

W4170 MULTISTATE RESEARCH COMMITTEE
RESPONSE TO USEPA OIG REPORT No. 19-P-0002¹

Prepared by
USDA National Institute of Food and Agriculture
Research Committee W4170

June 2020

¹EPA unable to assess the impact of unregulated pollutants in land-applied biosolids on human health and the environment

AUTHORS

Nicholas Basta, Professor of Soil and Environmental Science
School of Environmental Science & Natural Resources, Ohio State University, Columbus, OH

Ian Pepper, Professor of Environmental Microbiology
Director of the Water and Environmental Technology Center (WEST), University of Arizona, Tucson, AZ

Linda S. Lee, Professor of Environmental Chemistry
Purdue University, Department of Agronomy, West Lafayette, IN

Greg Kester, Director of Renewable Resource Programs
CA Association of Sanitation Agencies, Sacramento, CA

Alyssa Zearley, Research Associate
School of Environment and Natural Resources, Ohio State University, Columbus, OH

REVIEWERS

Gregory Evanylo, Professor and Extension Specialist
School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA

Jim Ippolito, Associate Professor, Soil Fertility and Environmental Soil Quality
Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO

Robert Brobst
Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO

Ganga M. Hettiarachchi, Professor of Soil and Environmental Chemistry
Department of Agronomy, Kansas State University, Manhattan, KS

Hui Li, Professor
Department of Plant, Soil and Microbial Sciences, Michigan State University East Lansing, MI

Kirill Kostyanovsky, PhD, CTO and Founder
New Agronomics, Seattle, WA

Table of Contents

Executive Summaries

Summary of response to chemical issues

Summary of response to antibiotic issues

Summary of response to pathogen issues

Response to Chemical Issues

The 63 “listed” biosolids chemicals of concern

The 317 “unlisted” biosolids chemicals of concern

Key exposure pathways of environmental fate and transport

Hormones and steroids

Brominated flame retardants

Pharmaceutical and personal care products (PPCPs) (not including antibiotics, antimicrobials, hormones, and steroids)

Other organics

Pesticides and metabolites

Metals and inorganics

Dioxins/Furans and dioxin-like compounds

PFAS: A challenging current concern

PFAS in the environment

PFAS in wastewater and biosolids

Response to Antibiotic Issues

Response to Pathogen Issues

Summary and Conclusions

Bibliography

Appendix 1: Response to Antibiotic Issues

- i) Impact of Land Application of Biosolids on Environmental Antibiotic Resistance**
- ii) Impact of Antibiotics Added to Soil via Land Application of Biosolids**
- iii) Fate of Antibiotics in Soil**

Appendix 2: Response to Pathogen Issues

- i) Incidence of Pathogens in Biosolids**
- ii) Fate and Transport of Pathogens in Land-Applied Biosolids**
- iii) Historical Allegations of Microbial Hazards Associated with Land Application of Biosolids**

Executive Summaries

On November 15, 2018 the USEPA Office of Inspector General (OIG) published “EPA Unable to Assess the Impact of Hundreds of Unregulated Pollutants in Land-Applied Biosolids on Human Health and the Environment,” Report No. 19-P-0002 (USEPA, 2018). The OIG report alleged that “...[EPA] lacked the data or risk assessment tools needed to make a determination on the safety of 352 pollutants found in biosolids...[including] 61 designated as acutely hazardous, hazardous or priority pollutants in other programs.” The response from USEPA Office of Water, which has regulatory oversight of the national biosolids program, in Appendix D stated “We are concerned about how the science is presented in the OIG report. It is biased and raises alarm...and is taken out of context”. Concern from USEPA Office of Water and widespread concern from practitioners led to the creation of this review and response.

This response has been co-authored by the USDA National Institute of Food and Agriculture (NIFA) Research Committee W4170: Beneficial Use of Residuals to Improve Soil Health and Protect Public and Ecosystem Health. This research group has more than a 45-year history of biosolids research used to support the regulatory community for promulgation of Part 503 of Chapter 40 of the Code of Federal Regulations (Title 40 CFR Part 503 – Standards for the Use or Disposal of Biosolids) and other science-based state and federal guidelines and regulations.

The objective of this report is to provide a science-based review of chemicals of concern highlighted in the OIG report. This review examines both (i) chemicals of concern that are federally regulated by their placement on the NIOSH hazardous drugs list, Priority Pollutant list, and/or the RCRA P-list (acutely toxic) and U-list (toxic) and (ii) the remaining “unlisted” chemicals that may be present in biosolids.

This response shows that the OIG report did not consider the concentration of chemicals found in the biosolids. Often, the bulk of human exposure to these chemicals is from domestic use of consumer goods and only trace amounts are found in biosolids. Overall, sufficient data and research are available to conclude that current biosolids regulations are protective of human health and the environment. Of course, as with any regulation intended to protect public health and the environment, they must always be dynamic and evolve with updated science. That fact does not imply that they are not protective while research is ongoing.

Summary of response to chemical issues

The OIG report (USEPA, 2018) alleged that “...[EPA] lacked the data or risk assessment tools needed to make a determination on the safety of 352 pollutants found in biosolids...[including] 61 designated as acutely hazardous, hazardous or priority pollutants in other programs.” Our literature review showed that extensive data and risk assessment, some conducted by USEPA, exist for the pollutants listed by OIG. Human exposure to these 352 contaminants in consumer products, food and food packaging, and ambient air is far greater than exposure through biosolids.

The 61 chemicals highlighted in the OIG report that are federally regulated by the NIOSH hazardous drugs list or as priority pollutants have been researched extensively. A hierarchical risk assessment using soil screening levels and persistence in this response found only a few of these chemicals in biosolids may require further study. The remaining “unlisted” biosolids chemicals in the OIG report and in the later-published 2016-2017 Biennial Review were also reviewed. Extensive environmental fate and transport data are available for most of these chemicals. In general, most of these chemicals either (i)

occur in biosolids below natural soil background levels, or (ii) are nontoxic and pose no risk at levels found in biosolids, or (iii) occur in biosolids at concentrations well below risk-based levels, or (iv) will not persist in the environment. These chemicals do not pose risk to human health when in the biosolids matrix. However, a few persistent pharmaceuticals may require further study to determine their potential ecological impact (Verlicchi & Zambello, 2015).

The USEPA Office of Water is currently retooling models for deterministic screening, and probabilistic risk assessments will then be used for many of the chemicals in the OIG report using existing data. Results from these assessments will inform new regulatory standards, if necessary. Data gaps exist where actual biosolids with realistic concentrations of the chemical of concern are land applied. Realistic actual land application of biosolids research data should be used by USEPA to conduct its risk assessment and to promulgate regulation of these and future chemicals of concern.

Summary of response to antibiotic issues

The presence of antibiotics in biosolids is identified as an issue in the OIG report. Specifically, the concern is over the new concept of environmental antibiotic resistance. However, numerous laboratory and field studies have been conducted on the fate and transport of antibiotics, which demonstrate low risk. Examination of peer-reviewed publications on the topic leads to the conclusion that although many antibiotics have been detected in land-applied biosolids, this poses negligible risk of adverse effects to human or environmental health for multiple reasons. First, the vast majority of existing antibiotics are naturally produced in soils by soil microorganisms that have existed in soils for at least a billion years. This means that antibiotics and antibiotic-resistant bacteria (ARBs) with antibiotic-resistant genes (ARGs) have also been in existence for at least a billion years. This, in turn, means that all known effects of antibiotics on soil microbial numbers, activity, and diversity (including horizontal gene transfer) have also been ongoing during this period. All soils, even pristine soils, contain naturally produced antibiotics. Inputs of antibiotics into soil from land application of biosolids are minimal relative to the concentrations already present in the soil. The major fate of antibiotics in soil is degradation or sorption to soil colloids, and in either case antibiotic activity is lost. Any ARBs introduced into soil via land application typically die within 2 to 3 months. In the literature, the majority of studies demonstrate that although concentrations of ARBs and ARGs may increase after biosolids land application, they typically decrease to pre-land-application levels within 3 months. Based on these facts, the presence of antibiotics in biosolids is unlikely to influence environmental antibiotic resistance.

Summary of response to pathogen issues

Biosolids treated to meet Class A, and with appropriate vector attraction controls, contain no detectable microbial pathogens; therefore, no pathogen issues are associated with this material. Factors in destruction of pathogens in biosolids begin in the biosolids treatment process generally following the wastewater treatment process. Public health engineered processes are able to produce Class A biosolids that are virtually free of pathogens. Class A processes generally require thermophilic temperatures over periods of time to accomplish that. USEPA developed a linear model based on extensive research, using a logarithmic scale for time, at various temperatures, and incorporated a margin of safety to assure that biosolids received the appropriate treatment. This model is similar to a nonlinear model developed by Strauch (1991, 1998). This method is also similar to the FDA requirements for pasteurization of egg nog, a food product with flow characteristics similar to those of liquid sewage sludge (see Fig. 4.1, USEPA, 2003a).

Class A material still needs treatment to minimize further purification and therefore must meet additional vector attraction reduction requirements. Class A material meeting both the pathogen and the appropriate vector attraction reduction process may be land applied without site restrictions related to pathogenic organisms. Other Class A processes currently are approved and listed in 40 CFR 503; however, the pathogen stressors such as temperature, pH, and moisture have not changed.

Class B treatments were implemented with the construction of the first wastewater treatment in the early part of the last century in the United States. Then during the 1970s to early 1980s federal construction grants were available to construct wastewater treatment plants as well as treatment for sewage sludge. The primary purpose at that time was to stabilize the sewage sludge, reducing the ability of the sludge to putrefy. In 1978 the federal regulation (40 CFR 257) placed pathogen controls and vector controls on sewage sludge that were tied to the treatment process (e.g., anaerobic and aerobic digestion). Many wastewater treatment plants, including the sludge treatment, were constructed prior to 1993 utilizing Class B processes (i.e., anaerobic, aerobic digesters). Research has demonstrated that Class B pathogen reduction from the treatment process ranges from 2 to 4 log₁₀. When 40 CFR 503 was implemented in 1993, the treatment requirement remained a process plus time for pathogens and was separated from the stabilization process, which in some cases was a numeric requirement (e.g., 38% volatile solids reduction). To assure that public health and the environment were protected, site restrictions were added as a requirement for land application of Class B biosolids. Those sludge treatment systems in addition to stabilizing were effective at pathogen removal.

Class A biosolids contain no detectable microbial pathogens; therefore, no pathogen issues are associated with this material. Class B biosolids do contain low levels of microbial pathogens, and the OIG report lists nine viral and eight bacterial pathogens that have been detected in biosolids. However, the direct threat of human exposure to pathogens within Class B biosolids is mitigated by site restrictions required by the Part 503 Federal Regulations. These site restrictions are of sufficient duration to ensure die-off and inactivation of pathogens within land-applied biosolids. This precludes human exposure to infectious agents. Indirect routes of human exposure to biosolids-associated pathogens have also been studied, including the potential for groundwater contamination and bioaerosol transport to off-site communities. In both scenarios, data show that the risk of infection from these indirect routes of exposure is minimal. Historically, allegations have been made of microbial hazards associated with land application of biosolids, including new exotic biologicals such as the SARS and Ebola viruses and infectious prions. However, when these allegations were investigated via peer-reviewed research, these allegations were shown to be exaggerated and/or false. The validity of these research findings is further endorsed by the fact that in the U.S. there has never been any peer-reviewed evidence of adverse public health effects resulting from microbial hazards or pathogens associated with biosolids land application. This is remarkable given the hundreds of thousands of land applications of biosolids in the U.S. over the past half century. Based on this record, one can conclude that the presence of pathogens in Class B biosolids does not adversely affect public health.

Response to Chemical Issues

Key exposure pathways of environmental fate and transport

Most research on potential exposure and risk from many of the unlisted chemicals uses pure chemicals under laboratory bioassay conditions. Studies determining the persistence and transport of these

chemicals from land-applied biosolids are limited but essential because chemicals generally are much less available when within a biosolids matrix. Environmental fate and transport of chemicals in land-applied biosolids will be further reduced by soil biochemical processes in soil (e.g., sorption, biotic, and abiotic degradation) (Fig. 1). Risk associated with land-applied chemicals in biosolids is determined from specific exposure pathways for humans and ecological receptors (Fig. 2). Select research studies that determined the environmental fate and transport of each group of chemicals follows.

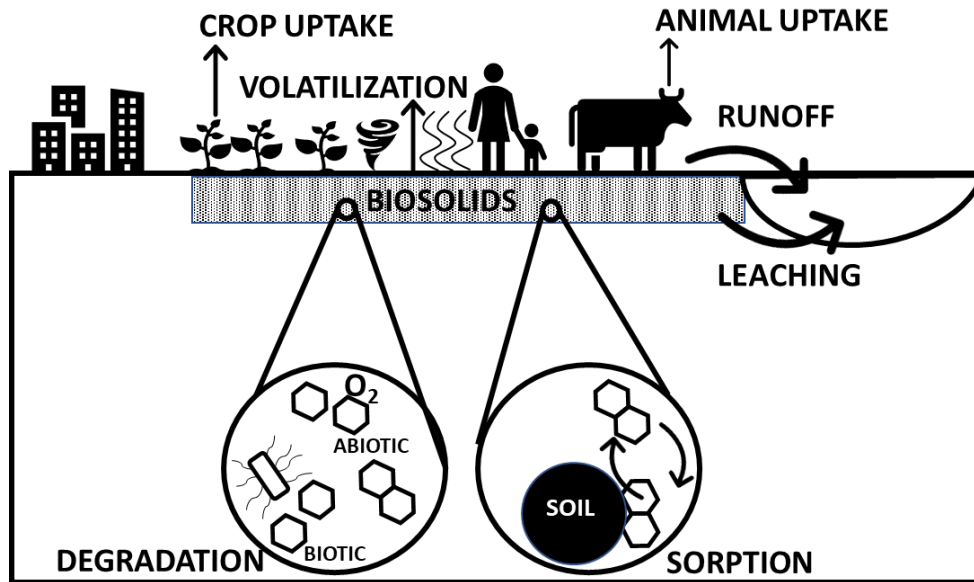


Fig. 1. Environmental fate and transport pathway for chemicals in biosolids.

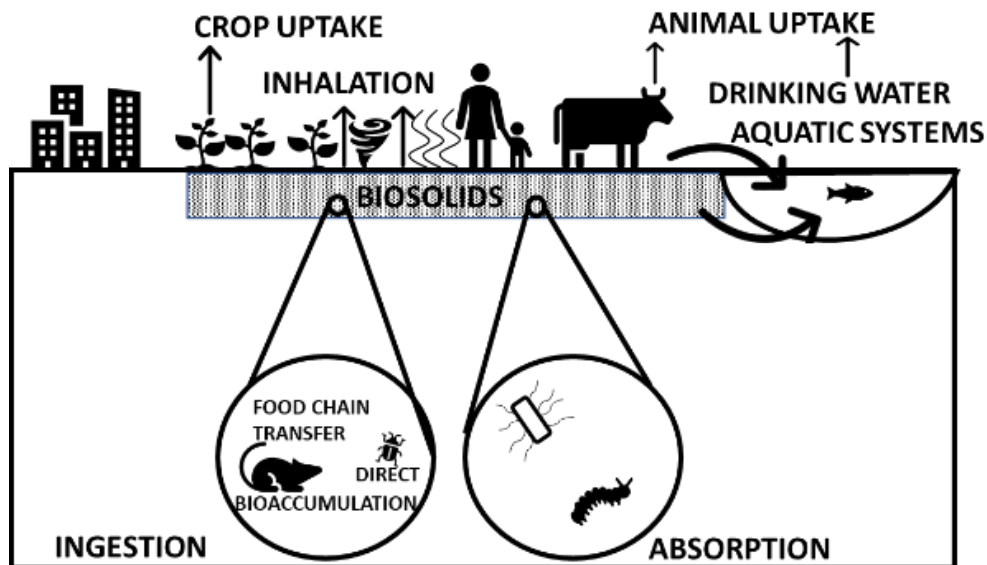


Fig. 2. Human and ecological exposure pathways for chemicals in biosolids.

The 63 “listed” biosolids chemicals of concern

The OIG reported on 352 chemicals and pathogens identified in the 2015 Biennial Review and/or the 2007-2008 Targeted National Sewage Sludge Survey (2009a). This report addresses these 352 chemicals as well as 28 additional chemicals identified in the 2016-2017 Biennial Review published after the OIG report. The OIG report focused on a group of 61 chemicals regulated by the NIOSH Hazardous Drugs list, Priority Pollutant list, and the RCRA P-list (acutely toxic) and U-list (toxic). Two additional “listed” chemicals were identified in the 2016-2017 report, bringing the total “listed” chemicals to 63 (Table 1).

Table 1. 63 chemicals found in biosolids that are regulated on the RCRA, Priority Pollutant, and NIOSH Hazardous Drugs lists; 61 chemicals are addressed in the OIG report, and 2 chemicals were identified in the later 2017 Biosolids Biennial Review.

