Risks Associated with Application of Municipal Biosolids to Agricultural Lands in a Canadian Context

Literature Review

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Prepared by:
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Canadian Municipal Water Consortium

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P.I.:

Dr. Lynda H. McCarthy, Ryerson University

Co-P.I.:

Dr. Jorge E. Loyo-Rosales, Ryerson University

Contributors:

Melanie Raby, Ryerson University

Michael Payne, Black Lake Environmental

Dr. Ann Huber, Soil Resources Group

John Lavery, Sylvis Environmental

Kasia Caputa, Sylvis Environmental

Laura Taylor, Ryerson University

Alicia Falls, Ryerson University

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ABBREVIATIONS AND ACRONYMS

ACM – Acetaminophen (a.k.a. paracetamol)
AD – Anaerobic digestion (sludge)
AhR – Aryl hydrocarbon receptor
AHTN – Tonalide
AMF – Arbuscular mycorrhizal fungi community
AOX – Adsorbable organohalogens
AP – Alkylphenol
APE – Alkylphenol ethoxylate
ARB – Antibiotic resistant bacteria
ARG – Antibiotic resistant genetic material
ARISA – Automated ribosomal intergenic spacer analysis
ATN – Atenolol
AZM – Azithromycin
BFR – Brominated flame retardant
BHT – Butylhydroxytoluene
BNQ – Bureau de Normalisation du Québec
BPA – Bisphenol A
BTBPE – 1,2-bis(2,4,6-tribromophenoxy)ethane
CBZ – Carbamazepine
CCME – Canadian Council of Ministers of the Environment
CFIA – Canadian Food Inspection Agency
CFU – Colony forming unit
CIM – Cimetidine
CIP – Ciprofloxacin
CLM – Clarithromycin
CMP – Chemicals Management Plan (Environment Canada)
CMWC – Canadian Municipal Water Consortium
CTP – Citalopram
CTZ – Clotrimazole
CP – Chlorinated paraffin (a.k.a. polychlorinated alkane, PCA)
CWN – Canadian Water Network
DBDPE – Decabromodiphenyl ethane
DCF – Diclofenac
DDE – Dichlorodiphenyldichloroethylene
DDT – Dichlorodiphenyltrichloroethane
DEET – N,N-diethyl-3-methylbenzamide
DEHP – Di(2-ethylhexyl) phthalate
DES – Diethylstilbestrol
DTC – Doxycycline
dw – Dry weight

DZP – Diazepam
E1 – Estrone
E2 – 17β-estradiol (also E2b)
E2a – 17α-estradiol
E2b – 17β-estradiol (also E2)
E3 – Estriol
EDC – Endocrine disrupting compound / Endocrine disruptor
EE2 – 17α-ethinyl estradiol
EL-FAME – Ester-linked fatty acid methyl ester
ER-CALUX – Estrogen-receptor-mediated chemically-activated luciferase-gene-expression bioassay
ERY – Erythromycin
ESOC – Emerging substance of concern
4-ETC – 4-Epitetracycline
EU – European Union
FISH – Fluorescent in situ hybridization
FLX – Fluoxetine
FRS – Furosemide
GFB – Gemfibrozil
GU – Genomic units (qPCR)
H2RA – H2 receptor agonists
HBCD – Hexabromocyclododecane
HEI – Highly exposed individual
HHCB – Galaxolide
IBP – Ibuprofen
KFN – Ketoprofen
LAS – Linear alkylbenzene sulfphonate
LCIA – Life cycle impact assessment
MAD – Mesophilic anaerobic digestion
MAH – Monocyclic aromatic hydrocarbon
MCZ – Miconazole
MeEE2 – Mestranol
MeTCS – Methyl-triclosan
MOX – Moxifloxacin
MPN – Most probable number
MTB-2 – Methylbenzothiazole
MTP – Metoprolol
NPB – National Biosolids Partnership
NOEC – No-observed-effect concentration
NOEL – No-observed-effect level
NOR – Norfloxacin
NP – Nonylphenol
NP1EO – Nonylphenol monoethoxylate

NP2EO – Nonylphenol diethoxylate
NPE – Nonylphenol ethoxylate
NPX – Naproxen
NRC – United States National Research Council
NSAID – Non-steroidal anti-inflammatory drugs
OC – Organochlorine
OFX – Ofloxacin
OP – 4-tert-Octylphenol
OPE – Octylphenol ethoxylate
OT – Organotin
OTNE – [1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethylnaphthalen-2yl]ethan-1-one
PAE – Phthalate acid ester
PAH – Polycyclic aromatic hydrocarbon
PBDE – Polybrominated diphenyl ether
PBEB – Pentabromoethyl benzene
PCA – Polychlorinated alkane (a.k.a. chlorinated paraffin, CP)
PCB – Polychlorinated biphenyl
PCDD/Fs – Dioxins and furans
PCN – Polychlorinated naphthalene
PCPs – Personal care products
PCR – Polymerase chain reaction
PDMS – Polydimethylsiloxane
PEC – Predicted environmental concentration
PFCs – Perfluorochemicals / Perfluorinated compounds
PFOA – Perfluorooctanoic acid
PFOS – Perfluorooctanesulfonic acid
PFRP – Process to further reduce pathogens
PFU – Plaque forming unit
PiE – Pharmaceuticals in the environment
PLFA – Phospholipid fatty acid
PNEC – Predicted no effect concentration
POP – Persistent organic pollutant
PPCPs – Pharmaceuticals and personal care products
PRP – Propranolol
PSRP – Process to significantly reduce pathogens
PXR – Pregnane X receptor
QAC – Quaternary ammonium compound
QMRA – Quantitative Microbial Risk Assessment
REACH – European regulation for the Registration, Evaluation, Authorisation and Restriction of Chemicals
RQ – Risk quotient
SER – Sertraline

SMZ – Sulfamethoxazole
SPY – Sulfapyridine
SRT – Sludge retention time
SUL – Sulphanilamide
TBBPA – Tetrabromobisphenol A
TC – Tetracycline
TCC – Triclocarban
TCEP – Tris(2-chloroethyl) phosphate
TCPP – Tris(2-chloro-1-methylethyl) phosphate
TCS – Triclosan
TiBP – Triisobutyl phosphate
TnBP – Tributyl phosphate
TNSSS – USEPA's Targeted National Sewage Sludge Survey
TOrCs – Trace organic compounds
TPP – Triphenyl phosphate
TSCA – United States Toxic Substances Control Act
UK – United Kingdom
US – United States of America
USEPA – United States Environmental Protection Agency
USGS – United States Geological Survey
USNAS – United States National Academy of Sciences
VEN – Venlafaxine
VOC – Volatile organic compound
WEAO – Water Environment Association of Ontario
WEF – Water Environment Federation
WERF – Water Environment Research Foundation
WWTP – Wastewater treatment plant
YES – Yeast estrogen screen bioassay

EXECUTIVE SUMMARY

The increasing number of wastewater treatment facilities in Canada and around the world has led to a concomitant growth in the amount of municipal sewage sludge and biosolids produced. These by-products can be landfilled or incinerated, but they can also be used for beneficial purposes. The main beneficial use is land application to soils, which allows the reuse of the biosolids main components, namely organic matter and nutrients, with agricultural or land reclamation purposes.

However, land application of biosolids has to be conducted appropriately in order to avoid public and environmental health risks. This is done by regulating application practices to avoid excess nutrient loading, and by consistently assessing biosolids quality to prevent public and environmental health risks from pathogens and toxic substances. Since the 1970s, regulations have been in place to limit pathogen and heavy metal content in biosolids intended for land application. However, advances in analytical chemistry in the last couple of decades have allowed the detection of a large number of substances in biosolids that may be present in relatively small amounts, but whose potential impact to public and environmental health is not well understood. These substances are known, amongst other terms, as emerging substances of concern (ESOCs).

Although pathogens as a group have been studied and addressed in wastewater treatment and biosolids land application for much longer than ESOCs, new pathogens continue to emerge, in part due to the development of new detection methods (e.g., PCR) that allow the detection of difficult-to-culture pathogens and a better identification of the pathogenic agents during disease outbreaks. But also due to changes in water treatment technologies, and the introduction of new pathogenic organisms, either from other geographical locations or through evolution of the microorganisms themselves (Nwachcuku and Gerba, 2004).

This report summarizes current knowledge on the occurrence, fate, and potential risks of ESOCs and pathogens present in biosolids after application to agricultural land, especially in conditions relevant to Canada.

Risk assessment of ESOCs and pathogens after biosolids land application

Risk assessment methodologies have been used to evaluate ESOCs and pathogens in the biosolids land application context. In the case of ESOCs, the few existing risk assessments suggest that their presence poses a low risk to human and environmental health. In the case of pathogens, for which more assessments are available, risk has been evaluated from low or practically inexistent to the general population, to high in worst-case conditions, which are generally related to occupational contexts, such as biosolids applicators with no personal protective equipment and high pathogen density in the biosolids.

The main reason behind the limited number of risk assessments is lack of data. For ESOCs, toxicity and ecotoxicity data are generally not available, and there is also a limited amount of information on their fate in the distinct environmental conditions specific to biosolids-amended soils. For pathogens, especially viruses and protozoa, more information is needed on their fate and behaviour in biosolids, inactivation during sludge treatment and in the environment, and dose-response relationships. Additionally, there is a need to incorporate the use of alternative indicator organisms that better represent the resistance to inactivation of some of

the more recalcitrant emerging pathogens. For both ESOCs and pathogens, there is a need for standardized analytical methods that allow their reliable detection and quantification (and viability in the case of pathogens) in biosolids and soils, which are difficult matrices to analyze.

Effects of sludge treatment on ESOCs and pathogens

Research on the effects of sludge treatment has concentrated on a few ESOCs (e.g., NPEs) and pathogens (fecal indicators and *Salmonella*), and one type of treatment (anaerobic digestion). In general, sludge treatment results in a net reduction of ESOCs mass and effects (i.e., estrogenicity), and in the number of pathogenic organisms. However, the magnitude of the reduction depends on the specific compounds or organisms, and the type and conditions of treatment, with some ESOCs (e.g., CBZ) and pathogens (e.g., enteroviruses) being recalcitrant to some or most treatments.

Fate of ESOCs and pathogens after biosolids land application

The fate of both biosolids-related ESOCs and pathogens after land application is a complex, site-specific phenomenon driven by the combination of a large number of factors, from the properties of the ESOCs or pathogens and the soils, to environmental variables such as temperature and soil moisture content, and the biosolids application methods. Therefore, the ultimate fate of different ESOCs and pathogens is very variable, with some (e.g., TCS, *Cryptosporidium*) persisting in the soil for long periods of time, while others are very mobile (e.g., iopromide). However, studies generally conclude that most of the compounds typically found in biosolids do not reach the groundwater when applied to soils, and that their concentrations in tile drainage and surface runoff tend to be much lower than typical concentrations found in WWTP effluents.

ESOCs uptake by plants and bioaccumulation by earthworms have been clearly demonstrated, although it might have been overestimated by the use of proof-of-concept methodologies that do not reflect relevant environmental and agricultural conditions. Additionally, although accumulation of ESOCs in soil, crops, or soil organisms might not be desirable, especially in the case of chemicals used unnecessarily in high amounts (e.g., TCS), their sole presence does not constitute proof of negative impact.

Although the presence of antibiotic resistant bacteria (ARB) in sludge and biosolids is well documented, incidence tends to be lower than in other compartments. The risk of antibiotic-resistance gene transfer in soil is considered to be low, and land-applied biosolids are not expected to affect the incidence of antibiotic resistance in pathogens.

Impact studies and biological endpoint testing in biosolids-amended soils

Impact studies in plants generally show that amending the soil with biosolids at appropriate rates has a positive impact. Studies on invertebrates (earthworms, springtails, and nematodes) show more mixed results, with negative impact being associated (in the cases it was determined) to the presence of high heavy metal concentrations, ammonia, pH or salinity levels. Invertebrate studies also conclude that springtails are a more sensitive indicator of toxicity than earthworms, and reproduction a more sensitive endpoint than lethality. Impact studies on microbial communities generally find that application of biosolids at relevant agronomic rates results in increases to microbial biomass, respiration rate, and enzymatic

activity, unless high concentrations of heavy metals are present, but the ecological impact of these findings has not been elucidated.

Use of biomarkers and omics

Biomarkers and omics have only started to be used in the soil ecotoxicology context. Because of their capacity to generate large amounts of genetic, metabolic, and protein expression data, omics has the potential to contribute in the study of the toxicity effects resulting from simultaneous exposure to multiple chemicals, the development of antibiotic resistance, and in ecosystem health assessment, but the use of these technologies in the field is still in its infancy. The use of biomarkers, such as estrogenicity, can provide valuable information on the possible effects of biosolids to biota without the need for exhaustive chemical analysis. However, research is still necessary to relate the responses of such biomarker tests to actual impact to individuals and populations, especially in the biosolids land application context.

Public perception

In contrast to wastewater treatment, public perception of biosolids tends to be negative for several reasons, including lack of awareness, the presence of pathogens and industrial sewage, odour emissions during land application, and reports of health effects associated with biosolids land application. This negative perception has already influenced public policy in some jurisdictions. Insufficient information and lack of community involvement in the decision-making progress for the design of biosolids management programs have also been cited as causes for public distrust. Biosolids managers have recognized these issues and they have adopted risk communication processes to develop public participation and trust in current approaches for the design of biosolids management programs. Additionally, WERF and USEPA commissioned the development and field-testing of a response protocol for the investigation of health symptoms attributed to biosolids land application.

Risk assessment and sustainability in the Canadian context

A ‘typical’ Canadian land application scenario was used to exemplify the quantitation of environmental risk from selected ESOCs using risk quotients. However, due to the lack of terrestrial toxicity data, these values are only relevant for preliminary, comparative purposes, and have little value as indicators of actual environmental risks. The elements needed to develop complete environmental risk assessments for ESOCs are discussed.

Additionally, the overall conclusion of this review is that it is necessary to develop a different strategy to address the ultimate goal of assessing the risk of the application of biosolids to agricultural land in Canada, which is to determine whether ESOCs as a group affect human and/or environmental health. The design of such Canadian risk evaluation strategy for ESOCs in biosolids requires the participation of representatives from the different stakeholder groups, and it should be part of the national consultation constituting Part B of this project.

Although open for debate by the stakeholders, the strategy should be centered on the evaluation of ecosystem health, such as Burton *et al.* (2012) proposed, and the following elements should be incorporated:

- Definition of the environmental protection goals of the strategy.

- Establishment of a prioritization process for ESOCs.
- Development of an endpoint-monitoring plan.
- Identify and address research gaps.

Ideally, the strategy to evaluate the risks, and especially the strategy for risk management, should take into account ESOCs in contexts other than biosolids land application in order to provide a more holistic perspective that helps to optimize resources; e.g., if a chemical is banned or its use restricted, its concentrations in biosolids will eventually decrease and investment in additional treatment to eliminate such chemical might be unnecessary. The creation of a centralized data 'clearinghouse' for ESOCs-related issues, as suggested by Kleywegt *et al.* (2007), could contribute to improve the exchange of information.

From a public health perspective, conducting scientifically based inquiries following reports of adverse health effects, especially by residents neighbouring biosolids-amended fields, would be a significant contribution to elucidate the possible links of these health effects to biosolids exposure.

Finally, the strategy should also consider that the sustainability of biosolids land application involves factors other than the potential environmental and public health risks. Examples of these factors include energy demand, the contribution to global warming (in the form of green house gas emissions), and ozone depletion potential. Evaluation of all sustainability factors is especially important when comparing land application to other beneficial uses for biosolids, such as gas co-generation and recovery from anaerobic digestion, and power generation from incineration in so-called waste-to-energy (WtE) schemes.

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1. INTRODUCTION

Currently, land application is one of the most common end destinations for biosolids, and the only one that allows for the beneficial reuse of its nutrients. If produced according to quality standards to limit the presence of toxic substances and pathogens, biosolids are a valuable resource for agricultural and land reclamation applications, rather than just an unavoidable by-product of wastewater treatment.

Starting in the 1970s, regulations were introduced to restrict the disposal of heavy metals into sewers, thus decreasing their concentrations in biosolids, and treatment standards were set that limited their pathogen load. In addition, agricultural management practices were developed for biosolids and other fertilizers to prevent runoff of excess nutrients into surface water bodies and groundwater and the accumulation of heavy metals in the soil. Although current regulations are generally considered to be protective of human and environmental health, the detection of an increasing number of chemicals in biosolids, labelled generically 'emerging substances of concern' (ESOCs), and the emergence of new pathogenic organisms have prompted interest in potential risks to humans and biota after the land application of these biosolids.

The main objective of this report is to review and summarize current understanding of the risks posed by ESOCs and emerging pathogens to human and environmental health, especially in the Canadian context. The report will serve as the basis for a national consultation to be conducted by the Canadian Water Network's Canadian Municipal Water Consortium on the potential risks associated with the application of municipal biosolids on agricultural land.

Biosolids, ESOCs and pathogens are defined in Chapter 2, along with a brief overview of biosolids production, disposal and reuse. Chapters 3 and 4 recount the development of current regulations addressing toxic chemicals and pathogens in biosolids, and the conclusions of recent studies assessing the risk of ESOCs and pathogens in the biosolids land application context. The ESOCs and pathogens considered for this review and the reasons why they were selected are presented in Chapter 5—further details on each compound and pathogen are presented as annexes. The effects of municipal sludge treatment on ESOCs and pathogens are discussed in Chapter 6, and their fate in soils after biosolids land application is reviewed in Chapter 7. Additionally, ESOCs uptake by plants and accumulation by earthworms, as well as the development of microbial antibiotic resistance in biosolids-amended soils, are also described in this chapter.

Chapter 8 is a review of studies evaluating impact to terrestrial plants, invertebrates, and microbial communities after biosolids application to the soil. This chapter also includes an overview of the use of biomarkers and 'omics' in the context of biosolids land application. Public perception of the use of biosolids is discussed in Chapter 9, and in Chapter 10, risk quotients are used to exemplify the quantitation of environmental risk in a 'typical' Canadian land application scenario, followed by a discussion on the needs to develop a formal environmental risk assessment. The chapter concludes with recommendations to address the issue of the risk evaluation of biosolids land application in the Canadian context during the national consultation constituting Part B of this project, including the design of a new strategy to evaluate if ESOCs are having an effect on human or environmental health.

2. BACKGROUND

2.1. Biosolids

2.1.1. Definition

The term biosolids was originally proposed by the Water Environment Federation (WEF) in the early 1990s to name treated municipal sewage sludge intended for use as fertilizer and/or soil amendment, and to differentiate the treated product from the non-treated (raw) sludge (NEBRA, 2008). The term is also used in the present work with the same meaning, unless noted otherwise.

The popularity of the term is evidenced by its extensive adoption throughout the wastewater-related industry, by government agencies, engineers and scientists. For example, the USEPA (2013a) defines biosolids as "the nutrient-rich organic materials resulting from the treatment of sewage sludge … When treated and processed, sewage sludge becomes biosolids which can be safely recycled and applied as fertilizer to sustainably improve and maintain productive soils and stimulate plant growth."

In Canada, where definitions vary by province, the Canadian Council of Ministers of the Environment (CCME, 2012a) defined municipal biosolids as "organic-based products which may be solid, semi-solid or liquid and which are produced from the treatment of municipal sludge which has been treated to meet jurisdictional standards, requirements or guidelines including the reduction of pathogens and vector attraction".

2.1.2. Production, disposal and beneficial reuse

In the last century, progressively stringent environmental quality standards and an increasing number of wastewater treatment facilities have led to a concomitant growth in the amounts of municipal sewage sludge and biosolids produced, especially in large urban centres within industrialized regions. In Canada, more than 660,000 metric tons (dry weight, dw) are generated per year (CCME, 2012b), with almost half of them being produced in southern Ontario (Michael Payne, personal communication). In comparison, approximately 6.5 million metric tons (dw) of biosolids were either disposed or reused in the US in 2004 (NEBRA, 2007).

After production, biosolids can be disposed of or reused for beneficial purposes. Due to a number of factors, including the potential hazards posed by biological and chemical components of these by-products and a natural aversion to faecal material, sewage sludge and biosolids have been considered as waste in some communities in spite of their use as agricultural fertilizers in diverse countries around the globe, and the long history of human excreta use in agriculture.

The most common disposal processes are incineration, with or without energy recovery, and land disposal, including landfilling. Although still practiced in some locations, ocean dumping of raw sewage, sewage sludge and biosolids has either been banned or is being abandoned.

Incineration is the controlled burning of the waste material and is the preferred alternative for waste disposal in some densely industrialized regions, such as Japan. Its main advantages are the large reduction of the waste's volume and the elimination of any pathogenic

organisms. However, modern incinerators require a large capital investment, including the necessary air pollution controls, additional fuel to initiate and sustain combustion, and the remaining incombustible materials usually require to be landfilled (Beecher, 2008).

Land disposal practices range from the uncontrolled surficial dumping of untreated sewage sludge or biosolids to their deposition in modern engineered landfills. The former practice is the cheapest in pure monetary costs, but it clearly poses the largest risk to public and environmental health. These risks can be greatly reduced by the construction and appropriate management of landfills, although the cost might be prohibitive for some communities (Beecher, 2008).

The prevalent beneficial reuse methods involve the incorporation of biosolids to soils in order to make use of their components such as nutrients (nitrogen and phosphorus) and micronutrients (e.g., copper, iron, nickel, zinc), especially for agricultural and/or horticultural applications, and organic matter, in land reclamation projects for example (Beecher, 2008). A more detailed description of the benefits of biosolids land application can be found in the recently published review by Lu *et al.* (2012), and the Guidance Document by the Canadian Council of Ministers of the Environment (CCME, 2012a). Recycling of phosphorus in the biosolids is of especial interest given the finite mineral phosphate supplies, which are expected to be depleted by the end of this century (Stadelmann, 2002; Gonzalez and Miller, 2013).

The land application of biosolids for beneficial reuse has to be conducted in an appropriate manner in order to avoid public and environmental health risks, and unpleasant odours, which are an important factor in the lack of public acceptance of this practice (Lu *et al.*, 2012). More importantly, poor application practices can result in the excess loading of nutrients and their discharge into local aquifers or surface waters. Additionally, poor practices combined with the lack of quality control in the biosolids production process may result in the presence of pathogens and toxic substances, which might pose a risk to humans and biota.

The percentage of biosolids destined to each of the processes described above varies geographically due to factors such as population density and public acceptance of the different practices. In Canada, a 2001 survey of 49 treatment plants indicated that over 40% of the biosolids produced was land applied, 30% was landfilled, and 15% incinerated (CWWA, 2001). These percentages suggest a rapid shift in management practices at the time, from incineration to landfilling, because an earlier survey had shown that most biosolids (~48%) in Canada were incinerated, especially in big cities, and only 7% was landfilled—the proportion being land applied did not change significantly (CH2M, 2000). More recently, the percentage of landfilled biosolids has decreased in favour of land application for agricultural and reclamation purposes in the Prairies and the West Coast, and of incineration in Ontario and Québec (Michael Payne and Denise Vieira, personal communication).

The situation in the US is similar to that described for Canada: 55% of the biosolids was land applied in 2004, mainly for agricultural purposes, and 45% was disposed in landfills or incinerated (NEBRA, 2007). In some European countries, the percentage of biosolids reuse tends to be even higher, reaching 71% in the UK (Eljarrat *et al.*, 2008) and 82% in Norway (Eriksen *et al.*, 2009). A review by Kelessidis and Stasinakis (2012) summarizes the trends in sludge reuse and disposal in 27 EU countries.

2.2. Emerging substances of concern (ESOCs)

At present, there is no consensus definition of "emerging substances of concern" (ESOCs) (Diamond *et al.*, 2011). Furthermore, there is no general agreement on the name for this heterogeneous group of substances either; they are also called "emerging pollutants," and "contaminants of emerging concern," among other similar names. Even the definition of "emerging" is elusive, as pointed out by Field *et al.* (2006), "because what is emerging is a matter of perspective as well as timing."

In many cases, these chemicals are also grouped under terms highlighting characteristics other than their "emerging" nature, such as "trace organic compounds" (TOrCs), "microconstituents", and "micropollutants", all of which emphasize their relatively low concentrations in the environment; or "pharmaceuticals in the environment" (PiE) and "pharmaceuticals and personal care products" (PPCPs), which stress the chemicals' intended uses (Daughton and Ruhoy, 2010).

In spite of the lack of consensus on the name and definition, common characteristics of chemicals identified as ESOCs are (Diamond *et al.*, 2011; Jensen *et al.*, 2012; SFEI, 2013):

- They have been detected in at least one environmental compartment, and they tend to be present in relatively low—event trace—amounts.
- They are generally not being regulated—or at least not for a recent concern, such as possible endocrine disruption effects of PCBs, currently banned because of their persistent and toxic nature (Diamond *et al.*, 2011).
- They are believed to have potential deleterious impact on human and/or environmental health, but these risks have not been thoroughly evaluated.

The chemical nature of ESOCs is very diverse and includes organic and inorganic compounds; natural and anthropogenic substances, including some of their transformation products; newly synthesized chemicals and others that have been released into the environment for a relatively long time but could not be measured until the appropriate analytical technology was available. Many ESOCs fall in more than one of these categories, as exemplified by the compounds listed in Table 2.1.

Furthermore, an ESOC may be considered emerging for long periods of time, in the order of years (Field *et al.*, 2006). This is due to the lengthy and costly process required to assess the human and ecological toxicity of ESOCs, and to the continuous discovery and/or improvement in our understanding of toxic effects, such as endocrine disruption.

The problem of defining a substance as an ESOC is further compounded by the large number of chemicals used in modern life, their ubiquity in the household, urban and industrial environments, and the relative ease with which they can be detected in the environment in relation to current knowledge of their potential deleterious effects. There are currently over 84,000 chemicals in the US Toxic Substances Control Act (TSCA) inventory (USEPA, 2013b), of which 10% are used in "significant amount" (Hogue *et al.*, 2007). Advances in analytical chemistry enable the detection of an increasing number of these chemicals in the environment, even when they are present in trace concentrations. However, the present understanding of their possible ecological risks lags markedly behind the ability to detect their

presence (Field *et al.*, 2006; Diamond *et al.*, 2011). Hence, prioritizing the chemicals for further study has become an important concern for regulatory agencies.

Table 2.1. Examples of ESOCs, their classification, and the reason(s) for concern.

ESOC	Function/ Use	Category/ Classification	Concerns
Silver nanoparticles	Antimicrobial	Inorganic; natural (Ag); anthropogenic (nano)	Microbial resistance; enhanced aquatic toxicity
Estradiol (E2)	Hormone	Organic; natural; always present, but only recently measured in the environment	Endocrine disruption
Ethinylestradiol (EE2)	Hormone	Organic; anthropogenic	Endocrine disruption
Dichlorodiphenyldichloroethylene (DDE)	None	Organic; transformation product of a banned anthropogenic compound (DDT)	Endocrine disruption

2.3. Pathogens

In contrast to ESOCs, pathogens are better defined. The term encompasses different classes of organisms capable of causing disease, especially in humans, but also in animals and plants (USEPA, 2007). Most of the organisms defined as pathogens are bacteria, viruses, fungi, and protozoa, although some definitions include cellular components, such as plasmids (USEPA, 2007). Because they were a concern earlier than ESOCs, pathogens were targeted since the first biosolids land application regulations were introduced (USEPA, 1989).

Most pathogens affecting humans originate from other animals or humans themselves. More than 150 have been identified in farm animal waste, which is considered the most likely source of waterborne disease outbreaks (Gerba and Smith, 2005). In centralized wastewater treatment, the source of municipal biosolids, pathogens are mainly of human origin and their concentration is directly proportional to the incidence of enteric infections in the contributing population (Gerba and Smith, 2005).

Table 2.2 lists the principal pathogens of concern in wastewater and sewage sludge compiled by Gerba and Smith (2005). With the exception of enteric viruses, which stem exclusively from humans, most of these pathogens can originate from animal as well as human sources as stated above.

Table 2.2. Principal pathogens of concern in municipal wastewater and sewage sludge (USEPA, 1989; Gerba and Smith, 2005).

