Reference Abstracts

Andrade, N. A., et al. (2015). "Long-term trends of PBDEs, triclosan, and triclocarban in biosolids from a wastewater treatment plant in the Mid-Atlantic region of the US." *J Hazard Mater* 282: 68-74.

In the US, land application of biosolids has been utilized in government-regulated programs to recycle valuable nutrients and organic carbon that would otherwise be incinerated or buried in landfills. While many benefits have been reported, there are concerns that these practices represent a source of organic micropollutants to the environment. In this study, biosolids samples from a wastewater treatment plant in the Mid-Atlantic region of the US were collected approximately every 2 months over a 7-year period and analyzed for brominated diphenyl ethers (BDE-47, BDE-99, and BDE-209), triclosan, and triclocarban. During the collection period of 2005-2011, concentrations of the brominated diphenyl ethers BDE-47+BDE-99 decreased by 42%, triclocarban decreased by 47%, but BDE-209 and triclosan remained fairly constant. Observed reductions in contaminant concentrations could not be explained by different seasons or by volumetric changes of wastewaters arriving at the treatment plant and instead may be the result of the recent phaseout of BDE-47 and BDE-99 as well as potential reductions in the use of triclocarban.

Balasubramani, A. and H. S. Rifai (2015). "Occurrence and distribution of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs) in industrial and domestic sewage sludge." *Environ Sci Pollut Res Int* 22(19): 14801-14808.

Sewage sludge samples collected from 43 different domestic and industrial wastewater treatment plants and petrochemical industries that discharge to the Houston Ship Channel (HSC) were analyzed for polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs), which are highly toxic and carcinogenic towards humans and animals. The measured total PCDD/F toxic equivalency (TEQ) ranged between 0.73 and 7348.40 pg/g dry weight. The mean TEQ of PCDD/Fs in industrial sludge was approximately 40 times higher than that in sewage sludge. The PCDD homolog concentrations in the industrial samples were higher than those observed at the wastewater treatment plants by a factor of 10, with total heptachlorodibenzodioxin (HpCDD) exhibiting the maximum concentration in most of the samples. Among the PCDF homologs, total heptadichlorodibenzofuran (HpCDF) dominated the total homolog concentration in sludge from the wastewater treatment plants, whereas total tetradichlorodibenzofuran (TeCDF) dominated the industrial sludge samples. Overall, the total PCDD/F TEQ in sludge samples was much higher than that in effluent samples from the same facility. A linear correlation ($R^2=0.62$, $p < 0.068$) was found indicating that sludge sampling can be used as a surrogate for effluent concentrations in wastewater treatment plants but not for industrial discharges.

Bircher, S., et al. (2015). "Sorption, uptake, and biotransformation of 17beta-estradiol, 17alpha-ethinylestradiol, zeranol, and trenbolone acetate by hybrid poplar." *Environ Toxicol Chem* 34(12): 2906-2913.

Hormonally active compounds may move with agricultural runoff from fields with applied manure and biosolids into surface waters where they pose a threat to human and environmental health. Riparian zone plants could remove hormonally active compounds from agricultural runoff. Therefore, sorption to roots, uptake, translocation, and transformation of 3 estrogens (17beta-estradiol, 17alpha-ethinylestradiol, and zeranol) and 1 androgen (trenbolone acetate) commonly found in animal manure or biosolids were assessed by hydroponically grown hybrid poplar, *Populus deltoides* x *nigra*, DN-34, widely used in riparian buffer strips. Results clearly showed that these hormones were rapidly removed from 2 mg L⁻¹ hydroponic solutions by more than 97% after 10 d of exposure to full poplar plants or live excised poplars (cut-stem, no leaves). Removals by sorption to dead poplar roots that had been autoclaved were significantly less, 71% to 84%. Major transformation products (estrone and estriol for estradiol; zearalanone for zeranol; and 17beta-trenbolone from trenbolone acetate) were detected in the root tissues of all 3 poplar treatments. Root concentrations of metabolites peaked after 1 d to 5 d and then decreased in full and live excised poplars by further transformation. Metabolite concentrations were less in dead poplar treatments and only slowly increased without further transformation. Taken together, these findings show that poplars may be effective in controlling the movement of hormonally active compounds from agricultural fields and avoiding runoff to streams.

Dodgen, L. K., et al. (2014). "Transformation and removal pathways of four common PPCP/EDCs in soil." *Environ Pollut* 193: 29-36.

Pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) enter the soil environment via irrigation with treated wastewater, groundwater recharge, and land application of biosolids. The transformation and fate of PPCP/EDCs in soil affects their potential for plant uptake and groundwater pollution. This study examined four PPCP/EDCs (bisphenol A, diclofenac, naproxen, and 4-nonylphenol) in soil by using (14)C-labeling and analyzing mineralization, extractable residue, bound residue, and formation of transformation products. At the end of 112 d of incubation, the majority of (14)C-naproxen and (14)C-diclofenac was mineralized to (14)CO₂, while a majority of (14)C-bisphenol A and (14)C-nonylphenol was converted to bound residue. After 112 d, the estimated half-lives of the parent compounds were only 1.4-5.4 d. However, a variety of transformation products were found and several for bisphenol A and diclofenac were identified, suggesting the need to consider degradation intermediates in soils impacted by PPCP/EDCs.

Fairbrother, A, et al. (2015). "Characterization of Ecological Risks from Environmental Releases of Decamethylcyclopentasiloxane (D5)." *Env. Toxic. and Chem.* 34(12):2715-22.

Decamethylcyclopentasiloxane (D5) is used in personal care products and industrial applications. The authors summarize the risks to the environment from D5 based on multiple lines of evidence and conclude that it presents negligible risk. Laboratory and field studies show that D5 is not toxic to aquatic organisms or benthic invertebrates up to its solubility limit in water or porewater or its sorptive capacity in sediment. Comparison of lipid-normalized internal concentrations with measured concentrations in benthos indicates that field-collected organisms do not achieve toxic levels of D5 in their tissues, suggesting negligible risk. Exposure to D5 resulted in a slight reduction of root biomass in barley at test concentrations 2 orders of magnitude greater than measured D5 levels in biosolids-amended soils and more than twice as high as the maximum calculated sorptive capacity of the soil. No effects were observed in soil invertebrates exposed to similar concentrations, indicating that D5 poses a de minimis risk to the terrestrial environment. High rates of metabolism and elimination of D5 compared with uptake rates from food results in biodilution in the food web rather than biomagnification, culminating in de minimis risk to higher trophic level organisms via the food chain. A fugacity approach substantiates all conclusions that were made on a concentration basis.

Federle, T., et al. (2014). "Probabilistic assessment of environmental exposure to the polycyclic musk, HHCB and associated risks in wastewater treatment plant mixing zones and sludge amended soils in the United States." *Sci Total Environ* 493: 1079-1087.

The objective of this work was to conduct an environmental risk assessment for the consumer use of the polycyclic musk, HHCB (CAS No. 1222-05-5) in the U.S. focusing on mixing zones downstream from municipal wastewater treatment plants (WWTPs) and sludge amended soils. A probabilistic exposure approach was utilized combining statistical distributions of effluent and sludge concentrations for the U.S. WWTPs with distributions of mixing zone dilution factors and sludge loading rates to soil to estimate HHCB concentrations in surface waters and sediments below WWTPs and sludge amended soils. These concentrations were then compared to various toxicity values. Measured concentrations of HHCB in effluent and sludge from a monitoring program of 40 WWTPs across the U.S. formed the basis for estimating environmental loadings. Based upon a Monte Carlo analysis, the probability of HHCB concentrations being below the PNEC (predicted no effect concentration) for pelagic freshwater organisms was greater than or equal to 99.87% under both mean and low flow regimes. Similarly, the probability of HHCB concentrations being less than the PNEC for freshwater sediment organisms was greater than or equal to 99.98%. Concentrations of HHCB in sludge amended soils were estimated for single and repeated annual sludge applications with tilling of the sludge into the soil, surface application without tilling and a combination reflecting current practice. The probability of soil HHCB concentrations being below the PNEC for soil organisms after repeated sludge applications was 94.35% with current sludge practice. Probabilistic estimates of HHCB exposures in surface waters, sediments and sludge amended soils are consistent with the published values for the U.S. In addition, the results of these analyses indicate that HHCB entering the environment in WWTP effluent and sludge poses negligible risk to aquatic and terrestrial organisms in nearly all exposure scenarios.

Fisher, J. C., et al. (2014). "Population dynamics and ecology of *Arcobacter* in sewage." *Front Microbiol* 5.