Chemical	RCRA list number	Priority Pollutant list	NIOSH Hazardous Drugs list	Category
2,3,7,8 tetrachlorodibenzo-P-dioxin		X		Organics
2-Propanone	U002			Solvents
Antimony		X		Metals
Benz(a)anthracene	U018	X		PAHs
Benzo(a)pyrene	U022	X		PAHs
Benzo(b)fluoranthene		X		PAHs
Benzo(k)fluoranthene		X		PAHs
Beryllium	P015	X		Metals
Bis (2-ethylhexyl) phthalate	U028	X		SVOCs
Carbamazepine			X	Other drugs
Carbon tetrachloride	U211	X		Organics
Chloroaniline, 4-	P024			SVOCs
Chloroform	U044	X		Organics
Chlorophthalene, 2-	U047	X		Organics
Chrysene	U050	X		PAHs
Cresol, p- (4-methylphenol)	U052			Preservative
Cyanide	P030	X		Organics
Cyclophosphamide	U058		X	Other drugs
Dichlorobenzene, 1,3-	U071	X		Pesticides
Dichlorobenzene, 1,4-	U072	X		Pesticides
Dimethoate	P044			Pesticides
Dimethyl phthalate	U102	X		Organics
Di-n-butyl phthalate	U069	X		Plasticizers
(Butoxyphosphate ethanol, 2-)				
Di-n-octyl phthalate	U107	X		Organics
Endosulfan, α	P050	X		Pesticides
	(Endosulfan)			

Table 1, continued

Chemical	RCRA list number	Priority Pollutant list	NIOSH Hazardous Drugs list	Category
Endosulfan, β	P050 (Endosulfan)	X		Pesticides
Estradiol, 17 α -			X	Hormones
Estradiol, 17 β -			X	Hormones
Estradiol-3-benzoate, β -			X	Hormones
Estriol (estradiol)			X	Hormones
Estrone			X	Hormones
Ethylbenzene		X		Organics
Ethynyl estradiol, 17 α -			X	Hormones
Fluoranthene	U120	X		PAHs
Heptachlor epoxide	P050	X		Pesticides
Mestranol			X	Other drugs
Methylene Chloride (Dichloromethane)	U080	X		Solvents
phthalene	U165	X		PAHs
Nitrophenol, p-	U170	X		Organics
N-nitrosodibutylamine (NDBA) 924-16-3	U172			Nitrosamines
N-nitrosodiethylamine (NDEA) 55- 18-5	U174			Nitrosamines
N-nitrosodimethylamine (NDMA) 62-75-9	P082	X		Nitrosamines
N-nitroso-di-n-propylamine (NDPA) 621-64-7	U111	X		Nitrosamines
N-nitrosodiphenylamine (NDPhA) 86-30-6	P082			Nitrosamines
N-nitrosopiperidine (NPIP) 100-75-4	U179			Nitrosamines
N-nitrosopyrrolidine (NPYR) 930-55- 2	U180			Nitrosamines
Norethindrone (norethisterone)			X	Hormones
Norgestimate			X	Other drugs
Norgestrel (levonorgestrel)			X	Hormones
Pentachloronitrobenzene	U185			Pesticides
Phenthrene		X		PAHs
Progesterone			X	Hormones

Table 1, continued

Chemical	RCRA list number	Priority Pollutant list	NIOSH Hazardous Drugs list	Category
Pyrene		X		PAHs
Silver		X		Metals
Testosterone			X	Hormones
Tetrachloroethylene	U210	X		Solvents
Thallium	P113-P115	X		Metals
Toluene	U220	X		Solvents
Trichlorophenol, 2,4,5-	(On U list with note to see F027)			Antimicrobial
Warfarin	P001		X	Other drugs

The USEPA Office of Water has conducted risk assessments on many of these chemicals associated with land application of biosolids (USEPA, 2018, Appendix D). Another comprehensive risk assessment is beyond the scope of this review and is unnecessary. The approach in this response is to compare concentration of the 63 chemicals of concern with known environmental toxicity, soil background levels, soil screening limits, contaminant biodegradation, and mobility data to determine the likelihood of the chemicals of concern accumulating in soil to potential levels of concern. We reviewed reported biosolids concentrations for all 63 contaminants of concern from (i) Targeted National Sewage Sludge Survey (2009), (ii) U.S. and Canadian literature, and (iii) recent testing results from biosolids produced in the U.S. Biosolids chemical concentrations were evaluated by the following decision hierarchy tree.

Decision hierarchy/tree:

1. Residential Soil Screening Limit (Target risk, TR=1E-06; Target hazard quotient, THQ=1.0); if higher, compared with
2. Part 503 Recommendations: List of 200, List of 50, Risk-based screening limit; if higher, compared with
3. Other risk-based screening limits (Ohio EPA Voluntary Action Plan); if higher, compared with
4. Background level. Any remaining chemicals that were above background levels were compared with
5. Persistence (half-life). If half-life >3 months, then remaining chemicals may accumulate to hazardous levels and require further investigation.

Results of the comparison are shown in Table 2. In short, cyanide, naphthalene, pentachloronitrobenzene, and carbamazepine of the 63 chemicals were not eliminated by the 5-step

decision hierarchy above and had biosolids concentrations that exceeded USEPA’s residential soil screening limit. Carbamazepine had no residential soil screening level, but biosolids concentrations are well below human therapeutic doses. However, the compound is persistent and may pose an ecological risk (discussed in “Pharmaceuticals” section below). These four chemicals in biosolids should be given research priority, while the remaining were found through the decision tree to pose little risk to human health. These findings agree with USEPA Office of Water, which in their response to the OIG report recommended that

Reference to the 61 pollutants designated as hazardous, acutely hazardous or priority pollutants in other EPA programs should be deleted in this section and throughout the report. This reference to the 61 designated pollutants serves to alarm the reader. The statement speaks to hazard, and hazard alone does not indicate risk. While OW will use toxicity and occurrence data to prioritize pollutants that need to be assessed for risk, there is no direct relationship between these designations and the CWA requirements for biosolids (USEPA, 2018, Appendix D).

Table 2. Results of literature review of 63 compounds in the OIG report and 2016-2017 Biennial Review.

Chemical	Results of literature review	Source
Chrysene	Concentration data not available from TNSSS* or NSSS†. All concentration data located in the literature review was below the RSSL‡	Bright & Healey, 2003 Kohli et al., 2006 USEPA, 2020
Dichlorobenzene, 1,4-	Concentration data not available from TNSSS or NSSS. All concentration data located in the literature review was below the RSSL	Bright & Healey, 2003 USEPA, 2020
Dimethyl phthalate	Concentration data not available from TNSSS or NSSS. All concentration data located in the literature review was below the RSSL	Khosravi & Price, 2015 USEPA, 2020
Di-n-butyl phthalate (Butoxyphosphate ethanol)	Concentration data not available from TNSSS or NSSS. All concentration data located in the literature review was below the RSSL	Bright & Healey, 2003 USEPA, 2020
N-nitrosodibutylamine (NDBA) 924-16-3	Concentration data not available from TNSSS or NSSS. All concentration data located in the literature review was below the RSSL	USEPA, 2020 Venkatesan et al., 2014
N-nitrosodiethylamine (NDEA) 55-18-5	Concentration data not available from TNSSS or NSSS. All concentration data located in the literature review was below the RSSL	USEPA, 2020 Venkatesan et al., 2014
Tetrachloroethylene	Concentration data not available from TNSSS or NSSS. All concentration data located in the literature review was below the RSSL	Bright & Healey, 2003 USEPA, 2020

Norgestrel (levonorgestrel)	Detection limit in the TNSSS <5%	USEPA, 2009a
Benzo a)pyrene	Mean TNSS concentration exceeds RSSL. Half-life data suggests compound is not likely to persist for more than 1 year	PubChem, 2019a USEPA, 2009b
Benzo(b)fluoranthene	Concentration data not available from TNSSS or NSSS. Concentration data in literature exceeds soil screening limits [§] . Half-life data suggests compound is not likely to persist for more than 1 year	Bright & Healey, 2003 Kohli et al., 2006
Benzo(k)fluoranthene	Concentration data not available from TNSSS or NSSS. Concentration data in literature exceeds soil screening limits [§] . Half-life data suggests compound is not likely to persist for more than 1 year	Bright & Healey, 2003 Kohli et al., 2006
N-nitrosopiperidine (NPIP) 100-75-4	Concentration data not available from TNSSS or NSSS. Concentration data in literature exceeds soil screening limits [§] . Half-life data suggests compound is not likely to persist for more than 1 year	PubChem, 2019e Venkatesan et al., 2014
N-nitrosodimethylamine (NDMA) 62-75-9	Not detected in the NSSS. Concentration data in literature exceeds soil screening limits [§] . Half-life data suggests compound is not likely to persist for more than 1 year.	USEPA, 1989 USEPA, 2020 Venkatesan et al., 2014
Ethylbenzene	Maximum concentration reported in the NSSS is below Ohio EPA's Voluntary Action Program Brownfields use standard	Ohio Environmental Protection Agency, 2019 USEPA, 1989
N-nitrosopyrrolidine (NPYR) 930-55-2	Concentration data not available from TNSSS or NSSS. Maximum concentration reported in the literature is below the RSSL.	USEPA, 2020 Venkatesan et al., 2014
Heptachlor epoxide	Concentration data not available from TNSSS or NSSS. Maximum concentration reported in the NSSS is below Part 503 recommendations	USEPA, 1989 USEPA, 1995, p. 83
2-Propanone	Concentration data not available from TNSSS or NSSS. Maximum concentration reported in the NSSS is below the RSSL	USEPA, 1989 USEPA, 2020
Antimony	Maximum concentration reported in the NSSS is below the RSSL	USEPA, 1989 USEPA, 2020

Beryllium	Maximum concentration reported in the TNSSS is below the RSSL	USEPA, 2009a USEPA, 2020
Chloronaphthalene, 2-	Maximum concentration reported in the NSSS is below the RSSL	USEPA, 1989 USEPA, 2020
Cresol, p- (4-methylphenol)	Maximum concentration reported in the NSSS is below the RSSL	USEPA, 1989 USEPA, 2020
Di-n-octyl phthalate	Maximum concentration reported in the NSSS is below the RSSL	USEPA, 1989 USEPA, 2020
Endosulfan, α	Maximum concentration reported in the NSSS is below the RSSL	USEPA, 1989 USEPA, 2020
Endosulfan, β	Maximum concentration reported in the NSSS is below the RSSL	USEPA, 1989 USEPA, 2020
Fluoranthene	Maximum concentration reported in the TNSSS is below the RSSL	USEPA, 2009a USEPA, 2020
N-nitrosodimethylamine (NDMA) 62-75-9	Not detected in the NSSS. Concentration data located in the literature review exceeded soil screening limits [§] . Half-life data suggests compound is not likely to persist for more than 1 year.	USEPA, 1989 USEPA, 2020 Venkatesan et al., 2014
N-nitroso-di-n-propylamine (NDPA) 621-64-7	Concentration data not available from TNSSS or NSSS. Concentration data located in the literature review exceeded soil screening limits [§] . Half-life data suggests compound is not likely to persist for more than 1 year.	USEPA, 2020 Venkatesan et al., 2014
Pyrene	Maximum concentration reported in the TNSSS is below the RSSL	USEPA, 2009a USEPA, 2020
Silver	Maximum concentration reported in the TNSSS is below the RSSL	USEPA, 2009a USEPA, 2020
Toluene	Maximum concentration reported in the NSSS is below the RSSL	USEPA, 1989 USEPA, 2020
Warfarin	Maximum concentration reported in the TNSSS is below the RSSL	USEPA, 2009a USEPA, 2020
Cyanide	Mean concentration in TNSSS is below RSSL but maximum exceeded RSSL and half-life >3 months. This compound may need further investigation	PubChem, 2020c USEPA, 2009a USEPA, 2020
Naphthalene	Mean concentration in literature is below RSSL but maximum exceeded RSSL and half-life >3 months. This compound may need further investigation	Bright & Healey, 2003 Kohli et al., 2006 PubChem, 2019d USEPA, 2020

Pentachloronitrobenzene	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a
Dichlorobenzene, 1,3-	Not detected in the NSSS. No concentration data was found in the literature.	USEPA, 1989
Trichlorophenol, 2,4,5-	Not detected in the NSSS. No concentration data was found in the literature.	USEPA, 1989
Carbamazepine	No soil screening limit for the compound. Persistent. Further addressed in the “Pharmaceuticals” section.	PubChem, 2020b USEPA, 2009a
Cyclophosphamide	No soil screening limit for the compound. Persistent. Sewage influent contains very low concentrations of the compound well below ecological or human health thresholds. This likely holds true for biosolids as the compound does not readily sorb to biosolids.	Buerge et al., 2006
Estradiol, 17α-	No soil screening limit for the compound. Half-life data suggests compound is not likely to persist for more than 1 year. Further addressed in the “Hormones and Steroids” section.	PubChem, 2019b USEPA, 2009a
Estradiol-3-benzoate, β-	No soil screening limit for the compound. Half-life data is not available. Further addressed in the “Hormones and Steroids” section.	USEPA, 2009a
Estriol (estradiol)	No soil screening limit for the compound. Half-life data suggests compound is not likely to persist for more than 1 year. Further addressed in the “Hormones and Steroids” section.	PubChem, 2019b USEPA, 2009a
Estrone	No soil screening limit for the compound. Half-life data suggests compound is not likely to persist for more than 1 year. Further addressed in the “Hormones and Steroids” section.	PubChem, 2019c USEPA, 2009a
Ethinyl estradiol, 17α-	Not detected in the TNSSS. Further addressed in the “Hormones and Steroids” section.	USEPA, 2009a
Mestranol	No soil screening limit for the compound. Half-life data suggests compound is not likely to persist for more than 1 year. Further addressed in the “Hormones and Steroids” section.	Clarke & Smith, 2011a

Nitrophenol, p-	No soil screening limit for the compound. Half-life data suggest compound is not likely to persist for more than 1 year.	PubChem, 2020a
Norethindrone (norethisterone)	No soil screening limit for the compound. Found in 6% of TNSSS samples. Half-life data is not available. Further addressed in the “Hormones and Steroids” section.	PubChem, 2019f USEPA, 2009a
Norgestimate	Not detected in the TNSSS	USEPA, 2009a
Progesterone	No soil screening limit for the compound. Half-life data suggests compound is not likely to persist for more than 1 year. Further addressed in the “Hormones and Steroids” section.	PubChem, 2020d USEPA, 2009a
Testosterone	No soil screening limit for the compound. Half-life data suggests compound is not likely to persist for more than 1 year. Further addressed in the “Hormones and Steroids” section.	PubChem, 2019g USEPA, 2009a
Thallium	Only 1 sample exceeded the RSSL in the TNSSS	USEPA, 2009b
Chloroaniline, 4-	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a
Dimethoate	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a
N-nitrosodiphenylamine (NDPhA) 86-30-6	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a
2,3,7,8 Tetrachlorodibenzo-P-Dioxin	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a
Benz a anthracene	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a
Bis 2-ethylhexyl phthalate	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a
Carbon tetrachloride	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a
Chloroform	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a
Methylene Chloride Dichloromethane	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a
Phenanthrene	Risk assessment conducted for Part 503a and deferred from regulation	USEPA, 1995a

*TNSSS =USEPA’s Targeted National Sewage Sludge Survey (2009).

†NSSS= USEPA’s National Sewage Sludge Survey (1989).

‡RSSL= USEPA’s Residential Soil Screening Limit. Regional Screening Level (RSL) Resident Soil Table (Target Risk, TR=1E-06, target hazard quotient, THQ=1) (2020).

§Screening limits= USEPA RSSL, Ohio EPA Voluntary Action Program (VAP) Standards (Brownfields), and Part 503 limits.

The 317 “unlisted” biosolids chemicals of concern

The OIG report lists 291 contaminants of concern in biosolids as “unlisted.” These contaminants are not included in the NIOSH, Priority Pollutant, or RCRA P and U lists. Additionally, 26 “unlisted” chemicals were identified in the 2016-2017 Biennial Review for a total of 317 “unlisted” contaminants that are addressed in the following sections. Many of these chemicals are nontoxic (e.g., calcium, potassium); and many are “emerging contaminants,” which may or may not have future regulatory standards (e.g., pharmaceuticals, personal care products). The chemicals can be categorized by group (Table 3).

Table 3. Chemical groups of 317 “unlisted”* chemicals in OIG report and 2016-2017 Biennial Review.

Group	Number of chemicals
Antibiotics and antimicrobials	58
Pharmaceuticals (not including antibiotics, antimicrobials, hormones, and steroids)	69
Hormones and steroids	15
Pesticides and metabolites	8
Metals and inorganics	23
Brominated flame retardants	36
Dioxins and dioxin-like compounds	38
PFASs and surfactants	22
Other organics (solvents, SVOCs, plastics, preservatives, odorants, fragrance, emollients)	31
Pathogens	17

*The above chemicals do not include the 61 OIG report chemicals or the 2 chemicals in the 2016-2017 Biennial Review that appear on the NIOSH, RCRA P-list (acutely toxic) and U-list (toxic), or Priority Pollutant lists (Table 1).

Hormones and steroids

Hormones and steroids are physiology- and behavior-altering chemicals produced and excreted by all organisms, resulting in a background level of hormones in environmental media. However, human and farm animal waste recycling practices can concentrate natural and synthetic hormones in the environment to abnormally high levels. Human activities that increase hormone loading include WWTP (wastewater treatment plant) effluent, biosolids recycling, and manure application. Since the 1960s, synthetic hormones from contraceptives, hormone replacement therapy, and animal agriculture have

also been released into the environment. High levels of exogenous hormones activate receptors in all organisms, leading to endocrine disruption.