Pathogen of Concern	Disease or Symptoms Caused
Bacteria	

Salmonella spp.	Salmonellosis (food poisoning), typhoid fever
Shigella spp.	Bacillary dysentery
Yersinia spp.	Acute gastroenteritis (diarrhoea, abdominal pain)
Vibrio cholerae	Cholera
Campylobacter jejuni	Gastroenteritis
Escherichia coli (pathogenic strains)	Gastroenteritis
Viruses	
Poliovirus	Poliomyelitis
Coxsackievirus	Meningitis, pneumonia, hepatitis, fever
Echovirus	Meningitis, paralysis, encephalitis, fever
Hepatitis A virus	Infectious hepatitis
Rotavirus	Acute gastroenteritis with severe diarrhoea
Human caliciviruses	Epidemic gastroenteritis with severe diarrhoea
Reovirus	Respiratory infections, gastroenteritis
Hepatitis E virus	Hepatitis
TT hepatitis	Hepatitis
Astroviruses	Gastroenteritis
Adenoviruses	Respiratory tract infections, gastroenteritis
Protozoa	
Cryptosporidium	Gastroenteritis, cryptosporidiosis
Entamoeba histolytica	Acute enteritis
Giardia lamblia	Giardiasis (diarrhoea and abdominal cramps)
Balantidium coli	Diarrhoea, dysentery
Toxoplasma gondii	Toxoplasmosis
Helminth worms	
Ascaris lumbricoides	Digestive disturbances, abdominal pain
Ascaris suum	Can have symptoms including coughing, chest pain
Trichuris trichiura	Abdominal pain, diarrhoea, anaemia, weight loss
Toxocara canis	Fever, abdominal discomfort, muscle aches
Taenia sasginata	Nervousness, insomnia, anorexia
Taenia solium	Nervousness, insomnia, anorexia
Necator americanus	Hookworm disease
Hymenolepis nana	Taeniasis

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3. RISK ASSESSMENT OF ESOCS IN BIOSOLIDS-AMENDED SOILS

As described in Chapter 2.2, ESOCs are generally not regulated because of their "emerging" nature; i.e., they have only recently been brought to attention. Additionally, advances in environmental analytical chemistry have allowed the detection of an ever-increasing number of organic chemicals in all environmental compartments—including biosolids—with progressively lower detection limits. In many cases, however, the understanding of the environmental fate and potential environmental and human effects of these compounds is limited or even non-existent.

Therefore, the main purpose of this chapter was to review available studies and formal risk assessments of ESOCs produced by governments, academia, and/or professional organizations in order to define the current state of knowledge of the potential risks of ESOCs in biosolids-amended soils, and to help in the selection of the chemicals reviewed in Appendix A.1.

The first part of the chapter summarizes the development of the current North American regulations for toxic chemicals in biosolids-amended soils—other regulated compounds are nutrients, but they are not part of the scope of this review, and therefore not discussed. Because most ESOCs are organic compounds, and current North American regulations contemplate mostly metals, the second part of the chapter discusses the risk assessment process that led to the exclusion of organic compounds from such regulations. The third part summarizes the reviews for ESOCs mentioned in the preceding paragraph, and the last section discusses the limitations to the current risk assessment approach.

3.1. Development of regulations for chemicals in biosolids-amended soils

The potential deleterious effects of chemicals, including trace elements, heavy metals, ESOCs, and pathogens to human health and the environment have raised concerns over the use of biosolids for soil amendment, and they were the fundamental driver for the development of regulations managing the use of biosolids in agriculture, especially when growing crops for human consumption or in grazing fields for cattle also intended for human consumption.

Historically, these concerns started receiving increased public attention in the 1970s. In an account of the development of biosolids regulations in the US, Chaney *et al.* (1996) identified this decade as one of "intense searching for environmental risks, and for research on land application of biosolids." This search led to the realization that biosolids could contain high concentrations of a number of contaminants, including heavy metals (Dudas and Pawlukz, 1975; Furr *et al.*, 1976; Sterritt and Lester, 1980) and persistent organic pollutants (POPs) such as PCBs (Furr *et al.*, 1976; Bergh and Peoples, 1977), and that these contaminants could be transferred to crops grown in soils amended with such biosolids (Dudas and Pawlukz, 1975). These types of observations were also made in the Canadian context (Van Loon, 1973; Dudas and Pawlukz, 1975).

As a consequence, regulations were introduced to control the quality of biosolids applied to land, and to decrease pollutant concentrations in the biosolids themselves by requiring

reduced discharges to municipal sewage systems from industrial sources (Chaney *et al.*, 1996)—although domestic wastewater could have also represented a significant source of pollutants, as was the case for some heavy metals (Klein *et al.*, 1974). These measures, together with the ban of PCBs and other POPs, resulted in a significant improvement of biosolids quality, with lower amounts of POPs and heavy metals in American biosolids, as measured in the 1991 National Sewage Sludge Survey (Chaney *et al.*, 1996).

The regulations for biosolids land application adopted by different countries were set following two different models, either a risk assessment approach or some form of the precautionary principle (Schoof and Houkal, 2005). The latter was preferred in Europe, while the former was adopted in Canada, the US, and the UK (Smith, 2009). This divergence resulted in more stringent concentration limits (either proposed or enacted) in Europe than in the US for both metals (McGrath *et al.*, 1994) and organic compounds (European Commission, 2000; Smith, 2009).

The limits set for biosolids-derived pollutants in agricultural soils varied even between countries using the same general approach. For example, individual European countries set maximum annual metal loadings of metals to soils varying up to 2-3 orders of magnitude (McGrath *et al.*, 1994); this wide variation stemmed from differences in the choice and interpretation of toxicity data, and in the definition of acceptable levels of effects to soil ecosystems, which in some countries meant that no net accumulation of metals in soil beyond background levels was to be allowed—this position assumed that any level of accumulation was undesirable regardless of the absence of negative effects (McGrath *et al.*, 1994). Ultimately, these variations are the result of the lack of a precise and consensual definition of the precautionary principle (Marchant, 2003).

In contrast, risk assessment approaches are based on setting standards to prevent harmful effects in humans and the environment, although they can also incorporate the desired level of precaution, either as part of the risk assessment itself, or subsequently, as part of the risk management strategy (Schoof and Houkal, 2005). The USEPA's CFR 40 Part 503 rule (USEPA, 1993), which contains the regulations of biosolids land application for the US, is a classic example of a risk-assessment based regulation.

The Part 503 rule was developed in the 1980s using a "pathway approach" for its risk assessment; i.e., it evaluated the possibility of a pollutant being transferred from biosolids-amended soils to humans or biota via different pathways (Table 3.1), such as direct ingestion (Chaney *et al.*, 1996). The rule set the contaminant limits to protect highly exposed individuals (HEIs); i.e., a conservative approach assessing the risk to a subset of humans and biota directly and constantly exposed to biosolids, as a toddler ingesting pure biosolids, for example, or earthworms living in biosolids-amended soils. These limits were generally based on the exposure resulting from a single pathway, because for most of the pollutants studied exposure occurred through a single dominant pathway.

Table 3.1. Exposure pathways in the USEPA Part 503 rule risk assessment of biosolids-amended soils to human and biota (USEPA, 1989; Chaney *et al.*, 1996).

Target organism/Exposure pathway	Concern

Humans:	
Direct ingestion: Biosolids → Human	Human toxicity
Soil ingestion: Biosolids → Soil → Human	Human toxicity
Plant ingestion: Biosolids → Soil → Plant → Human	Human toxicity
Animal product ingestion: Biosolids → Animal → Human	Human toxicity
Animal product ingestion: Biosolids → Soil → Animal → Human	Human toxicity
Animal product ingestion: Biosolids → Soil → Plant → Animal → Human	Human toxicity
Volatilization: Biosolids → Soil → Air → Human	Human toxicity
Dust: Biosolids → Soil → Airborne dust → Human	Human toxicity
Runoff: Biosolids → Soil → Surface water → Human	Human toxicity
Groundwater: Biosolids → Soil → Groundwater → Human	Human toxicity
Animals:	
Direct ingestion: Biosolids → Animal	Animal toxicity
Soil ingestion: Biosolids → Soil → Animal	Animal toxicity
Plant ingestion: Biosolids → Soil → Plant → Animal	Animal toxicity
Plants:	
Biosolids → Soil → Plant	Plant toxicity
Biota:	
Biosolids → Soil → Soil biota	Ecotoxicity
Biosolids → Soil → Soil biota → Predator	Ecotoxicity

Subsequent risk assessments, such as the one for dioxins (RTI, 2002), used a multiple-pathway probabilistic approach that evaluated the exposure of the members of a conceptual farm family to the contaminant of interest from all potential routes of exposure (Schoof and Houkal, 2005). This approach also indicated that most of the risk for exposure came from a limited number of pathways; in the case of dioxins, most of the exposure resulted from the ingestion of two animal products, namely beef and milk.

In Canada, Ontario introduced guidelines for the application of biosolids in agricultural soils in the late 1970s. Because little information on the effects of biosolids on soils and crops was available at the time, the guidelines postulated that "sewage biosolids should cause no net degradation of soil and environmental quality" (WEAO, 2001). In practice, this meant that the maximum concentrations in biosolids-amended soils of eleven elements (nine metals, arsenic and selenium) were limited to 2 to 8 times the background levels in the province soils, depending on the element's toxicity and whether they were considered plant micronutrients (WEAO, 2001; Hébert, 2012). Currently, most provinces and territories limit the amount of these elements in biosolids and/or in the soils where they are applied (CCME, 2010).

3.2. Risk assessment of organic compounds in biosolids-amended soils

The chemicals targeted in regulations governing the use of biosolids for soil amendment are mainly inorganic, especially in North America. In the US, the list is limited to seven metals (cadmium, copper, lead, mercury, molybdenum, nickel, zinc), arsenic and selenium (USEPA, 1993), and does not include any organic compounds. This was a result of the risk assessment process that led to the Part 503 rule. (A detailed description of the process can be found in USEPA (1995) and NRC (2002).)

When the development of the Part 503 rule started in the 1980s, a list of over 200 potential "pollutants of concern" that included organic compounds was submitted for evaluation to four expert panels, which designated 50 of the pollutants for further analysis. This analysis included the identification of exposure pathways and a worst-case scenario hazard screening process that considered toxicity, occurrence in the environment and extreme exposure to HEIs for each chemical. As a result, the list was reduced to 22 chemicals to be further assessed for risks from biosolids land application, 16 for risks from surface disposal, and 14 for incineration (Table 3.2).

Table 3.2. List of chemicals selected for detailed risk assessment during the development of USEPA Part 503 rule in the 1980s. Chemicals in bold correspond to those evaluated for risks from land application and/or surface disposal of biosolids. (USEPA, 1995).

Aldrin/Dieldrin	Cobalt	**Lead**	Phenanthrene
Arsenic	**Copper**	**Lindane**	Phenol
Benzene	**Cyanide**	Malathion	**Selenium**
Benzo[*a*]anthracene	**DDT/DDE/DDD**	**Mercury**	Tetrachloroethylene
Benzo(*a*)pyrene	2,4-Dichloro-phenoxyacetic acid	Methyl ethyl ketone	**Toxaphene**
Bis(2-ethylhexyl) phthalate (DEHP)	Dioxins	Methylenebis(2-chloroaniline)	**Trichloroethylene**
Beryllium	**Fluoride**	Methylene chloride	Tricresyl phosphate
Cadmium	Furans	**Molybdenum**	Vinyl chloride
Carbon tetrachloride	**Heptachlor**	**Nickel**	**Zinc**
Chlordane	**Hexachlorobenzene**	***n*-Nitroso-dimethylamine**	
Chloroform	Hexachlorobutadiene	**Polychlorinated biphenyls**	
Chromium	**Iron**	Pentachlorophenol	

However, after a national survey of pollutant concentrations in American biosolids conducted in 1988-1989, all of the organic compounds in the list were eliminated for one of the following reasons: the chemical had been banned or restricted for use in the US, it had a low frequency of detection in sludge (<5%), or its concentration in sludge was not expected to exceed the exposure limits identified during the preliminary risk assessment (USEPA, 1995).

A second round of pollutant selection conducted after the Part 503 rule was enacted designated 29 dioxins, dibenzofurans, and PCBs for risk assessment (NRC, 2002), whose results led the USEPA to conclude that no regulation was necessary to protect human health and the environment from possible risks due to exposure to these chemicals after biosolids land application (Schoof and Houkal, 2005).

Additionally, the Clean Water Act requires the Part 503 rule to be reviewed at least every 2 years to identify additional pollutants requiring regulation. The USEPA has conducted these reviews in 2005, 2007, and 2009. In the last review, the USEPA identified 49 new pollutants to be considered for regulation, and it is in the process of evaluating 26 of those (USEPA, 2012).

Some European countries and the European Union have instituted or proposed limits to certain organic compounds, such as PCBs, dioxins and furans, PAHs, NP and NPEs, LAS,

and DEHP (European Commission, 2000). Besides the differences in the approach to the establishment of environmental regulations mentioned above (e.g., the predominance of the precautionary principle in some countries), evidence of possible toxicological effects was scarce when the standards were developed; both the approach and the lack of evidence contributed to variation between jurisdictions in the limits set for the different contaminants in biosolids (Smith, 2009).

As previously mentioned, most Canadian provinces and territories currently limit the amount of mainly inorganic compounds in biosolids and/or in amended soils. In most cases, the compounds are eleven elements: nine metals (cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, zinc), arsenic and selenium. Only Nova Scotia and Québec set maximum concentrations for organic compounds, dioxins and furans in both instances (CCME, 2010). The Canadian Food Inspection Agency (CFIA) and the Bureau de Normalisation du Québec (BNQ) also set limits for dioxins and furans in biosolids, but for no other organic compounds (CCME, 2010). The inclusion of these compounds and their concentration limits stemmed from different regulations from Germany, the European Union, and the state of Maine in the US (MDDEP, 2012).

3.3. Risk assessment of ESOCs in biosolids-amended soils

Increasing awareness in the last two decades of the presence of a broader suite of organic compounds in biosolids that were not considered when regulations were enacted, has led to a widespread consensus that the potential risks posed by these substances, or ESOCs, need to be assessed (NRC, 2002; Schoof and Houkal, 2005; WEAO, 2010). This task has proven difficult, however, because toxicity and fate data are insufficient for many ESOCs (Higgins *et al.*, 2010) and because of the large number of compounds present in biosolids—as an example, the most recent USEPA's Targeted National Sewage Sludge Survey (TNSSS) included 145 analytes, most of them pharmaceuticals (USEPA, 2009). Even the attempts at prioritizing ESOCs for risk and/or toxicity assessment are complicated by the lack of data, which has been used as a factor in prioritization approaches, such as the one proposed by Clarke and Smith (2011) and discussed below.

Recent risk assessments and prioritization exercises include some by academic groups (Smith, 2009; Clarke and Smith, 2011), governments (Eriksen *et al.*, 2009; Jensen *et al.*, 2012), and professional organizations (Higgins *et al.*, 2010; WEAO, 2010). The organic compounds present in biosolids and considered for these studies are listed in

Table 3.3, and the recommendations stemming from these prioritization exercises are summarized in Table 3.4. The rest of this section describes the studies and summarizes their findings.

3.3.1. Norwegian risk assessment

The Norwegian Food Safety Authority conducted a risk assessment of 27 compounds or families of compounds in the context of biosolids land application (Eriksen *et al.*, 2009). The compounds included seven metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn), six families of organic compounds (phthalates, OP and OPEs, NP and NPEs, LASs, PCBs, PAHs), and 14 pharmaceuticals (atorvastatin, carisoprodol, chlorprothixene, ciprofloxacin, dipyridamole,

fexofenadine, gabapentin, levetiracetam, losartan, mesalazine, metoprolol, ranitidine, sotalol, tetracycline).

The metals and the organic compounds were chosen according to the availability of data on their toxicity and occurrence in Norwegian sludge—other compounds considered initially but not included in the risk assessment due to lack of data are listed in

Table 3.3. The pharmaceuticals selected were those out of the 1,414 drugs marketed in Norway that were expected to exceed 10 (for hormones, antibacterial and anticancer drugs) or 100 µg/kg in soil after biosolids application.

The risk assessment was performed using the pathway approach for the compounds above and 12 different exposure routes in order to evaluate the risks to "soil living organisms, the aquatic environment, grazing animals, animals eating feed based on plants from sludge-treated soil, children eating soil, and humans consuming drinking water, crop plants and/or meat affected by the use of sludge as soil conditioner" (Eriksen *et al.*, 2009).

The overall conclusion of the risk assessment suggested that exposure to the chemicals analyzed resulting from biosolids land application in Norway is likely to occur at levels well below PNEC values in most cases, and is not expected to constitute a significant risk to humans and biota. The possible exceptions to this conclusion were:

- The possibility of a 2–4 times increase in the total concentrations of cadmium, copper, mercury and zinc after applying the maximum allowed amount of sludge for 100 years. This led the authors of the study to recommend monitoring of these metals in biosolids used for agricultural land applications and to continue decreasing their concentration in sewage sludge.
- The possibility of a relatively high human intake of cadmium and copper—exceeding exposure limits—in cases where lifetime vegetable consumption consists exclusively of vegetables grown in soils receiving the maximum amount of biosolids for 100 years.
- Estimated OP and LASs concentrations exceeded PNEC values after biosolids application to agricultural soils. However, the high biodegradability and rapid dissipation of these compounds led the authors to consider them a low risk to the soil ecosystem.

As part of this study, the risk of the promotion of antibiotic resistance in biosolids-amended soils was also evaluated and it was deemed unlikely for most antibiotics analyzed, with the possible exception of ciprofloxacin because of its persistence and mobility in soils (Eriksen *et al.*, 2009).

3.3.2. Imperial College reviews

An early review by Smith (2009) identified over a dozen organic compounds or classes of compounds present in sewage sludge and biosolids ranging in concentrations from ng/kg to g/kg (

Table 3.3). The author also conducted a literature-based qualitative assessment of the potential risks to human health and the environment from the use of biosolids in agricultural lands. He concluded that the overall risk for impact to crop yields, animal health, groundwater, surface water, and air quality is low when applying the biosolids according to European and British regulations.

Smith (2009) also identified several issues as "possible risks" to human health and soil fertility from land application of biosolids. Identification as possible risk occurred "where there is some reported evidence that current operational practice may result in a potential impact on the environment on the basis that one or [both] of the following conditions apply: there is uncertainty about the environmental implications of particular sludge components, [and/or] effects may occur under certain extreme 'worst-case' conditions, given the current regulations and codes of practice." The issues were:

- The high concentrations in biosolids of PCAs, which are persistent and have the potential to bioaccumulate. Their potential for transfer to the food chain and their potential impact on human health are unknown.
- Lack of experimental confirmation through a microbial risk assessment that antibiotic-resistant microorganisms do not pose a risk to human health.
- The potential impact of TCS and other personal-care products to soil fertility has not been evaluated even though these chemicals have been shown to impact soil microbes.

In a later assessment, Clarke and Smith (2011) ranked a slightly different series of organic compounds or families of compounds (

Table 3.3) to determine research priorities in the effects of chemicals after biosolids land application. The following criteria were used to calculate a priority index ranging from 0 to 11 points, with a higher index representing a higher priority for research and monitoring:

- Persistence in soil (> 6 months) (0-2 points)
- Potential for human health impacts resulting from biosolids land application (0-2 points)
- Evidence of bioaccumulation in humans or the environment (0-2 points)
- Evidence of ecotoxicity (0-2 points)
- Extent, quality, and consistency of empirical data (0-3 points)

The full list of chemicals and their indices are presented in Table 3.4. The three highest scores were given to PFCs, PCAs, and PCNs. The main concerns specified by Clarke and Smith (2011) regarding PFCs were their ubiquity in the environment, including human blood, and their unique chemical behaviour, especially their high water solubility relative to other POPs, which could allow them higher mobility in the environment and, consequently, to accumulate in plants and/or contaminate water sources.

PCAs were singled out due to their relatively high concentrations in biosolids, reaching the low g/kg levels in some cases. Both PCAs and PCNs were considered persistent in soil, and both their introduction to the human food chain and bioaccumulation in biota were deemed possible. Lastly, there were few or no empirical data available for these three families of chemicals, precluding the development of formal risk assessments in the biosolids land application context.

3.3.3. Danish government risk assessment

The Danish Environmental Protection Agency commissioned an evaluation of the risk posed by five families of organic pollutants present in sewage sludge to soil-dwelling organisms (Jensen *et al.*, 2012). These five groups, comprising BFRs, musks, pharmaceuticals, PCBs, and PFCs, were selected based on the results of the three studies summarized above

(Eriksen *et al.*, 2009; Smith, 2009; Clarke and Smith, 2011), the availability of data to conduct risk assessments, and the concerns of the Danish public.

The risk assessments were based on the calculation of margins of safety (MoS), defined as the quotient of the predicted soil concentration and the lowest no-observed-effect level (NOEL). An MoS value between 10 and 1,000—depending on the nature and amount of available toxicity data for different trophic levels—was considered to be "sufficient for protection of the soil environment" as defined by the European Union's REACH program (Jensen *et al.*, 2012).

The results of the assessment led the authors to conclude that BFRs, musks, pharmaceuticals, and PCBs were unlikely to pose a "significant risk to soil dwelling organisms and soil quality in general" at the concentrations present in Danish biosolids and assuming that the relevant application guidelines were followed. In contrast, the relatively low MoS obtained for one of the PFCs evaluated, PFOS, suggests that this substance "may pose a long term risk to soil ecosystems" (Jensen *et al.*, 2012). However, the listing of PFOS in Annex B of the Stockholm Convention (Stockholm Convention, 2009) and the resulting restrictions of its production and use in Denmark were expected to decrease its concentration in biosolids and, consequently, the risks to soil organisms.

Although they were not as thoroughly evaluated, the authors also recommended the assessment of PCAs, PCNs, TCC[1], TCS, and parabens as a result of their literature search.

3.3.4. WEAO reports

In 2010, the Water Environment Association of Ontario (WEAO) published an update to their 2001 report on the fate and significance of contaminants in biosolids (WEAO, 2001, 2010). The main objectives of the 2001 report were to review the literature to assess the fate and significance of contaminants in biosolids applied to agricultural soils. These contaminants were divided in two groups (WEAO, 2001):

- Group I – Contaminants for which *sufficient* credible scientific information exists to assure the public that the current agricultural land application program/guidelines are adequate to protect the well-being of soils, crops, animals, human health, ground and surface water qualities.
- Group II – Contaminants for which *insufficient* credible scientific information exists to assure the public that the current agricultural land application program/guidelines are adequate to protect the well-being of soils, crops, animals, human health, ground and surface water qualities.

The 2010 update analyzed the information generated in the period between the two reports and assessed whether the contaminants still belonged in the original groups or there was enough information to reclassify them. The 2010 report also reviewed chemicals or families of chemicals that had not been considered in 2001. A list of the compounds reviewed in both reports is presented in

Table 3.3, and their current classification as Group I or Group II can be found in Table 3.4.

[1] Jensen *et al.* (2012) actually referred to "trichloban", which was assumed by the authors of the present report to be a reference for triclocarban.

Additionally, as part of the final recommendations, the 2010 report suggested biosolids components that should receive priority for future research efforts (WEAO, 2010):

- Perfluorinated organic compounds
- Personal care products, including fluorescent whitening agents, QACs, siloxanes and UV filters
- Concentrations and viability of protozoans such as *Cryptosporidium*
- Pathogens of recent concern, such as the swine (H1N1) and avian (H5N1 and H5N2) influenza viruses.

The prioritization was based only on the availability of environmental fate and toxicity information on the contaminants; i.e., the compounds chosen are those for which little information exists. The report acknowledged that a proper prioritization would require full risk assessments that were not available at the time (WEAO, 2010).

3.3.5. WERF studies

The Water Environment Research Foundation (WERF) published in 2010 a study on trace organic chemicals in biosolids-amended soils in the United States (Higgins *et al.*, 2010). The report included a prioritization based on biosolids occurrence data from the USEPA's TNSSS (USEPA, 2009) and the USGS biosolids survey (Kinney *et al.*, 2006), and on toxicity and bioaccumulation properties when available. Higher priority was given to compounds present at high maximum mean or median concentrations (>1,000 µg/kg) in one of the two occurrence reports. PFCs and their precursors, BFRs, and synthetic steroids were also considered high priority in spite of their relatively low concentrations in American biosolids due to their potential for bioaccumulation and/or endocrine disruption.

Although conducting risk assessments was not a goal of the study, the authors sought to identify the missing information necessary to perform such assessments for the high priority compounds. Therefore, the decision was made to exclude from the study compounds for which such information was already available, including those previously identified as priority pollutants by the USEPA, compounds or families of compounds whose presence in biosolids was already regulated, and/or for which risk assessments existed, and those present at relatively low frequencies and/or concentrations in the two occurrence surveys that served as a basis for the study.

The list of chemical families considered for the study can be found in

Table 3.3; of these, nine groups of chemicals encompassing over 50 compounds were considered high priority: brominated flame retardants (BFRs), perfluorochemicals and precursors (PFCs), antimicrobials, antibiotics, musks, other pharmaceuticals and personal care products (PPCPs), plasticizers, steroidal chemicals, and surfactants (Table 3.4).

This study also compiled and summarized current knowledge on the fate, transport and toxicity of the high priority compounds. Because the prioritization was based partially on occurrence data, the presence of these chemicals in biosolids was well documented, with the notable exception of the PFCs. Their mobility in soils was also found to be relatively well understood or at least amenable to modelling, although not necessarily in the context of biosolids land application; less information was available on biodegradation and

bioaccumulation. In contrast, there was limited information on the human toxicity of these chemicals, and virtually no ecotoxicology data.

The main conclusion of this study was that even when data for the high priority chemicals was available, few were generated through research specifically designed to address the distinct environmental conditions specific to biosolids-amended soils, resulting in high uncertainty in the risk assessment of ESOCs in this context. Furthermore, the study also concluded that the lack of toxicological and ecotoxicological data is the most significant data gap preventing proper risk assessments for ESOCs in biosolids-amended soils.

WERF also commissioned a subsequent study to compile unpublished data for these compounds (Pittinger *et al.*, 2012) to complement and update the original report. This project identified empirical data for almost half of the 61 chemicals researched, with most of the data being pharmacological or related to mammalian toxicity. Environmental fate and ecotoxicological data were also identified for 29 of the chemicals, but they were mainly modelling estimates rather than experimental values. The authors determined that information on biological transference rates (uptake, accumulation and depuration), ecological toxicity endpoints, and impact on soil ecosystems was insufficient for all chemical classes studied.

This work also drew attention to the need for a collective prioritization of the ESOCs to be studied in the biosolids land application context, because the different studies currently available differ in the selection process of the ESOCs to be targeted (Pittinger *et al.*, 2012). As part of this prioritization, the authors proposed a retrospective re-evaluation of the reasons that led to select the chemicals studied to date. They also suggested taking into consideration market trends in the use of ESOCs, such as the diminished use of PBDEs, and "to identify the endpoints of highest impact and value for risk assessment or prioritization" (Pittinger *et al.*, 2012).

3.4. Limitations of the current risk assessment approach and considerations for future work

Considering the studies reviewed in the last section (Chapter 3.3) as a whole, the following common themes can be discerned on the evaluation of the potential risks of ESOCs in the biosolids land application context:

- Only a few of the hundreds of ESOCs have been subject to thorough risk assessments, and the existing risk assessments concluded that the presence of the studied ESOCs poses a low risk to human and environmental health.
- The main reason behind the limited number of risk assessments is the lack of data to conduct them. Especially lacking are toxicity and ecotoxicity data, but there is also a limited amount of information on the fate of ESOCs in the distinct environmental conditions specific to biosolids-amended soils.
- The ESOCs considered in risk assessments and prioritization studies are chosen from existing information, usually a combination of occurrence in biosolids and known toxicity properties. Comprehensive evaluations of chemicals that would draw attention to

compounds not routinely monitored, such as the systematic analysis of the pharmaceuticals marketed in Norway (Eriksen *et al.*, 2009), are rarely conducted.
- Risk assessments have typically been conducted using a chemical-by-chemical approach, which does not take into account the effects of simultaneous exposure to numerous chemicals, or to their transformation products. Additionally, this approach has been mainly reactive; i.e., it considered ESOCs that were already known to be present in biosolids and/or toxic, and it did not include provisions to deal with unknown chemicals.

Although the current risk evaluation approach has generated valuable information on the possible effects of individual chemicals, it does not address the ultimate goal of determining whether ESOCs as a group affect human and/or environmental health when present in biosolids applied to agricultural land for beneficial reuse, specifically in the Canadian context. Therefore, it is necessary to develop a different strategy, designed to address this goal, and with this outcome "always in mind" as defined by Daughton (2004).

The design of such Canadian risk evaluation strategy for ESOCs in biosolids requires the participation of representatives from the different stakeholder groups, and it should be part of the national consultation discussed in Part B of this project.

Although open for debate by the stakeholders, this new strategy should incorporate the following elements:

- Definition of the environmental protection goals of the strategy – The creation of a Canadian risk evaluation strategy must begin by establishing the desired level of protection for human and environmental health, which necessitates a clear definition of the parameters to be monitored to ensure that the level of protection is achieved. For example, one of the stated goals of the Ontario guidelines for use of sewage biosolids in agriculture was that "sewage biosolids should cause no net degradation of soil and environmental quality," and they set clear limits to the permissible increments to the baseline concentrations of certain inorganic compounds in soils (WEAO, 2001). Similar limits could be set for environmental health parameters; e.g., increase in chemical stress biomarkers in earthworms.
- Establishment of a prioritization process for ESOCs – For historical and practical reasons, the current list of ESOCs was not the result of a systematic analysis of all chemicals that may be present in biosolids. Target analytes were chosen for reasons such as production volume or the endocrine disrupting properties of some of them. Therefore, different institutions have used different criteria to set research priorities independent from each other. A revised list of priority ESOCs should be compiled, tailored to Canadian needs and perspectives, and incorporating economic knowledge, specifically changes in ESOCs utilization trends as suggested by Pittinger *et al.* (2012). This list, which would require continual updating, will contribute to focus ESOCs research and to avoid duplicating efforts. The list update strategy should include provisions to incorporate chemicals that are not currently a concern, but could be considered ESOCs in the future; e.g., chemicals that are not currently monitored in biosolids, but that may be expected to accumulate in them (Higgins *et al.*, 2010). A modeling approach such as the one developed by McLachlan *et al.* (2014) for organosilicon chemicals in the environment could be used for this purpose.
- Development of an endpoint-monitoring plan – As discussed above, the chemical-by-chemical approach is insufficient to evaluate possible negative effects of ESOCs on

environmental health; consequently, this strategy should include monitoring such effects. Although there are research gaps in this area that can only be addressed by long-term field studies (Higgins *et al.*, 2010), in order to plan such studies it is necessary to define first which biological responses to ESOCs can be expected, whether they are considered acceptable, and what kind of biological endpoints, such as stress indicators (Daughton, 2004), could be monitored as early predictors of negative effects resulting from exposure to ESOCs.