Arcobacter species are highly abundant in sewage where they often comprise approximately 5-11% of the bacterial community. Oligotyping of sequences amplified from the V4V5 region of the 16S rRNA gene revealed *Arcobacter* populations from different cities were similar and dominated by 1-3 members, with extremely high microdiversity in the minor members. Overall, nine subgroups within the *Arcobacter* genus accounted for >80% of the total *Arcobacter* sequences in all samples analyzed. The distribution of oligotypes varied by both sample site and temperature, with samples from the same site generally being more similar to each other than other sites. Seven oligotypes matched with 100% identity to characterized *Arcobacter* species, but the remaining 19 abundant oligotypes appear to be unknown species. Sequences representing the two most abundant oligotypes matched exactly to the reference strains for *A. cryaerophilus* group 1B (CCUG 17802) and group 1A (CCUG 17801(T)), respectively. Oligotype 1 showed generally lower relative abundance in colder samples and higher relative abundance in warmer samples; the converse was true for Oligotype 2. Ten other oligotypes had significant positive or negative correlations between temperature and proportion in samples as well. The oligotype that corresponded to *A. butzleri*, the *Arcobacter* species most commonly isolated by culturing in sewage studies, was only the eleventh most abundant oligotype. This work suggests that *Arcobacter* populations within sewer infrastructure are modulated by temperature. Furthermore, current culturing methods used for identification of *Arcobacter* fail to identify some abundant members of the community and may underestimate the presence of species with affinities for growth at lower temperatures. Understanding the ecological factors that affect the survival and growth of *Arcobacter* spp. in sewer infrastructure may better inform the risks associated with these emerging pathogens.

Guerra, P., et al. (2015). "Occurrence and Fate of Trace Contaminants during Aerobic and Anaerobic Sludge Digestion and Dewatering." *J Environ Qual* 44(4): 1193-1200.

Digestion of municipal wastewater biosolids is a necessary prerequisite to their beneficial use in land application, in order to protect public health and the receiving environment. In this study, 13 pharmaceuticals and personal care products (PPCPs), 11 musks, and 17 polybrominated diphenyl ethers were analyzed in 84 samples including primary sludge, waste activated sludge, digested biosolids, dewatered biosolids, and dewatering centrate or filtrate collected from five wastewater treatment plants with aerobic or anaerobic digestion. Aerobic digestion processes were sampled during both warm and cold temperatures to analyze seasonal differences. Among the studied compounds, triclosan, triclocarban, galaxolide, and BDE-209 were the substances most frequently detected under different treatment processes at levels up to 30,000 ng/g dry weight. Comparing aerobic and anaerobic digestion, it was observed that the levels of certain PPCPs and musks were significantly higher in anaerobically digested biosolids, relative to the residues from aerobic digestion. Therefore, aerobic digestion has the potential advantage of reducing levels of PPCPs and musks. On the other hand, anaerobic digestion has the advantage of recovering energy from the biosolids in the form of combustible gases while retaining the nutrient and soil conditioning value of this resource.

Holzem, R. M., et al. (2014). "Determining the Ecological Impacts of Organic Contaminants in Biosolids Using a High-Throughput Colorimetric Denitrification Assay: A Case Study with Antimicrobial Agents" *Environ Sci Technol* 48: 1646-1655.

Land application accounts for ~50% of wastewater solid disposal in the United States. Still, little is known regarding the ecological impacts of nonregulated contaminants found in biosolids. Because of the myriad of contaminants, there is a need for a rapid, high-throughput method to evaluate their ecotoxicity. Herein, we developed a novel assay that measures denitrification inhibition in a model denitrifier, *Paracoccus denitrificans* Pd1222. Two common (triclosan and triclocarban) and four emerging (2,4,5 trichlorophenol, 2-benzyl-4-chlorophenol, 2-chloro-4-phenylphenol, and bis(5-chloro-2-hydroxyphenyl)methane) antimicrobial agents found in biosolids were analyzed. Overall, the assay was reproducible and measured impacts on denitrification over 3 orders of magnitude exposure. The lowest observable adverse effect concentrations (LOAECs) were 1.04 μM for triclosan, 3.17 μM for triclocarban, 0.372 μM for bis-(5-chloro-2-hydroxyphenyl)methane, 4.89 μM for 2-chloro-4-phenyl phenol, 45.7 μM for 2-benzyl-4-chlorophenol, and 50.6 μM for 2,4,5-trichlorophenol. Compared with gene expression and cell viability based methods, the denitrification assay was more sensitive and resulted in lower LOAECs. The increased sensitivity, low cost, and high-throughput adaptability make this method an attractive alternative for meeting the initial testing regulatory framework for the Federal Insecticide, Fungicide, and Rodenticide Act, and recommended for the Toxic Substances Control Act, in determining the ecotoxicity of biosolids-derived emerging contaminants.

Judy, J. D., et al. (2015). "Effects of silver sulfide nanomaterials on mycorrhizal colonization of tomato plants and soil microbial communities in biosolid-amended soil." *Environ Pollut* 206: 256-263.

We investigated effects of Ag₂S engineered nanomaterials (ENMs), polyvinylpyrrolidone (PVP) coated Ag ENMs (PVP-Ag), and Ag(+) on arbuscular mycorrhizal fungi (AMF), their colonization of tomato (*Solanum lycopersicum*), and overall microbial community structure in biosolids-amended soil. Concentration-dependent uptake was measured in all treatments. Plants exposed to 100 mg kg⁻¹ PVP-Ag ENMs and 100 mg kg⁻¹ Ag(+) exhibited reduced biomass and greatly reduced mycorrhizal colonization. Bacteria, actinomycetes and fungi were inhibited by all treatment classes, with the largest reductions measured in 100 mg kg⁻¹ PVP-Ag ENMs and 100 mg kg⁻¹ Ag(+). Overall, Ag₂S ENMs were less toxic to plants, less disruptive to plant-mycorrhizal symbiosis, and less inhibitory to the soil microbial community than PVP-Ag ENMs or Ag(+). However, significant effects were observed at 1 mg kg⁻¹ Ag₂S ENMs, suggesting that the potential exists for microbial communities and the ecosystem services they provide to be disrupted by environmentally relevant concentrations of Ag₂S ENMs.

Judy, J. D., et al. (2015). "Nanomaterials in Biosolids Inhibit Nodulation, Shift Microbial Community Composition, and Result in Increased Metal Uptake Relative to Bulk/Dissolved Metals." *Environ Sci Technol* 49(14): 8751-8758.

We examined the effects of amending soil with biosolids produced from a pilot-scale wastewater treatment plant containing a mixture of metal-based engineered nanomaterials (ENMs) on the growth of *Medicago truncatula*, its symbiosis with *Sinorhizobium meliloti*, and on soil microbial community structure. Treatments consisted of soils amended with biosolids generated with (1) Ag, ZnO, and TiO₂ ENMs introduced into the influent wastewater (ENM biosolids), (2) AgNO₃, Zn(SO₄)₂, and micron-sized TiO₂ (dissolved/bulk metal biosolids) introduced into the influent wastewater stream, or (3) no metal added to influent wastewater (control). Soils were amended with biosolids to simulate 20 years of metal loading, which resulted in nominal metal concentrations of 1450, 100, and 2400 mg kg⁻¹ of Zn, Ag, and Ti, respectively, in the dissolved/bulk and ENM treatments. Tissue Zn concentrations were significantly higher in the plants grown in the ENM treatment (182 mg kg⁻¹) compared to those from the bulk treatment (103 mg kg⁻¹). Large reductions in nodulation frequency, plant growth, and significant shifts in soil microbial community composition were found for the ENM treatment compared to the bulk/dissolved metal treatment. These results suggest differences in metal bioavailability and toxicity between ENMs and bulk/dissolved metals at concentrations relevant to regulatory limits.

Koupaie, E. H. and C. Eskicioglu (2015). "Health risk assessment of heavy metals through the consumption of food crops fertilized by biosolids: A probabilistic-based analysis." *J Hazard Mater* 300: 855-865.

The objective of this study was to perform a probabilistic risk analysis (PRA) to assess the health risk of Cadmium (Cd), Copper (Cu), and Zinc (Zn) through the consumption of food crops grown on farm lands fertilized by biosolids. The risk analysis was conducted using 8 years of historical heavy metal data (2005-2013) of the municipal biosolids generated by a nearby treatment facility considering one-time and long-term biosolids land application scenarios for a range of 5-100 t/ha fertilizer application rate. The 95th percentile of the hazard index (HI) increased from 0.124 to 0.179 when the rate of fertilizer application increased from 5 to 100 t/ha at one-time biosolids land application. The HI at long-term biosolids land application was also found 1.3 and 1.9 times greater than that of one-time land application at fertilizer application rates of 5 and 100 t/ha, respectively. Rice ingestion had more contribution to the HI than vegetable ingestion. Cd and Cu were also found to have more contribution to the health risk associated to vegetable and rice ingestion, respectively. Results indicated no potential risk to the human health even at long-term biosolids land application scenario at 100 t/ha fertilizer application rate.

Lee, H., et al. (2014). "Fate of Polyfluoroalkyl Phosphate Diesters and Their Metabolites in Biosolids-Applied Soil: Biodegradation and Plant Uptake in Greenhouse and Field Experiments." *Environ Sci Technol* 48(1): 340-349.