However, the vast majority (>90+) of influent hormones are degraded in WWTPs (Fleming et al., 2016). Remaining hormones primarily sorb to biosolids. Biodegradation of hormones and synthetic hormones in biosolids and soils (half-lives days to weeks; Clarke & Smith, 2011b; Mina et al., 2016) are sufficient to prevent accumulation. Human exposure to hormones in biosolids is insignificant compared to the body's natural hormone production. However, the main concern is potential endocrine disruption to aquatic organisms (Fig. 2), which are especially sensitive to hormones. Several factors, including rainfall intensity, soil properties, and contribution of runoff to the waterbody influence whether biosolids hormones in runoff could exceed concentrations associated with endocrine disruption in aquatic organisms (Yang et al., 2012) (Fig. 1). Site restrictions imposed on Class B biosolids are intended to prevent runoff and minimize potential impacts to aquatic ecosystems. In addition, incorporating or injecting manures decreases potential hormone runoff from agricultural land to surface water significantly (Mina et al., 2016).

In the U.S., 90% of hormones present in the environment come from livestock manures, particularly pregnant and cycling dairy cows (Khanal et al., 2006; Pollard & Morra, 2017). Approximately 4 million dry tons of biosolids are applied across 0.1% of U.S. cropland annually (Lu et al., 2012), while more than 350 million dry tons of manure are applied across 5% of U.S. cropland (EST, 2015; MacDonald, 2009). A smaller footprint, combined with lower hormone concentrations, suggests that biosolids hormones have a smaller environmental impact than manure application. These findings agree with a comprehensive report by the Water Environment Association of Ontario, which concluded further research on risk from hormones from land-applied biosolids was not a priority.

Conclusions:

- Hormones do not persist in soil after land application of biosolids.
- Biosolids is a minor source of hormones compared to animal manure applications.
- Hormones in biosolids are not a human health risk.
- Site restrictions imposed on Class B biosolids are designed to prevent runoff and minimize negative impacts to aquatic ecosystems.

Table 4. Key studies on hormones in biosolids.

Citation	Title	Conclusion
Khanal et al., 2006	Fate, transport, and biodegradation of natural estrogens in the environment and in engineered systems	90% of environmental hormone load is from manure application. Hormones break down very quickly in WWTPs, and remainder in biosolids breaks down within days-weeks.
Clarke & Smith, 2011	Review of emerging organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids	Degradation rates of synthetic hormones are slower than natural but still sufficient to prevent buildup. Steroids from biosolids are unlikely to be human or environmental health risk.
Yang et al., 2012	Steroid hormone runoff from agricultural test plots applied with municipal biosolids	Hormone concentrations from biosolids-amended field plots were above endocrine-disrupting thresholds in aquatic organisms up to 35 days post-application. More information is needed to assess potential environmental impacts.

Brominated flame retardants

Brominated flame retardants, or BFRs, are organobromide compounds that became widely used in the 1970s in response to California legislation requiring flame retardants in fabrics. The BFRs are now used in electronics, fabrics, furniture, and carpets. These chemicals have come under scrutiny as they are *universally* distributed and persistent in the environment and are present in human blood. One class of BFRs, polybrominated diphenyl ethers (PBDEs) are commonly found in biosolids. Polybrominated diphenyl ethers (PBDE) exposure is associated with neuro-, hepato-, immuno-, and immunotoxicity and reproductive effects, endocrine disruption, and carcinogenicity in laboratory studies, though the few small studies of human exposure have not conclusively linked health impacts to environmental exposure. The BFRs enter the wastewater stream through residential and industrial pathways. The majority (60-90%) of brominated flame retardants (BFRs) sorb to sludge in WWTPs because these compounds are hydrophobic and poorly soluble. They have limited degradability in WWTPs and soils (Kim et al., 2017). Half-life estimates range from weeks to months (USEPA, 2008) to years (Kim et al., 2017). Volatilization, photodegradation, and microbial breakdown are proposed mechanisms of BFR breakdown and/or inactivation in soil (Gorgy et al., 2013; Kwak et al., 2017; Sellström et al., 2005) (Fig. 1). PBDE transfer out of the soil has been observed, but this is relatively minor: Gottschall et al. (2017) found that a maximum of 1.7% PBDE mass was lost to tile in biosolids-amended soil. Tile drainage concentrations were similar to WWTP effluent concentrations.

Indoor dust and food are recognized as the most important pathways for adult and child PBDE exposure (Daso et al., 2010). Indoor dust is believed to be contaminated with BFRs through contact with and degradation of BFR-treated materials. Concentration of BFR in biosolids-amended soil is less than house dust (Andrade et al., 2010; Jones-Otazo et al., 2005; Rauert & Harrad, 2015). PBDE uptake was observed in vegetable crops grown in factory-contaminated soil with comparable PBDE levels to biosolids-

amended soils (Andrade et al., 2010; Yang et al., 2018), but Gottschall et al. (2017) and Li et al. (2015) did not find PBDE uptake in wheat and corn grain grown in biosolids-amended soils. Rigby found that dairy cows fed biosolids at a 5% feed rate (representing an unlikely worst-case grazing after surface application scenario) had increased BFRs in their milk, but cows fed biosolids-amended soil at 5% feed (representing the typical scenario of grazing after biosolids are washed into the soil by rain) had no increase in BFR milk concentrations (Fig. 2). A risk assessment for consuming tomatoes on biosolids-amended soil showed no significant risk to human health (Navarro et al., 2018). Both the Navarro and Rigby studies were conducted in the EU, the biosolids had PBDE concentrations an order of magnitude lower than U.S. biosolids, and accumulation in U.S. crops may differ. Human exposure to PBDEs and other flame retardants is primarily through indoor dusts and the numerous household objects that are coated in flame retardants (Andrade et al., 2010; Jones-Otazo et al., 2005; Rauert & Harrad, 2015). Biosolids contributes little of an individual's exposure.

A review conducted by Kwak et al. (2017) found that the ecological impacts of PBDEs in soil include bioaccumulation in earthworms and changes in soil microbe community structure and enzyme activity (Kwak et al., 2017). However, toxicological impacts on higher soil organisms and plants were not noted even at high concentrations (1,000 mg/kg). Zhang et al. (2015) found decreased lysosomal membrane integrity in earthworms exposed to 10 and 100 mg/kg BDE-209 in soil. In general, these levels far exceed BDE-209 concentrations in biosolids-amended agricultural soils (Davis et al., 2015). Navarro et al. (2018) found BFRs in biosolids-amended soils posed low risk to soil, sediment, and freshwater organisms or to their predators.

The BFRs and PBDEs have been recognized by Clarke and Smith (2011b) and Higgins et al. (2010) as high research priorities; but Eriksen et al. (2009), Jensen et al. (2012), and Smith (2009) deemed the compounds low priority and low risk. Most countries are currently phasing out traditional BFRs and PBDEs, which are expected to degrade almost completely in the soil within the next 100 years (2012). Novel brominated flame retardants (NBFRs) are replacement compounds for traditional BFRs and are used largely without restriction. These compounds are not acutely toxic, and their biodegradability is not well known. They have been detected in the environment in recent studies, and concentrations are sometimes similar to traditional BFRs (Ezechiáš et al., 2014; Yadav et al., 2017). Though little research is available on novel BFRs, they likely pose little risk as human exposure from biosolids remains low and they are less acutely toxic than traditional BFRs.

Conclusions:

- BFRs are widely distributed, persistent in the environment, and are reported in human blood. The main exposure pathways are indoor dust and food. Biosolids do not appear to contribute largely to either pathway.
- BFRs in indoor dust are primarily from household objects, and concentrations are higher in dust than in biosolids-amended soil.
- Phased-out flame retardants will degrade in soil.

Table 5. Key studies on brominated flame retardants in biosolids.

Citation	Title	Conclusion
Navarro et al., 2018	Environmental risk assessment of perfluoroalkyl substances and halogenated flame retardants released from biosolids-amended soils	An ecological risk assessment found no environmental risk from flame retardants released in biosolids-amended soil.
Gottschall et al., 2017	Brominated flame retardants and perfluoroalkyl acids in groundwater, tile drainage, soil and crop grain following a high application of municipal biosolids to a field	A 22 Mg dw ha ⁻¹ biosolids application did not increase PBDE concentrations in tile drainage and groundwater above ecological toxicological thresholds for acute exposure.

Pharmaceutical and personal care products (PPCPs) (not including antibiotics, antimicrobials, hormones, and steroids)

Pharmaceuticals and personal care products (PPCPs) are a large, inhomogeneous category of chemicals that can induce physiological changes at low concentrations. These chemicals and their metabolites are continuously introduced into sewage systems through excretion and flushing, and current WWTP do not fully remove many PPCPs. Human health risks from PPCPs in biosolids are considered low as concentrations in biosolids are much lower than therapeutic, lowest observable adverse effect levels (LOELs) and no observable adverse effect levels (NOELs) (Brown et al., 2019; Zenker et al., 2014). Wu et al. (2015) reviewed concentration of PPCPs in crops grown on biosolids-amended soil. In all studies reviewed by Wu et al., uptake into edible plant parts was not present or was very low. Crop tissue concentrations where uptake did occur were a fraction of acceptable daily limits or therapeutic doses, typically by several orders of magnitude. Sabourin et al. (2012) measured 118 PPCPs in vegetable crops grown in an 8 mt/ha biosolids-amended soil. PPCP chemicals were not found in crop tissue in all replicates. Human PPCP exposure from biosolids exposure or ingestion of crops grown on biosolids-amended soil is negligible compared with average PPCP use. Pharmaceutical exposure from biosolids is orders of magnitude lower than minimum effective doses, which are the minimum doses at which significant impacts are observed. These minimum effective doses are vetted for safety as part of the pharmaceutical registration process.

Soil and aquatic organisms are more sensitive to low concentrations of PPCPs, and many studies have explored the ecological risks of biosolids application (Zenker et al., 2014). Their effects are varied and oftentimes resemble their therapeutic responses in humans. A review by Verlicchi and Zambello (2015) included environmental risk assessments of PPCPs in land-applied biosolids to aquatic organisms. Chemicals found by the reviewed papers that had RQ > 1 included NSAIDs ibuprofen and naproxen and salicylic acid, propranolol (beta-blocker), acetaminophen, triclosan and triclocarban, caffeine, and some hormones and antibiotics (discussed elsewhere in this paper). An edge-of-plot surface runoff study by Busalacchi (2012) calculated ecological hazard quotients <1.0 for several PPCPs except ibuprofen. The triclocarban (TCC) and triclosan (TCS) in soil amended with Chinese biosolids exceeded estimated PNECs for *Eisenia* spp. (Chen et al., 2014). Park et al. (2013) found that TCC and TCS in biosolids did not inhibit microbial density or diversity as microbes overcame the toxic effects of TCC and TCS when in the biosolids matrix.

The fate and transport of PPCPs in soil is variable (Fig. 1). Behavior is dependent on chemical, biosolids, and soil properties and is not currently well predicted by these. Morais et al. (2013) modelled fate and impact of PPCPs in biosolids runoff on freshwater ecosystems. Most PPCPs studied tended to remain in the soil system. Mefenamic acid (NSAID) had the highest probability of impacting aquatic organisms. Gottschall et al. (2012) evaluated the fate and transport of more than 80 PPCPs in a biosolids-amended field. Only miconazole, triclocarban, carbamazepine, and ofloxacin were present in soil after 1 year. Eight PPCPs were detected in tile after the first rain and only carbamazepine was detected in tile during subsequent rains. Ibuprofen, triclosan, triclocarban, and o-desmethyl venlafaxine moved to 2-m depth after the first rain, but none were observed at 4 or 6 m. Injection greatly decreased PPCP concentrations in surface runoff (Topp et al., 2008). Studies reviewed by McCarthy et al. (2015) found that most PPCPs did not reach groundwater, and surface runoff and tile drainage concentrations tended to be much lower than WWTP effluent (Fig. 2).

Conclusions:

- Field studies show loss of PPCP to surface water dissipates quickly after land application. Minimal downward movement of PPCP in field land application of biosolids studies.
- PPCP in tile and runoff tend to be much lower than WWTP effluent.
- Limited data shows that runoff concentration of PPCP in surface runoff from land-applied biosolids is below aquatic ecotoxicological endpoints.
- NSAIDs, triclosan, triclocarban, o-desmethyl venlafaxine, carbamazepine, miconazole, ofloxacin propranolol (beta-blocker), acetaminophen, and caffeine identified in the reviewed studies with RQ > 1 should be considered for future study focusing on ecological risk assessment.

Table 6. Key studies on pharmaceuticals and personal care products (PPCPs) in biosolids.

Citation	Title	Conclusion
McCarthy et al., 2015	Risks associated with application of municipal biosolids to agricultural lands in a Canadian context	Ecological risk of PPCPs in biosolids is low as most do not reach groundwater. Surface runoff and tile drainage concentrations are lower than WWTP effluent.
Sabourin et al., 2012	Uptake of pharmaceuticals, hormones, and parabens into vegetables grown in soil fertilized with municipal biosolids	Measured 118 PPCPs in vegetable crops grown in a biosolids-amended soil. No chemical was found in all replicates.
Verlicchi & Zambello, 2015	Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil—a critical review	Reviewed 169 compounds in biosolids and conducted ecological risk assessments. Seven compounds were deemed a critical research priority.

Other organics

The OIG report identified 37 “other organic” chemicals, which included solvents, SVOCs, plastics, fragrances, parabens, PAHs, and emollients (Table 7).

Table 7. Organic compounds found in biosolids in the OIG report and 2016-2017 Biennial Review.

Compound	Classification	Compound	Classification
Benzyl paraben	<i>Preservatives</i>	Methyl protocatechuate	<i>Parabens</i>
Bisphenol A	<i>Plastics</i>	Methylnaphthalene, 2-	<i>PAHs</i>
Butylparaben	<i>Parabens</i>	Nitrogen, organic	<i>Organics</i>
Decamethylcyclopentasiloxane (D5)	<i>Emollients</i>	Propyl paraben	<i>Parabens</i>
Dihydroxybenzoic acid, 3,4-	<i>Metabolites</i>	Skatole	<i>NA</i>
Dimethyl-3,5,-dinitro-4-tert-butylacetophenone, 2,6-	<i>Odorants</i>	Styrene	<i>Organics</i>
Ethanol, 2-butoxy-phosphate	<i>Organics</i>	Tetrabromobisphenol A	<i>Organics</i>
Ethyl paraben	<i>Parabens</i>	Tonalide (AHTN)	<i>Fragrance</i>
Galaxolide	<i>Fragrance</i>	Trichlorobenzene, 1,3,5-	<i>Organics</i>
Hexanoic Acid	<i>Semivolatile Organic</i>	Tris(2-chloroethyl) phosphate	<i>Organics</i>
Hydroxy benzoic acid, 4-	<i>Parabens</i>	Xylene, m-	<i>Solvents</i>
Indole	<i>Fragrance</i>	Xylene, musk	<i>Odorants</i>
Limonene, d-	<i>Fragrance</i>	Xylene, o-	<i>Solvents</i>
Methyl paraben	<i>Parabens</i>	Xylene, p	<i>Solvents</i>

A USEPA human health risk assessment of xylene in biosolids revealed no significant risk to ecological or human health (USEPA, 2003b).

Decamethylcyclopentasiloxane (D5) is an emollient used in lotions and other personal care products. It is persistent and widespread in the environment and shows potential for bioaccumulation, but it has low toxicity. The EU has regulated D5 in products in part due to contamination with toxic octamethylcyclotetrasiloxane (D4), which is not included in the OIG report (Committee for Risk Assessment (RAC), 2016). A Canadian risk assessment found D5 in biosolids did not pose significant ecological or human health risk, and the government has declined to regulate D5 (Giesy et al., 2011).

Parabens are preservatives that reduce microorganism growth in personal care products and foods. Methyl protocatechuate, hydroxy benzoic acid, 4-, and dihydroxybenzoic acid, 3,4- are paraben metabolites. Most cosmetics and personal care products contain parabens, usually at a concentration around 10,000 mg/kg (Błędzka et al., 2014). Parabens are primarily degraded in WWTP processing, and biosolids concentrations did not exceed 300 mg/kg dw in a survey of U.S. biosolids (Chen et al., 2017), far below concentrations in cosmetics and personal care products. Parabens in Canadian biosolids-amended soil were not taken up by vegetable crops in a study by Sabourin et al. (2012). Biosolids is an insignificant exposure pathway for parabens (Gosens et al., 2014). Alberio et al. (2012) conducted a preliminary ecological risk assessment of parabens in biosolids-amended soil to soil and aquatic organisms. Biosolids-amended soil paraben concentrations were 1000 times less than predicted no-effect concentrations (PNECs).

Fragrances limonene, d-, galaxolide, dimethyl-3,5,-dinitro-4-tert-butylacetophenone, 2,6- (musk ketone), and tonalide (AHTN) are commonly used in high concentrations in PPCPs and are found in human and

wild animal tissues, sediments, and surface waters (Cruz & Barceló, 2015). Some fragrances are not completely removed by WWTP and are found in the tens of mg/kg. Musks slowly degrade in soil and column studies suggest a low leaching potential. However, direct and indirect fragrance exposures from biosolids represent a minor human exposure pathway compared with PPCP use. Musks pose a risk to aquatic organisms if present in sufficient amounts (Heberer, 2002). An EU risk assessment found no major risks from tonalide in biosolids, and the conclusion of the complete environmental risk assessment was that tonalide did not require further regulation (Committee for Risk Assessment (RAC), 2008). Clarke and Smith (2011b), Eriksen et al. (2009), Jensen et al. (2012), Smith (2009), and WEAO (2010) gave low research priority to these compounds, though Higgins et al. (2010) gave it a high research priority.