- Identification and undertaking of research gaps – The studies discussed in this section have already enumerated many of the research gaps, and individual research groups around the world are working to address many of them. However, a concerted effort is necessary to fill the specific gaps in the Canadian context; it is noteworthy, for example, that from WEAOs original list of chemicals in Group II (WEAO, 2001), only hormones were able to be reclassified to Group I after a decade (WEAO, 2010). Moreover, there is a conspicuous lack of field studies incorporating long-term monitoring of agricultural and other ecosystems where biosolids are land applied. This kind of studies, while costly and complex, would help considerably to settle the question of possible effects of ESOCs in the application of biosolids to agricultural land (Higgins *et al.*, 2010).

3.5. Summary

ESOCs have not been regulated because of their “emerging” nature. They have only recently been brought to attention, because advances in environmental analytical chemistry have enabled their detection, or because concerns over relatively novel types of deleterious effects (e.g., endocrine disruption) have surfaced.

In contrast, interest in the pollutants currently regulated in biosolids started in the 1970s, when it became clear that a number of them, including heavy metals and persistent organic pollutants, could be transferred to crops grown in soils amended with biosolids.

In consequence, regulations were introduced to control the quality of biosolids applied to land, and to decrease pollutant concentrations in the biosolids themselves (Chaney *et al.*, 1996); additionally, a number of POPs were banned. These measures resulted in a significant improvement of biosolids quality.

In North America, these biosolids regulations are based on risk assessment, and the risk assessment process used by the USEPA led to the exclusion of the organic compounds considered at the time. However, increasing awareness of the presence of a large number of organic chemicals (ESOCs) in biosolids has led to widespread interest to assess their potential risks (NRC, 2002; Schoof and Houkal, 2005; WEAO, 2010). This task has proven difficult, however, because toxicity and fate data are insufficient for many ESOCs (Higgins *et al.*, 2010), and because of the large number of ESOCs present in biosolids.

Recent risk assessments and prioritization studies summarized in this chapter include some by academic groups (Smith, 2009; Clarke and Smith, 2011), governments (Eriksen *et al.*, 2009; Jensen *et al.*, 2012), and professional organizations (Higgins *et al.*, 2010; WEAO, 2010).

Considering these studies as a whole, the following common themes can be discerned:

- Only a few of the hundreds of ESOCs have been subject to thorough risk assessments, and the existing risk assessments concluded that the presence of the studied ESOCs poses a low risk to human and environmental health.
- The main reason behind the limited number of risk assessments is the lack of data to conduct them. Especially lacking are toxicity and ecotoxicity data, but there is also a limited amount of information on the fate of ESOCs in the distinct environmental conditions specific to biosolids-amended soils.
- The ESOCs considered in risk assessments and prioritization studies are chosen from existing information, usually a combination of occurrence in biosolids and known toxicity properties. Comprehensive evaluations of chemicals that would draw attention to compounds not routinely monitored, such as the systematic analysis of the pharmaceuticals marketed in Norway (Eriksen *et al.*, 2009), are rarely conducted.
- Risk assessments have typically been conducted using a chemical-by-chemical approach, which does not take into account the effects of simultaneous exposure to numerous chemicals, or to their transformation products. Additionally, this approach has been mainly reactive; i.e., it considered ESOCs that were already known to be present in biosolids and/or toxic, and it did not include provisions to deal with unknown chemicals.

Although the current risk evaluation approach has generated valuable information on the possible effects of individual chemicals, it does not address the ultimate goal of determining whether ESOCs as a group affect human and/or environmental health when present in biosolids applied to agricultural land for beneficial reuse, specifically in the Canadian context. Therefore, it is necessary to develop a different strategy, designed to address this goal.

The design of such Canadian risk evaluation strategy for ESOCs in biosolids requires the participation of representatives from the different stakeholder groups, and it should be part of the national consultation discussed in Part B of this project.

Although open for debate by the stakeholders, this new strategy should incorporate the following elements:

- Definition of the environmental protection goals of the strategy.
- Establishment of a prioritization process for ESOCs.
- Development of an endpoint-monitoring plan.
- Identification and undertaking of research gaps.

Table 3.3. Summary of the chemicals present in biosolids and considered for the different studies discussed in this chapter.

Imperial College Reviews		**Danish Risk Assessment**	**Norwegian Risk Assessment**	**WEAO Reports**	**WERF Studies**
Smith (2009)[a]	Clarke and Smith (2011)[a]	Jensen *et al.* (2012)	Eriksen *et al.* (2009)	WEAO (2001, 2010)	Higgins *et al.* (2010)[c]
>1,000: • LASs • PCAs >100 & <1,000: • NP/NPEs • MAHs • PAHs • DEHP >1 & <100: • TCC • Musks • TCS <1: • OTs • PCBs • PBDEs • PCNs • OC pesticides	>1,000: • Steroids: cholesterol, coprostanol, epicoprostanol • QACs >100 & <1,000: • PCAs • PDMSs • Steroids: campesterol, cholestanol, stigmasterol >1 & <100: • PAEs • TCC • Synthetic musks • TCS • Antibiotics and pharmaceuticals • PBDEs <1: • OTs • BPA • PCNs • PFCs • Steroids: E1, E2, E3, EE2	• BFRs • Musks • Pharmaceuticals • PCBs • PFCs	• Metals (Cd, Cr, Cu, Hg, Ni, Pb, Zn) • Phthalates • OP/OPEs • NP/NPEs • LASs • PCBs • PAHs • Pharmaceuticals: atorvastatin, carisoprodol, chlorprothixene, CIP, dipyridamole, fexofenadine, gabapentin, levetiracetam, losartan, mesalazine, MTP, ranitidine, sotalol, TC • Inorganic compounds (Ag, As, Be, Bi, Mo, Sb, Se, Sn, V, W)[b] • OTs[b] • BFRs (PBDEs, TBBPA, HBCD)[b] • Chlorophenols[b] • Chlorobenzenes[b] • PCAs[b] • PFCs (PFOS, PFOA)[b] • BPA[b] • TCS[b] • Musks (galaxolide, tonalide)[b] • BHT[b] • Pesticides (Irgarol, Diuron, glyphosate)[b] • DEET[b]	• Regulated inorganics (As, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Se, Zn) • Unregulated inorganics (Ag, Al, B, Ba, Be, CN^-, F^-, Mn, Sb, Sn, Tl, Ti, V) • Asbestos • Pathogens • VOCs • PCBs • PAHs • Pesticides • LASs • APs • Estrogenic hormones • PCDD/Fs • Pharmaceuticals • Radionuclides • Nutrients (N, P) • Plasticizers • PBDEs • BPA • PFCs • Musks • TCS • TCC • PCPs	• Aliphatics • Antibiotics • Antimicrobials • BFRs • Chlorobenzenes • Cyclics • Dioxins • Musks • Nitrosamines • Organotins • Other PPCPs • PAHs • PCBs • Pesticides • PFCs • Phenols • Phosphate esters • Plasticizers • Steroidal chemicals • Surfactants

[a] Values correspond to average concentrations in biosolids (mg/kg dry weight).
[b] These compounds were not included in the risk assessment due to lack of data.
[c] For the full list of individual compounds see Higgins *et al.* (2010).

Table 3.4. Summary of the recommendations and research priorities identified by the studies discussed in this chapter.

Imperial College Reviews		**Danish Risk Assessment**	**Norwegian Risk Assessment**	**WEAO Reports**	**WERF Studies**
Smith (2009)	Clarke and Smith (2011) [a]	Jensen *et al.* (2012)	Eriksen *et al.* (2009)	WEAO (2001, 2010)	Higgins *et al.* (2010)
More research necessary on: • PCAs toxicity • Antibiotic resistance • TCS and PPCPs effects on soil microorganisms	10/11: • PFCs 9/11: • PCAs • PCNs 7/11: • PBDEs • OTs • TCS • TCC 6/11: • Benzothiazoles 5/11: • Antibiotics and pharmaceuticals 3/11: • Synthetic musks 2/11: • BPA • QACs • Steroids 1/11: • PAEs • PDMSs	Low risk [b]: • BFRs • Musks • Pharmaceuticals • PCBs • PFCs [c] For future review [d]: • PCAs • PCNs • TCC • TCS • Parabens	Chemicals studied (Table 3.3) not expected to constitute a significant risk to humans and biota in Norway Recommendations: • Reduce levels of Cd, Cu, Hg, Zn in biosolids • Monitor new chemicals in biosolids • Generate more experimental data	Group I [e]: • Regulated inorganics (As, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, Se, Zn) • VOCs • PCBs • PAHs • Pesticides • LASs • APs • PCDD/Fs • Radionuclides • Nutrients (N, P) • Plasticizers • Hormones Group II [e]: • Unregulated inorganics (Ag, Al, B, Ba, Be, CN^-, F^-, Mn, Sb, Sn, Tl, Ti, V) • Asbestos • **Pathogens** • Pharmaceuticals • PBDEs • BPA • **PFCs** • Musks • TCS • TCC • **PCPs**	High priority: • BFRs (PBDEs, HBCD, TBBPA) • PFCs • Antimicrobials (TCC, TCS) • Antibiotics (4-ETC, CIP, DTC, MCZ, OFL, TC) • Musks (HHCB, AHTN) • Other PPCPs (Cimetidine) • Plasticizers (BPA) • Synthetic steroidal chemicals (EE2, MeEE2) • Surfactants (4-cumylphenol, OP) Low priority [f]: • Aliphatics • Organotins • Phenols • Phosphate esters • PPCPs • Steroidal chemicals • Surfactants

[a] Values correspond to the research priority index developed by Clarke and Smith (2011) as detailed in the text.
[b] Considered to pose very low risk, mainly due to the low levels found in Danish biosolids.
[c] PFOS might pose a risk to soils, but its banning will result in lower levels.
[d] Recommended for future study but not evaluated in this risk assessment.
[e] Group I: compounds with enough information to ensure adequate protection to human and environmental health. Group II: compounds without sufficient information. Compounds in bold: research priority.
[f] For full list of low priority compounds see Table 2-1 in Higgins *et al.* (2010)

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4. RISK ASSESSMENT OF PATHOGENS IN BIOSOLIDS-AMENDED SOILS

4.1. Historical and current regulations for pathogens in biosolids

In the United States, concerns regarding pathogens in sewage sludge disposed or applied to land were first addressed in 1979, when the USEPA adopted guidelines that required sewage sludge to be treated before application to reduce pathogen levels and vector attraction (Farrell, 1992). In 1982 an Intra-Agency Sludge Task Force was created, which recommended that a regulatory program be developed for sewage sludge management. This program required states to submit, review and approve sewage sludge management programs (Farrell, 1992). In 1980, the USEPA began developing the technical criteria for the use or disposal of sewage sludge (the name "biosolids" was introduced later, s. Chapter 2.1.1), which eventually became the CFR 40 Part 503 rule, more commonly known simply as the Part 503 rule (Farrell, 1992; USEPA, 1993). The pathogen standards of the Part 503 rule were not developed using a risk-based framework as the standards for the chemical pollutants (NRC, 2002); instead, they were based on the experimental work of Yanko (1988), who reported a good correlation between fecal coliform densities and the frequency of salmonellae detection, and who also demonstrated that the likelihood of *Salmonella* detection was rare if the density of fecal coliforms in sewage sludge was below 1,000 most probable number (MPN) per gram of total dry solids.

The USEPA Part 503 rule, issued in 1993, remains in effect today (USEPA, 1993). The pathogens it considers for regulation in biosolids are *Salmonella* and fecal coliforms (as an indicator organism; see section 4.2.1 below). Based on the content of one of these two types of organisms, the Part 503 rule defines two classes for biosolids intended for land application: Class A and Class B.

In order to be classified as Class A, the density of *Salmonella* in biosolids must be less than 3 MPN/4 g total solids (dw), or contain less than 1000 MPN/g of fecal coliforms, as defined by Yanko (1988). In addition, biosolids must be subject to one of the processes to further reduce pathogens (PFRP) defined in the Part 503 rule, which include digestion, composting, pH adjustment, pasteurization, and other thermal processes. Alternative treatments are allowed as long as the density of enteric viruses is reduced to less than 1 plaque forming unit (PFU)/4 g total solids (dw), and the viable helminth ova to less than 1 per 4 g total solids (dw).

Class B biosolids have no restriction on *Salmonella*, enteric viruses, or helminth ova, but the density of fecal coliforms should be less than 2,000,000 MPN/g total solids (dw) (or less than 2,000,000 colony forming units (CFU)/g total solids (dw)). Alternatively, one of the processes defined by the rule and collectively known as "processes to significantly reduce pathogens" (PSRP) can be utilized. The PSRPs include aerobic and anaerobic digestion, composting and lime stabilization, among others (USEPA, 1993).

In 2002, the US National Academy of Sciences evaluated the Part 503 rule in their document *Biosolids Applied to Land: Advancing Standards and Practices* (NRC, 2002).

The report found "no documented scientific evidence that the Part 503 rule has failed to protect public health," but it also acknowledged the continual need to update the scientific basis of the rule through research and risk-assessment methods. Additionally, most of the American states have enacted stricter land application rules compared to the Part 503 standards due to criticisms to the rule, including questions on its scientific bases and anecdotal evidence of health issues related to biosolids land application (Viau *et al.*, 2011).

In Canada, the end use of biosolids is governed at the provincial/territorial level through regulatory approvals, permits, and licences, whether it is disposal or land application. In some provinces (British Columbia, Québec, Manitoba, and Saskatchewan), USEPA standards for land application have been adopted. In other provinces (Alberta, Nova Scotia, and Ontario), standards were developed from pre-existing guidelines. Manitoba and New Brunswick use standards based on CCME guidelines (CCME, 2010).

Therefore, the pathogens and pathogen indicators currently regulated in Canada vary between provinces/territories. Some of them have standards for pathogens and pathogen indicators, while others, such as Alberta and Manitoba, have standards for treatments instead. As summarized in the review elaborated by the Canadian Council of Ministers of the Environment (CCME, 2010), the most commonly regulated pathogen is *Salmonella*. Most provinces require less than 3 MPN/4 g total solids (dw), although compost regulated under the Fertilizers Act and Regulations, governed by the Canadian Food Inspection Agency (CFIA) requires *Salmonella* to be not detectable. Pathogen indicators include fecal coliforms and *E. coli*. Most provinces require <1000 MPN/g fecal coliforms, although some provinces (Nova Scotia and British Columbia) allow Class B biosolids to have <2,000,000 MPN/g fecal coliforms.

Under Ontario's updated 2011 regulations, there are two pathogen reduction categories: CP1 and CP2. Besides containing less than 1,000 *E. coli* CFU/g total solids (dw) and less than 3 *Salmonella* CFU (or MPN)/4 g total solids (dw), CP1 biosolids (or other material containing human body waste), must show no detectable levels of viable helminth ova and total culturable enteric viruses in 4 g total solids (dw) (NMA, 2002; CCME, 2010). (For aqueous materials with less than 1% solids, the numerical limits are the same but measured in a 100 mL basis.)

In the European Union (EU), land application of biosolids is governed by a 1986 Directive (EEC, 1986). Although no specific pathogen limits are described, the Directive prohibits land application of untreated sludge unless it is injected or incorporated into the soil, and it also sets waiting periods for the use of sludge in fields intended for the production of fruits and vegetables, or for animal grazing. If desired, individual countries within the EU can set their own pathogen standards. The Directive is currently undergoing reassessment, and the inclusion of pathogen reduction goals has been proposed (European Commission, 2000), including both conventional and advanced (hygienisation) treatments. Conventional treatment would need to achieve at least a 2 $\log_{10}$ reduction in *E. coli*, whereas advanced treatment would require a minimum of a 6 $\log_{10}$ reduction in *E. coli* to less than 5×10^2 CFU/g, and no detectable *Salmonella* spp in 50 g (wet weight) of treated sludge (European Commission, 2000).

In summary, Canadian and American biosolids regulations are similar, with both regulating the densities of *Salmonella*, fecal coliforms and *E. coli*. Some Canadian provinces have also adopted helminth and enteric virus standards that are stricter than the USEPA standards. The European Union currently does not have a blanket pathogen standard for all member states.

4.2. Pathogens and indicator organisms in biosolids-amended soils

Pathogens found in biosolids can be divided into four major categories: bacteria (sub-divided into indicator bacteria, pathogenic bacteria, and 'emerging' bacteria, of which little is known), viruses, protozoa, and helminth worms. The main organisms of concern were listed in Table 2.2. Two comprehensive reviews of pathogens and indicator organisms found in biosolids have been published recently and are discussed below (Sidhu and Toze, 2009; WEAO, 2010), followed by a review of the published risk assessments of pathogens and indicator organisms in sludges and biosolids; individual emerging pathogens of concern are discussed in the appendix.

4.2.1. Indicator organisms

The concept of indicator organisms is based on the idea that their presence indicates that other organisms, usually pathogenic, will be present as well (Gerba, 2014). Indicator organisms should be present whenever pathogens are present, and should originate from the same source (Gerba, 2014).

The use of indicator organisms began in the United States in 1914, when the US Public Health Service adopted coliform bacteria as indicator organisms for fecal bacteria contamination (Gerba, 2014), because the former are also found in warm-blooded animals' lower digestive tract and are excreted in feces in large numbers, making them easier to detect than the usually smaller populations of pathogenic bacteria of concern (Gerba, 2014), which may not always be present (e.g. *Salmonella*), or are present in low numbers (e.g. *Ascaris*), and/or are difficult to measure accurately (e.g. *Cryptosporidium*). Additionally, quantification of all pathogenic organisms in biosolids would not be possible due to the lack of analytical techniques for all of them, in addition to cost and time constraints (Sidhu and Toze, 2009).

Therefore, Canadian and American biosolids regulations use fecal coliforms as indicator organisms to signal the potential presence of pathogenic organisms, and treatment guidelines for biosolids may be based solely on indicator populations as discussed in the last section; e.g., Ontario CP2 biosolids pathogen standards only require the analysis of *E. coli* (it can be present in densities up to $2x10^6$ CFU/g dw). Higher treatment criteria often include both indicator and pathogenic organisms; e.g., Ontario CP1 biosolids discussed above.

However, the use of fecal coliform indicators does not adequately represent the presence of all pathogens, especially during disinfection and inactivation processes, and might underestimate microbial risks (Harwood *et al.*, 2005; Viau *et al.*, 2011). Consequently, the use of alternative organisms and suites of indicator organisms, such as *Clostridium perfringens* and *Enterococcus* spp, has been proposed (Harwood *et al.*,

2005; Sidhu and Toze, 2009; Viau *et al.*, 2011). For a review of alternative indicators, see Sidhu and Toze (2009).

4.2.2. Literature reviews on pathogens in biosolids

Sidhu and Toze (2009) reviewed the literature on human pathogens and their indicators in biosolids. They summarized the enteric pathogens commonly found in biosolids and their population densities, as well as the potential health risks. They also reviewed traditional and emerging microbial indicator organisms. More importantly, the authors summarized the main knowledge gaps that have prevented a quantitative risk assessment (Sidhu and Toze, 2009):

- Lack of analytical methods for the detection, enumeration, and viability of pathogens, especially viruses, protozoa, and helminths.
- Lack of data on the fate and behaviour of pathogens in biosolids.
- Lack of standardized quantitation methods, especially for viruses and protozoa.

Additionally, they recommended the use of a suite of organisms instead of a single indicator, including the use of alternative indicators that could eventually be used as index and model microorganisms; i.e., to serve as surrogates of pathogen presence (index organisms) and behaviour (model organisms).

The WEAO (2010) update report on the fate and significance of contaminants in biosolids summarized in Chapter 3.3.4 also reviewed the literature on pathogens, including areas of recent research growth, including the fate and transport of pathogens in the terrestrial environment, as well as the transfer of pathogens in biosolids to soils, with a large portion of this work being completed and funded by Ontario institutions and governmental bodies. The review also identified a number of research gaps, which were consistent with those identified by Sidhu and Toze (2009). These gaps included the need for analytical procedures to quantify pathogens in biosolids, as well as to assess their viability; the occurrence and fate of known and emerging pathogens; and the potential human health risks of viruses found in biosolids.

The update report concluded that from 2001, when the original report was published (WEAO, 2001), to 2010, not enough information had been generated "to assure the public that the current agricultural land application program/guidelines are adequate to protect the well-being of soils, crops, animals, human health, ground and surface water qualities" (WEAO, 2010), and that pathogens should continue to be classified as a Group II contaminant requiring additional research, as noted in Chapter 3.3.4.

Typical population ranges for groups of indicator and pathogenic organisms in raw sludge are summarized in Table 4.1, and a more detailed set of data, incorporating data on microbial densities in biosolids, is presented in Table 4.2.

Table 4.1. Indicator and pathogenic organisms density in raw sludge; summarized from WEAO (2010), and Sidhu and Toze (2009).

Group	**Organisms**	**Population ranges** (log_{10}/g dw) (CFU, MPN, PFU)[1]; typically microscopic counts for protozoa and helminths

Indicator bacteria	*E. coli* Fecal coliforms Total coliforms Fecal *Streptococcus*	6-7 7-8 8 7
Pathogenic bacteria	*Enterococcus* *Clostridium perfringens* *Salmonella* *Shigella* *Campylobacter* *Leptospira* *Vibrio cholerae* *Yersinia* *Listeria monocytogenes*	5-7 5-6 0-3 1 Present ~28% 1
Emerging pathogens	Verotoxigenic *E .coli*, (e.g., O157-H7) Antibiotic resistant bacteria (ARB) *Clostridium difficile* *Helicobacter, Mycobacterium, Aeromonas, Legionella, Burkholderia* also of concern (NRC, 2002; WEAO, 2010)	 Present 100% Present 100%; ~80% toxigenic
Enteric viruses	Total Enterovirus Human adenovirus Polio, Coxsackie, Echo, Hepatitis A, Norwalk, Roto- and Reo-viruses also of concern (NRC, 2002; WEAO, 2010)	0-2 2 1-2
Protozoa	*Cryptosporidium* Viable *Giardia*	1-2 1 0-2
Helminths	Total Viable *Ascaris* *Trichuris*, *Toxocara*, *Taenia*, *Necator* and *Hymenolepis* also of concern (WEAO, 2010)	 -1
[1]CFU: colony forming units; MPN: most probable number; PFU: plaque forming unit.		

4.3. Risk assessment of pathogens in the biosolids land application context

Several researchers have elaborated a number of risk assessments for different pathogens in the biosolids land application context. Risk scenarios have included different exposure routes to the pathogens in the biosolids, including soil and vegetable ingestion, and exposure to aerosol particles. These 3 exposure routes are considered the most relevant for pathogen risk assessment related to biosolids land application along with the consumption of contaminated water (Viau *et al.*, 2011; Teng, 2012).

Gerba *et al.* (2002) performed a risk analysis to evaluate the survival of emerging pathogens to Class B level biosolids treatment. As part of this work, the authors reviewed the occurrence and fate of enteric viruses in biosolids, because they are resistant to inactivation by heat and pH and have long survival rates. Furthermore, the types of illnesses attributed to these organisms had increased in the decade prior to the review.

The results of the risk assessment indicated that the yearly risk of infection with 2 different enteric viruses from a single exposure would exceed the USEPA recommendation of 1:10,000 only if pure biosolids were ingested. Ingestion of soil amended with biosolids by injection would result in risks below the USEPA

recommendation. Additionally, the authors considered that these risk numbers were overestimated because they assumed no virus inactivation, and 8 hours-per-day exposures to the biosolids. Moreover, at biosolids land application sites in Arizona, the viruses were undetectable 24 h after application.

Gale (2003, 2005) studied the risk of infection with 7 different pathogens (*Salmonella* spp, *Listeria monocytogenes*, *Campylobacter* spp, *Escherichia coli* O157, *Cryptosporidium parvum*, *Giardia*, and enteroviruses) from root crops such as carrots and radish in the UK, and concluded that the risks were very low. The highest predicted risk was for *C. parvum*, and it was equivalent to 1 infection every 45 years in the entire UK population, when considering the required waiting period after biosolids application. The analysis also showed that the waiting periods are crucial in reducing the risk, and their proper implementation makes it unnecessary to improve efficiency of sludge treatment beyond 99%, at least in the case of mesophilic anaerobic digestion, which was the type of treatment considered in this analysis.

The importance of waiting periods was stressed by Rahube *et al.* (2014), who measured the abundance of pathogenic bacteria, and antibiotic resistant coliforms and genes in vegetables grown in soil amended with biosolids and untreated sludge. Although they did not observe any significant difference between treated and untreated soils with respect to viable bacteria, amended soils increased the abundance of antibiotic resistance genes in some cases. However, a 15-month waiting period was sufficient to significantly attenuate the genes' abundance.

Brooks *et al.* (2005) and Tanner *et al.* (2008) studied the risk of exposure to aerosols produced during biosolids land application. They concluded that the annual probability of infection from aerosols for the nearest residents downwind was no greater than 7:100,000 (Brooks *et al.*, 2005). In contrast, a higher level of risk was found for the workers applying the biosolids to the land, ranging from 0.0001% to 34%/yr for low and high exposure scenarios (Tanner *et al.*, 2008). The high exposure scenario assumed poor-quality biosolids were being applied without personal protection equipment. From the 2 model organisms chosen, the risks were generally lower for *Salmonella* than for Coxsackievirus A-21 (an enterovirus) by several orders of magnitude due to the virus' infectivity.

The type of biosolids application had a higher impact on the magnitude of the risk compared to environmental factors, such as wind speed. Risk from microbial aerosolization after application was considered insignificant, because air samples collected days after application had no detectable biosolids-related microorganisms. The authors also concluded that in general, risks to workers at land application sites seemed lower than risks to WWTP workers (Tanner *et al.*, 2008).

In Canada, Flemming *et al.* (2009) conducted a preliminary screening level quantitative microbial risk assessment (QMRA) for enteric pathogens in six Ontario WWTPs utilizing mesophilic anaerobic digestion as sludge treatment. They analyzed primary sludge, liquid digested biosolids, and dewatered cake biosolids, freshly dewatered and after storage. They detected *Salmonella* and *Listeria* in 70-100% of primary sludge and liquid digested biosolids, and in 50-60% of dewatered cake biosolids. *Yersinia* was

found in 20-30% of samples from all treatment stages. The protozoa *Giardia* was found in 80% of cake biosolids, and *Cryptosporidium* was found in only 20% of the biosolids.

QMRA was conducted for pathogens for which human dose-response data were available (*Clostridium perfringens*, *Salmonella*, *Giardia*, and *Cryptosporidium*), and two human exposure routes were chosen: direct ingestion of biosolids-amended soil by children, and ingestion of aerosolized particles occurring during or shortly after application (Flemming *et al.*, 2009).

The authors concluded that risk to children from direct ingestion of soil with fresh biosolids application was very low, with *Salmonella* risk at 10^{-13} per day, and *Clostridium perfringens* risk at 10^{-7}-10^{-9} per day. Risks for *Cryptosporidium* and *Giardia* were considerably (5-$\log_{10}$) higher than for bacterial pathogens; however, the authors noted that this risk was based on relatively few samples (n=9), which were derived from molecular detection methods that do not have the capability to distinguish viable and/or infectious organisms (Flemming *et al.*, 2009).

More recently, Brooks *et al.* (2012) used QMRA to characterize direct and indirect exposure risks to land applied biosolids and animal manure. They found that the largest single exposure risks were associated to ingestion of recently amended soil in the case of the general public, and to fomite for occupational exposure. Enteric viruses and *Campylobacter jejuni* were the organisms associated to the greatest risk in most exposure scenarios. Their study showed similar results to Gale (2003, 2005) in relation to microbial dilution and inactivation with time, concluding that risks decreased significantly after application, and that waiting periods in agricultural amended-soils reduced risks from food crops to low by 4 months, and to insignificant after a year.

Comparing biosolids to manure land application, Brooks *et al.* (2012) concluded that their risks are driven by the high number of bacteria in the case of manure, and by the high infectivity of lower density viruses in the case of biosolids. For risks related to bacteria and other shared pathogens, manure generally ranked higher than biosolids. Although the relatively risks were dynamic; immediately after application, risk was high for all residuals in the following general order: bovine = poultry < swine ≤ biosolids. After a month, however, the risks from manure were higher, and in the long term, biosolids again showed the largest risk due to the presence of viruses.