Significant contamination of perfluoroalkyl acids (PFAAs) in wastewater treatment plant (WWTP) sludge implicates the practice of applying treated sludge or biosolids as a potential source of these chemicals onto agricultural farmlands. Recent efforts to characterize the sources of PFAAs in the environment have unveiled a number of fluorotelomer-based materials that are capable of degrading to the perfluoroalkyl carboxylates (PFCAs), such as the polyfluoroalkyl phosphate diesters (diPAPs), which have been detected in WWTP and paper fiber biosolids. Here, a greenhouse microcosm was used to investigate the fate of endogenous diPAPs and PFCAs present in WWTP and paper fiber biosolids upon amendment of these materials with soil that had been sown with *Medicago truncatula* plants. Biodegradation pathways and plant uptake were further elucidated in a separate greenhouse microcosm supplemented with high concentrations of 6:2 diPAP. Biosolid-amended soil exhibited increased concentrations of diPAPs (4-83 ng/g dry weight (dw)) and PFCAs (0.1-19 ng/g dw), as compared to control soils (nd-1.4 ng/g dw). Both plant uptake and biotransformation contributed to the observed decline in diPAP soil concentrations over time. Biotransformation was further evidenced by the degradation of 6:2 diPAP to its corresponding fluorotelomer intermediates and C4-C7 PFCAs. Substantial plant accumulation of endogenous PFCAs present in the biosolids (0.1-138 ng/g wet weight (ww)) and those produced from 6:2 diPAP degradation (100-58 000 ng/g ww) were observed within 1.5 months of application, with the congener profile dominated by the short-chain PFCAs (C4-C6). This pattern was corroborated by the inverse relationship observed between the plant soil accumulation factor (PSAF, C-plant/C-soil) and carbon chain length ($p < 0.05$, $r = 0.90-0.97$). These results were complemented by a field study in which the fate of diPAPs and PFCAs was investigated upon application of compost and paper fiber biosolids to two farm fields. Together, these studies provide the first evidence of soil biodegradation of diPAPs and the subsequent uptake of these chemicals and their metabolites into plants.

Luo, F., et al. (2014). "Characterization of contaminants and evaluation of the suitability for land application of maize and sludge biochars." *Environ Sci Pollut Res Int* 21(14): 8707-8717.

Prior to the application of biochar as an agricultural improver, attention should be paid to the potential introduction of toxicants and resulting unintended impacts on the environment. In the present study, the concentrations of polycyclic aromatic hydrocarbons (PAHs), heavy metals, and mineral elements were determined in maize and sludge biochars produced at 100 degree C increments between 200 and 700 degree C. The concentration ranges of total PAHs were 358-5,136 $\mu\text{g kg}^{-1}$ in maize biochars and 179-70,385 $\mu\text{g kg}^{-1}$ in sludge biochars. The total heavy metals were detected at the following concentrations (mg kg^{-1}): Cu, 20.4-56.7; Zn, 59.7-133; Pb, 1.44-3.50; Cd, <0.014; Cr, 8.08-21.4; Ni, 4.38-9.82 in maize biochars and Cu, 149-202; Zn, 735-986; Pb, 54.7-74.2; Cd, 1.06-1.38; Cr, 180-247; Ni, 41.1-56.1 in sludge biochars. The total concentrations of PAHs and heavy metals in all maize biochars and most sludge biochars were below the control standards of sludge for agricultural use in China, the USA, and Europe. The leachable Mn concentrations in sludge biochars produced at below 500 degree C exceeded the groundwater or drinking water standards of these countries. Overall, all the maize biochars were acceptable for land application, but sludge biochars generated at temperatures between 200 and 500 degree C were unsuitable for application as soil amendments due to their potential adverse effects on soil and groundwater quality.

Luo, K., et al. (2015). "Efficiency of repeated phytoextraction of cadmium and zinc from an agricultural soil contaminated with sewage sludge." *Int J Phytoremediation* 17(1-6): 575-582.

Long-term application of sewage sludge resulted in soil cadmium (Cd) and zinc (Zn) contamination in a pot experiment conducted to phytoextract Cd/Zn repeatedly using *Sedum plumbizincicola* and *Apium graveolens* in monoculture or intercropping mode eight times. Shoot yields and soil physicochemical properties changed markedly with increasing number of remediation crops when the two plant species were intercropped compared with the unplanted control soil and the two monoculture treatments. Changes in soil microbial indices such as average well colour development, soil enzyme activity and soil microbial counts were also significantly affected by the growth of the remediation plants, especially intercropping with *S. plumbizincicola* and *A. graveolens*. The higher yields and amounts of Cd taken up indicated that intercropping of the hyperaccumulator and the vegetable species may be suitable for simultaneous agricultural production and soil remediation, with larger crop yields and higher phytoremediation efficiencies than under monoculture conditions.

Ma, R., et al. (2014). "Fate of Zinc Oxide and Silver Nanoparticles in a Pilot Wastewater Treatment Plant and in Processed Biosolids." *Environ Sci Technol* 48(1): 104-112.

Chemical transformations of silver nanoparticles (Ag NPs) and zinc oxide nanoparticles (ZnO NPs) during wastewater treatment and sludge treatment must be characterized to accurately assess the risks that these nanomaterials pose from land application of biosolids. Here, X-ray absorption spectroscopy (XAS) and supporting characterization methods are used to determine the chemical speciation of Ag and Zn in sludge from a pilot wastewater treatment plant (WWTP) that had received PVP coated 50 nm Ag NPs and 30 nm ZnO NPs, dissolved metal ions, or no added metal. The effects of composting and lime and heat treatment on metal speciation in the resulting biosolids were also examined. All added Ag was converted to Ag₂S, regardless of the form of Ag added (NP vs ionic). Zn was transformed to three Zn-containing species, ZnS, Zn-3(PO₄)(₂), and Zn associated Fe oxy/hydroxides, also regardless of the form of Zn added. Zn speciation was the same in the unamended control sludge. Ag₂S persisted in all sludge treatments. Zn-3(PO₄)(₂) persisted in sludge and biosolids, but the ratio of ZnS and Zn associated with Fe oxy/hydroxide depended on the redox state and water content of the biosolids. Limited differences in Zn and Ag speciation among NP-dosed, ion-dosed, and control biosolids indicate that these nanoparticles are transformed to similar chemical forms as bulk metals already entering the WWTP.

Mackay, D., et al. (2015). "Decamethylcyclopentasiloxane (D5) environmental sources, fate, transport, and routes of exposure." *Environ Toxicol Chem* 34(12): 2689-2702.

The environmental sources, fate, transport, and routes of exposure of decamethylcyclopentasiloxane (D5; CAS no. 541-02-6) are reviewed in the present study, with the objective of contributing to effective risk evaluation and assessment of this and related substances. The present review, which is part of a series of studies discussing aspects of an effective risk evaluation and assessment, was prompted in part by the findings of a Board of Review undertaken to comment on a decision by Environment Canada made in 2008 to subject D5 to regulation as a toxic substance. The present review focuses on the early stages of the assessment process and how information on D5's physical-chemical properties, uses, and fate in the environment can be integrated to give a quantitative description of fate and exposure that is consistent with available monitoring data. Emphasis is placed on long-range atmospheric transport and fate in water bodies receiving effluents from wastewater treatment plants (along with associated sediments) and soils receiving biosolids. The resulting exposure estimates form the basis for assessments of the resulting risk presented in other studies in this series. Recommendations are made for developing an improved process by which D5 and related substances can be evaluated effectively for risk to humans and the environment.

Mamindy-Pajany, Y., et al. (2014). "Impact of lime-stabilized biosolid application on Cu, Ni, Pb and Zn mobility in an acidic soil." *Environ Sci Pollut Res Int* 21(6): 4473-4481.

A soil column leaching study was conducted on an acidic soil in order to assess the impact of lime-stabilized biosolid on the mobility of metallic pollutants (Cu, Ni, Pb and Zn). Column leaching experiments were conducted by injecting successively CaCl₂, oxalic acid and ethylenediaminetetraacetic acid (EDTA) solutions through soil and biosolid-amended soil columns. The comparison of leaching curves showed that the transport of metals is mainly related to the dissolved organic carbon, pH and the nature of extractants. Metal mobility in the soil and biosolid-amended soils is higher with EDTA than with CaCl₂ and oxalic acid extractions, indicating that metals are strongly bound to solid-phase components. The single application of lime-stabilized biosolid at a rate ranging from 15 to 30 t/ha tends to decrease the mobility of metals, while repeated applications (2 x 15 t/ha) increase metal leaching from soil. This result highlights the importance of monitoring the movement and concentrations of metals, especially in acid and sandy soils with shallow and smaller water bodies.

Markiewicz, M., et al. (2015). "Mobility and biodegradability of an imidazolium based ionic liquid in soil and soil amended with waste sewage sludge." *Environ Sci Process Impacts* 17(8): 1462-1469.