Organic nitrogen is a slow release plant nutrient. Farmers do not apply biosolids in excess of crop requirements and take mineralization rates into account when determining plant available nitrogen. Organic nitrogen offers groundwater protection because the plant can mineralize and use it at optimal times as opposed to inorganic nitrogen, which flushes through the system with large amounts unable to be taken up by the crop.

Methylnaphthalene was found in British biosolids below the USEPA RSSL and does not persist in soil (2011). The primary exposure pathway for trichlorobenzene, 1,3,5- is inhalation of ambient air and ingestion of food. This compound was listed in the 2005 Biennial Review but was not detected in biosolids studied by da Silva Souza et al. (2020), Justina and Skoronski (2018), Mazzeo et al. (2015), and Pihno et al. (2014); while $4 \mu\text{g kg}^{-1}$ was detected by Bittencourt et al. (2016). The authors calculated that soil amended with these biosolids would have trichlorobenzene, 1,3,5- concentrations 5 orders of magnitude below permissible concentrations set in Brazil. Production and use of tris(2-chloroethyl) phosphate continues to decline, and concentrations found by Cristale et al. (2016) and Woudneh et al. (2015) were well below the USEPA RSSL. Hexanoic acid and ethanol are both added to food and are not human health concerns in biosolids. Both are mobile in soil but readily biodegraded in soil and water, and the risk of bioconcentration in aquatic organisms is low). Bisphenol A exposure is primarily through food packaging, and the maximum concentration measured in archived biosolids from the TNSSS was 2 orders of magnitude lower than USEPA's RSSL (Xue et al., 2015). Styrene breaks down readily in soil. Human exposure to tri(2-butoxyethyl) phosphate occurs primarily from its use as a plasticizer and flame retardant in consumer goods such as food packaging, rubber washers, and textiles. It rapidly degrades in water and WWTPs and does not accumulate in aquatic organisms (Gramatica et al., 2016). The NOAEL in rats is 300-1000 mg/kg/d (PubChem, n.d.-c). Tri(2-butoxyethyl) phosphate concentrations in Canadian and Chinese biosolids were 1.6-581 $\mu\text{g kg}^{-1}$. Human exposure to tri(2-butoxyethyl) phosphate from biosolids is very low.

Conclusions:

- Many chemicals in this category are used in consumer and personal care products. Exposure through these pathways far exceeds that from biosolids.
- Research finds insignificant risk to human health from the remaining “organic chemicals” in the OIG report.

Pesticides and metabolites

Despite what the OIG report suggests, extensive data and risk assessments are available for pesticides. These data, including human and ecological risk assessments and fate and transport data, were developed as part of the federal registration process. Pesticides are routinely monitored and if undesirable impacts from a pesticide become known, action is taken to reduce its impact (limiting use, banning the material, etc.). The USEPA declined to regulate organic pesticides while developing Title 40 Part 503a as low NSSS concentrations, risk assessments, and pesticide phase outs indicated that these compounds posed low risk. Pesticides are land applied in much greater amounts than from land application of biosolids. Reviews by Eriksen et al. (2009), Higgins et al. (2010), Smith (2009), and WEAO (2001, 2010) did not deem pesticides in biosolids a research priority.

Conclusions:

- Toxicity and fate and transport data for pesticides are available as they are generated as part of the pesticide registration process.
- Few studies examine currently used pesticides in biosolids. Organochloride pesticide concentrations in biosolids have decreased with phase-out.
- Pesticides within biosolids are applied at concentrations 1-2 orders of magnitude lower than federally allowable pesticide treatment levels (Lang et al., 2005).

Metals and inorganics

The OIG report lists 24 metals and inorganics (Table 8). With the exception of phosphorus, nitrogen compounds, and molybdenum, the remaining inorganics and metals have natural abundance in soil that exceeds their concentration in biosolids (Kabata-Pendias, 2010; Smith et al., 2013; USEPA, 2009a). Of the elements listed in Table 8, B, Ca, Co, Fe, Mg, Mn, Mo, N, and P compounds, K, and Na are necessary for plant and animal growth and (re)productivity. Thus, the plant and human nutrition and ecological health benefits of the majority of elements listed in Table 8 are well established (Steffan et al., 2019; White and Brown, 2010). Insufficiencies, rather than excesses, of these elements are more likely to detrimentally impact plant and animal health in most of the world.

Of the elements listed in Table 8, only several are of minor concern: Mo, N, P, and As. Molybdenum does not pose a risk to human health. Land-applied Mo should be considered because of potential Mo-induced hypocuprosis in grazing ruminants. Extensive research in land-applied biosolids studies (O'Connor et al., 2001) were used to perform a comprehensive risk assessment to update federal regulations that establish Mo limits in biosolids and land-applied biosolids. O'Connor et al. (2001) concluded that the risk from Mo-induced hypocuprosis from land-applied biosolids is small. Their risk assessment supported numerical standards for Mo at a 40 kg/ha cumulative limit and a 40 mg/kg pollutant limit. Providing an adequate Cu mineral supplement, standard procedure in proper herd management (e.g., cattle, sheep, other domesticated ruminants), would augment the conservatism of these values (O'Connor et al., 2001).

Science-based federal regulations promulgated by the USEPA limit biosolids-based N and P application to rates approved by state nutrient management regulations. The USEPA has additionally provided guidance on how to calculate biosolids' rates to supply plant available N for various land management

systems (USEPA, 1995b). Environmental fate and risk from land-applied biosolids P has been thoroughly researched and is mitigated by several factors (Barbarick & Ippolito, 2003; Elliott & O'Connor, 2007; Ippolito et al., 2007). Water extractable phosphorus (WEP) is the significant form of P for environmental consequence, and it is minimal in biosolids that contain iron and aluminum oxides (e.g., Ippolito et al., 2007).

Conclusions:

- Many of the inorganics and metals have natural abundance in soil that exceeds their concentration in biosolids.
- Risk-based standards for arsenic and molybdenum are addressed by federal rules for land application of biosolids.
- Science-based federal regulations promulgated by USEPA limit biosolids-based N and P application to rates approved by state nutrient management regulations.

Table 8. Inorganic compounds identified in OIG report.

Pollutant	CAS No.*	Category
Aluminum	7429-90-5	Metals
Barium	7440-39-3	Metals
Boron	7440-42-8	Metals
Calcium	7440-70-2	Inorganics
Cerium	7440-45-1	Metals
Cobalt	7440-48-4	Metals
Fluoride	16984-48-8	Inorganics
Iron	7439-89-6	Metals
Magnesium	7439-95-4	Metals
Manganese	7439-96-5	Metals
Molybdenum	7439-98-7	Metals
Nitrate	14797-55-8	Inorganics
Nitrite	14797-65-0	Inorganics
Nitrogen	7727-37-9	Inorganics
Phosphate (total)	14265-44-2	Inorganics
Phosphorus	7723-14-0	Inorganics
Potassium	2023695	Metals
Rubidium	7440-17-7	Metals
Sodium	7440-23-5	Metals
Tin	7440-31-5	Metals
Titanium	7440-32-6	Metals
Vanadium	7440-62-2	Metals
Yttrium	7440-65-5	Metals

*CAS: Chemical Abstracts Registry Number.

Dioxins/Furans and dioxin-like compounds

There has been extensive research on dioxins and dioxin-like compounds in biosolids. The USEPA and the Association of Metropolitan Sewage Agencies conducted nationwide biosolids surveys in the early 2000s. Based on these surveys and risk assessments, USEPA found that dioxins and dioxin-like compounds did not pose a significant risk to human or environmental health. The announcement of USEPA's decision not to regulate along with supporting documents can be found at <https://www.epa.gov/biosolids/dioxins-sewage-sludge>.

PFAS: A challenging current concern

Per- and polyfluorinated alkyl substances (PFAS) are a highly versatile and widely utilized family of synthetic surfactants with a diverse and unique set of characteristics including thermal and chemical stability, surface-tension lowering and stain, oil, and water repellency (OECD, 2013). These properties have led to a plethora of uses including in firefighting foams, non-stick cookware, textiles, carpets, furniture, paper products, food packaging, cosmetics, painting materials, lubricants, greases, pesticide formulations, and chrome and other plating operations as well as in electronics, automotive, and aerospace industries (Buck et al., 2011; Lindstrom et al., 2011; Schaider et al., 2017). Some of the unique properties that make PFAS so attractive in commerce is what makes them problematic in the environment.

Within the PFAS family are several subclasses defined by different functional groups or other unique structural characteristics with the total number of individual compounds produced for commerce approaching 5000 (Wang et al., 2017). In the OIG report, PFAS were mentioned only once and specifically referred to only perfluorooctanoate (PFOA), which is one of several PFAS in the subclass known as perfluoroalkyl acids (PFAAs). PFOA along with perfluorosulfonate (PFOS) are the two PFAS that garnered the most attention at the time of the OIG report, but since then several other PFAAs of concern have surfaced. Over the past two decades, animal and epidemiological studies raised concerns about human health impacts from PFAS exposures (ATSDR, 2020; USEPA, 2016a, 2016b) and led to increasing attention by public health agencies and advocates. The PFAS are persistent and have relatively high water solubility but may also bioaccumulate depending on their specific structures (chain length and head groups). PFOA and PFOS are known to bioaccumulate and have been reported in the blood serum and breast milk of almost all humans throughout the globe. Notably, while PFOA and PFOS are found in every American person's blood stream in the parts per billion range, those concentrations have decreased by 70% for PFOA and 84% for PFOS between 1999 and 2014, which roughly coincides with the end of the production and phase out of PFOA and PFOS in the U.S. (ATSDR, 2020). PFAS research has since broadened to include shorter-chain PFAAs. PFAS replacements and precursor PFAS refer to PFAS that can break down to the more mobile PFAAs (Brandsma et al., 2019; Buck et al., 2011; Hopkins et al., 2018; Munoz et al., 2019).

PFAS in the environment

PFAS can enter the environment in numerous ways, including through atmospheric deposition, wastewater discharge or irrigation, pesticide application, firefighting foams, and land application of biosolids. This has led to PFAS being detected across the globe and dispersed across various

environmental media and biota (Ahrens et al., 2011) even in areas not subject to direct release of PFAS (Rankin et al., 2014; Vedagiri et al., 2018). For example, a global soil survey of 32 PFAS indicated that every soil sample tested (62 locations) had some PFAS detected (Rankin et al., 2016). Likewise, PFAS were found in every sample of soil collected at random background sites in Vermont.

PFAS are frequently detected contaminants in source water and in treated drinking water (Boone et al., 2018) and are measured in precipitation (National Atmospheric Deposition Program, 2020).

The growing discovery of widespread PFAS contamination led to the adoption of a life-time drinking water public health advisory by USEPA of 70 ng/L or parts per trillion (ppt) for the sum of PFOS and PFOA in May 2016 (USEPA, 2016a, 2016b). Several states have developed more stringent drinking water and groundwater standards. For example, Massachusetts and Vermont have adopted groundwater standards at 20 ng/L for the sum of the concentrations of five or six individual PFAS. California has set drinking water notification levels at 5.1 and 6.5 ng/L for PFOA and PFOS, respectively (ECOS, 2019). Michigan has set drinking water standards for six PFAAs (e.g., 8 ng/L PFOA and 16 ng/L PFOS) as well as Gen-X but has deferred setting soil screening levels until PFAS soil-water partitioning is better understood.

PFAS in wastewater and biosolids

Historically, PFOA and PFOS were the most frequently detected in biosolids and at the highest concentrations (Clarke & Smith, 2011b). The preponderance of historical PFAS sludge analytical results report data for these two compounds. As would be expected, wastewater treatment facilities with PFAS industrial dischargers have higher PFAS concentrations in their biosolids (Clarke & Smith, 2011b; Lindstrom et al., 2011). However, the presence of PFOA, PFOS, and other PFAAs in sludge from facilities without industrial inputs underscores the importance of domestic sources of PFAS (Clarke & Smith, 2011b).

Land-applied biosolids inevitably transfers some PFAS to soils with the total conveyed dependent on the concentration in the biosolids applied and the number of applications. Levels typically found in biosolids-amended site soils are lower than in soils impacted by industrial and/or firefighting releases of PFAS. Clarke & Smith (2011b) reported PFOS in biosolids-amended soil at short-term application rates (2–11 µg/kg) and long-term application rates (5.5–483 µg/kg). Soils on Maine farm fields that have used biosolids annually for 15 or more years had a range of PFOAs from 1.1 to 12.9 µg/kg and from 2.1 to 6.1 µg/kg PFOS.

The presence of PFAS in foods for human consumption has been well established (Genualdi & de Jager, 2019; Herzke et al., 2013; Smith, 2001). The uptake of PFAS by plants grown in contaminated soil or consumption of these plants as animal feed has long been recognized as a potential contributor of PFAS to the human diet (Ghisi et al., 2019; Liu et al., 2019; Perez et al., 2017). However, overall, plant uptake rates appear minimal in biosolids-amended field studies; and no evidence is available to date that shows exposure from plant uptake at municipal-derived biosolids sites is significant or impacts human health (Blaine et al., 2014; Kowalczyk et al., 2013; Lupton et al., 2011; Perez et al., 2017).

Therefore, in general, significant PFAS exposure to the general public through diet seems unlikely given that a very small proportion of nationwide crop production is fertilized by municipal biosolids compared to production associated with chemical fertilizers and manure. Since federal harvesting restrictions

frequently make it impractical to use biosolids on food crops for direct human consumption, the potential exposure from biosolids use is decreased further. One concern voiced by some is in regard to a potential human exposure route through the use of crop residue and associated byproducts to produce cash crops used in animal feed. The latter could lead to elevated PFAS in meat and milk, particularly of the long chain PFAAs, which can bioaccumulate and biomagnify in livestock (Kowalczyk et al., 2013); however, this has yet to be shown for typical nonindustrially impacted biosolids.

Conclusion:

- Exposures associated with land application of typical biosolids that are not heavily industrially impacted are insignificant compared to the other direct and indirect exposures in modern living environments. Recycling of biosolids to soils has not been a significant contributor to the nearly ubiquitous presence of PFAS in the human population.
- When it comes to PFAS, multiple avenues exist for fugitive emissions to the environment and potential human exposure. Certainly, emissions from the production of these chemicals or their use in manufacturing of other products and in firefighting foams, as well as their presence in everyday household products, account for the vast majority and highest levels of exposures for local populations. The mobility and persistence of PFAS results in more diffuse, long-term exposure for wider populations. Diffuse, long-term PFAS presence in the environment and commerce ensure that PFAS will be discharged to society's waste management systems such as landfills and wastewater treatment facilities.
- PFAS are released to the environment via land application of biosolids, and evaluation of land application as a pathway for human exposure through the contamination of groundwater and/or surface water and uptake by plants and animals has occurred and is ongoing. Except for a few, rare worst-case scenarios involving industrially impacted biosolids, the literature does not show cases of excessive human exposure associated with the use of biosolids in agriculture (Blaine et al., 2013; Gottschall et al., 2017; Kowalczyk et al., 2013; Lindstrom et al., 2011; Lupton et al., 2012; X. Zhang et al., 2016).

Response to Antibiotic Issues

As antibiotic and antimicrobial concentrations in biosolids are well below therapeutic doses, direct exposure is not a human health issue. Ecotoxicological effects are reviewed by McCarthy et al. (2015) who concluded that antibiotics and antimicrobials in biosolids do not pose a major ecological risk. Soil microbiological impacts and antibiotic resistance from antibiotics in biosolids are thoroughly reviewed below and by Pepper in "Issue: Antibiotics Detected in Biosolids" (Appendix 1).

Response to Pathogen Issues

Class A in combination with the appropriate vector attraction reduction treatment processes meet the goal to reduce the level of pathogens in biosolids to below detectable levels and below the level at which they are infectious. Class B biosolids do contain microbial pathogens, and the OIG report lists nine viral and eight bacterial pathogens that have been detected in biosolids. However, the direct threat of human exposure to pathogens within Class B biosolids, with the appropriate vector attraction reduction

treatment processes, is mitigated by site restrictions designated by the Part 503 Federal Regulations. These site restrictions are of sufficient duration to ensure die-off and inactivation of pathogens within land-applied biosolids (USEPA, 2003a). No peer-reviewed evidence of adverse public health effects resulting from microbial hazards associated with land application of biosolids were found. This topic is thoroughly reviewed below and by Pepper in “Issue: Pathogens Detected in Biosolids” (Appendix 2).

Summary and Conclusions

The OIG report alleged that “[EPA] lacked the data or risk assessment tools needed to make a determination on the safety of 352 pollutants found in biosolids...[including] 61 designated as acutely hazardous, hazardous or priority pollutants in other programs.” Our review of literature showed extensive data and risk assessment, some conducted by USEPA, exists for the pollutants listed by OIG. In short, the above statement in the OIG is inaccurate and alarmist.