Similar conclusions to Brooks *et al.* (2012) were reached by Viau *et al.* (2011) in a comprehensive review and analysis of the existing literature on microbial risk assessment of biosolids land application. After evaluating the different exposure pathways, they ranked the overall risks as follows: accidental direct ingestion > aerosol inhalation >> contaminated groundwater ingestion > contaminated food ingestion.

As part of their study, Viau *et al.* (2011) also estimated aerosol inhalation risks from adenovirus and norovirus for the first time, and compared them to *Salmonella* and enterovirus. Their results showed that the former 2 increased the risk by approximately 2 orders of magnitude, leading support to previous observations that the current choice of indicator organisms can underestimate risk estimates.

They also concluded that improving biosolids treatment would be the option of choice to decrease risk, because treating biosolids to Class A grade, especially through composting, would result in lowering exposure to pathogens by several orders of magnitude compared to setting buffers around biosolids land application sites, or modifying application practices to reduce aerosol generation (Viau *et al.*, 2011).

Viau *et al.* (2011) also reviewed the few available epidemiological studies, with the most comprehensive conducted close to 3 decades ago (Dorn *et al.*, 1985). The 3-year study by Dorn *et al.* (1985) involved close to 50 farms in Ohio where biosolids were applied (2-10 dry metric tons/ha/year), and included monthly health questionnaires and quarterly serological testing. The study found neither increased incidence of illness or symptoms in humans or domestic animals, nor differences in serological conversion rates in farms applying biosolids compared to reference farms with no application.

As noted by Viau *et al.* (2011), a more recent study by Khuder *et al.* (2007), which relied solely on questionnaires, showed a higher incidence of respiratory and gastrointestinal ailments in people living close to biosolids-amended fields. Other surveys have also reported adverse health effects and symptoms in residents near application sites, although most of them cannot be considered well-designed epidemiological studies (Jenkins *et al.*, 2007; Viau *et al.*, 2011).

Both Viau *et al.* (2011) and Jenkins *et al.* (2007) conclude that further epidemiology evidence is necessary to evaluate the potential links between residence in the vicinity of biosolids application fields and health effects. Viau *et al.* (2011) observed that practically no scientific follow-up has been given by the USEPA to anecdotal health complaints, and both groups agree in that timely investigation and documentation of these potential health impacts would contribute to clarify the possible links with biosolids exposure. A protocol to set up such an investigation and documentation system was developed (Aitken *et al.*, 2007) and tested (Liang *et al.*, 2012) in the United States (see Chapter 9.4).

4.4. Summary

Similar to the ESOCs, pathogen-related risks are dependent on the individual organism identity and show wide variations that also depend on the routes of exposure. Risk assessments have demonstrated high variation in risk (Viau *et al.*, 2011), from very low, such as in the case of exposure resulting from vegetable consumption, to high, as in the case of occupational exposure for workers at biosolids land application sites under worst-case conditions (Tanner *et al.*, 2008).

Other factors affecting the magnitude of the risks from pathogens in the context of biosolids land application are the type of treatment that the biosolids received, the level of dilution and incorporation to the soil, and the time elapsed from application to exposure. Thus, the magnitude of the risk can shift over time and it is site specific (Brooks *et al.*, 2012). As an example, Teng (2012) ranked the risk of ingestion of contaminated vegetables from a specific site as higher than aerosol inhalation and

contaminated groundwater consumption, whereas Viau *et al.* (2011) had estimated that the general risk of contaminated food ingestion would be lower than the other two.

In general, overall risks to the general population from exposure to pathogens from biosolids amended soils are considered low. In the specific case of the possibility of infection from ingestion of crops grown in biosolids amended soils, the risks are considered to be very low, especially if the waiting periods between application and harvest are observed (Gale, 2003, 2005; Brooks *et al.*, 2012).

However, currently used indicator organisms might underestimate risks by not considering pathogens that might have greater infectivity or be more resistant to inactivation during treatment or in the environment (Harwood *et al.*, 2005; Viau *et al.*, 2011). Additionally, although risk estimates always have a certain degree of uncertainty, the estimates for pathogen infection in the biosolids land application context can be relatively large (several orders of magnitude) because some of the factors contributing to the magnitude of the risk are not well understood. Examples of factors requiring better characterization are pathogen concentrations in biosolids, effects of treatment, inactivation in the environment, frequency and duration of exposure, dose-response relationships, and differences in human sensitivity to pathogens.

Finally, there has been very limited epidemiology work on the possible health effects of biosolids land application, and most of it was based on self-reporting questionnaires (Jenkins *et al.*, 2007; Viau *et al.*, 2011). Conducting full epidemiology studies is a complex and expensive task requiring large amounts of data (NRC, 2002). However, conducting scientifically based inquiries following reports of adverse health effects, especially by residents neighbouring biosolids-amended fields, would be a significant contribution to elucidate the possible links of these health effects to biosolids exposure, and could be used to collect some of the data necessary to plan a complete epidemiological assessment if necessary (Aitken *et al.*, 2007). A protocol to set up such an investigation and documentation system was developed (Aitken *et al.*, 2007) and tested (Liang *et al.*, 2012) in the United States (see Chapter 9.4).

Table 4.2. Microorganism densities (MPN or CFU/g (dw) unless noted otherwise) in raw sludge and biosolids.

Organism	Raw sludge	Lime stabilized	Anaerobic Digestion			Aerobic Digestion	Drying			Compost	Lystek	Reference
			Standard	High rate thru-put	Temperature phased		Heat	Solar	Non-solar			
Escherichia coli												
			1.0E+05		1.3E+04					6.0E+01		[1]
	3.0E+06		6.0E+03									[6]
	8.2E+06		1.5E+05									[2]
	>1.6E+03		>1.6E+03								<1.8E+00	[3]
						2E+06 /100mL						[9]
Fecal coliforms												
	4.2E+07						0	4.1E+ 03	6.0E+05			[4]
	1.53E+08		1.08E+06									[5]
			6.3E+05		4.0E+02					5.0E+01		[1]
	2.6E+07				3.7-7.4E+02							[7]
	2.0E+07		1.0E+04									[6]
	1.1E+07		2.0E+05									[2]
	>1.6E+03		>1.6E+03								<1.8E+00	[3]
Total coliforms												
	4.58E+08		4.13E+06									[5]
Fecal *Streptococcus*												
	1.71E+07		1.51E+06									[5]
Enterococcus spp												
			2.0E+05		1.0E+04					3.2E+03		[1]
	5.0E+05		1.0E+04									[6]
	2.4E+06		1.1E+05									[2]
Salmonella spp												
	4.71E+02		1.01E+01									[5]
			4.0E+01		ND					4.0E+00		[1]
	5.1E+02- 1.1E+04		5.0E+00- 1.1E+02									[8]

Organism	Raw sludge	Lime stabilized	Anaerobic Digestion			Aerobic Digestion	Drying			Compost	Lystek	Reference
			Standard	High rate thru-put	Temperature phased		Heat	Solar	Non-solar			
	2.50E+02		<2E-01									[6]
	4.3E+01		1.0E+01									[2]
		<0.6-1.3 /100 mL	2.00E+00	1.6-6.2/100 mL						<2E+00-4.4E+01		[9]
	positive		positive								negative	[3]
Campylobacter jejuni, in CFU/g (dw)												
	2.6E+03-3.8E+03		1.6E+00									[8]
Yersinia enterocolitica, in MPN/g (dw)												
	2.5E+00		8.5E-01									[2]
Clostridium spp, in CFU/g (dw)												
			1.6E+06		1.0E+06					6.3E+03		[1]
Clostridium perfringens												
			3.1E+03							1.0E+03		[1]
	7.2E+05		1.1E+06									[2]
Clostridium difficile, in GU/g (dw)												
			2.0E+02		1.0E+02					ND		[1]
Listeria spp												
			1.7E+02							2.5E+00		[1]
Listeria monocytogenes												
			2.9E+01							1.2E+00		[1]
	4.7E+01		1.3E+00									[2]
	2.3E+02-1.5E+03		8.3E-01-2.6E+01									[8]
Enteric viruses, in PFU/4g												
	1.9E+01		2.2E+01									[5]
			7.8E+02 (seeded)								<1.0E+00	[3]
in MPN/4g	6.7E+01-2.2E+03		2.9E+00-4.8E+02									[6]
Enterovirus, in PFU/g (dw)												
in MPN/g			7.9E+00		1.6E+00					2.5E+00		[1]
in MPN/4g			2E-01-6E+01									[11]

Organism	Raw sludge	Lime stabilized	Anaerobic Digestion			Aerobic Digestion	Drying			Compost	Lystek	Reference
			Standard	High rate thru-put	Temperature phased		Heat	Solar	Non-solar			
	2E+00-2.15E+02		5.9/100 mL	138/100 mL	< 3.3/100 mL	3.35E+00				<2.3		[10]
	4.8E+00-3.5E+02		1.0E-01-1.3E+02									[8]
Norovirus, in PFU/g (dw)												
	3.6E+03-2.0E+07		1.0E+03-3.0E+03									[8]
Helminths ova, numbers/4 g												
			1.3E+02 (seeded)								<1.0E+00	[3]

1. Viau *et al.* (2011)
2. Flemming *et al.* (2009)
3. Singh *et al.* (2008)
4. Ogleni and Ozdemir (2010)
5. Pepper *et al.* (2010)
6. Wong *et al.* (2010)
7. Riau *et al.* (2012)
8. Brooks *et al.* (2012)
9. USEPA (1991)
10. USEPA (1992)
11. Gerba *et al.* (2002)

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5. SELECTION CRITERIA FOR THE ESOCS AND PATHOGENS IN THIS REVIEW

5.1. ESOCs

As discussed in Chapter 3.4, the current chemical-by-chemical approach to ESOCs risk assessment does not fully address the ultimate goal of evaluating the possible effects of ESOCs as a group to human and/or environmental health, both in the global environment and in the biosolids land application context, and a new evaluation strategy is needed. Although the strategy is yet to be defined, some of its elements were described in Chapter 3.4, including the need of a prioritization system for the ESOCs to be assessed.

The development of such a prioritization system is complicated by the lack of data on the fate of ESOCs in biosolids-amended soils and, more importantly, of toxicity information, which prevents the development of formal risk assessments (Higgins *et al.*, 2010). Largely due to this reason, there is no general agreement between regulatory entities as to which compounds should be monitored and/or regulated, and the scope of potential regulations (MDDEP, 2012). For example, the European Union proposed the adoption of limits for the concentrations of AOX, DEHP, LASs, NPEs, PAHs, PCBs, and PCDD/Fs in sludge intended for land application in 2000 (European Commission, 2000), but these limits have yet to be adopted and remain controversial (Smith, 2009).

Although the prioritization strategy will be developed in due course with the participation of all stakeholders, ideally as part of the national consultation presented in Part B, a preliminary list of ESOCs considered relevant in the current Canadian context was generated for the purposes of this review. The choice of ESOCs for inclusion in the present work relied upon the recommendations found in the reports described in Chapter 3.3, summarized in

Table 3.3 and Table 3.4, and the literature review for individual ESOCs (Appendix A.1).

Also considered were compounds detected frequently and/or in high concentrations in Canadian biosolids analyzed as part of Environment Canada's Chemicals Management Plan (CMP) wastewater monitoring program (Guerra *et al.*, 2014). Additionally, several Canadian experts from academia and the federal and Ontario provincial governments provided several recommendations based on their professional judgement (Shirley Anne Smyth, Sonya Kleywegt, Chris Metcalfe, and Shelly Bonte-Gelok, personal communication).

ESOCs considered for this review were:

- Antibiotics:
  - Azalides: azithromycin (AZM)
  - Fluoroquinolones: ciprofloxacin (CIP), moxifloxacin (MOX), norfloxacin (NOR), ofloxacin (OFX)
  - Sulfonamides: sulfamethoxazole (SMZ), sulphanilamide (SUL)
  - Tetracyclines: doxycycline (DTC), 4-epitetracycline (4-ETC), tetracycline (TC)

- Antimicrobials: triclocarban (TCC), triclosan (TCS)
- Brominated flame retardants: polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA), decabromodiphenyl ethane (DBDPE), pentabromoethyl benzene (PBEB), 1,2-bis(2,4,6-tribromophenoxy)ethane (BTBPE)
- Musks: galaxolide (HHCB), tonalide (AHTN)
- Nanoparticles
- Organotins
- Perfluorinated compounds
- (Other) PPCPs:
  - Analgesics: acetaminophen (ACM)
  - Antacids (H2RA): cimetidine (CIM)
  - Antidepressants: citalopram (CTP), sertraline (SER), venlafaxine (VEN)
  - Antiepileptic: carbamazepine (CBZ)
  - Antifungals: clotrimazole (CTZ), miconazole (MCZ)
  - Anti-inflammatories (NSAID): ibuprofen (IBP)
  - Beta-blockers: atenolol (ATN), metoprolol (MTP), propranolol (PRP)
  - Diuretics: furosemide (FRS)
  - Hypolipidemic agents: gemfibrozil (GFB)
- Plasticizers: bisphenol A (BPA)
- Polychlorinated alkanes
- Polychlorinated naphthalenes
- Quaternary ammonium compounds
- Steroidal chemicals: ethinyl estradiol (EE2), methyl ethinyl estradiol (MeEE2)
- Surfactants: 4-cumylphenol, nonylphenol ethoxylates (NPEs), octylphenol ethoxylates (OPEs)

From the list of compounds above, nanoparticles have only recently been brought to attention in the biosolids context. They are unique amongst the ESOCs in the list not only because they are inorganic compounds, but also because they pose a new risk assessment question. Although the toxicity of the nanoparticles' component elements (e.g., Ag) has been relatively well studied, the effects of particle size on their toxic effects have not been characterized. This uncertainty is one of the reasons why nanoparticles are considered an emerging issue, the other being their growing use in consumer products. For example, silver concentrations in Swedish wastewater sludge decreased steadily during the first decade of the 21st century due to the abandonment of film in favour of digital photography, but concentration declines stalled later in the decade and started to increase again around 2010 due to the increased use of silver nanoparticles in consumer products (KEMI, 2012).

5.2. Pathogens

Currently, the only regulated pathogens in biosolids in both Canada and the United States are *Salmonella*, helminths, enteric viruses, and fecal coliforms. However,

several relatively recent literature reviews and a group of American experts have identified a series of 'emerging' pathogens of concern in biosolids, which are summarized in Table 5.1.

Table 5.1. Emerging pathogens of concern in biosolids.

Category	Organism	Disease/Symptoms	Reference(s)
Bacteria	*Mycobacterium*	Tuberculosis, leprosy	NRC (2002)
	E. coli O157:H7	Gastroenteritis, hemorrhagic colitis, hemolytic uremic syndrome	Gerba *et al.* (2002); NRC (2002); Yanko (2004); Sidhu and Toze (2009)
	Legionella	Legionellosis, Pontiac fever	NRC (2002)
	Listeria	Listeriosis	Gerba *et al.* (2002); NRC (2002); Sidhu and Toze (2009)
	Helicobacter	Gastric disease (gastric cancer, ulcers)	Gerba *et al.* (2002); NRC (2002); Yanko (2004); Sidhu and Toze (2009)
	Aeromonas	Gastroenteritis, soft-tissue and systemic infections	NRC (2002)
	Clostridium difficile	Diarrhea, colitis	Xu *et al.* (2014)
	Burkholderia	Respiratory infections in cystic fibrosis patients	NRC (2002)
	Antibiotic resistant bacteria	Specific to the resistant bacterium	Pepper *et al.* (2006); WEAO (2010)
Fungi	Microsporidia	Gastroenteritis, acute and chronic diarrhea	NRC (2002)
Viruses	Adenovirus	Gastroenteritis and respiratory infection	NRC (2002); Sidhu and Toze (2009)
	Norovirus/Norwalk-like viruses	Gastroenteritis	NRC (2002); Sidhu and Toze (2009)
	Astrovirus	Gastroenteritis	NRC (2002)
	Hepatitis A, E	Infectious hepatitis	NRC (2002); Sidhu and Toze (2009)
	Rotavirus	Gastroenteritis	NRC (2002)
	Polyomavirus	Polyomavirus-associated nephropathy, hemorrhagic cystitis and multifocal leukoencephalopathy	Sidhu and Toze (2009)
	H5N1, H5N2 avian influenza	Influenza, respiratory distress	WEAO (2010)
	H1N1 swine influenza		
Other	Prions	Transmissible spongiform encephalopathies	Pepper *et al.* (2006)
	Endotoxins	Fever, asthma	NRC (2002); Pepper *et al.* (2006)

The USEPA and the USDA conducted a joint expert meeting in 2001, where several new organisms of concern were identified as discussed in Gerba and Smith (2005). A year later, the US National Academy of Sciences published a report (NRC, 2002) where they identified emerging pathogens that were likely to be present in biosolids, and which of these warranted further investigation based on then-current detection and quantification methodologies, and their probability of survival during biosolids treatment and in the environment.

Additionally, Pepper *et al.* (2006) and Sidhu and Toze (2009) reviewed the literature on human pathogens in biosolids, including several bacteria and viruses considered of emerging concern. Most recently, a report by WEAO (2010) summarized the emerging pathogens reiterated here, and identified several additional viral pathogens, including the H1N1 swine influenza virus, as potential emerging pathogens of concern.

Several of the reviews mentioned above have discussed the pathogens of concern in biosolids—including those considered as 'emerging'—in detail, and a brief overview of these organisms can be found in the appendix. Additionally, the potential for antibiotic resistance bacteria development is discussed in Chapter 7.3.

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6. EFFECTS OF SLUDGE TREATMENT (BIOSOLIDS PRODUCTION) METHODS ON ESOCS AND PATHOGENS

6.1. ESOCs

For a variety of reasons, it might be deemed necessary to limit the concentration of some ESOCs in biosolids, as it is already the case for some chemicals. And because the vast majority of ESOCs present in biosolids come from sewage—although some chemicals could potentially originate from additives used as part of the wastewater or sludge treatment processes, the two logical options to eliminate or decrease the concentrations of ESOCs in biosolids are source control and sludge treatment.

Source control was an effective tool to reduce the concentrations of heavy metals in sludge and biosolids in the 1970s and 1980s (Paulsrud and Nedland, 1997; CLABS *et al.*, 1999; Draeger *et al.*, 1999; Water UK, 2006). Outright bans of some chemicals, such as POPs, had the same effect or are expected to, as it was the case for PCBs and PCDD/Fs in most industrialized countries (Clarke and Porter, 2010; Olofsson *et al.*, 2012).

However, it is not always desirable to ban chemicals because of the benefits we derive from them, as it is the case for antibiotics. A ban is not even an alternative for substances such as natural hormones, considered ESOCs due to their endocrine-disrupting potential, especially for aquatic organisms (Mills and Chichester, 2005). For other chemicals, a ban might not achieve the reduction in biosolids concentrations at a rapid rate, as it was the case for DEHP in Sweden, whose usage has declined approximately 40% since 2000, but its concentrations in municipal sewage sludge have remained constant, presumably due to the ubiquitous presence of DEHP in materials such as vinyl flooring (Olofsson *et al.*, 2012).

Consequently, when source reduction is not an option, ESOCs concentrations in biosolids have to be controlled at the biosolids production (aka sludge treatment) stage, which is part of the wastewater treatment process. During this process, ESOCs are subject to two stages of treatment, and both can influence ESOCs concentrations: the wastewater treatment per se, and the sludge treatment that results in the biosolids themselves.

However, most treatment processes in place now, both for wastewater and sludge, were not originally designed to eliminate ESOCs. Numerous studies and literature reviews have addressed the effectiveness of wastewater treatment to eliminate ESOCs from the liquid phase (Petrović *et al.*, 2003; Lishman *et al.*, 2006; Stephenson and Oppenheimer, 2007; Miège *et al.*, 2009; Zabczynski *et al.*, 2010; Deblonde *et al.*, 2011; Bell *et al.*, 2012; Rojas *et al.*, 2012; Verlicchi *et al.*, 2012; Guerra *et al.*, 2014; Luo *et al.*, 2014). Additionally, Garcia-Rodríguez *et al.* (2014) recently published an extensive review on the removal of ESOCs in biologically-based wastewater treatment systems, such as constructed wetlands.

In general, the effectiveness of wastewater treatment is variable, with some ESOCs being almost completely removed from the liquid phase and others being released with

the effluent. ESOCs removal from the liquid phase might result from microbial degradation or from sorption to the solids and elimination with the residual sludge, especially in the case of hydrophobic compounds.

Research on the effects of the different sludge treatments on ESOCs has been typically conducted either by analyzing ESOCs concentrations or by measuring bioassay responses along the process. The first part of this chapter focuses on studies that used chemical concentrations to evaluate the efficiency of sludge treatment, and the second reviews the studies that used bioassays.

6.1.1. Effects of sludge treatment on ESOCs concentrations

Because the wastewater and sludge treatment systems in current use were not originally designed to remove trace chemicals, they do not necessarily eliminate ESOCs efficiently. In addition, many of the ESOCs are removed from the liquid phase of wastewater and accumulate in the sludge due to their hydrophobic nature (CCME, 2010).

Most of the existing studies on the fate of ESOCs during wastewater treatment focused on elimination from the liquid phase, mainly because of the link between WWTP effluents and endocrine disruption effects observed in biota in receiving waters (Furlong *et al.*, 2010), but also due to the difficulty of measuring ESOCs in sludge and biosolids, especially when present in relatively low concentrations (Citulski and Farahbakhsh, 2010; Furlong *et al.*, 2010).

However, interest in the effectiveness of treatment increased as evidence of the presence of ESOCs in biosolids emerged. Several studies have been published on this subject, either by reviewing available information, or by conducting experiments designed to elucidate the fate of different classes of ESOCs during sludge treatment.

Literature reviews

The Canadian Council of Ministers of the Environment (CCME) commissioned a comprehensive literature review, published in 2010, on the occurrence of ESOCs in Canadian biosolids and the effects of sludge treatment on ESOCs concentrations (CCME, 2010). Data published before mid 2009 for the following chemical categories were compiled and reviewed: industrial chemicals (e.g., plasticizers, PFCs); APs and APEs; flame retardants; hormones, steroids and sterols; pharmaceuticals; PCPs; metals; others, such as PAHs and PCDD/Fs.

The authors found that the occurrence of some chemicals in Canadian biosolids is well documented (e.g., NP, AHTN, HHCB), whereas no data exists for others, such as “many antibiotics and pharmaceuticals” (CCME, 2010). They also concluded that information on ESOCs removal during sludge treatment was even scarcer; most studies focused exclusively on ESOCs concentrations in the biosolids and did not report concentrations in the original sludge. Data were especially lacking for composting, alkaline stabilization, and drying, whereas anaerobic digestion was better documented. Their conclusions on the potential of the different sludge treatments for ESOCs reduction are summarized in Table 6.1.

The abundance of "NA" in Table 6.1 underscores the lack of data on the effectiveness of sludge treatment on ESOCs concentrations. Additionally, the authors remarked on the lack of consistency in the reporting of data in the literature, especially the inadequate description of the sludge studied, the failure to report whether it was treated or not, and the omission of its origin; e.g., from primary or secondary treatment (CCME, 2010). This might indicate that the majority of studies were not designed primarily to evaluate the efficacy of sludge treatment and were only interested in ESOCs abundance in the final biosolids.

Table 6.1. Potential of sludge treatment processes for ESOCs reduction according to CCME (2010).

Compound (class)	Aerobic digestion	Anaerobic digestion	Composting	Alkaline stabilization	Drying (heat)	Other drying (e.g. air or solar)
Industrial chemicals						
Bis(2-ethylhexyl phthalate)	NA	R	R	NA	R	NA
APs and APEs	R	X	R	R?	R?	NA
BPA	NA	X	NA	NA	NA	NA
Linear alkylbenzene surfactants	R	NA	NA	NA	NA	NA
PFCs	NA	NA	NA	NA	NA	NA
BFRs	NA	R	NA	NA	NA	NA
Personal Care Products						
Antimicrobials	NA	X	NA	NA	NA	NA
Nitro musk fragrances	R	R	NA	NA	NA	NA
Polycyclic musk fragrances	X	X	NA	NA	NA	NA
QACs	NA	NA	NA	NA	NA	NA
Siloxanes	NA	NA	NA	NA	NA	NA
Metals						
Organotins	NA	X	NA	NA	NA	NA
Other						
Polyaromatic hydrocarbons	NA	NA	NA	NA	NA	NA
PCDD/Fs	NA	NA	NA	NA	NA	NA

R: some reduction indicated; R?: mixed results; X: no reduction; NA: no data available

A contemporary review to CCME (2010) published by Citulski and Farahbakhsh (2010) on endocrine disrupting chemicals (EDCs) also emphasized the disproportionate number of studies for a few chemicals and the lack of studies for others. They found that the chemicals receiving most attention, such as the NPEs, were those present in relatively high concentrations in biosolids (ppm or ppb). In contrast, they found a lack of studies for compounds present in lower concentrations, such as the estrogenic hormones, even though the latter are considered more potent EDCs, presumably due to the difficulty of measuring low concentrations of these compounds in complex matrices. They also concluded that most studies have focused on anaerobic and aerobic digestion, whereas composting and alkaline stabilization are less studied.

Not surprisingly, more recent reviews have reached similar conclusions to CCME (2010) and Citulski and Farahbakhsh (2010), such as Hernandez-Raquet (2013), who authored an extensive review on the fate of APEs, hormones, and pharmaceuticals during aerobic and anaerobic sludge treatment, and Hamid and Eskicioglu (2012) who reviewed the fate of estrogenic hormones during wastewater and sludge treatment.

A review by Stasinakis (2012) focused on sludge anaerobic digestion, and remarked on the contradictory ESOCs removal results observed for certain chemicals, such as estrogens, LAS, and some PCPs. Stasinakis (2012) also reviewed the studies on the effects of ESOCs on the anaerobic digestion process itself; although most of the studies are limited to surfactants and antibiotics, the author concluded that these effects, if any, also vary widely with the nature of the compounds. However, the concentrations used for most of these studies, all laboratory scale, were high relative to those normally found in sludge (Carballa *et al.*, 2007).

Additionally, Stasinakis (2012) concluded that the few published studies on sludge pretreatment including thermal hydrolysis, ozone and ozone pretreatment, do not seem to be particularly beneficial for ESOCs degradation, whereas the use of additional treatment post AD (post-aeration, composting, post-ozonation) improved the removal of EDCs.

Experimental studies – Type of sludge treatment process

In addition to the literature reviews, several experimental studies addressing the fate of different ESOC classes during sludge treatment have been published after the extensive CCME review of 2010, and in general, the conclusions reached by these studies support the conclusions reached by the review. These studies also focus mainly on anaerobic digestion and are generally limited to a few chemicals, usually those considered endocrine disruptors and/or pharmaceuticals.

Furlong *et al.* (2010) conducted a two-year study involving four full-sized American WWTPs to assess the fate of estrogenic compounds during sludge treatment. The plants incorporated different sludge thickening, stabilization, dewatering, conditioning and other processes, from lime addition to composting and pelletization. However, the high variability in the results and the limited number of samples did not allow the formulation of definitive conclusions on the differences between treatments, but the study yielded relevant information on the ultimate fate of a subset of ESOCs during sludge treatment.

In all four plants, Furlong *et al.* (2010) observed a reduction in the total mass loads of estrogenic ESOCs from the influent entering the plants to the biosolids exiting the process. This observation held also in the two cases where they evaluated ESOCs loads in the liquid effluents, suggesting biodegradation as the main mechanism responsible for the reduction of estrogenic compounds, which was also observed by other researchers; e.g., Samaras *et al.* (2013).

The authors also concluded that hormones are greatly reduced during secondary treatment as opposed to simply accumulating in the solid phase, supporting Hernandez-

Raquet (2013) conclusion in her review that hormones are mainly eliminated through microbial degradation during wastewater treatment. They also observed a better removal of androgens compared to estrogens, which they attributed to a higher susceptibility to biodegradation due to the lack of the aromatic ring characteristic of estrogens.

In addition to estrogenic compounds, Furlong *et al.* (2010) also evaluated the fate of other ESOCs. As expected, they found that some of them were removed efficiently (e.g., caffeine, acetaminophen), whereas others were recalcitrant in both the liquid and solid phases (e.g., carbamazepine). Recalcitrant ESOCs, especially those with high log K_{ow} values, tended to accumulate in the solids as treatment progressed (e.g., from secondary to composted sludge).

The overall reduction in ESOCs concentration did not imply a reduction of every chemical in every part of the treatment process (Furlong *et al.*, 2010). For example, the concentration of nonylphenol mono- and di-ethoxylates increased during activated sludge treatment due to transformation of their parent products. Similarly, estrogenicity (as measured through bioassays) increased through most of the sludge treatment processes, although overall estrogenicity decreased when considering the wastewater treatment processes as a whole in the cases where it was evaluated (see Chapter 6.1.2 below for more details).

Another important conclusion of this study was that, although a positive correlation was observed between log K_{ow} values and partitioning to solids, even compounds with low log K_{ow} such as caffeine were present in the solid phase, suggesting that other factors also influence chemical partitioning between the liquid and solid phases. This phenomenon was also observed by Salveson *et al.* (2012) and by Guerra *et al.* (2014).