Sorption on solids and biodegradation are main phenomena that can mitigate the pollution of soil and water by ionic liquids (ILs). ILs sorbed on soil particles become immobilized (temporarily or permanently) which prevents them from spreading into deeper layers of soil or groundwater but which also makes them less bioavailable. In this study we attempt to examine if amendment of soil with waste sludge has a potential to mitigate the transport and enhance biodegradation of ILs using 1-methyl-3-octylimidazolium chloride ([OMIM][Cl]) as an example. We present the results of adsorption test (batch and column) and ultimate biodegradation of [OMIM][Cl] using microbial communities derived from soil. Finally, we combine all of these processes together to examine the fate of [OMIM][Cl] in a continuous column flow-through system in soil amended with waste sewage sludge. Addition of sludge serves two purposes: firstly, increasing soil organic matter (formerly proved to facilitate retardation), and secondly augmenting soil with versatile microbial communities previously shown to successfully degrade ILs.

McCall, C. A., et al. (2015). "Monitoring *Bacteroides* spp. markers, nutrients, metals and *Escherichia coli* in soil and leachate after land application of three types of municipal biosolids." *Water Res* 70: 255-265.

A lysimeter-based field study was done to monitor the transfer of culturable *Escherichia coli*, general (ALLBAC), human (Hf183) and swine (PIG-BAC-1) specific 16S rRNA *Bacteroides* spp. markers, nutrients and metals through soils and leachate over time following land application of a CP1/Class A as well as two CP2/Class B municipal biosolids (MBs). Hf183 markers were detected up to six days following application in soils receiving dewatered and liquid MBs, but not in leachate, suggesting their use in source tracking is better suited for recent pollution events. The CP2/Class B biosolids and swine manure contributed the highest microbial load with *E. coli* loads (between 2.5 and 3.7 log CFU (100 mL)⁻¹) being greater than North American concentration recommendations for safe recreational water. ALLBAC persisted in soils and leachate receiving all treatments and was detected prior to amendment application demonstrating its unsuitability for identifying the presence of fecal pollution. A significant increase in NO₃-N (for Lystek and dewatered MBs) and total-P (for dewatered and liquid MBs) in leachate was observed in plots receiving the CP1/Class A and CP2/Class B type MBs which exceeded North American guidelines, suggesting impact to surface water. Metal (As, Cd, Cr, Co, Cu, Pb, Mo, Ni, Se, Zn and Hg) transfer was negligible in soil and leachate samples receiving all treatments. This study is one of the first to examine the fate of *E. coli* and *Bacteroides* spp. markers in situ following the land application of MBs where surface runoff does not apply.

Miller, J. H., et al. (2014). "Elevation of antibiotic resistance genes at cold temperatures: implications for winter storage of sludge and biosolids." *Let Appl Microbiol* 59(6): 587-593.

Prior research suggests that cold temperatures may stimulate the proliferation of certain antibiotic resistance genes (ARGs) and gene transfer elements during storage of biosolids. This could have important implications on cold weather storage of biosolids, as often required in northern climates until a time suitable for land application. In this study, levels of an integron-associated gene (*intI1*) and an ARG (*sul1*) were monitored in biosolids subject to storage at 4, 10 and 20 degrees C. Both *intI1* and *sul1* were observed to increase during short-term storage (<2 months), but the concentrations returned to background within 4 months. The increases in concentration were more pronounced at lower temperatures than ambient temperatures. Overall, the results suggest that cold stress may induce horizontal gene transfer of integron-associated ARGs and that biosolids storage conditions should be considered prior to land application. SIGNIFICANCE AND IMPACT OF THE STUDY: Wastewater treatment plants have been identified as the hot spots for the proliferation and dissemination of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) to the environment through discharge of treated effluent to water bodies as well as application of biosolids to land. Identifying critical control points within the treatment process may aid in the development of solutions for the reduction of ARGs and ARB and curbing the spread of antibiotic resistance. This study found increases in ARGs during biosolids storage and identifies changes in operational protocols that could help reduce ARG loading to the environment when biosolids are land-applied.

Mohapatra, D. P., et al. (2014). "Analysis and advanced oxidation treatment of a persistent pharmaceutical compound in wastewater and wastewater sludge-carbamazepine." *Sci Total Environ* 470-471: 58-75.

Pharmaceutically active compounds (PhACs) are considered as emerging environmental problem due to their continuous input and persistence to the aquatic ecosystem even at low concentrations. Among them, carbamazepine (CBZ) has been detected at the highest frequency, which ends up in aquatic systems via wastewater treatment plants (WWTPs) among other sources. The identification and quantification of CBZ in wastewater (WW) and wastewater sludge (WWS) is of major interest to assess the toxicity of treated effluent discharged into the environment. Furthermore, WWS has been subjected for re-use either in agricultural application or for the production of value-added products through the route of bioconversion. However, this field application is disputable due to the presence of these organic compounds and in order to protect the ecosystem or end users, data concerning the concentration, fate, behavior as well as the perspective of simultaneous degradation of these compounds is urgently necessary. Many treatment technologies, including advanced oxidation processes (AOPs) have been developed in order to degrade CBZ in WW and WWS. AOPs are technologies based on the intermediacy of hydroxyl and other radicals to oxidize recalcitrant, toxic and non-biodegradable compounds to various by-products and eventually to inert end products. The purpose of this review is to provide information on persistent pharmaceutical compound, carbamazepine, its ecological effects and removal during various AOPs of WW and WWS. This review also reports the different analytical methods available for quantification of CBZ in different contaminated media including WW and WWS.

Oun, A., et al. (2014). "Effects of Biosolids and Manure Application on Microbial Water Quality in Rural Areas in the US." *Water* 6(12): 3701-3723.

Most of the waterborne disease outbreaks observed in North America are associated with rural drinking water systems. The majority of the reported waterborne outbreaks are related to microbial agents (parasites, bacteria and viruses). Rural areas are characterized by high livestock density and lack of advanced treatment systems for animal and human waste, and wastewater. Animal waste from livestock production facilities is often applied to land without prior treatment. Biosolids (treated municipal wastewater sludge) from large wastewater facilities in urban areas are often transported and applied to land in rural areas. This situation introduces a potential for risk of human exposure to waterborne contaminants such as human and zoonotic pathogens originating from manure, biosolids, and leaking septic systems. This paper focuses on waterborne outbreaks and sources of microbial pollution in rural areas in the US, characterization of the microbial load of biosolids and manure, association of biosolid and manure application with microbial contamination of surface and groundwater, risk assessment and best management practice for biosolids and manure application to protect water quality. Gaps in knowledge are identified, and recommendations to improve the water quality in the rural areas are discussed.

Parks, A. N., et al. (2014). "Environmental biodegradability of [(14)C] single-walled carbon nanotubes by *Trametes versicolor* and natural microbial cultures found in New Bedford Harbor sediment and aerated wastewater treatment plant sludge." *Environ Toxicol Chem* 34(2): 247-251.

Little is known about environmental biodegradability or biotransformations of single-walled carbon nanotubes (SWNT). Because of their strong association with aquatic organic matter, detailed knowledge of the ultimate fate and persistence of SWNT requires investigation of possible biotransformations (i.e., biodegradation) in environmental media. In the present study, [(14)C]SWNT were utilized to track biodegradation over 6 mo. by pure liquid culture of the fungus *Trametes versicolor* and mixed bacterial isolates from field-collected sediment or aerated wastewater treatment plant sludge. The mixed cultures were chosen as more environmentally relevant media where SWNT will likely be deposited under both aerobic and anaerobic conditions. Activity of [(14)C] was assessed in solid, aqueous, and (14)CO₂ gaseous phases to determine amounts of intact SWNT, partially soluble SWNT degradation products, and mineralized SWNT, respectively, during the 6 mo. of the experiment. Mass balances based on radiocarbon activity were approximately 100% over 6 mo., and no significant degradation of SWNT was observed. Approximately 99% of the [(14)C] activity remained in the solid phase, 0.8% in the aqueous phase, and less than 0.1% was mineralized to (14)CO₂, regardless of culture type. These results suggest that SWNT are not readily biodegraded by pure fungal cultures or environmental microbial communities, and are likely persistent in environmental media.

Picchioni, G. A., et al. (2014). "Nursery Crop Growth Response to Municipal Biosolids: Species Salt and Xeric Adaptation a Key Factor?" *Compost Science & Utilization* 22(3): 138.