The 63 chemicals, federally regulated by NIOSH hazardous drugs list, Priority Pollutants, and RCRA in the OIG report and the 2016-2017 Biennial Review have been researched and numerous risk assessments have been conducted by USEPA for land application of biosolids. A hierarchical risk assessment using soil screening levels and persistence in this response found only four of these chemicals in biosolids may require further study: cyanide, naphthalene, pentachloronitrobenzene, and carbamazepine.

The OIG report “unlisted” contaminants in biosolids were reviewed. Extensive environmental fate and transport data is available on most of these chemicals. In general, most of these chemicals either (i) occur in biosolids below natural soil background levels, or (ii) are nontoxic and pose no risk, or (iii) occur in biosolids at concentrations well below risk-based levels, or (iv) will not persist in the environment. These chemicals do not pose risk to human health. However, a few persistent pharmaceuticals may require further study to determine their potential ecological impact (Verlicchi & Zambello, 2015).

The USEPA Office of Water is currently conducting deterministic and probabilistic risk assessments for many of the chemicals in the OIG report using existing data. Results from these assessments will inform new regulatory standards, if necessary. Data gaps exist where actual biosolids, with realistic concentrations of the chemical of concern, are land applied. Field-realistic land application of biosolids research should be used to provide data by USEPA to conduct its risk assessment and promulgate regulation of these and future chemicals of concern.

Bibliography

- Ahrens, L., Shoeib, M., Harner, T., Lee, S.C., Guo, R., and Reiner, E.J. (2011). Wastewater treatment plant and landfills as sources of polyfluoroalkyl compounds to the atmosphere. *Environmental Science & Technology*. doi: 10.1021/es1036173
- Albero, B., Pérez, R.A., Sánchez-Brunete, C., and Tadeo, J.L. (2012). Occurrence and analysis of parabens in municipal sewage sludge from wastewater treatment plants in Madrid (Spain). *Journal of Hazardous Materials*, 239–240, 48–55. doi: 10.1016/j.jhazmat.2012.05.017
- Andrade, N.A., McConnell, L.L., Torrents, A., and Ramirez, M. (2010). Persistence of polybrominated diphenyl ethers in agricultural soils after biosolids applications. *Journal of Agricultural & Food Chemistry*, 58(5), 3077–3084. doi: 10.1021/jf9034496
- ATSDR (2020, June 24). *PFAS in the US population | ATSDR*. <https://www.atsdr.cdc.gov/pfas/health-effects/us-population.html>
- Baltz, R.H. (2010). Genomics and the ancient origins of the daptomycin biosynthetic gene cluster. *The Journal of Antibiotics*, 63(8), 506–511.
- Barbarick, K.A., and Ippolito, J.A. (2003). Termination of sewage biosolids application affects wheat yield and other agronomic characteristics. *Agronomy Journal*, 95, 1288–1294.
- Berendsen, B.J.A., Wegh, R.S., Memelink, J., Zuidema, T., and Stolker, A.A.M. (2015). The analysis of animal feces as a tool to monitor antibiotic usage. *Talanta*, 132, 258–268. doi: 10.1016/j.talanta.2014.09.022
- Bittencourt, S., Aisse, M.M., and Serrat, B.M. (2016). Agronomic and inorganic parameters of sewage sludge sanitized by alkaline stabilization: A case study of the State of Paraná, Brazil. *Scientia Agraria*, 17(2), 1–13.
- Blaine, A.C., Rich, C.D., Hundal, L.S., Lau, C., Mills, M.A., Harris, K.M., and Higgins, C.P. (2013). Uptake of perfluoroalkyl acids into edible crops via land applied biosolids: Field and greenhouse studies. *Environmental Science & Technology*, 47(24), 14062–14069. doi: 10.1021/es403094q
- Błędzka, D., Gromadzińska, J., and Wąsowicz, W. (2014). Parabens. From environmental studies to human health. *Environment International*, 67, 27–42. doi: 10.1016/j.envint.2014.02.007
- Brandsma, S.H., Koekkoek, J.C., van Velzen, M.J.M., and de Boer, J. (2019). The PFOA substitute GenX detected in the environment near a fluoropolymer manufacturing plant in the Netherlands. *Chemosphere*, 220, 493–500.
- Braschi, I., Blasioli, S., Fellet, C., Lorenzini, R., Garelli, A., Pori, M., et al. (2013). Persistence and degradation of new β -lactam antibiotics in the soil and water environment. *Chemosphere*, 93, 152–159. doi: 10.1016/j.chemosphere.2013.05.016
- Bright, D.A., and Healey, N. (2003). Contaminant risks from biosolids land application: Contemporary organic contaminant levels in digested sewage sludge from five treatment plants in Greater Vancouver, British Columbia. *Environmental Pollution*, 126(1), 39–49. doi: 10.1016/S0269-7491(03)00148-9
- Brooks, J.P., Tanner, B.D., Gerba, C.P., Haas, C.N., and Pepper, I.L. (2005a). Estimation of bioaerosol risk of infection to residents adjacent to a land applied biosolids site using an empirically derived transport model. *Journal of Applied Microbiology*, 98, 397–405.
- Brooks, J.P., Tanner, B.D., Josephson, K.L., Haas, C.N., Gerba, C.P., and Pepper, I.L. (2005b). A national study on the residential impact of biological aerosols from the land application of biosolids. *Journal of Applied Microbiology*, 99, 310–322.
- Brooks, J.P., Tanner, B.D., Gerba, C.P., and Pepper, I.L. (2006). The measurement of aerosolized endotoxin from land application of Class B biosolids in Southeast Arizona. *Canadian Journal of Microbiology*, 52, 150–156.

- Brooks, J.P., McLaughlin, M.R., Gerba, C.P., and Pepper, I.L. (2012). Land application of manure and Class B biosolids: An occupational and public quantitative microbial risk assessment. *Journal of Environmental Quality*, 41, 2009–2023.
- Brown, S., Kennedy, L., Cullington, M., Mihle, A., and Lono-Batura, M. (2019). Relating pharmaceuticals and personal care products in biosolids to home exposure. *Urban Agriculture*, 4(1). doi: 10.2134/urbanag2018.12.0005
- Buck, R.C., Franklin, J., Berger, U., Conder, J M., Cousins, I.T., Voogt, P.D., Jensen, A.A., Kannan, K., Mabury, S.A., and van Leeuwen, S.P.J. (2011). Perfluoroalkyl and polyfluoroalkyl substances in the environment: Terminology, classification, and origins. *Integrated Environmental Assessment & Management*. doi.org/10.1002/ieam.258
- Buerge, I.J., Buser, H.-R., Poiger, T., and Müller, M.D. (2006). Occurrence and fate of the cytostatic drugs cyclophosphamide and ifosfamide in wastewater and surface waters. *Environmental Science & Technology*, 40(23), 7242–7250. doi.org/10.1021/es0609405
- Busalacchi, D.M. (2012). *Evaluation of Biosolids as a Soil Amendment for Use in Ecological Restoration* The Ohio State University, Columbus OH. https://etd.ohiolink.edu/pg_10?0::NO:10:P10_ETD_SUBID:75809
- Castro-del Campo, N., Pepper, I.L., and Gerba, C.P. (2007). Assessment of *Salmonella typhimurium* growth in Class A biosolids and soil/biosolid mixtures. *Journal of Residuals Science & Technology*, 4, 83–88.
- Chen, F., Ying, G.-G., Ma, Y.-B., Chen, Z.-F., Lai, H.-J., and Peng, F.-J. (2014). Field dissipation and risk assessment of typical personal care products TCC, TCS, AHTN and HHCb in biosolid-amended soils. *Science of the Total Environment*, 470–471, 1078–1086. doi: 10.1016/j.scitotenv.2013.10.080
- Chen, J., Pycke, B.F.G., Brownawell, B.J., Kinney, C.A., Furlong, E.T., Kolpin, D.W., and Halden, R.U. (2017). Occurrence, temporal variation, and estrogenic burden of five parabens in sewage sludge collected across the United States. *Science of the Total Environment*, 593–594, 368–374. doi: 10.1016/j.scitotenv.2017.03.162
- Chen, Y.S., Zhang, H.B., Luo, Y.M., and Song, J. (2012). Occurrence and assessment of veterinary antibiotics in swine manures: A case study in East China. *Chinese Science Bulletin*, 57, 606–614. doi: 10.1007/s11434-011-4830-3
- Cheng, M., Wu, L., Huang, Y., Luo, Y., and Christie, P. (2014). Total concentrations of heavy metals and occurrence of antibiotics in sewage sludges from cities throughout China. *Journal of Soil Sediments*, 14, 1123–1135. doi: 10.1007/s11368-014-0850-3
- Chetochine, A., Brusseau, M.L., Gerba, C.P., and Pepper, I.L. (2006). Leaching of phage from Class B biosolids and potential transport through soil. *Applied Environmental Microbiology*, 72, 665–671.
- Clarke, B.O., and Smith, S.R. (2011a). Review of ‘emerging’ organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. *Environment International*, 37(1), 226–247. doi: 10.1016/j.envint.2010.06.004
- Committee for Risk Assessment (RAC), C. for S.-E. A. (SEAC), (2016). *Background Document to the Opinion on the Annex XV Dossier Proposing Restrictions on Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5)*. ECHA/RAC/RES-O-0000001412–86–97/D, EHCA/SEAC/RES-O-0000001412–86–109/F.
- Committee for Risk Assessment (RAC), (2008). *European Union Risk Assessment Report 1-(5,6,7,8-TETRAHYDRO-3,5,5,6,8,8-HEXAMETHYL-2-NAPHTHYL)ETHAN-1-ONE (AHTN)*.
- Crane, M., Boxall, A.B.A., and Barret, K. (2010). *Veterinary Medicines in the Environment*. CRC Press, Boca Raton, FL.

- Cristale, J., Ramos, D.D., Dantas, R.F., Machulek Jr., A., Lacorte, S., Sans, C., and Esplugas, S. (2016). Can activated sludge treatments and advanced oxidation processes remove organophosphorus flame retardants? *Environmental Research*, 144, 11–18. doi: 10.1016/j.envres.2015.10.008
- Cruz, S., and Barceló, D. (2015). *Personal Care Products in the Aquatic Environment*. Springer Publishing, New York.
- Cycoń, M., Orlewska, K., Markowicz, A., Zmijowska, A., Smolen-Dzirba, J., Bratosiewicz-Wasik, J. et al. (2018). Vancomycin and/or multidrug-resistant *Citrobacter freundii* altered the metabolic pattern of soil microbial community. *Frontiers in Microbiology*, 9, 1047. doi: 10.3389/fmicb.2018.01047
- Cycoń, M., Mroziak, A., and Piotrowska-Seget, Z. (2019). Antibiotics in the soil environment—Degradation and their impact on microbial activity and diversity. *Frontiers in Microbiology*, 10:338. doi: 10.3389/fmicb.2019.00338
- Cytryn, E. (2013). The soil resistome: The anthropogenic, the nature and the unknown. *Soil Biology & Biochemistry*, 63, 18–23.
- da Silva Souza, T., Lacerda, D., Aguiar, L.L., Martins, M.N.C., and Augusto de Oliveira David, J. (2020). Toxic potential of sewage sludge: Histopathological effects on soil and aquatic bioindicators. *Ecological Indicators*, 111, 105980. doi: 10.1016/j.ecolind.2019.105980
- Daso, A.P., Fatoki, O.S., Odendaal, J.P., and Okonkwo, J.O. (2010). A review on sources of brominated flame retardants and routes of human exposure with emphasis on polybrominated diphenyl ethers. *Environmental Reviews*, 18(NA), 239–254. doi: 10.1139/A10-010
- Davis, E.F., Gunsch, C.K., and Stapleton, H.M. (2015). Fate of flame retardants and the antimicrobial agent triclosan in planted and unplanted biosolid-amended soils. *Environmental Toxicology & Chemistry*, 34(5), 968 doi: 10.1016/j.ecolind.2019.105980976. doi: 10.1002/etc.2854
- Dela Justina, M., and Skoronski, E. (2018). Environmental and agronomical aspects of sludge produced from tannin-based coagulants in dairy industry wastewater treatment. *Waste & Biomass Valorization*. doi: 10.1007/s12649-018-0403-x
- DeVries, S.L., and Zhang, P. (2016). Antibiotics and the terrestrial nitrogen cycle: A review. *Current Pollution Reports*, 2, 51–67. doi: 10.1007/s40726-016-0027-3
- Ding, Y., Zhang, W., Gu, C., Xagorarakis, I., and Li, H. (2011). Determination of pharmaceuticals in biosolids using accelerated solvent extraction and liquid chromatography/tandem mass spectrometry. *Journal of Chromatography A*, 1218, 10–16. doi: 10.1016/j.chroma.2010.10.112
- Dolliver, H., Gupta, S., and Noll, S. (2008). Antibiotic degradation during manure composting. *Journal of Environmental Quality*, 37, 1245. doi: 10.2134/jeq2007.0399
- ECOS, (2019, September 13). *California Lowers PFAS Notification Levels, Initiates Regulatory Process*. The Environmental Council of the States (ECOS). <https://www.ecos.org/news-and-updates/california-lowers-pfas-notification-levels-initiates-regulatory-process/>
- Elliott, H.A., and O'Connor, G.A. (2007). Phosphorus management for sustainable biosolids recycling in the United States. *Soil Biology & Biochemistry*, 39(6), 1318–1327. doi: 10.1016/j.soilbio.2006.12.007
- Eriksen, G.S., Amundsen, C.E., Bernhoft, A., Eggen, T., Grave, K., Halling-Sørensen, B., Källqvist, T., Sogn, T., and Sverdrup, L. (2009). *Risk Assessment of Contaminants in Sewage Sludge Applied on Norwegian Soils*. Opinion of the panel on contaminants in the Norwegian Scientific Committee for Food Safety.
- Est, D.M.O. (2015, December 8). *Two Numbers: Animal Manure a Growing Headache in America*. Newsweek. <https://www.newsweek.com/2015/12/18/two-numbers-animal-manure-growing-headache-america-402205.html>

- Ezechiáš, M., Covino, S., and Cajthaml, T. (2014). Ecotoxicity and biodegradability of new brominated flame retardants: A review. *Ecotoxicology & Environmental Safety*, *110*, 153–167. doi: 10.1016/j.ecoenv.2014.08.030
- Fleming, M., Achari, G., and Hassan, Q.K. (2016). Modeling the loading and fate of estrogens in wastewater treatment plants. *Cogent Environmental Science*, *2*(1). doi: 10.1080/23311843.2016.1222690
- Ghisi, R., Vamerali, T., and Manzetti, S. (2019). Accumulation of perfluorinated alkyl substances (PFAS) in agricultural plants: A review. *Environmental Research*, *169*, 326–341.
- Giesy, J., Solomon, K., and Kacew, S. (2011). Report of the board of review for decamethylcyclopentasiloxane (D5). *Siloxane D5 Board of Review, Ottawa, ON, Canada*, 83 pp.
- Göbel, A., Thomsen, A., McArdell, C.S., Alder, A.C., Giger, W., Theiß, N., Löffler, D., et al. (2005). Extraction and determination of sulfonamides, macrolides, and trimethoprim in sewage sludge. *Journal of Chromatography A*, *1085*, 179–189. doi: 10.1016/j.chroma.2005.05.051
- Gorgy, T., Li, L.Y., Grace, J.R., and Ikononou, M.G. (2013). Migration of polybrominated diphenyl ethers in biosolids-amended soil. *Environmental Pollution*, *172*, 124–130.
- Gosens, I., Delmaar, C.J.E., Burg, W., de Heer, C., and Schuur, A.G. (2014). Aggregate exposure approaches for parabens in personal care products: A case assessment for children between 0 and 3 years old. *Journal of Exposure Science & Environmental Epidemiology*, *24*(2), 208–214. doi: 10.1038/jes.2013.33
- Gottschall, N., Topp, E., Edwards, M., Payne, M., Kleywegt, S., and Lapen, D.R. (2017). Brominated flame retardants and perfluoroalkyl acids in groundwater, tile drainage, soil, and crop grain following a high application of municipal biosolids to a field. *Science of the Total Environment*, *574*, 1345–1359. doi: 10.1016/j.scitotenv.2016.08.044
- Gramatica, P., Cassani, S., and Sangion, A. (2016). Are some “safer alternatives” hazardous as PBTs? The case study of new flame retardants. *Journal of Hazardous Materials*, *306*, 237–246. doi: 10.1016/j.jhazmat.2015.12.017
- Gundy, P.M., Gerba, C.P., and Pepper, I.L. (2009). Survival of coronaviruses in water and wastewater. *Food Environment Virology*, *1*, 10–14.
- Hammesfahr, U., Heuer, H., Manzke, B., Smalla, K., and Thiele-Bruhn, S. (2008). Impact of the antibiotic sulfadiazine and pig manure on the microbial community structure in agricultural soils. *Soil Biology and Biochemistry*, *40*, 1583–1591. doi: 10.1016/j.soilbio.2008.01.010
- Heberer, T. (2002). Occurrence, fate, and assessment of polycyclic musk residues in the aquatic environment of urban areas—A review. *Acta Hydrochimica et Hydrobiologica*, *30*(5–6), 227–243. doi: 10.1002/aheh.200390005
- Herrmann, R.F., Grosser, R.J., Farrar, D. et al. (2017). Field studies measuring the aerosolization of endotoxin during the land application of Class B biosolids. *Aerobiologia*, *33*, 417–434. doi: 10.1007/s10453-017-9480-8
- Higgins, C.P., Sharp, J.O., Sepulvado, J.G., Littrell, B., O’Connor, G., Snyder, E., and McAvoy, D. (2010). *Trace Organic Chemicals in Biosolids-Amended Soils: State-of-the-Science Review*. Water Environment Research Foundation, IWA Publishing. doi: <https://doi.org/10.2166/9781780403441>
- Hopkins, Z.R., Sun, M., DeWitt, J.C., and Knappe, D.R.U. (2018). Recently detected drinking water contaminants: GenX and other per- and polyfluoroalkyl ether acids. *Journal American Water Works Association*, *110*(7), 13–28. doi: 10.1002/awwa.1073
- Ippolito, J.A., Barbarick, K.A., and Norvell, K.L. (2007). Biosolids impact on soil phosphorus accountability, fractionation, and potential environmental risk. *Journal of Environmental Quality*, *36*, 764–772.