Some of the observations made by Furlong *et al.* (2010) were later confirmed by Salveson *et al.* (2012) in a study that evaluated the fate of a subset of ESOCs during anaerobic digestion, both in a full size WWTP and in a laboratory reactor.

ESOCs evaluated in the WWTP were atenolol (ATN), bisphenol A (BPA), caffeine, carbamazepine (CBZ), *N,N*-diethyl-3-methylbenzamide (DEET), fluoxetine, gemfibrozil (GFB), meprobamate, *tris*(2-chloroethyl) phosphate (TCEP), *tris*(2-chloro-1-methylethyl) phosphate (TCPP), triclocarban (TCC), and trimethoprim. ESOCs studied in the reactor were benzophenone, caffeine, cimetidine (CIM), DEET, diphenhydramine, fluoxetine, ibuprofen (IBP), naproxen (NPX), sulfamethoxazole (SMZ), TCEP, TCC, triclosan (TCS), and trimethoprim.

Salveson *et al.* (2012) found caffeine in both primary and secondary sludge from the WWTP, supporting Furlong *et al.* (2010) observation that even hydrophilic compounds can be present in sludge, especially when they are present in high concentrations in raw wastewater. Caffeine concentrations were higher in primary than secondary sludge, presumably due to the compound's high susceptibility to microbial degradation during the activated sludge treatment.

Salveson *et al.* (2012) also observed that hydrophobic compounds with high sorption potential to sludge and resistant to biodegradation, such as BPA, TCC, TCPP, CBZ, and fluoxetine, increased in concentration through anaerobic digestion because they were not degraded as the rest of the organic matter. Except for the most biodegradable compounds (ATN, caffeine, and trimethoprim), the concentration of the ESOCs measured in the WWTP did not decrease significantly during anaerobic digestion.

In general, the behaviour observed in the WWTP corresponded to that in the laboratory reactor for ESOCs that were highly (>90%; e.g. ATN, caffeine, trimethoprim) or poorly (<15%; e.g. BPA, CBZ, fluoxetine, GFB, TCEP, TCPP) removed. Compounds whose removal was between 15 and 90% (e.g., DEET and TCC) tended to show lower removals in the laboratory compared to the full size anaerobic digester.

Perhaps a more important proposal stemming from this research was the use of indicator compounds that would help predict the behaviour of a larger number of compounds during activated sludge treatment without necessarily having to test them. Salveson *et al.* (2012) selected 22 compounds based on their concentration and frequency of detection in wastewater, their physicochemical properties, and the availability of analytical methods. They classified these 22 chemicals in 9 groups based on their sorption (log K_d) and biotransformation (log k_b) parameters during activated sludge treatment (Table 6.2); e.g., chemicals with rapid biotransformation rates (high log k_b) and high sorption (high log K_d); chemicals with moderate biotransformation rates (intermediate log k_b) and low sorption (low log K_d).

The authors then evaluated the removal of the indicator compounds in seven WWTPs with different treatment processes and, in general, they were able to anticipate an expected range of removal for ESOCs based on their biotransformation and sorption characteristics. Not surprisingly, compounds with rapid biotransformation rates (high log k_b) and low sorption potential (low log K_d) showed the highest removals. Although these indicators were developed for removal from the liquid phase during activated sludge treatment, an analogous set of indicators could be developed for sludge treatment.

Table 6.2. Indicator compounds for activated sludge treatment classified by sorption and biotransformation properties according to Salveson *et al.* (2012).

		Biotransformation (k_b, L/g-d)		
		Slow, <0.1	**Moderate, 0.1-10**	**Rapid, >10**
Sorption (log K_d)	**Low, <2.5**	Carbamazepine Meprobamate Primidone TCEP Sucralose	DEET Sulfamethoxazole Gemfibrozil Iopromide Trimethoprim	Acetaminophen Caffeine Naproxen Ibuprofen Atenolol

	Moderate, 2.5-3	TCPP	Cimetidine	Benzophenone Diphenhydramine Bisphenol A
	High, >3	Triclocarban		Triclosan Fluoxetine

In a different study, Martín *et al.* (2012) studied the fate of 16 ESOCs (CBZ, caffeine, clofibric acid, DCF, E1, E2b, E3, EE2, GFB, IBP, KFN, NPX, PRP, salicylic acid, SMZ, and trimethoprim) in sludge during anaerobic digestion in 4 WWTPs in Seville, and in a composting facility. Except for caffeine and E2b, the concentration of all of the ESOCs decreased during anaerobic digestion, in contrast to the results reported in the studies above. The authors attributed the E2b increase to the cleavage of conjugated derivatives. Mean concentrations in compost (9.19 - 974 µg/kg dw) tended to be higher than in the digested sludge (3.29 - 636 µg/kg dw), with the exception of caffeine, CBZ, E2b, and NPX. The authors suggested that the increase in concentrations was mainly due to the loss of organic matter and the relative recalcitrance of the ESOCs, whereas those whose concentrations decreased would have been more amenable to aerobic degradation during composting.

In a more recent study, Martín *et al.* (2015) investigated the fate of the same ESOCs as in the previous study plus 6 additional compounds (ACM, ATN, bezafibrate, CIP, NOR, and OFX). They also expanded the reach of their study by including 10 WWTPs with different sludge treatments, including anaerobic and aerobic digestion, one anaerobic wastewater stabilization pond, and one composting facility. In general, they found lower chemical concentrations in the final products. The average concentrations for all detected chemicals in the sludge before treatment were 179, 310, and 142 µg/kg dw for primary, secondary and mixed (primary + secondary) sludge respectively, whereas the average concentration in the final products was 70 µg/kg dw in aerobically digested biosolids, 63 µg/kg dw in the stabilization pond sludge, 12 µg/kg dw in the compost, and 8 µg/kg dw in anaerobically digested biosolids.

Although the authors did not include mass balances and therefore did not calculate removals, the concentrations in the sludge, biosolids and compost suggest that anaerobic digestion and composting achieved a larger reduction in ESOCs concentrations than aerobic digestion and the anaerobic stabilization pond. Analyzing the concentrations of different classes of ESOCs independently, the stabilization pond sludge had the highest concentrations from the treated solids, in most cases as high as the untreated sludges (Martín *et al.*, 2015).

Similarly, Narumiya *et al.* (2013) studied the fate of 48 ESOCs during anaerobic digestion in 4 full-size Japanese WWTPs. Just as Furlong *et al.* (2010) and Salveson *et*

al. (2012), they found a wide range of removal efficiency. Some compounds were consistently removed by more than 90% (caffeine, trimethoprim, SMZ), while others proved to be recalcitrant (CBZ, DEET, sulfapyridine). Removals for the rest of the compounds detected in sludge were somewhere in between; e.g., around 50% for antibiotics such as CLM, ERY, NOR, OFX, and roxithromycin, but 30-40% for TCS and TCC.

Samaras *et al.* (2013) investigated the removal of several EDCs (NP, NP1EO, NP2EO, TCS, BPA) and NSAIDs (DCF, NPX, IBP, KFN) from mixed WWTP sludge during mesophilic anaerobic digestion in two full-size WWTPs in Greece. They found that the NSAIDs present above detection limits in the sludge (NPX and IBP) had removals above 80%, whereas removal for the EDCs ranged from 20 to 55%, with the exception of NP, which was actually formed during AD.

Less research has been conducted for other types of sludge treatment. Poulsen and Bester (2010) examined the fate of 10 ESOCs [AHTN, DEHP, HHCB, 2-methylbenzothiazole (MTB), [1,2,3,4,5,6,7,8-octahydro-2,3,8,8-tetramethylnaphthalen-2yl]ethan-1-one (OTNE), TCS, triisobutyl phosphate (TiBP), tributyl phosphate (TnBP), TCPP, and triphenyl phosphate (TPP)] and 2 transformation products (HHCB-lactone, and MeTCS) in an anaerobically digested sludge during the thermophilic phase of a full-scale composting process.

They observed a net loss of mass for all of the compounds, which was above 75% in all cases with the exception of AHTN, TPP, and the 2 metabolites (HHCB-lactone, and MeTCS). The relatively low degradation of the metabolites suggests they were formed during the process by microbial transformation of the parent products. However, statistical analysis showed that degradation rates were significantly larger than zero for only seven compounds (DEHP, MeTCS, MTB, OTNE, TCS, TiBP, and TnBP).

Patureau *et al.* (2012) studied the effects of a full-scale composting process at two different times of the year (spring and fall) on several families of pollutants (PAHs, PCBs, LAS, PAEs, PCDD/F, AOX), including 2 groups of ESOCs: NPEs (NP, NP1EO, NP2EO), and estrogens (E1, E2, E3, EE2). However, no conclusions could be drawn for the estrogens because they were only detected in the original sludge, but were below analytical detection limits in the final compost and all of the intermediary products.

As it was the case in the studies on anaerobic digestion discussed above, the concentrations of most compounds in the final compost were higher than in the initial composting mixture (dehydrated sludge mixed with green waste and sifting refuse from the end of the biodegradation phase), with the exception of AOX, LAS, PAEs, and NPEs in the fall, when concentrations decreased. Also analogous to previous observations in AD (Furlong *et al.*, 2010), a net increase in the total mass of NP occurred in one of the stages of the process (maturation), which was attributed to NP1EO and NP2EO degradation. However, the total mass for all of the compounds studied, including NP, was lower in the final compost than in the initial composting mixture (Patureau *et al.*, 2012).

Experimental studies – Operating parameters and other factors

Besides the type of sludge treatment, other operating parameters are expected to influence ESOCs removal, as it is the case in wastewater treatment (Lishman *et al.*, 2006). The most relevant of the operating parameters are generally considered to be temperature and sludge retention time (SRT) (Citulski and Farahbakhsh, 2010; Stasinakis, 2012), but their role is not clearly established because their influence is not the same for all compounds, and some studies report contradictory results (Samaras *et al.*, 2014).

Other parameters influencing the removal of ESOCs during sludge treatment include the bioavailability of the compounds, the acclimation of the microbial flora to the ESOCs, and cometabolic processes (Barret *et al.*, 2012; Stasinakis, 2012). Even the addition or spiking of ESOCs can influence removal because of the difference in interactions between organic matter and freshly-added versus originally-present ESOCs (Barret *et al.*, 2012).

Additionally, the operating parameters of the liquid stream in wastewater treatment also affect ESOCs concentrations in sludge; e.g., TCC accumulates in activated sludge as a function of sludge retention time in the activated sludge process (Salveson *et al.*, 2012).

Furthermore, most of the studies designed to analyze the effects of operating parameters on ESOCs concentrations are small scale by necessity in order to isolate the impact of individual parameters. Thus, the conclusions reached in laboratory experiments might not always translate to full-size reactors, where conditions might not be as homogeneous and controllable.

Carballa *et al.* (2007) studied the fate of 13 ESOCs (AHTN, CBZ, DCF, DZP, E1, E2b, EE2, HHCB, IBP, iopromide, NPX, Roxithromycin, and SMZ) in laboratory-scale sludge anaerobic digesters under mesophilic (37°C) and thermophilic (55°C) conditions. Just as the full-size WWTP studies described above, they also found a wide range of removals, from 0 for CBZ to 99% for SMZ, but they did not observe any effects of temperature or SRT on removal.

Samaras *et al.* (2014) investigated the removal during sludge anaerobic digestion of the same compounds analyzed in their 2013 study described above, but this time in a controlled laboratory-scale experiment. They used single-stage mesophilic (37°C), single-stage thermophilic (55°C), and two-stage thermophilic-mesophilic treatments operating at identical SRTs (20 d). Their results were in general agreement with their previous report (Samaras *et al.*, 2013). They observed high removal rates (>80%) for the NSAIDs, whereas removal of the EDCs ranged between 40 and 80%, and there was no difference between the 3 different treatments, although the two-stage treatment tended to show higher removal for TCS and the sum of NPEs, but it was not statistically significant due to the high variability in effluent concentrations.

The only compounds to show differences in removal due to temperature were the individual NPEs. NP was better removed under thermophilic conditions, whereas NP1EO showed higher removal under mesophilic temperatures. But these results were

complicated by the formation of NP from NP1EO and NP2EO, and of NP1EO and NP2EO from NPEs with longer ethoxylate chains, which were not analyzed (Samaras *et al.*, 2014). The formation of these compounds in the reactors results in negative removal rates in some cases, just as observed in the full size reactor (Samaras *et al.*, 2013). In general, NPEs are considered degradable by sludge anaerobic digestion, but the fate of NP is more controversial.

The same group also studied the influence of 2 different SRTs on the removal of the 5 EDCs and 4 NSAIDs in the single-stage thermophilic reactors (Samaras *et al.*, 2014). Although they observed slightly better removal at 20 d SRT vs 8 d, the difference was not statistically significant.

In their sludge composting study, Patureau *et al.* (2012) observed lower concentrations of total PAEs, PCBs, PCDD/Fs, and NPEs in dehydrated sludge in the spring than in the fall, but higher concentrations of estrogens, and no statistically significant differences for AOX, LAS, and PAHs. The thermophilic stage of the composting process was much longer in the fall than in the spring, but the temperature during the maturation stage was higher in the spring than in the fall. According to the authors, these temperature differences affected the fate of some of the chemicals, with NPE and LAS removals being favoured under mesophilic conditions, and a shift in the NPEs concentration ratios. NP represented only 33 and 23% (spring and fall respectively) of the total NPE concentration in the initial composting mixture, but it increased to 48 and 75% in the final compost. However, because of the limited number of experiments conducted, it was not clear that temperature was the principal factor determining the observed differences.

As for parameters other than temperature and SRT, Barret *et al.* (2012) summarized their group's body of work on theoretical and experimental approaches to assess other parameters affecting the removal of PCBs, PAHs, NPEs, and PAEs during sludge treatment. They found that long-term exposure to relatively low concentrations of certain compounds resulted in increased removal of PAHs, presumably due to microbial acclimation. They also demonstrated that cometabolism was one of the main mechanisms, if not the most important, behind the removal of low concentrations of hydrophobic compounds, and the relevance of dissolved and colloidal organic matter to bioavailability.

6.1.2. Effects of sludge treatment on biological endpoints

The effects of sludge treatment on ESOCs can also be evaluated indirectly by measuring changes in the response of bioassays. The majority of studies have focused on estrogenicity as the effect to assess through the treatment processes.

In their review on the fate of endocrine disrupters during biosolids treatment, Citulski and Farahbakhsh (2010) summarized different studies conducted to elucidate changes in estrogenicity using bioassays. The reviewers found that different bioassays can give different and inconsistent results, with the commonly used yeast estrogen screening (YES) bioassay (Routledge and Sumpter, 1996) giving lower values than the estrogen-receptor-mediated chemically-activated luciferase-gene-expression (ER-CALUX)

bioassay (Legler *et al.*, 1999) in one study (Kanda, 2004), but higher in another (Lorenzen *et al.*, 2004).

Additionally, some of the results differed not only in magnitude, but also in the observed trends, with one study (Kanda, 2004) reporting an increase in estrogenicity after heat-drying anaerobically-digested sludge when using YES, but a decrease when using ER-CALUX. In contrast, Lorenzen *et al.* (2004) found a good correlation between the responses of the 2 bioassays when analyzing biosolids from 17 Ontario biosolids samples.

The studies reviewed by Citulski and Farahbakhsh (2010), especially Lorenzen *et al.* (2004) and Holbrook *et al.* (2002), suggest that aerobic digestion was more effective than anaerobic digestion in reducing estrogenicity in the biosolids.

Citulski and Farahbakhsh (2010) also emphasized the difficulties of testing sludge and biosolids with bioassays due to the cytotoxicity elicited by some of these matrices to the cells that are at the core of the assays. The toxicity could stem from co-extracted non-estrogenic compounds; e.g., Frische *et al.* (2009). Biosolids and/or sludge cytotoxicity was observed by several of the studies cited in the review (Kanda, 2004; McNamara *et al.*, 2009), and was also observed in more recent studies, such as Furlong *et al.* (2010) and Patureau *et al.* (2012) discussed below.

Finally, Citulski and Farahbakhsh (2010) also noted that the estrogenicity of a sample might depend on the analytical method used to extract the EDs from the samples; e.g., in solid-phase extraction, different compounds are extracted with different solvents (Fernandez *et al.*, 2009). Therefore, the authors recommended the adoption of standard methods for the analysis of sludge and biosolids using bioassays.

Studies published after the Citulski and Farahbakhsh review reached similar conclusions. For example, one of the main objectives of the study by Furlong *et al.* (2010) described in Chapter 6.1.1 was to assess changes in estrogenicity during wastewater and sludge treatment. Estrogenicity was evaluated with the YES and T47D-KBluc bioassays (Routledge and Sumpter, 1996; Wilson *et al.*, 2004). In all cases, estrogenicity could be explained by the presence of 16 compounds, including estrogenic hormones (EE2, E2a, E2b, E1, E3, DES), alkylphenols (NP, OP, NPE_{1-2}, OPE_{1-2}), BPA, DEHP, and benzophenone. Of these, E1, E2, NP, and NPE_{1-2} were the highest contributors.

Furlong *et al.* (2010) found that estrogenicity in the biosolids increased during sludge treatment, with the exception of aerobic digestion. Anaerobic digestion and lime addition both increased estrogenicity. However, when evaluating estrogenicity behaviour in wastewater treatment as a whole, the increase in estrogenicity in the solids through anaerobic digestion was compensated by the decrease in estrogenicity in the liquid phase achieved during secondary (activated sludge) treatment, resulting in an overall decrease in estrogenicity when comparing the raw influent to the effluent and biosolids leaving the plant.

The increase in estrogenicity in the biosolids through sludge treatment occurred in spite of the decrease in estrogenic hormone concentrations, which are generally considered the main source of estrogenicity in liquid WWTP effluents. According to Furlong *et al.* (2010), the increase in estrogenicity could be explained by the much higher concentrations of estrogenic alkylphenolic compounds found in the biosolids. In the specific case of anaerobic digestion, the formation of NP from NPEs was considered the main cause of the increase. In contrast, estrogenicity decreased during aerobic digestion as a consequence of NP and NPEs removal in aerobic conditions.

When evaluated, both composting and pelletization reduced estrogenicity in the biosolids. In the case of lime addition, estrogenicity increased in spite of an important reduction in both hormones and alkylphenol concentrations. The authors were not able to determine the cause of this phenomenon, but they argued that the change in pH could have transformed non-targeted compounds to an estrogenic form, or desorbed them from the solids.

Finally, the authors (Furlong *et al.*, 2010) found a good correlation between the estrogenicity results obtained by the bioassays and the chemical analysis—when calculating estrogenicity by addition of the individual contribution of each estrogenic chemical measured. However, bioassays yielded lower estrogenicity values, ostensibly due to the presence of estrogenic antagonists, competitive binding to the estrogen receptors, and matrix interferences in the bioassays that are not taken into account in the concentration addition model.

Patureau *et al.* (2012) on the other hand did not always observe a good correlation between bioassays and chemical concentrations in their sludge composting study. Along the changes in chemical concentrations during the composting process, they used bioassays to measure the response of 3 different receptors: estrogen α (ERα), aryl hydrocarbon (AhR), and pregnane X (PXR). In contrast to Furlong *et al.* (2010), they observed an increase in estrogenicity through the composting process, but in this case, it could not be explained by estrogens or NPEs. Patureau *et al.* (2012) suggested that the estrogenicity might have been caused by phytoestrogens from the green waste used to compost the sludge.

AhR activity was better correlated with the chemical measurements, and the authors ascribed it to PAHs, PCBs, PCDD/F, and AOX. Overall, the authors concluded that dioxin-like activity was not significantly affected by composting. PXR activity decreased during the process, but the activity could not be correlated to chemical concentrations because the responses of PXR to chemicals such as APEs and PCBs were unknown (Patureau *et al.*, 2012).

6.2. Pathogens

Treatment of the sewage sludge generally reduces indicator and pathogenic organisms, but not all respond in the same manner, as the authors of several reviews on this topic have observed (Arthurson, 2008; Sidhu and Toze, 2009; WEAO, 2010). Some

generalized log reductions are given in Table 6.3, but information was not found for all treatment processes and all target organisms.

In general, standard mesophilic anaerobic digestion (MAD) reduces indicator organisms, *Listeria* and viruses by between 1- and 2-$\log_{10}$. *Salmonella* may be reduced by a somewhat lesser amount (1 $\log_{10}$). Not all treatment plants result in equally effective treatment. For example, Flemming *et al.* (2009) reported the reduction of *E. coli* after digestion at 6 WWTPs to be 1.7 $\log_{10} \pm 0.9$ (standard deviation). Rapid processing through digestion results in lower treatment effectiveness (Table 6.3). However, higher temperatures improve performance (temperature phased systems in Table 6.3; Viau *et al.*, 2011).

The most significant deviation from the 1-2 $\log_{10}$ reduction following MAD is for *Clostridium* species. Clostridia are obligate anaerobic, spore forming bacteria; *Clostridium* species will in fact be part of the bio-digestion process. Therefore, it is not surprising that anaerobic digestion has no net effect on *Clostridium* populations. This is important in light of the increasing concern about hospital- and community-acquired outbreaks of *Clostridium difficile* (aka *C. diff.* as reported in the media.)

Aerobic wastewater treatment has a greater effect on organisms resistant to MAD such as *Clostridium*, *Cryptosporidium* and *Giardia*; reductions of 0.96 $\log_{10}$, 2.96 $\log_{10}$, and 1.4 $\log_{10}$, respectively, were measured by Chauret *et al.* (1999) at an Ottawa WWTP.

Drying with heat produces material with very low numbers of indicator organisms, while drying without heat but in sunlight reduces numbers by about 3 $\log_{10}$; drying in the shade is much less effective (1 $\log_{10}$ reduction) (Öğleni and Özdemir, 2010). Composting biosolids can greatly reduce populations (e.g. 3-6 $\log_{10}$ for *E. coli*), but results can be variable. Heat resistant spores of organisms such as *Clostridium* may persist, whereas viruses appear to be highly susceptible to composting (WEAO, 2010).

Advanced alkali treatment is the combination of high pH and temperature to render sludges pathogen free. The Lystek process includes high shear mixing forces, while the N-Viro process includes drying. Both processes meet CP1 or Class A biosolids guidelines. The information provided in Table Table 6.3 is for Lystek-treated biosolids.

Table 6.3. Effect of sludge treatment methods on indicator and pathogenic organisms density (log_{10}) (Sidhu and Toze, 2009; WEAO, 2010).

Group / Organism	Raw	Lime stabilized	Anaerobic digestion			Aerobic	Drying			Composted	Advanced alkaline stabilization
			MAD	Rapid thru-put	Temp phased		Heat	Solar	Non-solar		
E. coli Fecal coliforms Total coliforms Fecal streptococci	6-7 7-8 8 7	-2	3-5 4-7 6 6	6	4 2		Bdl[1]	3-4	5-7	-2-4 1	Bdl
Enterococcus *Clostridium perfringens* *Salmonella* *Shigella* *Campylobacter* *Leptospira* *Yersinia* *Listeria monocytogenes*	5-7 5-6 0-3 1 3 0 1	Bdl to -1	4-5 6 -1-1 0 Bdl -1 0		<0	1 <0				3 0	Bdl
Verotoxigenic *E. coli*, (e.g. O157)		Expected to be at least as sensitive as generic *E. coli*									
Antibiotic resistant bacteria (ARB)	100%	Expected to have susceptibilities to treatment similar to their non-antibiotic resistant counterpart; however, transfer of resistance genes within the sludge matrix speculated.									
Clostridium difficile	100%	Expected to behave similarly to *C. perfringens*, but no information available.									
Total enteric virus Enterovirus Human adenovirus	0-2 2 1-2		-1-0 0 -1-2		<0						Bdl
Cryptosporidium Viable[2] *Giardia*	 1 0-2		1-2 0 -1-2			2.96 1.4					
Total Viable[2] Ascaris	-1		-1								Bdl

[1]Bdl: below detection level; [2]measured by germination tests

6.3. Bacterial reactivation and regrowth

Given suitable conditions, including nutrients, which are readily available in biosolids, reactivation and regrowth of bacteria may occur between treatment and land application or other beneficial reuse method. The term 'reactivation' implies a sudden increase of culturable bacteria immediately after dewatering, a concept introduced by Higgins *et al.* (2007). Reactivation is used in contrast to 'regrowth,' which signifies continued growth of bacteria upon further storage (Qi *et al.*, 2008). Regrowth can occur without preceding reactivation (Qi *et al.*, 2008). Regrowth of enteric viruses and ova is not of concern as they are parasites and are not able to grow in biosolids without a suitable host (Navab-Daneshmand *et al.*, 2014a).

Bacterial regrowth in biosolids is well documented (Gibbs *et al.*, 1997; Sidhu *et al.*, 2001; Zaleski *et al.*, 2005b; Zaleski *et al.*, 2005a; Iranpour and Cox, 2006; WEF, 2006; Chen *et al.*, 2011a; Navab-Daneshmand *et al.*, 2014a; Navab-Daneshmand *et al.*, 2014b), especially when centrifuge dewatering is employed (Qi *et al.*, 2004; Higgins *et al.*, 2007; Qi *et al.*, 2007; Chen *et al.*, 2011b). Reviews of reactivation and regrowth of bacteria in biosolids have been published by Zaleski *et al.* (2005b) and WEF (2006).

Fecal indicators such as fecal coliforms can be used as conservative indicators of bacterial regrowth as they are found in much higher initial (pre-treatment) densities than pathogenic bacteria, and therefore the likelihood of some survival through treatment, and subsequent regrowth, is higher (Farrell, 1992). Hardier bacterial species such as *Salmonella* have also been used as indicators of regrowth. Regrowth of fecal coliforms could imply growth of pathogens and an increased health risk (Chen *et al.*, 2011a). In addition, regrowth of regulated bacteria such as fecal coliforms and *Salmonella* could cause biosolids to fail associated regulatory standards (Iranpour and Cox, 2006; Chen *et al.*, 2011a).

Regrowth of indicator and pathogenic bacteria has been demonstrated for mesophilic and thermophilic digested biosolids, and it may be as high as 4 $\log_{10}$. Regrowth of bacteria in stored biosolids implies incomplete destruction during treatment, and subsequent residual microbial activity (Iranpour and Cox, 2006; Chen *et al.*, 2011a). Iranpour and Cox (2006) observed regrowth during storage of 2-3 $\log_{10}$ for residual fecal coliforms after thermophilic anaerobic digestion, before stabilizing at initial levels after 1-2 weeks.

Qi *et al.* (2004) compared *E. coli* regrowth across four biosolids treatment facilities. Although results were variable, regrowth was seen at two of the facilities up to 2 $\log_{10}$. Flemming *et al.* (2009) demonstrated >1 $\log_{10}$ regrowth for *E. coli* and *Salmonella*, and even limited regrowth of *Listeria* and *Yersinia*. Chen *et al.* (2011a) observed fecal coliform regrowth in centrifuge dewatered biosolids to a peak of 3 $\log_{10}$ after 4 days; however, secondary stabilization, in which the coliform levels were below initial levels, was reached after 1-2 weeks in storage. In contrast, when they analyzed regrowth in a belt filter pressed biosolids sample, they observed very little regrowth over a 7 day period. Higgins *et al.* (2007) also observed regrowth in cake samples during storage, for both mesophilic and thermophilic digested biosolids. They reported a 4 $\log_{10}$ increase in *E. coli* to a peak at <7 days, before decreasing to below initial levels.

Specific research has been conducted on reactivation and regrowth of bacteria in centrifuged dewatered biosolids. As centrifugation residence time is normally less than 15 minutes, which is less than the doubling time of 20 minutes for *E. coli*, it is unlikely that the observed growth

occurs solely during the centrifugation process (Qi *et al.*, 2008). Qi *et al.* (2007) reported fecal coliform regrowth after 24 hour incubation at 25 and 37°C in centrifuged dewatered biosolids on the order of 2 $\log_{10}$, and significant regrowth in digested biosolids. They also studied regrowth in soil: biosolids mixtures of 3:1 and 280:1 (w/w) and found the effect of dilution to decrease the regrowth curve by 2-3 $\log_{10}$ *E. coli* MPN/g dry soils; however, the regrowth curve still followed the same pattern of increase to a peak of 4 $\log_{10}$ after 5 days, followed by a decrease and secondary stabilization after 20 days. In a later experiment, Qi *et al.* (2008) showed that higher total solid (TS) levels of the dewatered biosolids led to greater magnitudes of fecal coliform increase. Increased TS also reduced methane production, which the authors suggested could mean fewer methanogens to compete with fecal coliforms. They also showed that TS had a greater impact on regrowth than the level of shearing during centrifugation.

Recent research conducted at McGill University (Navab-Daneshmand *et al.*, 2014a; Navab-Daneshmand *et al.*, 2014b) showed regrowth of *E. coli* in heat-treated and electro-dewatered biosolids of 4-5 $\log_{10}$ under both aerobic and anaerobic conditions. The same experiment conducted the following year elicited a different result. Regrowth of *E. coli* in electro-dewatered biosolids was minimal compared to regrowth in heat-treated biosolids of almost 4 $\log_{10}$. The authors suggested that this difference is due to biosolids characteristics, namely increased dewatering efficiency in the first year, and a faster temperature decrease in the second year.