Growth responses of potted ornamental crops to municipal biosolids in the semiarid southwestern USA are not adequately known. In 10- to 11-wk greenhouse pot studies, we evaluated the effects of dried biosolids-amended growing media on four ornamental crop species: Garden chrysanthemum (*Dendranthema Xgrandiflorum* 'Megan'), butterfly bush (*Buddleia davidii* 'Nanho Blue'), Japanese honeysuckle (*Lonicera japonica* 'Purpurea'), and blanket flower (*Gaillardia Xgrandiflora* 'Goblin'). The biosolids were composted without bulking agents (100% sewage sludge) and incorporated into growing media at rates ranging from 0 to 593 kg m^{super(-3)}, or 0 to 72% by volume. Biosolids increased substrate pH from 5.8 to 7.2 and electrical conductivity (EC) from 2.6 to 47.3 dS m^{super(-1)}. Any addition of biosolids (>30 kg m^{super(-3)}) reduced total plant dry matter (DM) of chrysanthemum. Conversely, shoot DM of blanket flower and butterfly bush increased by four- to five-fold at biosolids rates of 59 to 148 kg m^{super(-3)} (7 to 18% by volume) with corresponding increases in shoot N and P concentrations. Biosolids rates higher than 148 kg m^{super(-3)} reduced top growth of the latter two species and of Japanese honeysuckle. For all species, growth reductions with excessive biosolids rates likely resulted from osmotic stress and specific NH_{sub(4)} toxicity. However, based on the substantial growth stimulations at moderate biosolids rates, xeric and salt-adapted species, such as blanket flower and butterfly bush, may be ideally suited for expanding the use of highly saline biosolids at semiarid nursery production sites.

Prosser, R. S., et al. (2015). "Effect of biosolids-derived triclosan and triclocarban on the colonization of plant roots by arbuscular mycorrhizal fungi." *Sci Total Environ* 508: 427-434.

Arbuscular mycorrhizal fungi (AMF) form a symbiotic relationship with the majority of crop plants. AMF provide plants with nutrients (e.g., P), modulate the effect of metal and pathogen exposure, and increase tolerance to moisture stress. The benefits of AMF to plant growth make them important to the development of sustainable agriculture. The land application of biosolids is becoming an increasingly common practice in sustainable agriculture, as a source of nutrients. However, biosolids have been found to contain numerous pharmaceutical and personal care products including antimicrobial chemicals such as triclosan and triclocarban. The potential risks that these two compounds may pose to plant-AMF interactions are poorly understood. The current study investigated whether biosolids-derived triclosan and triclocarban affect the colonization of the roots of lettuce and corn plants by AMF. Plants were grown in soil amended with biosolids that contained increasing concentrations of triclosan (0 to 307 mug/g dw) or triclocarban (0 to 304 mug/g dw). A relationship between the concentration of triclosan or triclocarban and colonization of plants roots by AMF was not observed. The presence of biosolids did not have a significant ($p>0.05$) effect on percent colonization of corn roots but had a significant, positive effect ($p<0.05$) on lettuce roots. Biosolids-derived triclosan and triclocarban did not inhibit the colonization of crop plant roots by AMF.

Prosser, R. S., et al. (2014). "Toxicity of biosolids-derived triclosan and triclocarban to six crop species." *Environ Toxicol Chem* 33(8): 1840-1848.

Biosolids are an important source of nutrients and organic matter, which are necessary for the productive cultivation of crop plants. Biosolids have been found to contain the personal care products triclosan and triclocarban at high concentrations relative to other pharmaceuticals and personal care products. The present study investigates whether exposure of 6 plant species (radish, carrot, soybean, lettuce, spring wheat, and corn) to triclosan or triclocarban derived from biosolids has an adverse effect on seed emergence and/or plant growth parameters. Plants were grown in soil amended with biosolids at a realistic agronomic rate. Biosolids were spiked with triclosan or triclocarban to produce increasing environmentally relevant exposures. The concentration of triclosan and triclocarban in biosolids-amended soil declined by up to 97% and 57%, respectively, over the course of the experiments. Amendment with biosolids had a positive effect on the majority of growth parameters in radish, carrot, soybean, lettuce, and wheat plants. No consistent triclosan- or triclocarban-dependent trends in seed emergence and plant growth parameters were observed in 5 of 6 plant species. A significant negative trend in shoot mass was observed for lettuce plants exposed to increasing concentrations of triclocarban ($p < 0.001$). If best management practices are followed for biosolids amendment, triclosan and triclocarban pose a negligible risk to seed emergence and growth of crop plants.

Prosser, R. S., et al. (2014). "Bioaccumulation of triclosan and triclocarban in plants grown in soils amended with municipal dewatered biosolids." *Environ Toxicol Chem* 33(5): 975-984.

Biosolids generally contain the microbiocidal agents triclosan (TCS) and triclocarban (TCC) that are persistent during wastewater treatment and sorp to organic material. The present study investigated the concentration of TCS in tissues of radish, carrot, and soybean grown in potted soil amended with biosolids. Highest mean concentrations of TCS in radish, carrot, and soybean root tissue midway through the life cycle were 24.8 ng/g, 49.8 ng/g, and 48.1 ng/g dry weight, respectively; by the conclusion of the test, however, concentrations had declined to 2.1 ng/g, 5.5 ng/g, and 8.4 ng/g dry weight, respectively. Highest mean concentrations of TCS in radish and carrot shoot tissue were 33.7 and 18.3 ng/g dry weight at days 19 and 45, respectively, but had declined to 13.7 ng/g and 5.5 ng/g dry weight at days 34 and 69, respectively. Concentration of TCS in all samples of soybean seeds was below method detection limit (i.e., 2.8 ng/g dry wt). The present study also examined the concentration of TCS and TCC in edible portions of green pepper, carrot, cucumber, tomato, radish, and lettuce plants grown in a field amended with biosolids. Triclosan was detected only in cucumber and radish up to 5.2 ng/g dry weight. Triclocarban was detected in carrot, green pepper, tomato, and cucumber up to 5.7 ng/g dry weight. On the basis of the present study and other studies, we estimate that vegetable consumption represents less than 0.5% of the acceptable daily intake of TCS and TCC. These results demonstrate that, if best management practices for land application of biosolids in Ontario are followed, the concentration of TCS and TCC in edible portions of plants represents a negligible exposure pathway to humans.

Prosser, R. S. and P. K. Sibley (2015). "Human health risk assessment of pharmaceuticals and personal care products in plant tissue due to biosolids and manure amendments, and wastewater irrigation." *Environ Int* 75: 223-233.

Amending soil with biosolids or livestock manure provides essential nutrients in agriculture. Irrigation with wastewater allows for agriculture in regions where water resources are limited. However, biosolids, manure and wastewater have all been shown to contain pharmaceuticals and personal care products (PPCPs). Studies have shown that PPCPs can accumulate in the tissues of plants but the risk that accumulated residues may pose to humans via consumption of edible portions is not well documented. This study reviewed the literature for studies that reported residues of PPCPs in the edible tissue of plants grown in biosolids- or manure-amended soils or irrigated with wastewater. These residues were used to determine the estimated daily intake of PPCPs for an adult and toddler. Estimated daily intake values were compared to acceptable daily intakes to determine whether PPCPs in plant tissue pose a hazard to human health. For all three amendment practices, the majority of reported residues resulted in hazard quotients <0.1 . Amendment with biosolids or manure resulted in hazard quotients ≥ 0.1 for carbamazepine, diphenhydramine, salbutamol, triclosan, and sulfamethazine. Irrigation with wastewater resulted in hazard quotients of ≥ 0.1 for ambrettolid, carbamazepine, diclofenac, flunixin, lamotrigine, metoprolol, naproxen, sildenafil and tonalide. [corrected]. Many of the residues that resulted in hazard quotients ≥ 0.1 were due to exposing plants to concentrations of PPCPs that would not be considered relevant based on concentrations reported in biosolids and manure or unrealistic methods of exposure, which lead to artificially elevated plant residues. Our assessment indicates that the majority of individual PPCPs in the edible tissue of plants due to biosolids or manure amendment or wastewater irrigation represent a de minimis risk to human health. Assuming additivity, the mixture of PPCPs could potentially present a hazard. Further work needs to be done to assess the risk of the mixture of PPCPs that may be present in edible tissue of plants grown under these three amendment practices.

Pulicharla, R., et al. (2015). "Toxicity of chlortetracycline and its metal complexes to model microorganisms in wastewater sludge." *Science of the Total Environment* 532: 669-675.

Complexation of antibiotics with metals is a well-known phenomenon. Wastewater treatment plants contain metals and antibiotics, thus it is essential to know the effect of these complexes on toxicity towards microorganisms, typically present in secondary treatment processes. In this study, stability constants and toxicity of chlortetracycline (CTC) and metal (Ca, Mg, Cu and Cr) complexes were investigated. The calculated stability constants of CTC-metal complexes followed the order: Mg-CTC $>$ Ca-CTC $>$ Cu-CTC $>$ Cr-CTC. Gram positive *Bacillus thuringiensis* (Bt) and Gram negative *Enterobacter aerogenes* (Ea) bacteria were used as model microorganisms to evaluate the toxicity of CTC and its metal complexes. CTC-metal complexes were more toxic than the CTC itself for Bt whereas for Ea, CTC and its metal complexes showed similar toxicity. In contrast, CTC spiked wastewater sludge (WWS) did not show any toxic effect compared to synthetic sewage. This study provides evidence that CTC and its metal complexes are toxic to bacteria when they are biologically available. As for WWS, CTC was adsorbed to solid part and was not biologically available to show measurable toxic effects. (c) 2015 Elsevier B.V. All rights reserved.