- Ishii, S., Ksoll, W.B., Hicks, E., Sadowsky, M.J. (2006). Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. *Applied Environmental Microbiology*, 72, 612–621.
- Jensen, J., Ingvertsen, S.T., and Magid, J. (2012). *Risk Evaluation of Five Groups of Persistent Organic Contaminants in Sewage Sludge*. Environmental Project No. 1406, Environmental Protection Agency, Danish Ministry of the Environment, Copenhagen.
- Jones-Lepp, T.L., and Stevens, R. 2007. Pharmaceuticals and personal care products in biosolids/sewage sludge: The interface between analytical chemistry and regulation. *Analytical & Bioanalytical Chemistry*, 387, 1173–1183. doi: 10.1007/s00216-006-0942-z
- Jones-Otazo, H.A., Clarke, J.P., Diamond, M.L., Archbold, J.A., Ferguson, G., Harner, T., Richardson, G.M., Ryan, J.J., and Wilford, B. (2005). Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs. *Environmental Science & Technology*, 39(14), 5121–5130. doi: 10.1021/es048267b
- Kabata-Pendias, A. (2010). *Trace Elements in Soils and Plants*. CRC Press, Boca Raton, FL.
- Khanal, S.K., Xie, B., Thompson, M.L., Sung, S., Ong, S.-K., and van Leeuwen, J. (Hans), (2006). Fate, transport, and biodegradation of natural estrogens in the environment and engineered systems. *Environmental Science & Technology*, 40(21), 6537–6546. doi: 10.1021/es0607739
- Khosravi, K., and Price, G.W. (2015). Determination of phthalates in soils and biosolids using accelerated solvent extraction coupled with SPE cleanup and GC–MS quantification. *Microchemical Journal*, 121, 205–212.
- Kim, M., Li, L.Y., Gorgy, T., and Grace, J.R. (2017). Review of contamination of sewage sludge and amended soils by polybrominated diphenyl ethers based on meta-analysis. *Environmental Pollution*, 220, 753–765. doi: 10.1016/j.envpol.2016.10.053
- Kinney, C.A., Furlong, E.T., Zaugg, S.D., Burkhardt, M.R., Werner, S.L., Cahill, J.D., et al. (2006). Survey of organic wastewater contaminants in biosolids destined for land application. *Environmental Science & Technology*, 40, 7207–7215. doi: 10.1021/es0603406
- Kirchmann, H., Åström, H., and Jonsell, G. (1991). Organic pollutants in sewage sludge, 1: Effect of toluene, naphthalene, 2-methylnaphthalene, 4-n-nonylphenol and di-2-ethylhexyl phthalate on soil biological processes and their decomposition in soil. *Swedish Journal of Agricultural Research (Sweden)*, 21(3), 107–113.
- Kohli, J., Lee, H.-B., & Peart, T.E. (2006). Organic contaminants in Canadian municipal sewage sludge. Part II. Persistent chlorinated compounds and polycyclic aromatic hydrocarbons. *Water Quality Research Journal*, 41(1), 47–55. doi: 10.2166/wqrj.2006.005
- Kowalczyk, J., Ehlers, S., Oberhausen, A., Tischer, M., Fürst, P., Schafft, H., and Lahrssen-Wiederholt, M. (2013). Absorption, distribution, and milk secretion of the perfluoroalkyl acids PFBS, PFHxS, PFOS, and PFOA by dairy cows fed naturally contaminated feed. *Journal of Agricultural & Food Chemistry*, 61(12), 2903–2912.
- Kümmerer, K. (2009). Antibiotics in the aquatic environment—A review—Part I. *Chemosphere*, 75(4), 417–434.
- Kwak, J.I., Moon, J., Kim, D., and An, Y.-J. (2017). Soil ecotoxicity of seven endocrine-disrupting chemicals: A review. *European Journal of Soil Science*, 68(5), 621–649. doi: 10.1111/ejss.12467
- Lang, M., Forste, J., Goldstein, N., Johnston, T., and Brandt, R. (2005). *National Manual of Good Practice for Biosolids*. Water Environment Research Foundation, National Biosolids Partnership.
- Li, H., Qu, R., Yan, L., Guo, W., and Ma, Y. (2015). Field study on the uptake and translocation of PBDEs by wheat (*Triticum aestivum* L.) in soils amended with sewage sludge. *Chemosphere*, 123, 87–92. doi: 10.1016/j.chemosphere.2014.12.045

- Li, L.L., Huang, L.D., Chung, R.S., Fok, K.H., and Zhang, Y.S. (2010). Sorption and dissipation of tetracyclines in soils and compost. *Pedosphere*, 20, 807–816. doi: 10.1016/S1002-0160(10)60071-9
- Li, W., Shi, Y., Gao, L., Liu, J., and Cai, Y. (2013). Occurrence, distribution and potential affecting factors of antibiotics in sewage sludge of wastewater treatment plants in China. *Science of the Total Environment*, 445–446, 306–313. doi: 10.1016/j.scitotenv.2012.12.050
- Lillenberg, M., Yurchenko, S., Kipper, K., Herodes, K., Pihl, V., Löhmus, R., et al. (2010). Presence of fluoroquinolones and sulfonamides in urban sewage sludge and their degradation as a result of composting. *International Journal of Science & Technology*, 7, 307–312. doi: 10.1007/BF03326140
- Lin, K., and Gan, J. (2011). Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils. *Chemosphere*, 83, 240–246. doi: 10.1016/j.chemosphere.2010.12.083
- Lindstrom, A.B., Strynar, M.J., Delinsky, A.D., Nakayama, S.F., McMillan, L., Libelo, E.L., Neill, M., and Thomas, L. (2011). Application of WWTP biosolids and resulting perfluorinated compound contamination of surface and well water in Decatur, Alabama, USA. *Environmental Science & Technology*. doi: 10.1021/es1039425
- Liu, F., Ying, G.G., Tao, R., Zhao, J.L., Yang, J.F., and Zhao, L.F. (2009). Effects of six selected antibiotics on plant growth and soil microbial and enzymatic activities. *Environmental Pollution*, 157, 1636–1642. doi: 10.1016/j.envpol.2008.12.021
- Liu, J., Zhong, G., Li, W., and Mejia Avendaño, S. (2019). Isomer-specific biotransformation of perfluoroalkyl sulfonamide compounds in aerobic soil. *Science of the Total Environment*. doi: 10.1016/j.scitotenv.2018.09.214
- Lu, Q., He, Z.L., and Stoffella, P.J. (2012). Land application of biosolids in the USA: A review. *Applied and Environmental Soil Science*. doi: 10.1155/2012/201462
- Lupton, S.J., Huwe, J.K., Smith, D.J., Dearfield, K.L., and Johnston, J.J. (2012). Absorption and excretion of 14C-perfluorooctanoic acid (PFOA) in Angus cattle (*Bos taurus*). *Journal of Agricultural and Food Chemistry*, 60(4), 1128–1134.
- Ma, T., Pan, X., Chen, I., Liu, W., Christie, P., Luo, Y., et al. (2016). Effects of different concentrations and application frequencies of oxytetracycline on soil enzyme activities and microbial community diversity. *European Journal of Soil Biology*, 76, 53–60. doi: 10.1016/j.ejsobi.2016.07.004
- MacDonald, J.M. (2009). *Manure Use for Fertilizer and for Energy: Report to Congress*. Administrative Publication No. (AP-037), USDA Economic Research Service, 53 pp.
- Martínez-Carballo, E., González-Barreiro, C., Scharf, S., and Gans, O. (2007). Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. *Environmental Pollution*, 148, 570–579. doi: 10.1016/j.envpol.2006.11.035
- Massé, D.I., Saady, N.M.C., and Gilbert, Y. (2014). Potential of biological processes to eliminate antibiotics in livestock manure: An overview. *Animals*, 4, 146–163. doi: 10.3390/ani4020146
- Mazzeo, D.E.C., Fernandes, T.C.C., Levy, C.E., Fontanetti, C.S., and Marin-Morales, M.A. (2015). Monitoring the natural attenuation of a sewage sludge toxicity using the *Allium cepa* test. *Ecological Indicators*, 56, 60–69. doi: 10.1016/j.ecolind.2015.03.026
- McCarthy, L., Loyo-Rosales, J., Raby, M., Payne, M., Huber, A., Lavery, J., and Falls, A. (2015). *Risks associated with the application of municipal biosolids to agricultural lands in a Canadian context*. Canadian Water Network (CWN), Canadian Municipal Water Consortium (CMWC), Waterloo, Ontario. Available from Cwn-Rce: ca/Assets/Resources/Pdf/McCarthy-Risks-Biosolids-2015.
- Miles, S.L., Sun, W., Field, J.A., Gerba, C.P., and Pepper, I.L. (2013). Survival of infectious prions during anaerobic digestion of municipal sewage sludge and lime stabilization of Class B biosolids. *Journal of Residuals Science & Technology*, 10, 69–85.

- Mina, O., Gall, H.E., Saporito, L.S., and Kleinman, P.J.A. (2016). Estrogen transport in surface runoff from agricultural fields treated with two application methods of dairy manure. *Journal of Environmental Quality*, 45(6), 2007–2015. doi: 10.2134/jeq2016.05.0173
- Morais, S.A., Delerue-Matos, C., Gabarrell, X., and Blázquez, P. (2013). Multimedia fate modeling and comparative impact on freshwater ecosystems of pharmaceuticals from biosolids-amended soils. *Chemosphere*, 93(2), 252–262. doi: 10.1016/j.chemosphere.2013.04.074
- Munoz, G., Liu, J., Duy, S.V., and Sauvé, S. (2019). Analysis of F-53B, Gen-X, ADONA, and emerging fluoroalkylether substances in environmental and biomonitoring samples: A review. *Trends in Environmental Analytical Chemistry*, 23, e00066.
- Navarro, I., de la Torre, A., Sanz, P., Fernández, C., Carbonell, G., and Martínez, M. de los Á. (2018). Environmental risk assessment of perfluoroalkyl substances and halogenated flame retardants released from biosolids-amended soils. *Chemosphere*, 210, 147–155. doi: 10.1016/j.chemosphere.2018.07.007
- Nielson, J.W., Josephson, K.L., Pepper, I.L., Arnold, R.B., DiGiovanni, G.D., and Sinclair, N.A. (1994). Frequency of horizontal gene transfer of a large catabolic plasmid (pJP4) in soil. *Applied Environmental Microbiology*, 60, 4053–4058.
- O'Connor, G.A., Brobst, R.B., Chaney, R.L., Kincaid, R.L., McDowell, L.R., Pierzynski, G.M., Rubin, A., and Van Riper, G.G. (2001). A modified risk assessment to establish molybdenum standards for land application of biosolids. *Journal of Environmental Quality*, 30(5), 1490–1507. doi: 10.2134/jeq2001.3051490x
- OECD (2013). *OECD/UNEP Global PFC Group, Synthesis paper on per- and polyfluorinated chemicals (PFCs)*. Environment, Health and Safety, Environment Directorate, OECD.
- Ohio Environmental Protection Agency (2019). *Support Document for the Development of Generic Numerical Standards and Risk Assessment Procedures*. The Voluntary Action Program Division of Environmental Response and Revitalization Ohio Environmental Protection Agency July 2019. <https://epa.ohio.gov/portals/30/rules/July%202019%20VAP%20Support%20Document.pdf>
- Pan, X., Qiang, Z., Ben, W., and Chen, M. (2011). Residual veterinary antibiotics in swine manure from concentrated animal feeding operations in Shandong Province, China. *Chemosphere*, 84, 695–700. doi: 10.1016/j.chemosphere.2011.03.022
- Park, I., Zhang, N., Ogunyoku, T.A., Young, T.M., and Scow, K.M. (2013). Effects of triclosan and biosolids on microbial community composition in an agricultural soil. *Water Environment Research*, 85(12), 2237–2242. doi: 10.2175/106143012X13560205144335
- Pepper, I., Josephson, K., Bailey, R., Burr, M., and Gerba, C. (1993). Survival of indicator organisms in Sonoran Desert soil amended with sewage-sludge. *Journal of Environmental Science and Health Part A-Environmental Science and Engineering & Toxic and Hazardous Substance Control*, 28(6), 1287–1302. doi: 10.1080/10934529309375943
- Pepper, I.L., Zerzghi, H., Brooks, J.P., and Gerba, C.P. (2008). Sustainability of land application of Class B biosolids. *Journal of Environmental Quality*, 37, 58–67.
- Pepper, I.L., Brooks, J.P., Sinclair, R.G., Gurian, P.L., and Gerba, C.P. (2010). Pathogens and indicators in United States Class B biosolids: National and historic distributions. *Journal of Environmental Quality*, 39(6), 2185–2190. doi: 10.2134/jeq2010.0037
- Pepper, I.L., Brooks, J.P., and Gerba, C.P. (2018). Antibiotic resistant bacteria in municipal wastes: Is there reason for concern? *Environmental Science & Technology*, 52(7), 3949–3959. doi: 10.1021/acs.est.7b04360
- Perez, A.L., Poteete, C., Louie, F., Garner, L., and Scott, P.K. (2017). State of the science and meta-analysis of crop uptake of per-and polyfluoroalkyl substances (PFAS). Presented at the DIOXIN 2017 Symposium, Vancouver Canada, August 21, 2017.

- Pinho, G.P., Silvério, F.O., Evangelista, G.F., Mesquita, L.V., and Barbosa, É.S. (2014). Determination of chlorobenzenes in sewage sludge by solid-liquid extraction with purification at low temperature and gas chromatography mass spectrometry. *Journal of the Brazilian Chemical Society*, 25(7), 1292–1301. doi: 10.5935/0103-5053.20140109
- Pollard, A.T., and Morra, M.J. (2017). Estrogens: Properties, behaviors, and fate in dairy manure-amended soils. *Environmental Reviews*, 25(4), 452–462.
- PubChem (2019a, May 15). *Benzo[a]pyrene*. <https://pubchem.ncbi.nlm.nih.gov/compound/2336>
- PubChem (2019b, June 13). *Estradiol*. <https://pubchem.ncbi.nlm.nih.gov/compound/5757>
- PubChem (2019c, June 13). *Estrone*. <https://pubchem.ncbi.nlm.nih.gov/compound/5870>
- PubChem (2019d, June 13). *Naphthalene*. <https://pubchem.ncbi.nlm.nih.gov/compound/931>
- PubChem (2019e, June 13). *N-Nitrosopiperidine*. <https://pubchem.ncbi.nlm.nih.gov/compound/7526>
- PubChem (2019f, June 13). *Norethindrone*. <https://pubchem.ncbi.nlm.nih.gov/compound/6230>
- PubChem (2019g, June 13). *Testosterone*. <https://pubchem.ncbi.nlm.nih.gov/compound/6013>
- PubChem (2020a). *4-Nitrophenol*. <https://pubchem.ncbi.nlm.nih.gov/compound/980>
- PubChem (2020b). *Carbamazepine*. <https://pubchem.ncbi.nlm.nih.gov/compound/2554>
- PubChem (2020c). *Hydrogen cyanide*. <https://pubchem.ncbi.nlm.nih.gov/compound/768>
- PubChem (2020d). *Progesterone*. <https://pubchem.ncbi.nlm.nih.gov/compound/5994>
- PubChem (n.d.-a). *1-Methylnaphthalene*. Retrieved December 17, 2019, from <https://pubchem.ncbi.nlm.nih.gov/compound/7002>
- PubChem (n.d.-b). *Ethanol*. Retrieved December 17, 2019, from <https://pubchem.ncbi.nlm.nih.gov/compound/702>
- PubChem (n.d.-c). *Tris(2-butoxyethyl) phosphate*. Retrieved December 18, 2019, from <https://pubchem.ncbi.nlm.nih.gov/compound/6540>
- Rankin, K., Lee, H., Tseng, P.J., and Mabury, S.A. (2014). Investigating the biodegradability of a fluorotelomer-based acrylate polymer in a soil-plant microcosm by indirect and direct analysis. *Environmental Science & Technology*. doi: 10.1021/es502986w
- Rauert, C., and Harrad, S. (2015). Mass transfer of PBDEs from plastic TV casing to indoor dust via three migration pathways—A test chamber investigation. *Science of the Total Environment*, 536, 568–574. doi: 10.1016/j.scitotenv.2015.07.050
- Rusin, P., Maxwell, S., Brooks, J., Gerba, C., and Pepper, I. (2003). Evidence for the absence of *Staphylococcus aureus* in land applied biosolids. *Environmental Science & Technology*, 37, 4027–4030.
- Sabourin, L., Duenk, P., Bonte-Gelok, S., Payne, M., Lapen, D.R., and Topp, E. (2012). Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. *Science of the Total Environment*, 431, 233–236. doi: 10.1016/j.scitotenv.2012.05.017
- Sassi, H.P., Ikner, L.A., Abd-Elmaksoud, S., Gerba, C.P., and Pepper, I.L. (2018). Comparative survival of viruses during thermophilic and mesophilic anaerobic digestion. *Science of the Total Environment*, 615, 15–19.
- Schaidler, L.A., Balan, S.A., Blum, A., Andrews, D.Q., Strynar, M.J., Dickinson, M.E., Lunderberg, D.M., Lang, J.R., and Peaslee, G.F. (2017). Fluorinated compounds in U.S. fast food packaging. *Environmental Science & Technology Letters*. doi: 10.1021/acs.estlett.6b00435
- Schlüsener, M.P., Von Arb, M.A., and Bester, K. (2006). Elimination of macrolides, tiamulin, and salinomycin during manure storage. *Archives of Environmental Contamination & Toxicology*, 51, 21–28. doi: 10.1007/s00244-004-0240-8
- Sellström, U., de Wit, C.A., Lundgren, N., and Tysklind, M. (2005). Effect of sewage-sludge application on concentrations of higher-brominated diphenyl ethers in soils and earthworms. *Environmental Science & Technology*, 39(23), 9064–9070. doi: 10.1021/es051190m