Sidhu *et al.* (2001) proposed that regrowth of pathogenic bacteria can also be suppressed by indigenous microorganisms found in the biosolids. They seeded *Salmonella typhimurium* in sterile and non-sterile composted biosolids, and observed rapid colonization by *S. typhimurium* to a maximum population density of $>10^8$/g in sterilized biosolids, compared to a density of $<10^3$/g in non-sterile biosolids.

Regrowth of indicator organisms and *Salmonella* is dependent upon favourable conditions of moisture, temperature, and substrate availability (Zaleski *et al.*, 2005b). A study by Yeager and Ward (1981) explored the effect of moisture content on survival and regrowth of bacteria during long-term biosolids storage. They reported regrowth in samples with ≤75% total solids, and no growth in samples with ≥85% total solids. Similarly, Gibbs *et al.* (1997) observed regrowth of fecal coliforms and salmonellae in stored biosolids after rainfall at the beginning of the winter season, following by a decrease in bacteria during a hot, dry summer.

In general, regrowth of fecal coliforms in biosolids appears to occur in mesophilic and thermophilic digestion facilities that are coupled with centrifugation dewatering. Coliform populations can increase rapidly during the first 5-7 days of storage by up to 4 $\log_{10}$; however, populations then appear to decrease, and secondary stabilization occurs after several weeks.

6.4. Summary and recommendations

Most of the existing research on the fate of ESOCs during wastewater treatment focuses on removal from the liquid phase and generally fails to consider sludge treatment. This is mainly because the original interest on ESOCs fate in WWTPs stemmed from biological effects observed in biota living in effluent receiving waters (Furlong *et al.*, 2010), but also because of

the greater difficulty of measuring ESOCs—especially those present in relatively low concentrations, such as hormones—in biosolids (CCME, 2010; Citulski and Farahbakhsh, 2010; Furlong *et al.*, 2010).

Even less common are studies reporting mass balances along with concentrations, and studies looking at overall ESOCs fate in WWTPs; i.e., in both liquid and solid streams. In the cases where these kinds of studies have been conducted, treatment generally results in a net mass reduction of the ESOCs and effects (i.e., estrogenicity) measured; e.g., Furlong *et al.* (2010); Patureau *et al.* (2012).

In addition, only a relatively limited amount of ESOCs has been studied, such as the NPEs (Citulski and Farahbakhsh, 2010), and most research has concentrated on anaerobic digestion over other sludge treatment methods (CCME, 2010).

Just as it is the case for wastewater, the effects of sludge treatment on ESOCs vary widely, with some of the compounds being clearly and consistently removed; e.g., caffeine (Furlong *et al.*, 2010), while others are recalcitrant; e.g., carbamazepine (Furlong *et al.*, 2010). Furthermore, the concentration of recalcitrant compounds in biosolids tends to increase as treatment progresses (Furlong *et al.*, 2010; Salveson *et al.*, 2012). Additionally, for some chemicals, such as NP and estrogens, results are still contradictory (Martín *et al.*, 2012; Stasinakis, 2012).

Additionally, the effects of operating parameters such as temperature and sludge retention time are not clear, with some studies showing no significant effects and others yielding contradictory results (Carballa *et al.*, 2007; Samaras *et al.*, 2014).

Similarly, research on the effects of sludge treatment on pathogens has concentrated on relatively few organisms, namely fecal indicators and *Salmonella*, and has favoured anaerobic digestion over other types of sludge treatment. In general, treatment reduces the number of organisms, but the magnitude of the reduction also varies with organism and treatment, with some viruses and prions being resistant to most treatments, although higher temperatures improve inactivation of all organisms (Viau *et al.*, 2011).

Amongst the more studied pathogens, *Clostridium* stands out because of its recalcitrance to anaerobic digestion. *Clostridium*, along with other organisms resistant to anaerobic digestion, such as *Cryptosporidium* and *Giardia*, are more sensitive to aerobic wastewater treatment in the liquid phase (Chauret *et al.*, 1999).

Reactivation and regrowth of bacteria in biosolids has been well documented and appears to follow a consistent pattern, with bacterial densities increasing an peaking within one week, and then decreasing to the original levels or lower within one month.

Besides the effects of sludge treatment on ESOCs concentration, different studies have targeted the effects of treatment on the capacity of biosolids to induce certain biological effects, which are measured with bioassays. The most studied effect by far is estrogenicity, generally using YES as the bioassay.

In general, aerobic digestion is considered more effective than anaerobic digestion in reducing estrogenicity in the biosolids (Holbrook *et al.*, 2002; Lorenzen *et al.*, 2004). Furthermore, composting and pelletization have been found to reduce estrogenicity in the

solids, whereas anaerobic digestion and lime addition increased estrogenicity (Furlong *et al.*, 2010). However, when wastewater treatment was evaluated as a whole (i.e., considering both the liquid and the solid streams), overall estrogenicity decreased (Furlong *et al.*, 2010).

Estrogenicity is mainly attributed to estrogenic hormones and alkylphenols (Furlong *et al.*, 2010), and to phytoestrogens in the case of compost (Patureau *et al.*, 2012), but it was not always possible to correlate estrogenicity to changes in chemical concentrations, as in the case of lime addition (Furlong *et al.*, 2010).

The use of different types of bioassays sometimes yields contradictory results, and cytotoxicity caused by the biosolids in some cases prevents accurate measurement of the biological effects; additionally, the choice of extraction solvents can also affect bioassay results (Citulski and Farahbakhsh, 2010).

Finally, several of the authors noted that both bioassays and analytical methodologies to assess ESOCs concentration in biosolids need to be improved and standardized, and that they would also benefit from more rigorous quality control practices. For the bioassays, the cytotoxicity and other issues mentioned above have to be addressed, whereas the analytical methods of trace amounts of ESOCs in biosolids still presents some challenges, which were reviewed and summarized by Citulski and Farahbakhsh (2010), and include issues such as sample extraction and clean-up to improve sensitivity and reproducibility. Additionally, more method development might also be necessary; e.g., for the analysis of the conjugated forms of pharmaceuticals (Celiz *et al.*, 2009; Furlong *et al.*, 2010).

Recommendations

Existing research on the fate of ESOCs during sludge treatment shows that elimination of these chemicals is variable and compound-dependent. Testing the effects of every type of sludge treatment on all ESOCs would be both impractical and expensive, and it would not address future ESOCs. In addition, the ESOCs evaluated were not chosen taking into account whether these compounds constitute a risk to biota in the context of their intended use (e.g., land application), because most of the existing toxicity data were developed for the aquatic environment.

However, the most important consideration for future work on the fate of ESOCs in sludge treatment is that it should not be evaluated in isolation, but considered part of a general risk evaluation strategy for ESOCs, such as the one discussed in Chapter 3.4, and set the research agenda accordingly. Before spending the resources on improving the capacity of WWTPs to eliminate ESOCs, it should be determined whether current treatment practices are sufficiently protective, which requires a better understanding of the risks that ESOCs may pose to biota and ourselves as part of the land application of biosolids.

Other recommendations for future work on the fate of ESOCs in sludge treatment stemming from the review of the literature are:

- Use indicator (surrogate) compounds to facilitate analysis of ESOCs concentrations during sludge treatment – The work of Salveson *et al.* (2012), who used indicator (surrogate) compounds with different sorption and biotransformation potential to predict ESOCs behaviour during activated sludge treatment, could be extended to the different sludge treatment processes.

- Research should combine both changes in ESOCs concentrations and bioassay responses – Concentration analysis by itself is not sufficient to evaluate effects; for example, chemical analysis of a set of estrogenic compounds in biosolids will not reflect antagonistic or synergistic effects, and it will miss non-targeted compounds (Citulski and Farahbakhsh, 2010), including metabolites (Celiz *et al.*, 2009).
- Standardize both bioassays and analytical determination of ESOCs concentrations – As mentioned above, these methodologies need to be standardized, along with quality control practices, and method development is still required for the analysis of some compounds, especially conjugated derivatives of hormones and pharmaceuticals.
- Evaluate ESOCs overall fate in WWTPs – Most of the work to date has investigated the fate of ESOCs in either the liquid or the solids trains independently, but rarely in both. This work is important to understand the interactions between the two phases, and because it might be desirable to maximize ESOCs sorption to the solids in order to prevent their release into WWTP receiving waters, where impact to biota could be more immediate and the potential for negative effects higher.
- Evaluate the use of combinations of existing sludge treatments and/or additional treatment in the WWTP recycling streams – If it is considered necessary to decrease ESOCs concentrations in biosolids, the use of combinations of different sludge treatments and/or physicochemical processes should be investigated (Hernandez-Raquet, 2013). Alternatively, existing wastewater treatment could be supplemented with the use of side-stream treatment of sludge filtrate and centrate to decrease the concentrations of ESOCs in internal recycling streams and consequently their loads in biosolids (Salveson *et al.*, 2012).

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7. FATE OF ESOCS AND PATHOGENS AFTER BIOSOLIDS LAND APPLICATION

After the application of biosolids to agricultural soil, different environmental processes such as transport and degradation determine the ultimate fate of ESOCs and pathogens in the environment. Their fate is of interest because they have the potential to affect human and/or environmental health if they are transported to surface waters or groundwater, if they persist in the soil and are absorbed by plants, especially edible crops, or affect the soil biota, including the microbial community. The fate of ESOCs and pathogens in soils is the subject of this chapter, whereas the impact on terrestrial animals and plants is discussed in Chapter 8.

7.1. ESOCs

A significant portion of the existing research on the fate of ESOCs in biosolids-amended soils has been carried out in Ontario, which makes them more relevant for the purposes of this review, although the types of soil in Ontario are certainly not representative of the whole country.

7.1.1. Fate and transport in soils

The fate of the ESOCs in soils is determined by physicochemical characteristics, such as the chemical nature and the adsorption capacity (represented by the soil/water adsorption coefficient, K_d), of both the chemicals and the soils, and is therefore very variable. It is also difficult to predict because of the presence of different functional groups in a single molecule and the changes in speciation with pH (Xu *et al.*, 2009b).

Besides intrinsic molecular properties, the hydraulic properties of the soil and its macro structure have an impact in the movement of ESOCs throughout the soil and affect their capacity to reach surface waters and aquifers or to interact with soil particles. Additionally, environmental factors such as soil temperature and moisture affect the rates of ESOCs biodegradation.

Lapworth *et al.* (2012) reviewed the processes controlling ESOCs movement in the soil as part of a review on the fate and presence of ESOCs in groundwater. The processes considered for the review included physical and hydraulic characteristics, sorption processes, biodegradation, and the effect of redox conditions.

Lissemore *et al.* (2006) studied the factors that influenced the migration of pharmaceutical compounds from a watershed with known agricultural inputs to tributaries of a river in southern Ontario, relating the impact of both physical and chemical processes on the spatial and temporal distribution of the pharmaceuticals in surface waters.

Additionally, in agricultural settings, transport might be facilitated by the presence of macropores in the soil, such as earthworm burrows, that allow the rapid flow of water and thus prevent the interaction of the ESOCs with soil particles that is necessary for their retention (Lapen *et al.*, 2008; Larsbo *et al.*, 2009).

Migration of ESOCs is also affected by soil aging, as shown by Mueller *et al.* (2006), who found that the recoveries of BDE-47, 99, and 100 from spiked soils were significantly diminished (~90%) after a 10-week period due to sorption to soil components. However, the simultaneous presence of two plant species, radish and zucchini, increased these recoveries

by almost eight times, suggesting that certain plant interactions might result in enhanced bioavailability (Mueller *et al.*, 2006), even in the case of aged soils (Welsh *et al.*, 2009).

The form in which the biosolids are applied also has an effect. Irrigation with (un)treated wastewater, and even liquid biosolids, might result in a greater bioavailability of the ESOCs in comparison to the cases where the biosolids are applied in solid form. However, the potential for transport is also expected to be greater in the case of liquid applications (Edwards *et al.*, 2009; Sabourin *et al.*, 2009).

The following sections review field studies on the fate of ESOCs in soil after biosolids land application, both in dry and liquid forms, and laboratory studies on the fate of ESOCs designed to elucidate the influence of individual parameters on the fate of the compounds.

Field studies – Persistence in soils and vertical migration

Although they did not use biosolids, Kinney *et al.* (2006) studied the fate of 19 pharmaceuticals in 3 soils irrigated with reclaimed water (WWTP effluent with additional treatment). They analyzed soil samples at different depths (up to 30 cm) before and during irrigation season in a Western American city. Although most of the compounds did not accumulate in the soil, different pharmaceuticals showed different degrees of accumulation: CIM, warfarin, GFB, and codeine did not accumulate; ACM, FLX, caffeine, ERY, and CBZ were consistently retained in the soil; and the rest of the chemicals showed inconsistent behaviour. The authors attributed the differences in accumulation to the physical-chemical properties of the compounds (e.g., solubility and K_{ow}), and the soil characteristics, such as the organic matter content. Most of the chemicals were detected in the deepest core (25-30 cm) in at least one soil sample, suggesting potential leaching to lower soil layers.

Ternes *et al.* (2007) investigated the fate of 52 PPCPs in a German agricultural field that has been irrigated with a mixture of WWTP effluent and anaerobically-digested biosolids for over 45 years. Of the 22 compounds typically present in the effluent and the biosolids, only diatrizoate, iopamidol, CBZ, and SMZ were detected in groundwater monitoring wells installed in the field. The rest of the compounds were presumably biodegraded or sorbed, in spite of the soil's low organic matter and clay content, and the presence of a sandy aquifer.

Edwards *et al.* (2009) studied the fate of ten PPCPs (ACM, FLX, IBP, GFB, NPX, CBZ, ATN, SMZ, TCC, and TCS), and cotinine (a metabolite of nicotine) in field plots with tile drainage that received 2 biosolids applications, the first one in liquid form, followed a year later by an application of dewatered biosolids by either direct injection or surface spreading and incorporation to the topsoil by tilling.

Analysis of the drainage previous to the dewatered biosolids application, 9 months after the liquid biosolids were applied, showed that NPX, ATN, TCS, and cotinine were still present, indicating persistence in the soil during this period, when the fields were left unplanted. (It is worth noting that ATN and NPX were spiked into the liquid biosolids (Lapen *et al.*, 2008), which might have influenced their fate.)

In the case of dewatered biosolids application, tile drainage did not occur until the first substantial rain or irrigation event. In contrast, the first tile effluent occurred at the time of application in the case of the liquid biosolids. Most of the compounds analyzed were detected in the effluents, with the exception of TCC, GFB, and SMZ that were consistently below

quantification limits (Edwards *et al.*, 2009). Maximum concentrations occurred at the time of application when biosolids were applied in liquid form, and in the first three rain events, less than a month after application, for ACM, IBP, NPX, CBZ, and cotinine. An exception to this trend was TCS, whose peak concentration occurred 113 d after application.

In general, maximum concentrations in tile drainage were higher after liquid biosolids application than after dewatered biosolids were applied. Furthermore, maximum concentrations observed in tile effluent for both treatments tended to be lower than maximum WWTP effluent concentrations observed elsewhere. TCS was the only compound present in concentrations that might pose a risk to biota. The observed peak concentrations of TCS, 3.68 and 0.24 µg/L after liquid and dewatered biosolids application respectively, were above several ecotoxicological endpoints (Edwards *et al.*, 2009).

Finally, the authors also concluded that the dewatered biosolids application method (surface spreading or direct injection) did not make a significant difference in the mass loads of ESOCs in tile drainage (Edwards *et al.*, 2009).

Lapen *et al.* (2008), in the first part of the study described above (Edwards *et al.*, 2009), studied the fate of a slightly different set of ten PPCPs (ACM, FLX, IBP, GFB, NPX, CBZ, ATN, SMZ, SPY, and TCS), and cotinine (a metabolite of nicotine) in field plots with tile drainage where liquid biosolids enriched with some of the ESOCs (ACM, ATN, NPX, GFB, SMZ) were applied. Two different application methods were used, subsurface deposition and surface spreading with tilling for incorporation to the topsoil. Additionally, they used rhodamine WT to evaluate the possibility of using the dye as a surrogate for the ESOCs fate in tile effluent.

Their results showed that ESOCs appear in tile drainage as early as 3 and up to 39 minutes after application, indicating rapid movement through the soil, presumably through soil macropores such as earthworm burrows. This was observed in both application treatments. However, application by subsurface deposition resulted in significantly lower mass loads in tile drainage during the first rain event compared to surface spreading.

Most of the ESOCs were observed above quantification limits only in the tile effluent event following application. This was the case for the acidic drugs (ACM, NPX, IBP, GFB), FLX, and the sulfonamides (SMZ, SPY). CBZ, TCS, ATN, and cotinine were also observed in post-application tile discharge events, with CBZ and TCS still above quantification limits several weeks after the application event.

Rhodamine WT concentrations were able to predict ESOCs concentrations in tile effluent with accuracy, especially for the application event. In later events, the relationship is weaker but still significant, with the exception of the acidic drugs, which showed poor correlations.

The fate of PTBDEs and PFCs was also investigated as part of the study above (Gottschall *et al.*, 2010), but only during the liquid biosolids application event including both application methods. For the PBDEs measured (BDE-47, 99, 100, 153, 154, 183, and 209) surface spreading resulted in higher concentrations of the individual congeners in tile drainage than in both subsurface deposition (not statistically significant) and control (concentrations were up to approximately one order of magnitude higher) sites. Most of the PBDE mass, however, was retained in the soil. PBDEs lost in the tile drainage during application represented <0.1% and <1.7% of the total PBDE mass applied in the subsurface deposition and the surface

spreading sites respectively. From the 10 PFCs analyzed, only PFOS and PFOA were detected in tile effluent and in low concentrations, 17 and 12 ng/L respectively.

In a separate, more recent study, Gottschall *et al.* (2012) investigated the fate of a suite of PPCPs after a single application of dewatered anaerobically-digested biosolids to an agricultural field at the maximum rate allowed in Ontario (22 Mg dw ha^{-1}). More than 80 PPCPs were analyzed in the biosolids, but only 28 were selected for further evaluation, because the majority of the PPCPs were either non-detectable or were present in very low concentrations. In general, the compounds selected were present at concentrations over 100 ng/g dw. The compounds with the 5 highest concentrations were TCS, TCC, CIP, OFX, and NOR at 10,900, 4,940, 3,260, 1,400, and 1,010 ng/g dw respectively.

The PPCPs were analyzed in groundwater and tile effluent produced during rain events in the year following biosolids application (Gottschall *et al.*, 2012). CBZ, ACM, IBP, TCS, TCC, CTP, VEN and its metabolite *O*-desmethylvenlafaxine were detected in tile effluent from the first rain event, 22 days after application, in concentrations ranging from 13 to 74 ng/L. In contrast to the study described above (Edwards *et al.*, 2009), only one of the compounds (CBZ) was detected in tile effluent after the first rain event. In groundwater, only IBP, TCS, TCC and *O*-desmethylvenlafaxine were detected 2 d after the first rain event at 2 m depth in concentrations between 10 and 19 ng/L. None of the compounds analyzed were detected in any other groundwater samples on any sampling dates or depths (2, 4, and 6 m).

The PPCPs were also analyzed over the course of the experiment in both soil and biosolids aggregates incorporated to the soil (Gottschall *et al.*, 2012). Only a few compounds were detected in soil 405 d after application: MCZ, TCC, IBP, CBZ, and 2-hydroxy-ibuprofen, a metabolite of IBP in concentrations ranging from 22 to 174 ng/g. Quantitative analysis also showed the presence of OFX (OFX, NOR, and CIP were not quantified in soils due to analytical complications). Most of the PPCPs were still detected in the biosolids aggregates a year after application, although concentrations decreased for most of them; e.g., TCS and TCC concentrations were down to 1,750 and 3,100 ng/g respectively. The lowest reductions observed were for FLX and MCZ at around 50%.

Finally, wheat grain grown in the biosolids-amended soil was analyzed for the PPCPs (Gottschall *et al.*, 2012). The grain was spring wheat planted approximately 7 months after biosolids application and harvested 4 months later, and none of the PPCPs were detected in any of the wheat samples.

Gorgy *et al.* (2013) measured individual PBDE congeners in different layers of Canadian agricultural soil after application of biosolids to the top 0.05 m layer. A year after application, they observed an exponential decrease in PBDEs concentration in the top layer (0-0.05 m), while concentration in medium depth layers increased with time (0.05-0.25 and 0.25-0.45 m). (No PBDEs were detected under 0.45 m.) Although these changes in concentration were explained by vertical migration, this process could not account for all of the PBDE mass loss from the top layer; other processes such as volatilization, photolysis, and biodegradation were assumed to be the cause for the mass loss. In an earlier study (Gorgy *et al.*, 2012), the same group had observed PBDE migration to deeper layers—up to 1.05 m vs. 0.45 in the study referenced above. This difference was explained by the higher organic matter content and the lower permeability of the soils in the later study and the longer period between samplings for the earlier study (Gorgy *et al.*, 2013).

Limited loss of PBDEs through processes such as migration and biodegradation suggests that they are persistent in soils and would accumulate after repeated biosolids applications. Andrade *et al.* (2010) observed significantly higher PBDE concentrations in Virginia soils amended multiple times with biosolids than in soils receiving a single application, in agreement with earlier observations made in Arizona by Arnold *et al.* (2008), in Spanish soils by Eljarrat *et al.* (2008), and by Sellström *et al.* (2005) in Sweden.

Langdon *et al.* (2012) studied the dissipation of NP, OP, TCS, and BPA in agricultural field plots in Australia where either lagoon or centrifuge-dried anaerobically digested biosolids had been applied at approximately 45 and 25 t dw/ha respectively. NP, OP, and BPA concentrations in the biosolids amended soils decreased after 11 months, with half-lives of 257 and 248 d for NP for centrifuge- and lagoon-dried biosolids respectively, 231 and 75 d for OP, and 289 and 43 d for BPA. In contrast, TCS concentrations did not decrease during the study period. The persistence of the 4 compounds in the field was much higher than predicted by the laboratory experiments conducted by the same group and discussed in the following section (Langdon *et al.*, 2011).

The persistence of estrogenic properties of soils amended with biosolids has also been demonstrated in the field. Langdon *et al.* (2014) measured the YES bioassay response of soil from the plots in Australia described above (Langdon *et al.*, 2012). Although at low levels, the authors found estrogenic activity in the treated soils (non-treated plots showed no measurable estrogenic response) that persisted even after 4 months of application.

Field studies – Surface runoff

In two related studies (Topp *et al.*, 2008; Sabourin *et al.*, 2009), a group of Canadian researchers simulated precipitation events to induce surface runoff in experimental field plots amended with either liquid (Topp *et al.*, 2008) or dewatered (Sabourin *et al.*, 2009) biosolids in order to study the fate of several ESOCs (ATN, CBZ, cotinine, GFB, NPX, IBP, ACM, SMZ, and TCS in both studies; caffeine and TCC were also evaluated in the dewatered biosolids study). In the case of the liquid biosolids, some of the compounds (GFB, NPX, ACM, ATN, SMZ) were spiked to ensure detectable amounts during the experiment.

Similarly to their observations in tile drainage effluent after liquid biosolids application, where lower mass loads of the ESOCs were observed for subsurface biosolids application (Lapen *et al.*, 2008), the authors found that when the liquid biosolids were injected into the soil, ESOCs concentrations in the surface runoff were generally below quantitation limits (with the exception of ATN and TCS, which were quantifiable only after the first simulated rain event post-application), whereas surface application resulted in quantifiable concentrations in runoff from simulated rain events 1, 3, 7, 22 and 36 d after application, with several ESOCs being quantified even after 266 d (GFB, cotinine, CBZ, TCS, and SMZ) (Topp *et al.*, 2008).

In general, ESOCs concentrations declined in runoff after the first rain event, with the exceptions of IBP and ACM, whose concentrations increased several weeks after surface application of the liquid biosolids. The authors attributed the latter phenomenon to the release of the 2 chemicals from the solid matrix or to degradation of their conjugate forms (Topp *et al.*, 2008).

In the case of the dewatered biosolids application (Sabourin *et al.*, 2009), which were surface applied only, almost all ESOCs analyzed were quantifiable in runoff after simulated rain

events 1, 3, 7, 21, and 36 d after application. The exceptions were ATN, which was detected in only 2 events, NPX in one, and GFB, which was not detected in any runoff samples. In general, the percentage of the mass applied that was eliminated in the runoff was above 1% (and up to 29%) for the compounds with log K_{ow} values under 2.45 (ATN, CBZ, cotinine, caffeine, and ACM), whereas compounds with log K_{ow} values over 3.18 (GFB, NPZ, IBP, SMZ, TCS, TCC) lost less than 1% of the applied mass to runoff.

Similar to the conclusions of the study on tile drainage effluents (Edwards *et al.*, 2009), the maximum concentrations measured in surface runoff were much lower than typical WWTP effluent concentrations, and TCS was the only compound to be present in concentrations in the same order of magnitude as reported ecotoxicological endpoints (Sabourin *et al.*, 2009).

Although not intending to isolate surface runoff from groundwater transport, Lissemore *et al.* (2006), in their study of pharmaceuticals in an Ontario watershed, generally observed a correlation between economic activity and the presence of certain chemicals in surface waters; e.g., pharmaceuticals intended solely for animal use were present in water from sites adjacent to agricultural lands, and absent in downstream sites more influenced by an urban area. However, CBZ, which has no animal use, was consistently detected in receiving waters from several of the agricultural sites, and its presence was correlated to the application of biosolids in fields upstream these sites.

Pharmaceutical concentrations also showed temporal variations, including spikes that coincided with agricultural land application of manure and biosolids. The timing of the concentration spikes was delayed by sorption of the chemicals to the soil for more hydrophobic chemicals. Although pharmaceutical concentrations in water were not correlated to the concentrations of soluble ions used as markers of agricultural runoff (i.e., nitrate and phosphate), there was a moderate correlation to the levels of dissolved organic carbon, at least for some compounds, which could be a result of colloid-associated transport.

Laboratory studies and modelling

In the case of hydrophobic ESOCs, their affinity for solids and their low water solubilities limit their rapid vertical migration in soils. Using leaching column tests, Gorgy *et al.* (2011) observed that approximately 80% of the original mass of PBDEs present in the first layer, a mixture of soil and biosolids, remained after passing the equivalent to 40 times its volume of water. Most of the rest of the PBDEs were retained in the soil layer downstream the soil/biosolids mixed layer, and only 1% was found in the final leachate. However limited, migration of even the more hydrophobic congeners was observed; e.g., BDE-209. The lower congeners showed higher mobility as expected from their relatively high water solubilities, but the hydrophobic congeners have been shown to partition to fine particles, which allow them to be present in leachates in concentrations exceeding their water solubilities (Gorgy *et al.*, 2009).

Oppel *et al.* (2004) measured the potential for leaching of 6 pharmaceutical compounds (CBZ, clofibric acid, DZP, ivermectin, IBP, and iopromide) in 3 different soils in laboratory column experiments. They concluded that CBZ, DZP, IBP, and ivermectin were retained by the soils, whereas clofibric acid and iopromide showed a high degree of mobility. Because CBZ is often present in German groundwater, the authors hypothesized that groundwater

contamination does not generally occur from vertical transport through the soil, but from water exchange between the aquifers and receiving waters.

Xu *et al.* (2009b) measured sorption and degradation of 6 PPCPs (clofibric acid, NPX, IBP, TCS, DCF, and BPA) in 4 different agricultural soils (loamy sand, sandy loam, silty clay, and silt loam). They found that sorption capacity varied widely between chemicals, with TCS showing the highest affinity for the soil (average K_d = 117 L/kg) and IBP the lowest (average K_d = 1.5 L/kg) by almost two orders of magnitude. The type of soil also had an influence on sorption capacity; e.g., K_d values for TCS ranged from 9.7 L/kg in loamy sand soil to 273 L/kg in silt loam. They also observed a high positive correlation of K_d values with soil organic matter content for all chemicals, with the exception of clofibric acid, whose sorption capacity was highly correlated to clay content. These observations are consistent with the expected sorption behaviour for neutral and ionisable chemicals respectively, with the former expected to sorb more readily to organic matter.

Degradation of the chemicals also varied widely between chemicals and soil types, but it could be modelled in almost all cases as a first-order exponential decay. Half-lives ranged from 0.8 to 20 d, with IBP and BPA having the shortest. The authors hypothesized that the variation in degradation rates between soils was due to either differences in microbial populations and/or soil properties; e.g., higher sorption to clay or organic matter can decrease availability for microbial degradation, and higher organic matter content can also offer a more abundant and preferred source of nutrients for the organisms than the PPCPs (Xu *et al.*, 2009b).

In a similar set of experiments, Yu *et al.* (2013) studied sorption and degradation of BPA, CBZ, GFB, OP, and TCS in clay, sand and loam. They obtained similar results to the earlier study (Xu *et al.*, 2009b), with a wide variation in sorption affinity and degradation between chemicals and soil types. TCS also showed the highest affinity (expressed as K_d) for the soils, whereas CBZ and GFB had the lowest K_d values. CBZ was the most recalcitrant compound to biodegradation, with half-lives from 28 to 39 d, while the rest of the chemicals showed half-lives between 9.8 and 18 d.