Pycke, B. F., et al. (2014). "Transformation products and human metabolites of triclocarban and triclosan in sewage sludge across the United States." *Environ Sci Technol* 48(14): 7881-7890.

Removal of triclocarban (TCC) and triclosan (TCS) from wastewater is a function of adsorption, abiotic degradation, and microbial mineralization or transformation, reactions that are not currently controlled or optimized in the pollution control infrastructure of standard wastewater treatment. Here, we report on the levels of eight transformation products, human metabolites, and manufacturing byproducts of TCC and TCS in raw and treated sewage sludge. Two sample sets were studied: samples collected once from 14 wastewater treatment plants (WWTPs) representing nine states, and multiple samples collected from one WWTP monitored for 12 months. Time-course analysis of significant mass fluxes ($\alpha=0.01$) indicate that transformation of TCC (dechlorination) and TCS (methylation) occurred during sewage conveyance and treatment. Strong linear correlations were found between TCC and the human metabolite 2'-hydroxy-TCC ($r=0.84$), and between the TCC-dechlorination products dichlorocarbanilide (DCC) and monochlorocarbanilide ($r=0.99$). Mass ratios of DCC-to-TCC and of methyl-triclosan (MeTCS)-to-TCS, serving as indicators of transformation activity, revealed that transformation was widespread under different treatment regimes across the WWTPs sampled, though the degree of transformation varied significantly among study sites ($\alpha=0.01$). The analysis of sludge sampled before and after different unit operation steps (i.e., anaerobic digestion, sludge heat treatment, and sludge drying) yielded insights into the extent and location of TCC and TCS transformation. Results showed anaerobic digestion to be important for MeTCS transformation (37-74%), whereas its contribution to partial TCC dechlorination was limited (0.4-2.1%). This longitudinal and nationwide survey is the first to report the occurrence of transformation products, human metabolites, and manufacturing byproducts of TCC and TCS in sewage sludge.

Rahube, T. O., et al. (2014). "Impact of Fertilizing with Raw or Anaerobically Digested Sewage Sludge on the Abundance of Antibiotic-Resistant Coliforms, Antibiotic Resistance Genes, and Pathogenic Bacteria in Soil and on Vegetables at Harvest." *Appl Environ Microbiol* 80(22): 6898-6907.

The consumption of crops fertilized with human waste represents a potential route of exposure to antibiotic-resistant fecal bacteria. The present study evaluated the abundance of bacteria and antibiotic resistance genes by using both culture-dependent and molecular methods. Various vegetables (lettuce, carrots, radish, and tomatoes) were sown into field plots fertilized inorganically or with class B biosolids or untreated municipal sewage sludge and harvested when of marketable quality. Analysis of viable pathogenic bacteria or antibiotic-resistant coliform bacteria by plate counts did not reveal significant treatment effects of fertilization with class B biosolids or untreated sewage sludge on the vegetables. Numerous targeted genes associated with antibiotic resistance and mobile genetic elements were detected by PCR in soil and on vegetables at harvest from plots that received no organic amendment. However, in the season of application, vegetables harvested from plots treated with either material carried gene targets not detected in the absence of amendment. Several gene targets evaluated by using quantitative PCR (qPCR) were considerably more abundant on vegetables harvested from sewage sludge-treated plots than on vegetables from control plots in the season of application, whereas vegetables harvested the following year revealed no treatment effect. Overall, the results of the present study suggest that producing vegetable crops in ground fertilized with human waste without appropriate delay or pretreatment will result in an additional burden of antibiotic resistance genes on harvested crops. Managing human exposure to antibiotic resistance genes carried in human waste must be undertaken through judicious agricultural practice.

Rhodes, E. R., et al. (2015). "Determining pathogen and indicator levels in class B municipal organic residuals used for land application." *J Environ Qual* 44(1): 265-274.

Biosolids are nutrient-rich organic residuals that are currently used to amend soils for food production. Treatment requirements to inactivate pathogens for production of Class A biosolids are energy intensive. One less energy intensive alternative is to treat biosolids to Class B standards, but it could result in higher pathogen loads. Quantitative microbial risk assessments models have been developed on land application of Class B biosolids but contain many uncertainties because of limited data on specific pathogen densities and the use of fecal indicator organisms as accurate surrogates of pathogen loads. To address this gap, a 12-mo. study of the levels and relationships between, and human adenovirus (HAdV) with fecal coliform, somatic, and F-RNA coliphage levels in Class B biosolids from nine wastewater treatment plants throughout the United States was conducted. Results revealed that fecal coliform, somatic, and F-RNA coliphage densities were consistent throughout the year. More important, results revealed that HAdV ($= 2.5 \times 10$ genome copies dry g) and ($= 4.14 \times 10$ cysts dry g) were in all biosolids samples regardless of treatment processes, location, or season. oocysts were also detected (38% positive; range: 0-1.9 $\times 10$ oocysts dry g), albeit sporadically. Positive correlations among three fecal indicator organisms and HAdV, but not protozoa, were also observed. Overall, this study reveals that high concentrations of enteric pathogens (e.g., and HAdV) are present in biosolids throughout the United States. Microbial densities found can further assist management and policymakers in establishing more accurate risk assessment models associated with land application of Class B biosolids.

Ross, J. and E. Topp (2015). "Abundance of Antibiotic Resistance Genes in Bacteriophage following Soil Fertilization with Dairy Manure or Municipal Biosolids, and Evidence for Potential Transduction." *Appl Environ Microbiol* 81(22): 7905-7913.

Animal manures and municipal biosolids recycled onto crop production land carry antibiotic-resistant bacteria that can influence the antibiotic resistome of agricultural soils, but little is known about the contribution of bacteriophage to the dissemination of antibiotic resistance genes (ARGs) in this context. In this work, we quantified a set of ARGs in the bacterial and bacteriophage fractions of agricultural soil by quantitative PCR. All tested ARGs were present in both the bacterial and phage fractions. We demonstrate that fertilization of soil with dairy manure or human biosolids increases ARG abundance in the bacterial fraction but not the bacteriophage fraction and further show that pretreatment of dairy manure can impact ARG abundance in the bacterial fraction. Finally, we show that purified bacteriophage can confer increased antibiotic resistance to soil bacteria when combined with selective pressure. The results indicate that soilborne bacteriophage represents a substantial reservoir of antibiotic resistance and that bacteriophage could play a significant role in the horizontal transfer of resistance genes in the context of an agricultural soil microbiome. Overall, our work reinforces the advisability of composting or digesting fecal material prior to field application and suggests that application of some antibiotics at subclinical concentrations can promote bacteriophage-mediated horizontal transfer of ARGs in agricultural soil microbiomes.

Sivapatham, P., et al. (2014). "Chemical fractionation of Cu, Zn, Cd, Cr, and Pb in sewage sludge amended soils at the end of 65-d sorghum-sudan grass growth." *J Environ Sci Health A Tox Hazard Subst Environ Eng* 49(11): 1304-1315.

Heavy metals are potentially toxic to human life and the environment. Metal toxicity depends on chemical associations in soil. Understanding the chemical association of trace elements in soils amended with biosolids is very important since it determines their availability within rhizosphere and mobility beyond the rhizosphere. A sequential extraction method was used to determine the various chemical associations [labile (exchangeable + sorbed), organic, carbonates, and sulfides] of Cu, Zn, Cd, Cr, and Pb at the end of sorghum-sudan grass growth (65d) in Candler fine sand (pH = 6.8) and in Ogeechee loamy sand (pH = 5.2) amended with wastewater treatment sludge (WWTS) obtained from two different sources at application rates of 0, 24.7, 49.4, 98.8, and 148.2 Mg ha⁻¹. Results of this study indicated that irrespective of the soil type, Cu, Cd, Cr, and Pb in the labile fractions (exchangeable + sorbed) were in the range of 0-3.0 mg kg⁻¹ and the amount for Zn was in the range of 0.2-6.6 mg kg⁻¹. Therefore, their availability to plants and mobility beyond rhizosphere would be substantially low unless further transformations occur from other fractions. Results also indicated that the presence of substantial amounts of trace elements studied were in sulfide (HNO₃) fraction and in organic (NaOH) fraction irrespective of soil type with the exception of Pb which was mainly present as carbonate (Na₂EDTA) fraction and the remaining Pb equally as sulfide (HNO₃) and organic (NaOH) fractions. Furthermore, results indicated that Cd was mainly present as carbonate (Na₂EDTA) fraction. Irrespective of soil type, source and rate of WWTS application, summation of quantities of various fractions of all the trace elements studied through sequential extraction procedure were 1 to 25 % lower than that of total recoverable quantities of these trace elements determined on acid digestion described by US EPA method 3050 B. It was further evident that growing sorghum sudan grass for 65-d following the application of WWTS either depleted labile fractions or shifted the solid phases containing the trace elements in soils away from those extractable with more severe reagents, such as 4M HNO₃ to those extractable with milder reagents such as dilute NaOH and Na₂EDTA.