- Smith, D.B., Cannon, W.F., Woodruff, L.G., Solano, Federico, Kilburn, J.E., and Fey, D.L. (2013). *Geochemical and Mineralogical Data for Soils of the Conterminous United States*. U.S. Geological Survey Data Series 801, 19 pp. <https://pubs.usgs.gov/ds/801/>
- Smith, S.R. (2009). Organic contaminants in sewage sludge (biosolids) and their significance for agricultural recycling. *Philosophical Transactions of the Royal Society A: Mathematical, Physical & Engineering Sciences*, 367(1904), 4005–4041. doi: 10.1098/rsta.2009.0154
- Steffan, J.J., Brevik, E.C., Burgess, L.C., and Cerdá, A. (2018). The effect of soil on human health: An overview. *European Journal of Soil Science*, 69(1), 159–171. doi: 10.1111/ejss.12451
- Stevens, J.L., Northcott, G.L., Stern, G.A., Tomy, G.T., and Jones, K.C. (2003). PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musks, and polychlorinated n-alkanes in U.K. sewage sludge: Survey results and implications. *Environmental Science & Technology*, 37(3), 462–467. doi: 10.1021/es020161y
- Straub, T., Pepper, I., and Gerba, C. (1995). Comparison of PCR and cell-culture for detection of enteroviruses in sludge-amended field soils and determination of their transport. *Applied & Environmental Microbiology*, 61(5), 2066–2068. doi: 10.1128/AEM.61.5.2066-2068.1995
- Strauch, D. (1991). Microbiological treatment of municipal sewage waste and refuse as a means of disinfection prior to recycling in agriculture. *Studies in Environmental Science*, 42, 121–136.
- Strauch, D. (1998). Pathogenic micro-organisms in sludge. Anaerobic digestion and disinfection methods to make sludge usable as a fertiliser. *European Water Management*, 1(2), 12–26.
- Tanner, B.D., Brooks, J.P., Haas, C.N., Gerba, C.P., and Pepper, I.L. (2005). Bioaerosol emission rate and plume characteristics during land application of liquid class B biosolids. *Environmental Science & Technology*, 39(6), 1584–1590. doi: 10.1021/es0404466
- Topp, E., Monteiro, S.C., Beck, A., Coelho, B.B., Boxall, A.B.A., Duenk, P.W., Kleywegt, S., Lapen, D.R., Payne, M., Sabourin, L., Li, H., and Metcalfe, C.D. (2008). Runoff of pharmaceuticals and personal care products following application of biosolids to an agricultural field. *Science of the Total Environment*, 396(1), 52–59. doi: 10.1016/j.scitotenv.2008.02.011
- Topp, E., Chapman, R., Devers-Lamrani, M., Hartmann, A., Marti, R., Martin-Laurent, F., et al. (2013). Accelerated biodegradation of veterinary antibiotics in agricultural soil following long-term exposure, and isolation of a sulfamethazine degrading microbacterium sp. *Journal of Environmental Quality*, 42, 173–178. doi: 10.2134/jeq2012.0162
- Topp, E., Renaud, J., Sumarah, M., and Sabourin, L. 2016. Reduced persistence of the macrolide antibiotics erythromycin, clarithromycin and azithromycin in agricultural soil following several years of exposure in the field. *Science of the Total Environment*, 562, 136–144. doi: 10.1016/j.scitotenv.2016.03.210
- USEPA (1989). *Appendix F Estimates from the National 1988 Sewage Sludge Survey (NSSS)*.
- USEPA (1993). The standards for the use of disposal of sewage sludge. Final CFR Part 503 Rules. EPA 822/2-93/001. USEPA, Office of Water, Washington, DC.
- USEPA (1995a). *A Guide to the Biosolids Risk Assessments for the EPA Part 503 Rule*. <https://nepis.epa.gov/Exe/ZyPDF.cgi/200041MZ.PDF?Dockey=200041MZ.PDF>
- USEPA (1995b). *Process Design Manual: Land Application of Sewage Sludge and Domestic Sewage*. EPA/625/R-95-001. ORD, Washington, DC. <https://www.epa.gov/biosolids/process-design-manual-land-application-sewage-sludge-and-domestic-septage>
- USEPA (2003a). *Environmental Regulations and Technology Control of Pathogens and Vector Attraction in Sewage Sludge*. EPA/625/R-92/013. Revised July 2003. Office of Research and Development National Risk Management Research Laboratory Center for Environmental Research Information Cincinnati, OH 45268

- USEPA (2003b). *Technical Background Document for the Sewage Sludge Exposure and Hazard Screening Assessment* [Reports and Assessments]. USEPA. <https://www.epa.gov/biosolids/technical-background-document-sewage-sludge-exposure-and-hazard-screening-assessment>
- USEPA (2008). *Toxicological review of 2, 2', 4, 4'-tetrabromodiphenyl ether (BDE-47)*. EPA/635/R-07/005F, CAS No. 5436-43-1, USEPA, Washington, DC.
- USEPA (2009a). *Targeted National Sewage Sludge Survey Sampling and Analysis Technical Report*. <https://www.epa.gov/sites/production/files/2018-11/documents/tnsss-sampling-anaylsis-tech-report.pdf>
- USEPA (2009b). *Targeted National Sewage Sludge Survey Sampling and Analysis Technical Report Appendix B - Data Review and Preparation for Statistical Analysis*. https://www.epa.gov/sites/production/files/2015-04/documents/tnsss_stat_appendix_b_apr-20091.pdf
- USEPA (2016a). *Drinking Water Health Advisory for Perfluorooctane Sulfonate (PFOS)*. Report 822-R-16-004, USEPA, Washington, DC.
- USEPA (2016b). *Drinking Water Health Advisory for Perfluorooctanoic Acid (PFOA)*. EPA Document 822-R-16-005, USEPA, Washington, DC.
- USEPA (2018). *EPA Unable to Assess the Impact of Hundreds of Unregulated Pollutants in Land-Applied Biosolids on Human Health and the Environment* (No. 19-P-0002). https://www.epa.gov/sites/production/files/2018-11/documents/_epaog_20181115-19-p-0002.pdf
- USEPA (2020). *Regional Screening Level (RSL) Resident Soil Table (TR=1E-06, HQ=1) May 2020 (corrected)*. <https://semspub.epa.gov/work/HQ/200047.pdf>
- Vedagiri, U.K., Anderson, R.H., Loso, H.M., and Schwach, C.M. (2018). Ambient levels of PFOS and PFOA in multiple environmental media. *Remediation Journal*, 28(2), 9–51. doi: 10.1002/rem.21548
- Venkatesan, A.K., Pycke, B.F.G., and Halden, R.U. (2014). Detection and occurrence of N-Nitrosamines in archived biosolids from the Targeted National Sewage Sludge Survey of the U.S. Environmental Protection Agency. *Environmental Science & Technology*, 48(9), 5085–5092. doi: 10.1021/es5001352
- Verlicchi, P., and Zambello, E. (2015). Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil — A critical review. *Science of the Total Environment*, 538, 750–767. doi: 10.1016/j.scitotenv.2015.08.108
- Walters, E., McClellan, K., and Halden, R.U. 2010. Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms. *Water Research*, 44, 6011–6020. doi: 10.1016/j.watres.2010.07.051
- Wang, Q., Guo, M., and Yates, S.R. 2006. Degradation kinetics of manure-derived sulfadimethoxine in amended soil. *Journal of Agricultural & Food Chemistry*, 54, 157–163. doi: 10.1021/jf052216w.
- Wang, Z., Dewitt, J.C., Higgins, C.P., and Cousins, I.T. (2017). A never-ending story of per- and polyfluoroalkyl substances (PFASs)? *Environmental Science & Technology*. doi: 10.1021/acs.est.6b04806
- WEAO (2001). *Fate and Significance of Selected Contaminants in Sewage Biosolids Applied to Agricultural Land Through Literature Review and Consultation with Stakeholder Groups*. Water Environment Association of Canada, Mississauga, Ontario.
- WEAO (2010). *Assessing the Fate and Significance of Microconstituents and Pathogens in Sewage Biosolids*. Update of the 2001 WEAO Report on Fate and Significance. Water Environment Association of Canada, Mississauga, Ontario.
- White, P.J. and Brown, P.H. (2010). Plant nutrition for sustainable development and global health. *Annals of Botany*, 105, 1073–2010. doi: 10.1093/aob/mcq085

- Williams, D.L., Pepper, I.L., and Gerba, C.P. (2012). Survival of *Ascaris ova* in desert soils: A risk assessment. *Journal of Residuals Science & Technology*, 9, 151–157.
- Woudneh, M.B., Benskin, J.P., Wang, G., Grace, R., Hamilton, M.C., and Cosgrove, J.R. (2015). Quantitative determination of 13 organophosphorous flame retardants and plasticizers in a wastewater treatment system by high performance liquid chromatography tandem mass spectrometry. *Journal of Chromatography A*, 1400, 149–155. doi: 10.1016/j.chroma.2015.04.026
- Xu, Y., Yu, W., Ma, Q., Wang, J., Zhou, H., and Jiang, C. (2016). The combined effect of sulfadiazine and copper on soil microbial activity and community structure. *Ecotoxicology & Environmental Safety*, 134, 43–52. doi: 10.1016/j.ecoenv.2016.06.041
- Xue, J., Venkatesan, A.K., Wu, Q., Halden, R.U., and Kannan, K. (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. *Environmental Science & Technology*, 49(11), 6538–6544. doi: 10.1021/acs.est.5b01115
- Yadav, I.C., Devi, N.L., Li, J., and Zhang, G. (2017). Occurrence and source apportionment of halogenated flame retardants in the indoor air of Nepalese cities: Implication on human health. *Atmospheric Environment*, 161, 122–131. doi: 10.1016/j.atmosenv.2017.04.031
- Yang, C.-Y., Wu, S.C., Lee, C.-C., and Shih, Y. (2018). Translocation of polybrominated diphenyl ethers from field-contaminated soils to an edible plant. *Journal of Hazardous Materials*, 351, 215–223. doi: 10.1016/j.jhazmat.2018.02.037
- Yang, Y.-Y., Gray, J.L., Furlong, E.T., Davis, J.G., ReVello, R.C., and Borch, T. (2012). Steroid hormone runoff from agricultural test plots applied with municipal biosolids. *Environmental Science & Technology*, 46(5), 2746–2754. doi: 10.1021/es203896t
- Zaleski, K.J., Josephson, K.L., Gerba, C.P., and Pepper, I.L. 2005a. Potential regrowth and recolonization of *Salmonella* and indicators in biosolids and biosolids amended soil. *Applied Environmental Microbiology*, 71 3701–3708.
- Zaleski, K.J., Josephson, K.L., Gerba, C.P., and Pepper, I.L. 2005b. Survival, growth and regrowth of enteric indicator and pathogenic bacteria in biosolids, compost, soil, and land applied biosolids. *Journal of Residuals Science & Technology*, 2, 49–63.
- Zenker, A., Cicero, M.R., Prestinaci, F., Bottoni, P., and Carere, M. (2014). Bioaccumulation and biomagnification potential of pharmaceuticals with a focus to the aquatic environment. *Journal of Environmental Management*, 133, 378–387. doi: 10.1016/j.jenvman.2013.12.017
- Zerzghi, H., Gerba, C.P., Brooks, J.P., and Pepper, I.L. (2010). Long-term effects of land application of Class B biosolids on the soil microbial populations, pathogens and activity. *Journal of Environmental Quality*, 39, 402–408.
- Zhang, W., Li, J., Liu, K., and Lin, K. (2015). The behavior and toxicological effects of decabromodiphenyl ether (BDE209) in a soil–earthworm system. *Science of the Total Environment*, 537, 377–384. doi: 10.1016/j.scitotenv.2015.08.022
- Zhang, X., Lohmann, R., Dassuncao, C., Hu, X.C., Weber, A.K., Vecitis, C.D., and Sunderland, E.M. (2016). Source attribution of poly- and perfluoroalkyl substances (PFASs) in surface waters from Rhode Island and the New York Metropolitan Area. *Environmental Science & Technology Letters*. doi: 10.1021/acs.estlett.6b00255
- Zhao, L., Dong, Y.H., and Wang, H. 2010. Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. *Science of the Total Environment*, 408, 1069–1075. doi: 10.1016/j.scitotenv.2009.11.014

Appendix 1: Response to Antibiotic Issues

1) Impact of Land Application of Biosolids on Environmental Antibiotic Resistance

Soil microorganisms naturally produce and secrete antibiotic compounds, which can inhibit the growth of other microbes, including those that are the causative agents of infectious disease. However, the presence of antibiotics can result in antibiotic resistant bacteria (ARBs) with antibiotic resistant genes (ARGs). The conundrum then, is that the more an antibiotic is used to prevent infectious disease, the less effective it will become over time. Two perspectives on antibiotic resistance in soils are now well documented in the literature. The first is that antibiotic resistance is an ancient microbial attribute that has existed on earth for billions of years (Baltz, 2010). The second documented perspective is that ARBs and ARGs are found in all examined soils, even pristine soils (Cytryn, 2013). In fact, bacteria resistant to naturally produced antibiotics in soil results in an estimated $\approx 10^{16}$ ARB in an acre furrow slice of any given soil (Pepper et al., 2018). Based on the input of ARBs and ARGs from land application of biosolids and also on soil microbial principles, the potential impact of the biosolids on human health with respect to antibiotic resistance is negligible (Information Box 1).

2) Impact of Antibiotics Added to Soil via Land Application of Biosolids

Antibiotics consist of complex organic molecules that can be divided into several classes (Information Box 2). Antibiotics are used to treat infectious diseases in humans and they routinely end up in human sewage due to excretion of unmetabolized antibiotics (Cycoń et al., 2019). They are also prescribed to animals to treat infectious diseases and to enhance meat production in livestock, resulting in high concentrations in animal manures. In fact, biosolids typically contain lower concentrations of antibiotics than those found in animal manures (Information Box 2). This is significant since land application of animal manures is far more extensive than land application of biosolids. Overall, inputs of antibiotics from biosolids into soils are orders of magnitude less than inputs from manures. Antibiotic concentrations in soil typically range from ppb to ppm, with the highest concentrations usually found in areas where land application of manure has also occurred (DeVries & Zhang, 2016). Overall, antibiotics may affect the abundance of soil microorganisms (Xu et al., 2016), and overall microbial activity and diversity (Ma et al., 2016). However, since all soils have contained naturally produced antibiotics for millennia, such effects have likely been ongoing for 1000's or even millions of years.

3) Fate of Antibiotics in Soil

Antibiotics that enter soil or that are naturally produced in soil are subject to a number of abiotic, biotic or anthropogenic processes (Information Box 4). The overall persistence of antibiotics in soil is governed by the rate of transformation and is commonly estimated by the half-life ($T_{1/2}$) value, or the DT50 value (Information Box 5). Frequently, pesticides initially degrade faster at higher concentrations and subsequently slower at lower concentrations, such that $T_{1/2}$ values do not necessarily equate to DT50 values. This has resulted in the introduction of representative $T_{1/2}$ values, which reflect both the initial faster, and subsequent slower degradation rates.

Different classes of antibiotics have variable rates of degradation with half-lives ranging from <1 and >3000 days (Cycoń et al., 2019). Overall, there are many factors that influence the biotic and abiotic degradation of antibiotics (Cycoń et al., 2019). These include the enzymatic activity of the soil microorganisms; soil chemical and physical properties; abiotic factors including soil temperature and moisture; and the concentration of the antibiotic in the soil. Thus, half-life or DT50 values in a particular study only gives a general estimation of the persistence of the antibiotic. In particular, solubility and the sorption potential are critical parameters affecting the environmental fate of most antibiotics.