Langdon *et al.* (2011) studied the degradation of NP, OP, BPA, and TCS in the same soils and biosolids sources that they used to conduct their field studies (Langdon *et al.*, 2012), but under controlled laboratory conditions. They compared the ability of 2 degradation models to predict their experimental results, and found that modelling NP, BPA, and TCS degradation as a biphasic process incorporating a degradable and a recalcitrant fraction resulted in a better fit to the experimental data compared to the exponential first-order decay model. In contrast, OP degradation was sufficiently explained by the first-order model, indicating no recalcitrant fraction for this compound. The recalcitrant fraction is generally explained by decreased bioavailability due to limited oxygen concentrations within the biosolids, and/or to irreversible binding to the biosolids (Langdon *et al.*, 2012). Other authors have also observed that first-order decay might not be sophisticated enough to model chemical degradation in soils accurately (Das *et al.*, 2004).

More importantly, their laboratory results yielded much higher dissipation rates than those observed in the field (see Langdon *et al.* (2012) in the field studies section above); half-lives were approximately 10-20 times lower in the laboratory for NP and OP, and 2.5 times lower for BPA. Additionally, TCS did not degrade in the field, even though it was degraded in the

laboratory experiments. These differences were presumably due to environmental factors in the field, such as soil moisture and temperature, which were not accounted for in the laboratory experiments (Langdon *et al.*, 2011; Langdon *et al.*, 2012).

Walters *et al.* (2010) evaluated the fate of PPCPs in a 1:2 mixture of dewatered anaerobically digested biosolids and agricultural soil in outdoor mesocosms. Of the 72 PPCPs measured, only 15 were present in the initial biosolids-soil mixtures (AZM, CBZ, CIP, diphenhydramine, DTC, 4-ETC, FLX, GFB, MCZ, NOR, OFX, TC, thiabendazole, TCC, and TCS) in concentrations ranging from low ng/g to low µg/g for the TCC and TCS, which were present at the highest concentrations. Although most of the compounds' concentrations decreased during the experiment, 9 of the 15 compounds were still quantifiable in the soils after almost 3 years, and 3 of the chemicals showed virtually no concentration changes (TCC, FLX, diphenhydramine, and thiabendazole).

The half-lives calculated by Walters *et al.* (2010) from their mesocosms experimental data ranged from 187 d for TCS to over 2,310 d for CIP, and they were 1.6 to 33 times higher than the respective half-lives predicted by USEPA's EPI Suite software, reinforcing the necessity of improved models and the use of field studies suggested by Langdon *et al.* (2012) to properly evaluate ESOCs fate in soils.

Further modelling work by the same researchers (Walters and Halden, 2010) based on published half-lives of 16 ESOCs in biosolids-amended soils concluded that compounds with relatively short half-lives (40 d) can accumulate in the soil if applied at a frequency consistent with USEPA regulations (3 times per year). For compounds with longer half-lives, accumulation can occur if application occurs even once per year.

A vertical transport modelling software was used by Chen *et al.* (2013) to evaluate PPCPs leaching and accumulation in soils in a perennial turfgrass field after 10 years of irrigation with treated wastewater. The model was validated with experimental data obtained both in the field and laboratory (Xu *et al.*, 2009a), and their modelling results were in reasonable agreement with measured values.

Analysis of the modelling results concluded that all of the PPCPs studied would accumulate in the soil, with most of them reaching steady-state concentrations. The compounds with relatively low K_d (NPX, IBP, clofibric acid, and DCF) would present an oscillatory concentration pattern, with concentrations increasing during irrigation, and then decreasing after irrigation stopped, presumably due to losses from degradation, transport, and/or volatilization.

Compounds with higher K_d (E1, OP, BPA, TCS, and NP) would require longer to reach steady-state, although NP, TCS and E1 would not reach it in the 10 years modelled (i.e., they would keep accumulating in the soil). Their concentrations would also oscillate, but in a less pronounced manner. Losses of these chemicals were assigned to degradation and/or volatilization, because leaching would not be a significant elimination factor in their case.

Leaching of the chemicals beyond the top 20 cm would depend on the type of soil, with sandy loam retaining the chemicals more than loamy sand, therefore preventing downward migration. Even though some migration would be expected, the amount of chemicals migrating would be very low, especially in the loamy sand soil case where losses would be

negligible. In the loamy sand, their mass balances showed that, after 10 years of irrigation, none of the chemicals would loose more than 1.5% of their mass through vertical migration.

Overall, adsorption and degradation were determined by the authors (Chen *et al.*, 2013) to be the main fate for the chemicals when entering the soil through irrigation, and, together with concentration in the irrigation water, they were the variables driving the potential for migration into the groundwater, and accumulation in soil. Over-irrigation would also result in increased leaching for all chemicals, but was not considered a relevant factor for soil accumulation.

7.1.2. ESOCs uptake by plants

One of the main concerns related to biosolids land application is the possibility of ESOCs uptake by plants. As discussed in Chapter 3.1, the toxicity of heavy metals to plants, especially crops, and the uptake of both metals and POPs was one of the main reasons for the establishment of limits to the amounts of metals (and organic compounds in some jurisdictions) allowed in biosolids intended for land application.

Both the toxicity of heavy metals to plants (Nagajyoti *et al.*, 2010) and the uptake of these elements by some plant species have been well demonstrated, to the extent that plants are used to remove metals from contaminated water and soils (Lasat, 2000; Weis and Weis, 2004; Vamerali *et al.*, 2009).

Plant uptake of organic compounds has also been demonstrated (Ryan *et al.*, 1988; Paterson *et al.*, 1990; Simonich and Hites, 1995; Collins *et al.*, 2006), and a number of studies have also shown the accumulation of a variety of ESOCs by different plant species (Boxall *et al.*, 2006; Wu *et al.*, 2010; Aryal and Reinhold, 2011; Karnjanapiboonwong *et al.*, 2011; Pannu *et al.*, 2012; Wu *et al.*, 2012; Zarate Jr *et al.*, 2012; Prosser *et al.*, 2014).

Most of these studies were conducted with the goal of providing data to evaluate the risk to human health from edible plant products contaminated with ESOCs. However, in many cases the plants were exposed to unrealistically high concentrations of the compounds, or the exposure occurred in media that favoured uptake (e.g., soil-free aqueous media or sand) of the ESOCs, or that had been purposely enriched with the compounds; all scenarios that are not representative of standard agricultural practices (O'Connor, 1996; Prosser and Sibley, 2015). Although such studies are useful as proof-of-principle, it has long been shown that these practices might result in an overestimation of the availability of the chemicals for uptake by plants (O'Connor, 1996).

Prosser and Sibley (2015) recently collated and reviewed a number of studies on this topic to assess the risk to human health from the consumption of plant products grown in biosolids- or manure-amended soils, or with wastewater irrigation. Only studies that were conducted under relevant environmental and agronomical conditions were considered for the risk assessment, and they were limited to 10 in the case of biosolids-amended soils.

The risk assessment was conducted by estimating hazard quotients for the ESOCs measured in the plants reported in the chosen studies. Hazard quotients were calculated for adults and toddlers as the ratio of acceptable daily intake (ADI) to the estimated daily intake (EDI), and chemicals showing hazard quotients with values ≥ 0.1 were considered a potential human health hazard.

Only 10 of the 50 PPCPs analyzed in plants grown in biosolids-amended soils were detected in the edible portion (ATN, CBZ, CIP, diphenhydramine, NPX, NOR, salbutamol, triamterene, TCC, and TCS), and hazard quotients were ≥ 0.1 for only 4 (CBZ, diphenhydramine, salbutamol, and TCS). However, for CBZ, diphenhydramine, and TCS, the plants were exposed to concentrations in the higher end of the range of reported concentrations in biosolids, and in some cases even higher, thus probably exaggerating the risk. Fewer studies were found for plants grown with manure amendment or wastewater irrigation, and the risk analysis results were similar to those of the biosolids-amended soils.

Overall, the authors concluded that the risk to human health from the consumption of plants grown in biosolids- or manure-amended soils is minimal. However, they also noted that more information might be required because there have been relatively few studies, especially for manure and wastewater irrigation, and some ESOCs have not been considered at all. Additionally, the potential risk of exposure to mixtures of ESOCs needs to be evaluated (Prosser and Sibley, 2015).

7.1.3. ESOCs accumulation in earthworms

Because of their intimate contact, earthworms and other soil biota are directly exposed to chemicals present in the soil compartment. As a consequence, earthworms can accumulate both metals and organic compounds when present in the soil, and they have also been shown to accumulate some of the ESOCs (Sellström *et al.*, 2005; Markman *et al.*, 2007; Kinney *et al.*, 2008; Kinney *et al.*, 2012; Gaylor *et al.*, 2013; Carter *et al.*, 2014).

The degree of organic chemicals bioaccumulation by earthworms can show wide variations, depending on factors such as the nature of the chemical (Zhao *et al.*, 2013; Carter *et al.*, 2014) and its concentration (Gaylor *et al.*, 2013; Zhao *et al.*, 2013), the type of earthworm species (Kinney *et al.*, 2012; Gaylor *et al.*, 2013), and the type of soil (Nyholm *et al.*, 2010). Additionally, chemical uptake can be enantiomer-specific (Xu *et al.*, 2009c; Wang *et al.*, 2014).

The fate of the chemicals within the earthworms has not been as thoroughly studied, but existing evidence suggests that it is also variable and depends on the nature of the chemical. For example, Carter *et al.* (2014) studied the uptake of CBZ, DCF, FLX, and orlistat from spiked soils, and observed that all 4 compounds were absorbed by the worms, but CBZ and FLX were thoroughly eliminated from the worms when they were transferred to clean soil, whereas DCF and orlistat were only partially eliminated. Additionally, CBZ and FLX were absorbed as the parent compound, whereas DCF was degraded in the soil and unidentified metabolites were then absorbed. In a separate study, Wang *et al.* (2014) reported the presence of several metabolites of diniconazole in the earthworms after uptake that were not found in the soil, suggesting *in vivo* transformation.

Similar to ESOCs uptake by plants, several studies have shown that soil or biosolids aging can have an effect on accumulation by earthworms. Nyholm *et al.* (2010) observed lower accumulation in aged soil for several compounds, including PBDEs with 6 or less bromine atoms, whereas the congeners with 8 and 10 bromines showed no change. Liang *et al.* (2010) also saw a decrease in PBDEs bioaccumulation from aged soil, but it was the congeners with the larger number of bromine atoms that showed lower accumulation. A

major difference between the studies was the aging period, Nyholm *et al.* (2010) compared soils aged for 7 d and 2 years, whereas Liang *et al.* (2010) compared freshly spiked soils to 28 d aged soils.

Gaylor *et al.* (2013) also observed a decrease in PBDE bioavailability when comparing artificial soil amended with biosolids to the same soil spiked only with the PBDEs, and Higgins *et al.* (2011) observed lower bioavailability of TCC to *Eisenia fetida* when comparing their experimental results with biosolids-amended soils to literature data from freshly spiked sediments.

These results suggest that using spiked artificial soils might result in an overestimation of the bioaccumulation of ESOCs, as indicated by Nyholm *et al.* (2010). Additionally, Carter *et al.* (2014) showed that bioconcentration factors (BCFs) estimated with QSAR models overestimated the values calculated experimentally.

Finally, most of the studies reported no toxicity to the worms, with the exception of Kinney *et al.* (2012) and Gaylor *et al.* (2013), who observed mortality at the highest biosolids concentrations tested, 4% and ≥9% respectively, which are too high for the Canadian context. In general, however, these studies were not designed to test toxicity. Studies designed to assess the impact of biosolids amended-soils to earthworms are reviewed in Chapter 8.1.2.

7.2. Pathogens

After being applied to the soil, the fate of biosolids-derived pathogens is governed by a number of complex processes that ultimately also determine the level of human exposure to these organisms (Westrell *et al.*, 2004; Pachepsky *et al.*, 2006). These processes include:

- Transport in aerosols
- Survival and inactivation in the biosolids-amended soil and in plants following contact with contaminated soil and/or water
- Transport in soil

7.2.1. Aerosol generation from land application

As mentioned in Chapter 4.3, aerosolization is considered one of the most relevant routes for human exposure to pathogens after biosolids land application. The greatest risk from this route is considered to occur at locations close to biosolids loading operations. Brooks *et al.* (2005) demonstrated that during spreading operations (either spray irrigation or cake biosolids application with a manure spreader), air samples were not impacted (as measured by total heterotrophic plate counts above background levels) beyond 20-40 m depending on the site. Total coliforms, *Clostridium perfringens* and *E. coli* were isolated only occasionally and at distances of less than 5 m downwind from application; no aerosolized coliphages were found. Application of liquid biosolids by side slinging produces a much greater aerosol emission rate than liquid sludge spray application (Viau *et al.*, 2011).

Tang (2009) reviewed pathogen survival in aerosols, which depends on the nature of the organism itself and the surrounding environment. In general, low temperatures (7-8°C) promote survival of viruses and bacteria, with survival progressively decreasing as

temperatures increase; temperatures above 30°C prevent aerosol transmission. Viruses with lipid envelopes tend to survive longer at lower relative humidity (RH), while non-lipid enveloped viruses such as adenoviruses and influenza viruses survive longer at high RH (70-90%). Similarly, bacteria differ in their sensitivity to heat and humidity, with Gram-positive cells generally being more resistant than Gram-negative bacteria, but much depends on the state in which the cell was when aerosolized. UV is harmful to all microorganisms, with those bearing heavy cell coats tending to be more resistant (e.g. spore formers); high RH tends to be somewhat protective.

Therefore, application methods that generate minimal aerosols are preferred, e.g. injection or surface application followed by incorporation to the soil. These methods are also the preferred options for nutrient retention in the soil.

7.2.2. Survival and inactivation in soil

The rate of pathogen die-off depends on the interaction of several factors including: organism type, soil type, organic matter content, temperature, moisture, UV exposure, and indigenous microflora. Table 7.1 shows the estimated survival time of pathogens on soil and plants.

Much of the information on pathogen decline in soils used for assessing and modelling risks following land application of biosolids is based on data from manure-borne pathogens. This is likely a reasonable extrapolation. Furthermore, many models and risk assessments are based on linear extrapolation of decay rates from relatively short-term laboratory or, in a very few cases, field experiments. These extrapolations need to be used somewhat more cautiously.

Die-off of pathogenic or indicator bacteria in soil following application generally follows a two- or three-stage process. Immediately following application, a short period of apparent re-growth may occur for some organisms; this is followed by a rapid decline followed by a slower decline phase. Gale (2005) discussed the possible reasons for the two-phase decline pattern, which are related to a protective effect from microbial clustering on soil particles, as part of the development of a detailed method for a quantitative microbial risk assessment (QMRA) quantifying pathogen risks from consumption of root crops potentially impacted by biosolids land application. This QMRA model assumes straight line log-linear decay rates for pathogens in soil, but with the caveat that there is a great deal of uncertainty in the extrapolation of decay rates derived in 1-2 month studies to 12 months. There is a general lack of long-term pathogen survival information available. Warriner *et al.* (2009) recently reviewed the factors affecting the survival of pathogens and indicator organisms in soils.

Table 7.1. Survival times of pathogens on soil and plants (Gerba and Smith, 2005)

Pathogen	Soil		Plants	
	Absolute Maximum	Common Maximum	Absolute Maximum	Common Maximum
Bacteria	1 year	2 months	6 months	1 month
Viruses	6 months	3 months	2 months	1 month
Protozoa	10 days	2 days	5 days	2 days
Helminths	7 years	2 years	5 months	1 month

A study using a sentinel vial technique was conducted by the Soil Resource Group to examine the die-off of manure-borne pathogens and indicators under field conditions (SRG, 2011). Sentinel vials containing mixtures of soil and manure applied at typical application rates and spiked with cocktails of several strains each of *E. coli* O157, *Salmonella* spp and *Listeria monocytogenes* were set out in spring, late summer and fall. *E. coli* populations existing in the manure were monitored for comparison. Populations were monitored from the initiation of each field set up until the following spring, i.e. monitored for up to 12 months in the field. The effects of manure type, soil type, depth (surface and 10cm), and season (as affected by temperature and moisture) were examined. *E. coli, Salmonella, E. coli* O157 and *Listeria* underwent a 1-3 $\log_{10}$ reduction in the first 3 to 4 weeks following deployment in spring, late summer and fall. The pattern of die-off was typically a 2-phase pattern (rapid followed by slow decline rates); initial re-growth was common for *E. coli*, but also occurred occasionally with *E. coli* O157, *Salmonella* and *Listeria*. *E. coli* O157 and *Salmonella* were no longer detectable by 120 days under all conditions tested (method detection limit 10^2/g soil). However, manure-borne *E. coli* was occasionally isolated until the following spring (method detection limit 10/g soil). *Listeria* followed the same general patterns but results were somewhat more ambiguous, often due to difficulties in selectively culturing these organisms from a soil/manure matrix. *E. coli, Salmonella* and *E. coli* O157 survived longer at the 6" depth placement than at the surface, particularly in the fall; *Listeria* survival was unaffected by depth. Die-off rates were highest in warm dry conditions. Studies are on-going, including the use of enrichment procedures to better quantify the persistence of low levels of pathogens in soils.

Similar to the study above, Schwarz *et al.* (2014) studied the decay of enteric microorganisms, including adenovirus and *E. coli*, in biosolids-amended soil seeded with these microorganisms and under wheat cultivation. They found *E. coli* and *Salmonella enterica* had a short decay time (T_{90}=4-56 days) compared to un-amended soil (T_{90}=8-83 days). Comparatively, adenoviruses had a much longer decay time (T_{90}=180 days).

While initial populations of protozoan contaminants in sludges and biosolids are much lower than many bacterial contaminants, their survival is enhanced by the production of resistant oocysts. Measurement of die-off rates for protozoa such as *Cryptosporidium* in soils is complicated by the available methods: viability direct counts with immunofluorescence assay (IFA; most common), PCR (common), and infectivity counts (least common). Nasser *et al.* (2007) demonstrated that the degradation of the *Cryptosporidium parvum* oocyst cell walls by extracellular proteinases from *Pseudomonas aeruginosa* resulted in loss of infectivity without reducing enumeration by IFA or PCR. Furthermore, 10-day exposure of oocysts to dry soil at a mean temperature of 32°C resulted in a 2.5 $\log_{10}$ reduction in infectivity compared with a 0.5 $\log_{10}$ reduction in viability measured by IFA. They found no decrease in infectivity or viability in oocysts incubated for 10 days in distilled water or saturated loamy soil at 15°C, no decrease in viability measured at 30°C, but a 0.93 $\log_{10}$ reduction in infectivity.

Virus survival in soils is strongly affected by temperature and adsorption to the soil (Hurst *et al.*, 1980; Sobsey *et al.*, 1980). Temperatures above 30°C enhance inactivation. Viruses are strongly adsorbed onto clays, and adsorption increases survival, thus soil type is a primary factor. Parashar *et al.* (2011) demonstrated a 5 $\log_{10}$ reduction in soils spiked (to $10^{7\text{-}8}$) with Hepatitis A and E virus over 9 weeks in fluctuating environmental temperatures; constant temperatures increased survival times up to 13 weeks. In general, virus survival can be

expected to exceed enteric bacterial survival. Gale (2005) and Brooks *et al.* (2012) both use 4 $\log_{10}$ decay in 90 days for QMRAs.

Among the helminths, *Ascaris lumbricoides*, a roundworm, is the most commonly referred to helminth associated with biosolids, perhaps because of its reported longevity. Ensink and Fletcher (2009) summarized available information on the survival of *Ascaris* as follows: "eggs have shown the greatest survival time in soil, though the variability in survival times is great. Studies conducted during hot dry summers have shown a survival ranging from 27-35 days, while studies conducted during the winter season in Japan have shown a survival of up to 5-6 months. In rare instances, *Ascaris* eggs have shown to survive up to 7 years in soil, though the 'normal' maximum survival time is set by the WHO guidelines for the safe use of wastewater in agriculture at 2 years. At 20°C, it is estimated that it takes 15-100 days for all *Ascaris* eggs to be inactivated in soil." Survival in soils is higher at greater depths because of shielding from UV light and desiccation.

There is limited information on the effect of long-term biosolids application on pathogen loads. Zerzghi *et al.* (2010) did not detect any enterovirus, phage, or *Salmonella* following 20 years of annual applications of 8 and 24 tonnes/ha of liquid Class B biosolids onto a clay loam soil. Analyses were done 10 months after the last application. They did find elevated microbial activity as measured by dehydrogenase and sulfur oxidation activities, indicating improved soil health for both application rates.

7.2.3. Transport

Transport of surviving pathogens away from the site of application is very complex, and there is less relevant information available compared to information on pathogen survival. Significant work has been done on the development of models comprising various stages of movement of microorganisms through the environment. Pachepsky *et al.* (2006) critically evaluated available information on processes and controlling factors governing the transport of manure-borne pathogens including:

- Release from source,
- Survival and inactivation in manure, soil and surface water,
- Partitioning and attachment of pathogenic and indicator organisms to solid particles in runoff, soil and sediment, and
- Transport with straining or entrapment in overland flow and in streams

The authors identified challenges and research needs, and in particular the lack of information on most aspects of pathogen transport. No equivalent review of transport processes of biosolids-borne pathogens was found, but it is reasonable to assume that the processes will be similar. A possible exception to this may be when land-applied biosolids are of a significantly different nature than manure such as material with high fats/oils/grease content (FOG); however, the nature of the pathogen load will be different from typical MAD biosolids. MAD-treated biosolids that have undergone further treatments such as pelletization or high temperature/shear force are Class A biosolids with negligible pathogen loads.

In general, the transport of pathogens is driven by the hydrology of storm events. Teng *et al.* (2012) developed predictions of critical rainfall events for QMRAs based on intensity and duration of events and soil texture (which dictates the partitioning of runoff to infiltration for

any event). The authors concluded that the maximum runoff is produced by intermediate storms (short intense storms do not saturate the soil and longer duration events tend to be of lower intensity). Infiltration and runoff partitioning can be calculated based on the analysis and used for QMRAs. However, the impacts of slope, antecedent moisture, and cover were not considered in the analysis, but should also be taken into account according to Pachepsky *et al.* (2006).

The factors affecting the release of pathogens from source material and/or binding to solids being transported by water away from the site of application are dependent on the chemical and physical characteristics of both the organism, and the particulate matter, and the surrounding matrix. Jamieson *et al.* (2002) and Ferguson *et al.* (2003) published detailed reviews on movement and factors affecting movement of pathogens.

Information regarding the transport of pathogens is limited; the bulk of relevant information is derived from studies of manure-borne fecal coliforms and/or *E. coli* transported over field plots. Factors affecting the overland and sub-surface transport of pathogens and indicator organisms in soils were reviewed by Warriner *et al.* (2009).

Muirhead *et al.* (2006) studied the effect of flow intensity and tillage on the transport of *E. coli* down 5 m long field plots. Approximately 10% and 40% of the effluent *E. coli* were removed at flow rates of 2 L/min and 20 L/min, respectively, over the 5 m length of the field plots. Cultivation significantly reduced the overland transport of *E. coli* from the plots. It is reasonable to expect that *E. coli* in liquid biosolids would behave similarly.

Data on pathogen overland transport is even more limited, but Atwill *et al.* (2002) used planted soil boxes to assess the efficiency of vegetated buffer strips (VBS) to remove *Cryptosporidium* oocysts from surface and shallow subsurface flow. They determined that VBS at slopes ≤20% and lengths ≥3m should remove ≥99.9% of oocysts. However, under field conditions, the effectiveness of VBS to remove microorganisms is much more variable, and effective filtration depends largely on the state of vegetation and sheet flow (Dosskey *et al.*, 2002). There is also the possibility that VBS can act as a potential source of contamination if accumulated loads are released before die-off occurs.

The impact of application timing was examined in a long-term Ontario study by Culley and Barnett (1984) who measured total and fecal coliforms and fecal streptococci concentrations in runoff and subsurface discharge waters following manure applications. They found that neither the rate nor application time affected the bacterial content of spring surface and subsurface discharge unless the manure was applied on frozen soil. In this case, spring snowmelt dominated runoff and significantly decreased discharge water quality, indicating survival over winter and subsequent transport to surface waters.

Rapid leaching of indicators and pathogens by preferential flow and the impact of application method has been frequently demonstrated. Studies relating to the movement of manure-borne *E. coli*, other indicators and pathogens have been reviewed by Jamieson *et al.* (2002). Akhand *et al.* (2008) demonstrated preferential flows and measurable *E. coli* and *Clostridium perfringens* in tile water shortly following liquid municipal biosolids application to silt loam soils either by injection or surface application and incorporation, but not for surface application over aerator-tilled soils (Akhand *et al.*, 2006; Lapen *et al.*, 2008a). Ramirez *et al.* (2009) demonstrated the impact of tillage and rainfall on transport of *Cryptosporidium* through intact

soil blocks. Leaching of oocysts through macropores of no-till soils occurred even before rainfall treatments, and following rainfall events a greater number of oocysts leached through the no-till soils compared with conventional tillage (which breaks up macropores). Higher intensity rainfall events produced more oocysts in leachate samples. Forslund (2012) demonstrated higher recoveries of added *Cryptosporidium* oocysts and bacteriophage in rainfall-induced leachates from injection of manure slurries compared to surface application onto intact sandy clay loam soil columns; indigenous *E. coli* was not affected by application method. Experiments were done in the cold months (increasing survival), and leaching of bacteriophage persisted beyond 148 days. On the other hand, Horswell *et al.* (2010) found that survival and transport of human adenovirus spiked into sewage sludge in intact soil cores were minimal (up to 2 months at 16°C).

Movement of pathogens from manure or biosolids fertilized cropland to surface or groundwater has the potential to be a source of contamination of water that may be used for irrigation purposes. This could result from over application of liquid manures or biosolids and direct overland flow, but it would more commonly occur as preferential flow through soils to tiles, or in response to heavy rainfall shortly following manure or biosolids application. Potential also exists for rainfall-induced surface transport from treated cropland directly to fruit and vegetable fields.

7.2.4. Survival in crops

Persistence of pathogens on plant surfaces is generally short if they are exposed to sunlight, drying, etc., but can be enhanced if they are present in biofilms or collected within groves of epidermal cells (Warriner *et al.*, 2003). There have been relatively few studies with regard to the survival of human pathogens on the surface of leaves over long periods. Gerba and Smith (2005) reported the common maximum survival times for pathogens on plants to be 1 month for bacteria, viruses and helminths, and 2 days for protozoa; absolute maximum were 2 to 6 times longer (Table 7.1). However, the cited survival time for protozoa (2 days to absolute maximum of 5 days) seems particularly short for some protozoa in view of the known resistance of cysts and oocysts of *Giardia* and *Cryptosporidium*. However, studies using *C. jejuni*, *E. coli* O157:H7, and *Salmonella* would suggest that their survival on plant surfaces is low. Nevertheless, contamination of edible leaves immediately prior to harvest would represent a significant food safety hazard (Warriner *et al.*, 2009), and close monitoring of irrigation water quality is warranted.

Some pathogens have been shown to survive longer in the rhizosphere than in bare soil, but the impact appears to be plant-specific. For example, *E. coli* O157 persisted longer in the presence of rye and alfalfa plants, but not in the presence of other legumes or corn (Gagliardi and Karns, 2002). Natvig *et al.* (2002) found survival of manure-borne *E.coli* and *Salmonella* in soils 3 months post spring application, but not on vegetables planted 3 months following manure application. In contrast, they found some survival 2.5 months following summer application in soils and on harvested plants, demonstrating the impact of seasonal conditions on survival and required withholding times.

7.3. Development of antibiotic resistance

Antibiotic resistant bacteria (ARB) and genetic material (ARG) are being found increasingly in the environment, and products from WWTPs are potential sources. There are three issues to

consider with antibiotic resistance: the first is the release of the resistant organisms themselves to the environment, the second is the transfer of antibiotic resistant genes from one organism to another in the sludge matrices, and the third is the selective enrichment of antibiotic resistant populations due to the presence of antibiotics in sewage sludge. The presence of ARB, ARG and antibiotics in WWTP sludge and biosolids is common and has been studied by several groups. For example, Reinthaler *et al.* (2010) found that 74% of 27 samples of activated sludge samples (Austria) were positive for extended spectrum β-lactamases (ESBLs) that confer resistance to penicillin, cephalasporin and related antibiotics.

As reported in Pepper *et al.* (2006), Brooks (2004) evaluated the incidence of ARB in biosolids, pristine soil, ground water, tap water, and foodstuffs and found the percentage of ARB in biosolids to be nearly always less than that of pristine soil, ground water, or tap water. Munir and Xagoraraki (2011) examined ARG and ARB in the effluent and biosolids at 5 WWTPs in Michigan. Reductions in both resulted from advanced treatment methods (anaerobic digestion and lime stabilization) and conventional (dewatering and gravity thickening), though advanced systems were significantly better. Membrane bioreactor treatment systems reduced ARB and ARG by 1-3 $\log_{10}$ more than either advanced or conventional treatments. Chlorine and UV disinfection of effluents appeared not to affect either ARG or ARB. The daily release loads of ARG and ARB to the environment were higher through biosolids than through effluents.