Sridhar, B. B. M., et al. (2014). "Effect of Biosolid Amendments on the Metal and Nutrient Uptake and Spectral Characteristics of Five Vegetable Plants." *Water Air and Soil Pollution* 225(9).

The accumulation of metals and nutrients in biosolid-amended soils and the risk of their excess uptake by plants is a topic of great concern. This study examines the elemental uptake and accumulation in five vegetable plants grown on biosolid-applied soils and the use of spectral reflectance to monitor the resulting plant stress. Soil, shoot, root, and fruit samples were collected and analyzed for several elemental concentrations. The chemical concentrations in soils and all the plant parts increased with increase in applied biosolid concentrations. The Cu and Zn concentrations in the plant shoots increased in the order of collard < radish < lettuce < tomato < pepper. The Cu and Zn concentrations accumulated significantly in the fruits of the tomato plants compared to other plants. Among all the plants, the shoot concentration factor (SCF) of Zn was significantly higher for pepper plants, indicating increase in uptake of Zn. The shoot relative uptake index (SRUI) of Cu and Zn increased in the order of collard < radish < lettuce < tomato < pepper. The shoot dry weight and spectral reflectance of the radish plants in the near-infrared (NIR) region (800-1,300 nm) decreased significantly with increase in biosolid concentration compared to other plants. Increase in plant stress with increase in biosolid dose was evident in radish plants through significant reduction in Normalized Difference Vegetative Index (NDVI). This study indicates the potential use of spectral reflectance as a tool for the screening and monitoring of stress-sensitive plant species and their physiology and as a result, indirectly assesses the chemical concentrations in soils and plants.

Venkatesan, A. K. and R. U. Halden (2014). "Brominated flame retardants in U.S. biosolids from the EPA national sewage sludge survey and chemical persistence in outdoor soil mesocosms." *Water Res* 55: 133-142.

We determined national baseline levels and release inventories of 77 traditional and novel brominated flame retardants (BFRs) in biosolids composites (prepared from 110 samples) from the U.S. Environmental Protection Agency's 2001 national sewage sludge survey (NSSS). Additionally, analyses were performed on archived samples from a 3-year outdoor mesocosm study to determine the environmental persistence of BFRs in biosolids-amended soil. The total polybrominated diphenylether (PBDE) concentration detected in biosolids composites was 9400 +/- 960 mug/kg dry weight, of which deca-BDE constituted 57% followed by nona- and penta-BDE at 18 and 13%, respectively. The annual mean loading rate estimated from the detected concentrations and approximate annual biosolids production and disposal numbers in the U.S., of the sum of PBDEs and non-BDE BFRs was calculated to be 47,900-60,100 and 12,900-16,200 kg/year, of which 24,000-36,000 and 6400-9700 kg/year are applied on land, respectively. Mean concentration of PBDEs were higher in the 2001 samples compared to levels reported in EPA's 2006/7 Targeted NSSS, reflecting on-going efforts in phasing-out PBDEs in the U.S. In outdoor soil mesocosms, >99% of the initial BFRs mass in the biosolids/soil mixtures (1:2) persisted over the monitoring duration of three years. Estimates of environmental releases may be refined in the future by analyzing individual rather than composited samples, and by integrating currently unavailable data on disposal of biosolids on a plant-specific basis. This study informs the risk assessment of BFRs by furnishing national inventories of BFR occurrence and environmental release via biosolids application on land.

Venkatesan, A. K. and R. U. Halden (2014). "Contribution of polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) to the toxic equivalency of dioxin-like compounds in archived biosolids from the U.S. EPA's 2001 national sewage sludge survey." *Environ Sci Technol* 48(18): 10843-10849.

The World Health Organization recently proposed the inclusion of brominated congeners in addition to chlorinated congeners when computing the toxic equivalency (TEQ) of dioxin-like compounds (DLCs) in assessments of human health risks. In the present study, 12 polybrominated dibenzo-p-dioxins and furans (PBDD/Fs) were analyzed by gas chromatography/high resolution mass spectrometry in the composited, archived biosolids that were collected in 32 U.S. states and the District of Columbia from 94 wastewater treatment plants by the United States Environmental Protection Agency in its 2001 national sewage sludge survey. Two PBDDs and five PBDFs were detected in the biosolids composites at varying frequencies (40-100%) with a total mean concentration of 10,000 ng/kg dry weight (range: 630-42,800), of which 1,2,3,4,6,7,8-hepta-BDF constituted about 95% by mass. Relative to commercial polybrominated diphenyl ether (PBDE) formulations, the ratio of PBDD/Fs to PBDEs in biosolids was 55-times higher (approximately 0.002% vs approximately 0.11%), which indicates potential PBDE transformation or possibly additional sources of PBDD/Fs in the environment. The TEQ contribution of PBDD/Fs was estimated at 162 ng/kg 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (range: 15-672), which is equivalent to 75% (range: 12-96%) of the total TEQ in biosolids. The TEQ of DLCs released annually to U.S. soils as a result of the land application of biosolids was estimated at 720 g (range: 530-1600 g). Among all known DLCs determined in biosolids, brominated analogs contributed 370% more TEQ than did chlorinated congeners, which indicates the need to include brominated DLCs in the exposure and risk assessment of land-applied biosolids.

Venkatesan, A. K. and R. U. Halden (2014). "Loss and in situ production of perfluoroalkyl chemicals in outdoor biosolids-soil mesocosms." *Environ Res* 132: 321-327.

An outdoor mesocosm study was conducted in Baltimore, Maryland, to explore the fate of thirteen perfluoroalkyl substances (PFASs) over the course of three years in biosolids/soil mixtures (1:2) exposed to ambient outdoor conditions. Analysis by liquid chromatography tandem mass spectrometry showed perfluorooctanoate (PFOA) to be the most abundant analyte found early in the soil weathering experiment at 24.1ng/g dry weight (dw), followed by perfluoroundecanoate (PFUnDA) and perfluorodecanoate (PFDA) at 18.4 and 17.4ng/g dw, respectively. Short-chain perfluorinated carboxylates (PFCAs; C4-C8) showed observable loss from biosolids/soil mixtures, with experimentally determined first-order half-lives in soil ranging from 385 to 866 days. Perfluorooctane sulfonate (PFOS), perfluorononanoate (PFNA) and PFUnDA levels in biosolids/soil mixtures remained stable, while other long-chain PFCAs [PFDA, perfluorododecanoate (PFDoDA)] and perfluorooctane sulfonamide (PFOSA) levels increased over time, presumably due to the breakdown of unidentified precursors in a process analogous to that reported previously for wastewater treatment plants. This study informs risk assessment initiatives by furnishing data on the environmental persistence of PFASs while also constituting the first report on in situ production of long-chained PFASs in terrestrial environments.

Venkatesan, A. K., et al. (2014). "Detection and occurrence of N-nitrosamines in archived biosolids from the targeted national sewage sludge survey of the U.S. Environmental Protection Agency." *Environ Sci Technol* 48(9): 5085-5092.

The occurrence of eight carcinogenic N-nitrosamines in biosolids from 74 wastewater treatment plants (WWTPs) in the contiguous United States was investigated. Using liquid chromatography-tandem mass spectrometry, seven nitrosamines [(N-nitrosodimethylamine (NDMA), N-nitrosomethylethylamine, N-nitrosodi-n-propylamine (NDPA), N-nitrosodibutylamine, N-nitrosopyrrolidine, N-nitrosopiperidine (NPIP), and N-nitrosodiphenylamine (NDPhA)] were detected with varying detection frequency (DF) in 88% of the biosolids samples (n = 80), with five of the seven being reported here for the first time in biosolids. While rarely detected (DF 3%), NDMA was the most abundant compound at an average concentration of 504 +/- 417 ng/g dry weight of biosolids. The most frequently detected nitrosamine was NDPhA (0.7-147 ng/g) with a DF of 79%, followed by NDPA (7-505 ng/g) and NPIP (51-1185 ng/g) at 21% and 11%, respectively. The DF of nitrosamines in biosolids was positively correlated with their respective n-octanol-water partition coefficients ($R(2) = 0.65$). The DF and sum of mean concentrations of nitrosamines in biosolids increased with the treatment capacity of WWTPs. Given their frequent occurrence in nationally representative samples and the amount of U.S. biosolids being applied on land as soil amendment, this study warrants more research into the occurrence and fate of nitrosamines in biosolids-amended soils in the context of crop and drinking water safety.

Xue, J., et al. (2015). "Occurrence of Bisphenol A Diglycidyl Ethers (BADGEs) and Novolac Glycidyl Ethers (NOGEs) in Archived Biosolids from the U.S. EPA's Targeted National Sewage Sludge Survey." *Environ Sci Technol* 49(11): 6538-6544.