The more soluble the antibiotic, the greater the potential for transport via runoff or leaching. However, as solubility increases, bioavailability increases and with it the potential for rapid degradation. In contrast, less soluble antibiotics sorb to soil colloids and organic matter and are less mobile. Sorbed antibiotics are more resistant to degradation and thus more persistent, but these are also unavailable for plant uptake. Antibiotics with K_{oc} values > 4000 L/kg sorb strongly, are non-mobile and have low degradation rates (very persistent) (Crane et al., 2010). DT50 values for such antibiotics are >60 days. In contrast antibiotics with K_{oc} values < 15 L/kg sorb less, are highly mobile and degrade rapidly with DT50 values <5 days. It is also important to note that sorbed antibiotics often cannot be detected and may lose their antibacterial activity (Kümmerer, 2009).

K_{oc} values for representative antibiotics in different classes are shown in Information Box 3, while available $T_{1/2}$ or DT50 values of selected antibiotics are shown in Information Box 6. Many of these selected antibiotics are those identified in the EPA OIG November 2018 Report. Several of the antibiotics listed as detected in biosolids by the OIG report have very high K_{oc} values in excess of 4000 L/kg, indicating that they would sorb strongly to colloids and resist degradation. Examples include: Ciprofloxacin; Enrofloxacin; Azithromycin; Tylosin; Oxytetracycline; and Tetracycline. Notably, other antibiotics with very short half-lives such as Amoxicillin have not been detected in biosolids. Based on this it would appear that the combination of sorption and degradation prevent a permanent build-up of antibiotics in soil despite daily natural production by soil microbes and intermittent introduction of antibiotics via biosolids.

**Information Box 1. Soil Factors Influencing the Residual Impact of Land Application
on Antibiotic Resistance**

- Minimal ARBs added relative to what is already in soil (Pepper et al., 2018)
- Number of enteric pathogens (e.g. *E.coli*) introduced via effluent and biosolids is less than pathogens indigenous to soil (e.g. *Bacillus anthracis* or *Clostridium perfringens*) (Pepper et al., 2018)
- Enteric pathogens and ARBs introduced into soil normally die-off quickly (Zaleski et al., 2005b)
- When *E.coli* adapts to a soil environment, pathogenicity is lost (Ishii et al., 2006)
- Horizontal gene transfer in soil is limited due to spatial separation of cells (Nielson et al., 1994)

**Information Box 2. Maximum Reported Concentrations of Selected Antibiotics
Detected in Manure, Sewage Sludge, and Biosolids**

Class	Antibiotic	Concentration	References
MANURE, µg/kg			
Fluoroquinolones	Ciprofloxacin	45,000	Zhao et al., 2010
	Enrofloxacin	1,420	
	Fleroxacin	99,000	
	Norfloxacin	225,000	
Macrolides	Tylosin	7,000-8,100	Dolliver et al., 2008; Berendsen et al., 2015
Sulfonamides	Sulfadiazine	91,000	Martinez-Carballo et al., 2007
	Sulfadimidine	20,000	
Tetracyclines	Chlortetracycline	764,000	Massé et al., 2014 Chen et al., 2012 Pan et al., 2011
	Oxytetracycline	354,000	
	Tetracycline	98,000	
SEWAGE SLUDGE, µg/kg dw			
Diaminopyrimidines	Trimethoprim	133	Göbel et al., 2005
Fluoroquinolones	Ciprofloxacin	426	Lillenberg et al., 2010; Li et al., 2013
Macrolides	Azithromycin	1.3-158	Göbel et al., 2005; Li et al., 2013
Sulfonamides	Sulfadimethoxine	0-20	Lillenberg et al., 2010; Li et al., 2013
Tetracyclines	--	8,326	Cheng et al., 2014
BIOSOLIDS, µg/kg dw			
Lincosamides	Lincomycin	2.6	Ding et al., 2011
Macrolides	Azithromycin	14	Jones-Lepp and Stevens, 2007 Kinney et al., 2006
	Erythromycin	41	
Sulfonamides		650	US EPA, 2009
Tetracyclines	Oxytetracycline	743.6	US EPA, 2009; Ding et al., 2011

Adapted from Cycoń et al. (2019).

Information Box 3. K_{oc} values of Selected Antibiotics

Class	Antibiotic	K _{oc} (L/kg)
Aminoglycosides	Streptomycin	580-11,000
Diaminopyrimidines	Trimethoprim	4,600
Fluoroquinolones	Ciprofloxacin	1,127-61,000
	Enrofloxacin	39-768,740
	Norfloxacin	310
	Ofloxacin	44,140
Lonophores	Lasalocid	2.9-4.2
	Monensin	2.1-3.8
B-Lactams	Amoxicillin	865.5
	Cefuroxime	12.4-15.5
	Penicillin G	2.68
Lincosamides	Clindamycin	70
	Lincomycin	59
Macrolides	Azithromycin	59,900
	Clarithromycin	150
	Erythromycin	10
	Tylosin	110-95,532
Sulfonamides	Sulfahloropyridazine	41-170
	Sulfadiazine	37-125
	Sulfadimethoxine	89-323
	Sulfadoxine	1.8-31.3
	Sulfamethoxazole	1.2-94.9
	Sulfamethazine	60-208
	Sulfamonomethoxine	60-200
	Sulfapyridine	80-308
Tetracyclines	Chlortetracycline	794
	Oxtetracycline	2,872-93,317
	Tetracycline	400-93,320

Adapted from Cycoń et al. (2019).

Information Box 4. Evaluating the Persistence of Antibiotics in Soil via $T_{1/2}$ and DT50

<u>Parameter</u>	<u>Definition</u>
$T_{1/2}$	Time required to reduce the concentration of the compound by 50% from any concentration point in time.
DT50	Time required for the concentration of the compound to decline to half of the initial value.

Information Box 5. Degradation Rates of Specific Antibiotics

Antibiotic	$T_{1/2}$ DT50 (Days) or Specific Comments	References
Amoxicillin	$T_{1/2}$ = 0.5	Braschi et al., 2013
Clarithromycin - No history of CLA application - With history of CLA application	$T_{1/2}$ = 365 $T_{1/2}$ = 9.5	Topp et al., 2016
Chlortetracycline	DT50 = 25-30	Li et al., 2010a
Doxycycline	$T_{1/2}$ = 550	Walters et al., 2010
Erythromycin	$T_{1/2}$ = 20	Schlüsener et al., 2006
Sulfadimethoxine	DT50 = <10	Wang et al., 2006
Sulfadiazine	DT50 = 5-15	Hammesfahr et al., 2008
Sulfamethoxazole	$T_{1/2}$ = 10-15	Lin and Gan, 2011
Sulfamethazine	DT50 = <5	Topp et al., 2013
Tetracycline	$T_{1/2}$ = 578	Walters et al., 2010
Trimethoprim	DT50 = 5	Liu et al., 2009
Vancomycin	DT50 = 16	Cycoń et al., 2018

Adapted from Cycoń et al. (2019).

Appendix 2: Response to Pathogen Issue

1) Incidence of Pathogens in Biosolids

The OIG Report lists 352 constituents of concern that have been identified in biosolids. The sources of these constituents are: i) The 2015 Biennial Biosolids Review; and ii) The 1989 and 2001 National Sewage Sludge Surveys. Of the 352 constituents, 21 are listed as pathogens and are shown in Table 1. Of these enteric entities, 9 are viruses, 8 are bacteria, 2 are phage and one is listed as “aerobic endospores.” The term “aerobic endospore” is generic in nature and does not necessarily indicate a human pathogenicity. Likewise, the 2 phages listed as pathogens actually only infect bacteria and not humans. In contrast, all viruses listed are capable of infecting humans. Of the bacteria, all are enteric organisms, but *Clostridium perfringens* and *Listeria* are also commonly found in soils.

By definition, Class A biosolids contain no detectable microbial pathogens, including viruses, bacteria and helminths. In contrast, Class B biosolids significantly reduce pathogens but can still contain a variety of enteric pathogens. However, concentrations of enteric pathogens in Class B biosolids are typically low. For example, a national study of biosolids reported *E.coli*, *Salmonella* and enteric virus concentrations to be less than 1 per gram of biosolids (Pepper et al., 2010). Additionally, data collected both prior to, and after the issuance of the federal regulations, USEPA Part 503 Rule in 1993, indicate lower pathogen concentrations following the implementation of the rule. Thus, the Part 503 Rule has been effective in reducing potential public exposure to pathogens (Pepper et al., 2010; USEPA, 1993).

2) Fate and Transport of Pathogens in Land Applied Biosolids

There are multiple potential fates of pathogens within land-applied biosolids including: regrowth, or die-off and inactivation within soil; transport through soil and vadose zone into groundwater; aerosolization and transport off-site. Many studies have evaluated regrowth of bacterial pathogens such as *Salmonella* in both soil and/or biosolids. Regrowth in soil does not occur due to biotic and abiotic stress factors. However, regrowth within biosolids can occur if biosolids are stored prior to land application but only under saturated conditions (Zaleski et al., 2005a, 2005b). Regrowth of human pathogenic viruses cannot occur since there are no human hosts in soil. Survival studies of pathogenic microorganisms and fecal indicators introduced into soil have been conducted in the US for over half a century. Bacterial pathogens such as fecal indicators and *Salmonella* within biosolids normally survive only 2 to 3 weeks in soil. Once the biosolids are land applied, die-off occurs due to competition with billions of indigenous soil microbes and abiotic stress (Pepper et al., 1993; Zaleski, 2005b). Viruses in land-applied biosolids typically survive 2-3 months (Straub et al., 1995).

Because Class B biosolids are known to contain microbial pathogens, the Part 503 Rule provides site restrictions for land application of biosolids. The idea behind the restrictions is to allow sufficient time for pathogen die-off and inactivation within the soil, prior to any potential human exposure. The duration of each site restriction depends on the land use management and the potential for public exposure. These vary from a restriction of 30 days following land application to sites with a low potential for public exposure, to 14-20 months for land application sites intended for food crop production (USEPA, 1993). Indirect routes of human exposure to biosolid-associated pathogens include groundwater contamination and bioaerosol transport to off-site communities. The potential for transport of viruses through soil and the vadose zone into groundwater has been a concern. However, it is important to note that the presence of biosolids itself impacts the extent of transport. Specifically, viruses within a soil-biosolid matrix become associated and attached to the solid phase, which severely inhibits movement of virus out of the soil-biosolid matrix (Chetochine et al., 2006). Transport of pathogens via bioaerosols has also been evaluated in several studies (Brooks et al., 2005a, 2000b;

Tanner et al., 2005). The open-air environment for microbes is hostile and results in rapid die-off of bacteria and inactivation of viruses. Viruses are capable of surviving in aerosols longer than bacteria, but even for viruses, the risk of infection for off-site communities is exceedingly low at $\approx 1:10,000,000$.

3) Historical Allegations of Microbial Hazards Associated with Land Application of Biosolids

Most residential complaints about land application have been fueled by the odor of biosolids. Historically odors have been associated with disease, which has resulted in multiple allegations of microbial hazards associated with the land application of biosolids. Many of these allegations were initiated at the turn of the century, but isolated cases have continued to this day. As each allegation arose, it initiated intense public and scientific interest. These allegations are presented in Table 2, along with the findings of research conducted on each issue, and the resultant peer reviewed publications from each study. In all cases, the allegations proved to be false, when evaluated in a scientific manner. However, it is important to recognize that a large body of knowledge on the various issues was collected, including data on new and emerging biological entities of concern such as the SARS and Ebola viruses and infectious prions. Finally note that there is no documented peer-reviewed evidence of adverse public health effects resulting from microbial hazards associated with land application of biosolids. This is truly remarkable since there have been hundreds of thousands of land applications conducted in the US, on agricultural land over the past half century.

Table 1. Pathogens referenced in the OIG report*.

Pathogens (21)	
ARBs and ARGs	Enterovirus
Coronavirus	<i>E.coli</i>
Cosavirus	<i>Giardia</i>
Klassevirus	HAV human adenovirus
Norovirus	Human polyomaviruses
Adenovirus	<i>Listeria monocytogenes</i>
Clostridia	Murine norovirus
<i>Clostridium perfringens</i>	<i>Salmonella senftenberg</i>
<i>Enterococci</i>	

*Bacteroides fragilisphage, somatic coliphage, and aerobic endospores also mentioned; but these are not human pathogens.

Table 2. Land Application Microbial Allegations and Science Based Study Findings.

Year	Allegation	Study	Findings
2003	<i>Staphylococcus aureus</i> is found in land-applied biosolids and results in infections in members of the community.	Evidence for the absence of <i>Staphylococcus aureus</i> in land applied biosolids. <i>Environ. Sci. Technol.</i> 37:4027-4030.	<i>Staphylococcus</i> found in raw sewage, but does not survive wastewater treatment.
2004	Aerosolized pathogens from land applied biosolids results in community infections in neighboring residential areas.	Bioaerosol emission rate and plume characteristics during land application of liquid Class B biosolids. <i>J. Appl. Microbiol.</i> 99:310-322. <i>J. Appl. Microbiol.</i> 98:397-405. <i>Environ. Sci. & Technol.</i> 39:1584-1590.	Community risk of infection insignificant, occupational risk low but greater than community risk.
2005	Regrowth of <i>Salmonella</i> occurs following land application of biosolids.	Potential regrowth and recolonization of <i>Salmonellae</i> and indicators in biosolids and biosolid-amended soil. <i>Environ. Microbiol.</i> 71:3701-3708, <i>J. Residuals Sci. & Technol.</i> 4:83-88.	Regrowth of <i>Salmonella</i> only in Class A biosolids during storage if they become saturated with water, and not after land application.
2006	Land application of biosolids results in aerosolized endotoxin with adverse health effects on neighboring residents.	The measurement of aerosolized endotoxin from land application of Class B biosolids in Southeast Arizona. Brooks et al., 2006. <i>Can. J. Microbiol.</i> 52:150-156.	Most aerosolized endotoxin is of soilborne origin.

2006	Land application of biosolids results in transport of viruses through soil and the vadose zone and subsequent contamination of groundwater.	Leaching of phage from Class B biosolids and potential transport through soil. <i>Appl. Environ. Microbiol.</i> 72:665-671.	Viruses are firmly bound and embedded in biosolids and are less available for transport.
2008	Long-term land application is hazardous to soil.	Sustainability of land application of Class B biosolids. <i>J. Environ. Qual.</i> 37(5), Supplement S, S58-S67.	Land application of Class B biosolids is sustainable over two decades.
2009	The SARS virus (severe acute respiratory syndrome) following land application of biosolids poses a health threat to communities.	Survival of corona viruses in water and wastewater. <i>Food Environ. Virology</i> , 1(1):10-14.	The SARS viruses die off rapidly in wastewater and are less hardy than other human pathogenic viruses.
2010	Federal Regulations (USEPA Part 503 Rule, 1993) do not protect communities from pathogens in biosolids.	Pathogens and indicators in United States Class B biosolid: national and historic distributions. <i>J. Environ. Qual.</i> 39:2185-2190.	Bacterial and viral human pathogen levels in biosolids have decreased since federal regulations were introduced.
2010	Pathogens resulting from long-term land applications increase over time and are a health risk to communities.	Long-term effects of land application of Class B biosolids on the soil microbial populations, pathogens, and activity. <i>J. Environ. Qual.</i> 39:402-408.	Twenty years of continuous land application of Class B biosolids showed no increases in pathogen incidence and no long-term adverse effects of land application.
2012	Land application of animal manures in a healthy organic process compared to land application of biosolids, which is dangerous to human health.	Land application of manure and Class B biosolids: an occupational and public quantitative microbial risk assessment. <i>J. Environ. Qual.</i> 41:2009-2023.	Risk of bacterial infection from animal manures greater than the risk from biosolids.
2012	Helminths survive in soil for many years.	Survival of <i>Ascaris</i> in desert soils: A risk assessment. <i>J. Residuals Sci. & Technol.</i> , 9:151-157.	Helminths were inactivated in soil within 6 months.
2013	Prions in land-applied biosolids pose a health threat to animals and humans.	Survival of infectious prions during anaerobic digestion of municipal sewage sludge and lime stabilization of Class B biosolids. <i>J. Residuals Sci. & Technol.</i> 10:69-85.	Prions inactivated during wastewater treatment and land application of biosolids is not a viable route of exposure to prions.

2017	Land application of biosolids results in aerosolized endotoxin with adverse health effects on neighboring residents	Herrmann, R.F., Grosser, R.J., Farrar, D. et al. Field studies measuring the aerosolization of endotoxin during the land application of Class B biosolids. <i>Aerobiologia</i> , 33:417–434. doi: 10.1007/s10453-017-9480-8	The mean levels of TWA endotoxin detected in this study were much lower than these levels (<43 EU/m ³), and it is predicted that exposure to nearby residents is miniscule. Onsite workers could reach a threshold level of endotoxin exposure over a work shift and would be at greatest risk.
2018	Environmental antibiotic resistance enhanced by sewage and land application of biosolids.	Antibiotic resistant bacteria in municipal wastes: is there reason for concern? <i>Environ. Sci. Technol.</i> 52:3949-3959.	Either no increase in antibiotic resistant bacteria in soil after land application, or only a temporary increase for 2-3 months before decreasing to pre-land application levels.
2018	Emerging human pathogenic viruses including Ebola survive wastewater treatment and end up in biosolids.	Comparative survival of viruses during thermophilic and mesophilic anaerobic digestion. <i>Sci. Tot. Environ.</i> 615:15-19.	Viruses with a lipid-based envelope including Ebola are inactivated during aerobic digestion.