Epoxy resins incorporating bisphenol A diglycidyl ether (BADGE) and novolac glycidyl ether (NOGE) are used in a wide range of applications, including adhesives, structural and electrical laminates. However, little is known about the occurrence of BADGE, NOGE, and their derivatives in the environment. Using liquid chromatography-tandem mass spectrometry, BADGE, bisphenol F glycidyl ether (BFDGE), 3-ring NOGE, and eight of their derivatives (BADGE.2 H₂O, BADGE.H₂O, BADGE.HCl.H₂O, BADGE.2 HCl, BADGE.HCl, BFDGE.2 H₂O, and BFDGE.2 HCl) were determined in archived biosolid samples collected from 68 wastewater treatment plants (WWTPs) from the northeastern, midwestern, western, and southern regions of the USA. BADGE.2 H₂O was the most frequently detected (DR = 99%) and the most abundant compound found (median: 93.6 ng/g dry weight [dw]) in this family. The highest total concentrations of target chemicals, ranging from 83.6 to 2490 ng/g dw, were found in biosolids collected from the northeastern United States. The sum of geometric mean (GM) concentration of BADGE, NOGE, and their derivatives in biosolids increased with the treatment capacity of WWTPs. Based on the measured concentrations in biosolids and predicted mass in wastewater, it was estimated that approximately 3.5% of the total production of BADGEs was emitted through WWTP discharges.

Yang, Y., et al. (2014). "Metal and nanoparticle occurrence in biosolid-amended soils." *Science of the Total Environment* 485: 441-449.

Metals can accumulate in soils amended with biosolids in which metals have been concentrated during wastewater treatment. The goal of this study is to inspect agricultural sites with long-term biosolid application for a suite of regulated and unregulated metals, including some potentially present as commonly used engineered nanomaterials (ENMs). Sampling occurred in fields at a municipal and a privately operated biosolid recycling facilities in Texas. Depth profiles of various metals were developed for control soils without biosolid amendment and soils with different rates of biosolid application (6.6 to 74 dry tons per hectare per year) over 5 to 25 years. Regulated metals of known toxicity, including chromium, copper, cadmium, lead, and zinc, had higher concentrations in the upper layer of biosolid-amended soils (top 0-30cm or 0-15cm) than in control soils. The depth profiles of unregulated metals (antimony, hafnium, molybdenum, niobium, gold, silver, tantalum, tin, tungsten, and zirconium) indicate higher concentrations in the 0-30cm soil increment than in the 70-100cm soil increment, indicating low vertical mobility after entering the soils. Titanium-containing particles between 50nm and 250nm in diameter were identified in soil by transmission electron microscopy (TEM) coupled with energy dispersive x-ray spectroscopy (EDX) analysis. In conjunction with other studies, this research shows the potential for nanomaterials used in society that enter the sewer system to be removed at municipal biological wastewater treatment plants and accumulate in agricultural fields. The metal concentrations observed herein could be used as representative exposure levels for eco-toxicological studies in these soils.

Youngquist, C. P., et al. (2014). "Ciprofloxacin residues in municipal biosolid compost do not selectively enrich populations of resistant bacteria." *Appl Environ Microbiol* 80(24): 7521-7526.

Biosolids and livestock manure are valuable high-carbon soil amendments, but they commonly contain antibiotic residues that might persist after land application. While composting reduces the concentration of extractable antibiotics in these materials, if the starting concentration is sufficiently high then remaining residues could impact microbial communities in the compost and soil to which these materials are applied. To examine this issue, ciprofloxacin was added to biosolid compost feedstock to achieve a total concentration of 19 ppm, approximately 5-fold higher than that normally detected by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (1 to 3.5 ppm). This feedstock was placed into mesh bags that were buried in aerated compost bays. Once a week, a set of bags was removed and analyzed (treated and untreated, three replicates of each; 4 weeks). Addition of ciprofloxacin had no effect on the recovery of resistant bacteria at any time point ($P = 0.86$), and a separate bioassay showed that aqueous extractions from materials with an estimated 59 ppm ciprofloxacin had no effect on the growth of a susceptible strain of *Escherichia coli* ($P = 0.28$). Regression analysis showed that growth of the susceptible strain of *E. coli* can be reduced given a sufficiently high concentration of ciprofloxacin ($P < 0.007$), a result that is consistent with adsorption being the primary mechanism of sequestration. While analytical methods detected biologically significant concentrations of ciprofloxacin in the materials tested here, the culture-based methods were consistent with the materials having sufficient adsorptive capacity to prevent typical concentrations of ciprofloxacin residues from selectively enriching populations of resistant bacteria.

Yu, X., et al. (2015). "Occurrence and estrogenic potency of eight bisphenol analogs in sewage sludge from the U.S. EPA targeted national sewage sludge survey." *J Hazard Mater* 299: 733-739.

As health concerns over bisphenol A (BPA) in consumer products are mounting, this weak estrogen mimicking compound is gradually being replaced with structural analogs, whose environmental occurrence and estrogen risks are not well understood yet. We used high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) to determine the concentrations of eight bisphenol analogs in 76 sewage sludge samples collected by the U.S. Environmental Protection Agency (EPA) in 2006/2007 from 74 wastewater treatment plants (WWTPs) in 35 states. Bisphenols were detected at the following concentration ranges (ng/g dry weight) and detection frequencies: BPA (6.5-4700; 100%); bisphenol S (BPS; <1.79-1480; 84%); bisphenol F (BPF; <1.79-242; 68%); bisphenol AF (BPAF; <1.79-72.2; 46%); bisphenol P (BPP; <1.79-6.42; <5%), bisphenol B (BPB; <1.79-5.60; <5%), and bisphenol Z (BPZ; <1.79--66.7; <5%). Bisphenol AP (BPAP) was not detected in any of the samples (<1.79 ng/g dw). Concentrations of BPA in sewage sludge were an order of magnitude higher than those reported in China but similar to those in Germany. The calculated 17beta-estradiol equivalents (E2EQ) of bisphenols present in sludge samples were 7.74 (0.26-90.5) pg/g dw, which were three orders of magnitude lower than the estrogenic activity contributed by natural estrogens present in the sludge. The calculated mass loading of bisphenols through the disposal of sludge and wastewater was <0.02% of the total U.S. production. As the usage of BPA is expected to decline further, environmental emissions of BPS, BPF, and BPAF are likely to increase in the future. This study establishes baseline levels and estrogenic activity of diverse bisphenol analogs in sewage sludge.

Yuan, L., et al. (2015). "Lead Toxicity to the Performance, Viability, And Community Composition of Activated Sludge Microorganisms." *Environ Sci Technol* 49(2): 824-830.

Lead (Pb) is a prominent toxic metal in natural and engineered systems. Current knowledge on Pb toxicity to the activated sludge has been limited to short-term (≤ 24 h) toxicity. The effect of extended Pb exposure on process performance, bacterial viability, and community compositions remains unknown. We quantified the 24-h and 7-day Pb toxicity to chemical oxygen demand (COD) and NH₃-N removal, bacterial viability, and community compositions using lab-scale experiments. Our results showed that 7-day toxicity was significantly higher than the short-term 24-h toxicity. Ammonia-oxidizing bacteria were more susceptible than the heterotrophs to Pb toxicity. The specific oxygen uptake rate responded quickly to Pb addition and could serve as a rapid indicator for detecting Pb pollutions. Microbial viability decreased linearly with the amount of added Pb at extended exposure. The bacterial community diversity was markedly reduced with elevated Pb concentrations. Surface analysis suggested that the adsorbed form of Pb could have contributed to its toxicity along with the dissolved form. Our study provides for the first time a systematic investigation of the effect of extended exposure of Pb on the performance and microbiology of aerobic treatment processes, and it indicates that long-term Pb toxicity has been underappreciated by previous studies.

Zahaba, M., et al. (2015). "Isolation and characterization of luminescent bacterium for sludge biodegradation." *Journal of Environmental Biology* 36(6): 1255.

Microtox is based on the inhibition of luminescence of the bacterium *Vibrio fischeri* by the toxicants. This technique has been accepted by the USEPA (United States Environmental Protection Agency) as a biomonitoring tool for remediation of toxicants such as hydrocarbon sludge. In the present study, a luminescent bacterium was isolated from yellow striped scad (*Selaroides leptolepis*) and was tentatively identified as *Vibrio* sp. isolate MZ. This aerobic isolate showed high luminescence activity in a broad range of temperature from 25 to 35 degree C. In addition, optimal conditions for high bioluminescence activity in range of pH 7.5 to 8.5 and 10 gl super(-1) of sodium chloride, 10 gl super(-1) of peptone and 10 gl super(-1) of sucrose as carbon source. Bench scale biodegradation 1% sludge (w/v) was set up and degradation was determined using gas chromatography with flame ionised detector (GC-FID). In this study, *Rhodococcus* sp. strain AQ5NOL2 was used to degrade the sludge. Based on the preliminary results obtained, *Vibrio* sp. isolate MZ was able to monitor the biodegradation of sludge. Therefore, *Vibrio* sp. isolate MZ has the potential to be used as a biomonitoring agent for biomonitoring of sludge biodegradation particularly in the tropical ranged environment.