In contrast, Rizzo *et al.* (2013) concluded that biological processes did very little to reduce ARG or ARB, but the authors only looked at information up to the activated sludge process stage. Burch *et al.* (2013) examined the effect of aerobic digestion under semi-continuous flow mode, and found 90% reduction of some ARG, but increases in others; reduction rates for all increased under batch reactor flow, demonstrating the importance of the process design. Diehl and LaPara (2010) found little reduction in ARG under aerobic digestion at 22-55°C, but effective removal under anaerobic systems at >37°C. However, other studies showed increases in temperature to 37°C to be ineffective (Ma *et al.*, 2011).

Antibiotic resistance frequencies increase in response to the selective pressure of the presence of antibiotics. Gao *et al.* (2012) demonstrated the presence and reduction of 15 pharmaceuticals (primarily antibiotics) in a conventional WWTP in Michigan. About half were reduced by 90 to >99%, while others were reduced by 50% or less. Therefore, selective pressure increasing ARB frequencies is unlikely to be large. Tong *et al.* (2013) selectively tested for populations resistant to tetracyclines and β-lactam antibiotics. The authors showed no significant change in resistance rate for tetracyclines but a slight increase in the rate for some β-lactam RB, but with reduction in total ARB over the WWTP treatment processes.

Since antibiotic resistance is an energy-costly trait to maintain, organisms tend to lose resistance genes when there is no selective advantage to maintaining them. Antibiotics remaining in land-applied biosolids will be broken down in the soils, thus removing the need to maintain resistance genes. Based on this rationale, The Committee on Toxicants and Pathogens in Biosolids Applied to Land concluded that land-applied biosolids would not have "any substantial potential to alter the prevalence of antibiotic resistance among pathogenic microorganisms" (NRC, 2002).

Indeed, Brooks *et al.* (2007) were not able to measure any increase in ARB populations above background levels following single, and at one site long-term, biosolids applications.

Rahube *et al.* (2014) measured the abundance of ARG in vegetables grown in soil amended with biosolids and untreated sludge, and concluded that although both treatments had the potential to increase ARG presence, a 15-month waiting period was sufficient to attenuate their abundance.

7.4. Summary

The fate of ESOCs in soils after biosolids land application is a very site-specific phenomenon, because it is driven by the combination of a large number of factors, from the physicochemical properties of the chemicals and the soils, to environmental variables such as temperature and soil moisture content, and even the biosolids application methods. All these factors will influence the processes determining the ultimate of the ESOCs, including biodegradation, sorption to soils, and transport out of the soil column into groundwater with percolating water, or into surface water bodies with precipitation or irrigation runoff.

The list of factors that may influence the fate of ESOCs is vast and includes the compound's properties, such as water solubility and octanol/water partition coefficient (K_{ow}) (Lapworth *et al.*, 2012), soil characteristics, such as texture (Gottschall *et al.*, 2012), oxygen availability, temperature, pH, organic matter content (Lapen *et al.*, 2008b) and type (Rauch-Williams *et al.*, 2010), the presence of macropores (e.g., earthworm borrows) in the soil (Lapen *et al.*, 2008b; Larsbo *et al.*, 2009), the amount and timing of rain and/or irrigation events (Edwards *et al.*, 2009), soil aging (Mueller *et al.*, 2006), the presence of plants (Mueller *et al.*, 2006; Welsh *et al.*, 2009), and even the presence of other ESOCs (Monteiro and Boxall, 2009)

Taking into account the large number of ESOCs and their wide range of physicochemical properties, as well as the different types of soil and variations in the factors listed above, it is not surprising that the ultimate fate of the ESOCs is also very variable. For example, some compounds generally persist for long periods of time in the soil; e.g., TCS (Edwards *et al.*, 2009), and the PBDEs (Gorgy *et al.*, 2011), while others are very mobile; e.g., iopromide (Oppel *et al.*, 2004). Or in cases where tile drainage is used, ESOCs concentrations in the effluent can show wide variations as observed by Edwards *et al.* (2009) and Gottschall *et al.* (2012).

From the articles reviewed in this chapter, it is clear that field studies are still necessary to accurately evaluate the fate of ESOCs in soils, because laboratory studies and models tend to overestimate dissipation by sorption and biodegradation (Walters *et al.*, 2010; Langdon *et al.*, 2011; Langdon *et al.*, 2012), and that the processes that control migration of ESOCs through the soils and groundwater, including sorption to colloidal matter, are not well understood (Lissemore *et al.*, 2006; Lapworth *et al.*, 2012).

However, the studies above also reveal that most of the PPCPs typically found in effluent and biosolids do not reach the groundwater when applied to soils (Ternes *et al.*, 2007), and that the concentrations of most PPCPs in tile drainage (Edwards *et al.*, 2009) and surface runoff (Sabourin *et al.*, 2009) tend to be much lower than typical concentrations found in WWTP effluents.

Additionally, the potential for surface or groundwater contamination with ESOCs from biosolids land application has to be evaluated along with other ESOCs sources such as

manure, landfill leachate, or septic tanks (Larsbo *et al.*, 2009; Eggen *et al.*, 2010; Lapworth *et al.*, 2012). In the case of groundwater, surface water exchange might be a more likely source of ESOCs than vertical migration (Lapworth *et al.*, 2012) and, although the ESOCs might originate from runoff of the land-applied biosolids, effluents from WWTPs are considered to be the main source of PPCPs in surface waters (Larsbo *et al.*, 2009).

ESOCs uptake by plants has been clearly demonstrated. However, many of the published studies rely on the use of unrealistic conditions to evaluate this phenomenon, which might overestimate the concentrations found in plants grown under more relevant environmental and agricultural conditions (O'Connor, 1996; Prosser and Sibley, 2015). The risk to human health from the consumption of plants grown in biosolids-amended soils under relevant conditions was considered minimal, although available data for ESOCs in such plants is relatively scarce, and more information is necessary, especially in the case of exposure to ESOCs mixtures (Prosser and Sibley, 2015).

Similarly, bioaccumulation by earthworms has also been observed, and soil or biosolids aging was identified as a factor decreasing bioavailability of the ESOCs. Therefore, the use of artificial soils or biosolids, or spiking the samples can result in overestimating the potential for bioaccumulation (Nyholm *et al.*, 2010; Gaylor *et al.*, 2013).

Finally, although accumulation of ESOCs in soil, crops, or soil organisms might not be desirable, especially in the case of chemicals used unnecessarily in high amounts (e.g., TCS), the sole presence of the chemicals does not constitute proof of negative impact to the soil ecosystem, which has to be evaluated by other means.

In a similar way, the fate of pathogens after land application of biosolids is also a complex and site-specific phenomenon governed by a combination of processes and environmental factors. The most relevant processes to the ultimate fate of biosolids-related pathogens in the soil are aerosolization, survival/inactivation and transport in the soil (Westrell *et al.*, 2004; Pachepsky *et al.*, 2006).

During application, pathogens are subject to aerosolization, which is considered one of the most relevant routes for human exposure in this context. However, the risk for exposure occurs mainly at short distances from the application site (Brooks *et al.*, 2005), and it is mainly considered an occupational risk for biosolids operators in the land application context. Additionally, survival of pathogens in aerosols is affected by environmental conditions, including temperature, relative humidity, and UV exposure, with the magnitude of the effect being dependent on the nature of the pathogen (Tang, 2009).

The rate of pathogen survival in the soil and on plant surfaces also depend on the interaction of several factors, including the types of organism and soil, organic matter content, temperature, moisture, UV exposure, and indigenous microflora (Warriner *et al.*, 2009). In general, virus, helminth eggs and oocyst-forming protozoa (e.g., *Cryptosporidium*) have longer survival rates than bacteria.

Transport of pathogens in the soil is generally governed by the hydrology of the specific soil and site. The intensity and duration of rain events, the slope of the terrain, and soil characteristics such as texture, saturation, and vegetable cover are all factors that influence pathogen transport at the watershed level (Pachepsky *et al.*, 2006; Teng *et al.*, 2012).

However, at the particle level, pathogen adsorption and desorption interactions with the soil also affect their fate (Ferguson *et al.*, 2003).

Besides the risk of infection with biosolids-borne pathogens, the potential development and transfer of antibiotic resistance is one of the main public health concerns in the biosolids land application context. Although the presence of antibiotic resistant bacteria (ARB) in sludge and biosolids is well documented, incidence tends to be lower than in other compartments, such as pristine soil. The risk of antibiotic-resistance gene transfer in soil is also considered low (Pepper *et al.*, 2006), and land-applied biosolids are not expected to affect the incidence of antibiotic resistance in pathogens (NRC, 2002).

7.5. References

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8. IMPACT STUDIES AND BIOLOGICAL ENDPOINT TESTING IN BIOSOLIDS-AMENDED SOILS

As mentioned in previous chapters, most risk assessments conducted to date have concluded that there is no risk to human health, but the risks to biota have not been properly assessed (Clarke and Porter, 2010). However, a series of studies have investigated the impact of soil amendment with biosolids to different organisms, especially plants, but also to earthworms and other soil biota, including the microbial community. These impact studies are the main subject of this chapter, which also includes a discussion on the use of "omics" and biomarkers for toxicity testing in the context of biosolids land application.

8.1. Impact studies

8.1.1. Terrestrial plants

Studies assessing biosolids application to agricultural lands as a means of fertilizer and soil amendment have overwhelmingly found a positive impact on plant growth, biomass, and yield, although impact varied with soil and crop type. Early studies such as Cunningham *et al.* (1975) and Sabey and Hart (1975) applied biosolids at extreme rates of over 100 t dw/ha; although these rates still led to higher yields, they also resulted in unacceptable accumulation of heavy metals (Olness *et al.*, 1998). More recent studies such as Cogger *et al.* (2001), Cooper (2005), and Mantovi *et al.* (2005) used lower biosolids application rates, 10 to 25 t dw/ha.

Most authors attribute the observed yield increases to large additions of nutrients such as nitrogen, phosphorus and potassium, as well as increases in organic carbon and changes to water holding capacity, bulk density, aeration, pH, and nutrient availability of the soil (Olness *et al.*, 1998). Typical terrestrial plants used for these studies include fast-growing plants such as lettuce (*Lactuca sativa*) and various grass species, as well as more economically relevant crops, such as corn (*Zea mays*) and wheat (*Triticum aestivum*). Table 8.1 summarizes impact studies of land-applied sewage sludge and biosolids and the observed effects on terrestrial crops.

Table 8.1. Summary of impact studies of land application of sewage sludge and biosolids to crops, 1972 - present.

Species	Sludge/Biosolids Origin & Treatment	Chemical Analysis of Biosolids	Application Rate(s)	Field/Green-house/Lab; Duration	Effects on Growth &/or Crop Yield	Reference
Cynodon dactylon **(coastal Bermudagrass)**	Georgia; anaerobically digested	Nutrients, some metals	0, 0.63, 1.25, 2.5, 5.0 cm	Field; 2 years	Increase in dry biomass up to 2.5 cm application rate; smothering effect at higher rates	King and Morris (1972)
Hordeum vulgare **(barley)**	Lethbridge, Alberta; air-dried, digested	Nutrients	0, 34, 67, 101 t/ha	Field; 1 growing season	Increase in yield up to highest application rate for first crop, but decreased yield due to low remaining N levels for second crop	Milne and Graveland (1972)
Secale cereale **(rye),** ***Zea mays*** **(corn)**	Ontario; anaerobically digested	Nutrients, some metals	0, 2.3 cm	Field; 1 growing season	Increase in rye yield, no increase in corn yield over control	King *et al.* (1974)
Festuca arundinacea **Schreb. (fescue)**	Georgia; secondary treated, digested, vacuum filter cake	Nutrients, some metals	0, 5.6 t dw/ha, 5.6 t dw/ha + microelements	Field; 2 years	No increase in total yield over control	Boswell (1975)
Zea mays **(corn),** ***Seceale cereal*****e (rye)**	Wisconsin; anaerobically digested	Metals	0, 63, 125, 251, 502 t dw/ha	Greenhouse; 6 weeks	Increase in dry biomass up to 125 t/ha; decrease at higher rates	Cunningham *et al.* (1975)
Sorghum bicolour ***x S. sudanense*** **(sorhum sudangrass),** ***Panicum miliacium*** **(millet),** ***Triticum aestivum*** **(wheat)**	Colorado; liquid sludge, 50/50 mix aerobic and anaerobic digestion	Nutrients, metals	0, 25, 50, 100, 125 t dw/ha	Field; 1 growing season	Severe impact to germination at all application rates, likely due to organic contaminants Increase in yields up to 50 t/ha; decrease at higher rates	Sabey and Hart (1975)
Glycine max **(soybean)**	Unknown; anaerobically digested, air dried, 1-mm ground	Nutrients	0, 34, 67, 134 t/ha alone and with inorganic metals	Growth chamber; 30 days	Increase in dry biomass greatest at 34 t/ha; decrease at higher rates.	Dowdy and Ham (1977)
Secale cereale **(rye),** ***Zea mays*** **(corn),** ***Sorghum bicolor*** **x** ***S. sudanese*** **(sorghum-sudan)**	Unknown; anaerobically digested, liquid	Nutrients, some metals	0, 3.75, 7.5, 15, 30, 60 t/ha	Field; 3 years	Generally an increase in yield up to 15 t/ha; decrease at higher rates.	Kelling *et al.* (1977)
Phaseolus vulgaris **(snap bean)**	Unknown; anaerobically digested	P, K, Ca, Na, Mg	0 to 1400 t/ha, multiple applications	Field; 4 years	Increase in yield greatest at highest application rate.	Dowdy *et al.* (1978)
Glycine max **(soybean)**	Unknown; anaerobically digested and air-dried	Nutrients, metals	0, 25, 50, 100, 200 t/ha + metal salts	Field; 2 years	No significant increase in seed yield with increasing application rate.	Ham and Dowdy (1978)

Species	Sludge/Biosolids Origin & Treatment	Chemical Analysis of Biosolids	Application Rate(s)	Field/Green-house/Lab; Duration	Effects on Growth &/or Crop Yield	Reference
***Zea mays* (corn), hay (various species)**	Vermont; aerobically digested secondary sludge	Nutrients	0 – 4.56 cm	Field; 2 years	Increase in yield over inorganic fertilizer, except at highest application rate.	Magdoff and Amadon (1980)
***Allium cepa* (onion), *Lactuca sativa* (lettuce), *Antirrhinum majus* (snapdragon), *Festuca arundinacea* (turfgrass)**	San Diego; anaerobically digested secondary sludge; composted with 60% v/v chipped *Eucalyptus* trimmings	Nutrients, some metals	0, 37, 74 t dw/ha/yr	Field; 2 years	Increase in onion, turf and lettuce yield compared to control. Snapdragon yield not increased by compost addition.	Bevacqua and Mellano (1993)
***Zea mays* (corn), *Phalaris arundinacea* (reed canarygrass)**	Minnesota; liquid sludge, both anaerobic and aerobically digested	Nutrients, metals	Corn: mean 12 t dw/ha/year Grass: mean 14 t dw/ha/year	Field; 20 years	Significant increase in corn and grass yields compared to fertilized controls most years.	Linden *et al.* (1995)
***Capsicum annuum* (bell peppers), *Cucumis sativus* (cucumber)**	Unknown; yard trimming-biosolids compost, treated unknown	Nutrients, some metals	134 t ww/ha	Field; 1 growing season	Increase in pepper yield with co-application of biosolids compost and fertilizer	Roe *et al.* (1997)
***Artemisia* spp. (sagebrush)**	Colorado; municipal biosolids, treatment unknown	Nutrients, metals	0, 5, 10, 15, 20, 25, 30, 35, 40 t/ha	Field; 2 years	300% increase in biomass at 25 t/ha rate; decrease at higher levels	Pierce *et al.* (1998)
***Zea mays* (corn), various weed species**	Florida; polymer and lime-dewatered biosolids, anaerobically co-processed with municipal solid waste	Nutrients, metals	Extract (20 g dw to 50 mL distilled water)	Lab; 8 weeks	Immature (aged <8 weeks) delayed and reduced germination	Ozores-Hampton *et al.* (1999)
***Festuca arundinacea* Schreb. (tall fescue)**	Washington; Class A (1) thermophilically digested, dewatered, (2) mesophilically digested, heat-dried	Total N, some metals	6.7, 13.4, 20.2 t dw/ha/year for 7 years, split into three applications per year	Field; 7 years	Yields increased with biosolids rate	Cogger *et al.* (2001)
***Gossypium hirsutum* (cotton)**	Greece; secondary treatment unknown	Nutrients, some metals	0, 2:1, 10:1 (v/v) soil: sludge	Conditions not stated; 154 days	Highest increase in above ground biomass and plant height at 2:1 application rate	Tsakou *et al.* (2001a)
***Gossypium hirsutum* (cotton)**	Greece; secondary treatment unknown	Metals	0, 2:1, 10:1 (v/v) soil: sludge	Conditions and duration not stated	Increase in seed and fibre production up to 10:1 application rate	Tsakou *et al.* (2001b)
***Brassica napus* (canola)**	California; class B, aerobically digested	Total and water extractable elements, including metals	0, 1.9, 5.8, 11.7 metric ton dw/ha	Field; 2 years	Increase in total dry matter forage (leaf) yield of 1.5-3.8x above reference	Bañuelos *et al.* (2004)

Species	Sludge/Biosolids Origin & Treatment	Chemical Analysis of Biosolids	Application Rate(s)	Field/Green-house/Lab; Duration	Effects on Growth &/or Crop Yield	Reference
***Triticum aestivum* (wheat), *Tritico secale* (triticale)**	Australia; (1) dewatered sewage sludge cake, (2) N-Viro (lime-amended biosolids)	Nutrients, metals	Dewatered cake: 0, 6, 12, 24 t dw/ha; N-VIro: 0, 1.5, 3.0, 4.5 t dw/ha	Field; 3 years	Increase in yield over 50%; largest increase at the highest rate of dewatered sludge	Cooper (2005)
***Triticum aestivum* (winter wheat), *Beta vulgaris* (sugar beet), *Zea mays* (corn)**	Italy; (1) anaerobically digested liquid, (2) dewatered by belt filtration, (3) composted with wheat straw	Nutrients, metals	0, 5, 10 t dw/ha/year	Field; 12 years	Increase in yields similar to high mineral fertilizer Highest liquid and dewatered sludge rate led to wheat lodging and poor crop quality; quality better with compost at same rates	Mantovi *et al.* (2005)
***Brassica rapa*, *Avena sativa* (oat)**	Portugal; anaerobic co-digestion of primary and secondary sludge	Nutrients, some metals	0, 6, 12 t dw/ha	Lab; 19-21 days	No impact on germination Increase in biomass at both rates	Moreira *et al.* (2008)
***Brassica rapa*, *Lolium perenne* (ryegrass), *Trifolium pretense* (red clover)**	Spain; (1) aerobically digested, partially dewatered, (2) anaerobically digested, partially dewatered; both composted and thermal dried	Nutrients, metals, some organic pollutants	Various, 0 – 1000 g biosolids/kg soil	Lab; 15 days	Anaerobic- treated biosolids had a decreased inhibitory effect compared to aerobic-treated Moderate stimulatory effect on shoot length at low rates	Ramírez *et al.* (2008)
***Zea mays* (corn)**	Egypt; treatment not stated	N, P, K, Al, P	0, 1, 2, 3% biosolids co-applied with up to 4% water treatment residuals (WTRs)	Greenhouse; 105 days	Increase in yield at co-application 3% biosolids, 4% WTRs; decrease at higher rates	Mahdy *et al.* (2009)
***Brassica rapa*, *Avena sativa* (oat)**	Portugal; treatment not stated	Nutrients, some metals	0, 6, 15, 25, 45 t dw/ha	Lab; 42 days	No effect on germination of growth of *B. rapa* Impaired *A. sativa* emergence at 45 t/ha, no effect on growth	Natal-da-Luz *et al.* (2009)
***Pennisetum purpureum* Schum. (elephantgrass)**	Florida; Class A; anaerobic and undigested waste-activated sludge, dewatered by belt press and thermally-dried	Nutrients, some metals	0, 33, 67, 100% N from biosolids; remainder as ammonium nitrate fertilizer	Field; 2 years	Decrease in yield as N from inorganic fertilizer replaced with N from biosolids	Castillo *et al.* (2010)
Brassica rapa	Taiwan; treatment not stated	Cd and Pb	0, 5%	35 days; growth conditions not stated	Increase in dry biomass 1.3-1.6x above reference	Chen *et al.* (2010)

Species	Sludge/Biosolids Origin & Treatment	Chemical Analysis of Biosolids	Application Rate(s)	Field/Green-house/Lab; Duration	Effects on Growth &/or Crop Yield	Reference
***Zea mays* (corn)**	New Zealand; biochar from pyrolysed biosolids (treatment not stated) and vegetation	None	0, 2.5, 5.0, 10.0 t/ha	Lab; 5 days	No effect on germination or early growth	Free *et al.* (2010)
***Lactuca sativa* (lettuce)**	New York; treatment not stated	Personal care products and pharmaceuticals	0, 1, 2, 3, 4% (w/w)	Lab; 14 days	66% decrease in emergence relative to control for 4% amended soils aged 2 weeks; 60% decrease for soils aged 8 weeks	Kinney *et al.* (2012)
***Brassica napus* (canola), *Cannabis sativa* (hemp), *Zea mays* (corn)**	Finland; treatment not stated	None	0, 10, 20% (w/w) biosolids, 10% biosolids + 10% peat	Greenhouse; 75 days	Increase in leaf area and biomass and net photosynthesis in highest rates	Seleiman *et al.* (2012)
***Triticum aestivum* (wheat)**	Washington; anaerobically digested, dewatered	Nutrients, some metals	4.8, 6.9, 9.0 t/ha	Field; 16 years	Equivalent or increased yield over standard fertilizer	Cogger *et al.* (2013)
***Brassica napus* (canola)**	Greece; anaerobically digested	Nutrients and metals	0, 20, 50, 100 t dw/ha	Greenhouse; 2 months	Increase in biomass	Shaheen and Tsadilas (2013)

The majority of the studies in Table 8.1 (75%) reported an increase in the endpoint measured (e.g., germination, yield, dry biomass) relative to the control when using sewage sludge or biosolids. Of these studies, one quarter reported a decrease in the endpoint measured (usually yield) at the highest application rates (King and Morris, 1972; Cunningham *et al.*, 1975; Sabey and Hart, 1975; Dowdy and Ham, 1977; Kelling *et al.*, 1977; Pierce *et al.*, 1998; Mahdy *et al.*, 2009). Most of these were early studies that applied treated sewage sludge at extremely high rates, which are not allowed by current regulations, and the negative effects, when attributed, were due to different factors, such as smothering of the plants due to the excessive amount of sludge applied (King and Morris, 1972), and toxicity due to the large amounts of salts present in the sludge (Kelling *et al.*, 1977).

Additionally, three of the 33 studies reported neutral results, with the addition of biosolids showing no difference in the endpoint(s) measured compared to the controls. The results of these studies show that the use of biosolids as a fertilizer has a positive impact on crop endpoints, such as yield and germination, when applied at appropriate rates.

8.1.2. Terrestrial invertebrates and microbial communities

Typically, toxicity of ESOCs and biosolids land application to terrestrial organisms is measured in the laboratory using short-term (<1 month) exposure methods in artificial and field-collected soils. Well-studied endpoints, or impacts, include lethality, impaired growth and reproduction, and avoidance/preference behaviours. Common terrestrial invertebrates used for testing include springtails (Collembolans), earthworms, and nematodes. (For a review of soil organisms used in ecotoxicology, see Donker *et al.* (1994).)

Because of the importance of the microbial population to soil ecosystems, impact to microbial activity is also of interest and is measured by quantifying microbial numbers and biomass, as well as changes to microbial community structure and diversity. Because the traditional toxicity bioassays require relatively long periods of time and measure "drastic" endpoints, there is a need for rapid evaluation of early biomarkers of toxicity. Several "omics" (collective term used to designate genomics, proteomics, metabolomics, etc.) methodologies have been used with this purpose, and they include DNA/RNA fingerprinting and protein biomarkers.

Springtails

Springtails (Collembola) are a group of small (<6 mm) terrestrial arthropods that play a key role in soil decomposition and fertility (Hopkin, 1997; Fountain and Hopkin, 2005). Springtails have been studied from a biological and taxonomic standpoint since the early 1700s (Hopkin, 1997) and have been used in ecotoxicological studies since the 1960s (Fox, 1967; Scopes and Lichtenstein, 1967; Tomlin, 1975). For reviews of springtail biology, see Hopkin (1997) and Environment Canada (2007).

Parthenogenetic species that can reproduce without a male population, such as *Folsomia candida*, are used in terrestrial ecotoxicological studies due to their ecological

relevance and ease of culturing. Several regulatory bodies including Environment Canada (2007), ISO (2011, 2014), and OECD (2009) have produced standardized survival and reproductive bioassay protocols for springtail species. Springtails, in particular *F. candida*, have been used extensively in terrestrial ecotoxicology studies (see Fountain and Hopkin (2005) and Environment Canada (2007) for reviews), and several studies have employed springtails to assess impact of treated sewage sludge and biosolids land application.

Domene *et al.* (2007) investigated impact to *Folsomia candida* from seven organic wastes, including dewatered, thermally dried and composted sewage sludges. Impact, measured as lethality and reproductive impairment, increased as the lack of stability, and presence of toxic end products such as ammonium, increased. Composted sludge was found to be the most stable and had the least impact to springtail reproduction. It should be noted that this study applied treated sludge in a concentration series up to 1,000 g/kg ww artificial soil, a rate that is not relevant in the Canadian context.

Similar to Domene *et al.* (2007), Moreira *et al.* (2008) used springtails to assess impact of compost and digested sewage sludge; however, they used more relevant application rates, 6 and 12 ton dw/ha. Compost was produced from anaerobically digested biosolids that were composted with mixtures of grape leaves, grass clippings and corncobs. Both reproduction and avoidance were assessed. Avoidance bioassays did not show impact of the compost or treated sludge; however, some impact was seen to springtail reproduction at the highest amendment rate of compost. Interestingly, this impact was not observed when soil was amended with only the digested biosolids, which was the starting material for all composts.

In contrast, Natal-da-Luz *et al.* (2009) found no springtail reproductive impairment when springtails were exposed to a soil amended with municipal sewage sludge at rates up to 45 t dw/ha, but Artuso *et al.* (2011) found negative impact to springtail reproduction with biosolids application. Artuso *et al.* (2011) tested five biosolids from different WWTPs mixed with artificial soil at rates up to 20 t dw/ha and found a significant decrease in reproduction as the amendment rate increased.

The aforementioned springtail studies were all conducted in the laboratory, but Andrés *et al.* (2011) and Cole *et al.* (2001) studied Collembolan populations in the field after sludge application. Andrés *et al.* (2011) surveyed microarthropod density, including springtail density, three years after treated sewage sludge was applied to forest plots. Sludge application did not change the total abundance or diversity of collembolans, but it did increase the density of *T. velatus* (oribatid mite), a species that is commonly found in heavy metal-polluted soils. Cole *et al.* (2001) sampled Collembolan populations two years after beginning continuous dewatered sludge cake application. Sludge application increased the abundance of total Collembola compared to the control plots. In addition to the 'normal' dewatered sludge, cadmium and zinc-rich sludges were also applied, which did not increase Collembola abundance, suggesting a negative impact of these 2 metals on the Collembolan populations.

Earthworms

Earthworms (Lumbricidae) are a family of soil dwelling organisms that represent a significant component of the invertebrate biomass of most soils and the diet of many species of birds, small mammals, reptiles, and amphibians (Environment Canada, 2004). Earthworm biology was first described in the 1800s, and early ecological and behaviour studies include work on mechanical and light stimuli (Harper, 1905), and burrowing patterns (Evans, 1947).

Toxicological studies started in the 1960s with work on the impact of pesticides and herbicides in soils on earthworm survival (Caseley and Eno, 1966; Stenersen *et al.*, 1973; Stringer and Lyons, 1974). Similar to springtails, several regulatory bodies including Environment Canada (2004), ISO (2008, 2012a, 2012b), and OECD (1984, 2004) have produced standardized survival, reproductive and avoidance bioassay protocols for use with earthworm species, mainly *Lumbricus terrestris* and *Eisenia* sp. For a review of the use of earthworms in ecotoxicological studies, see Sheppard *et al.* (1998) and Environment Canada (2004).

A few studies have used earthworms to evaluate impact of biosolids land application. Banks *et al.* (2006) used *Eisenia foetida* as part of their multi-species impact assessment. They measured changes in earthworm biomass and reproduction over 7 days and 7 weeks, respectively. Two of the seven biosolids-amended soils tested showed significant reductions in earthworm biomass gain, and this effect was attributed to high salinity levels and low pH. One of the soils showed a significant decrease in reproduction, as measured by cocoon production, which was attributed to elevated copper levels.

Along with springtails, Moreira *et al.* (2008) also used *Eisenia andrei* to evaluate the impact of several composts and a digested sewage sludge, applied at 6 and 12 t dw/ha, to earthworm reproduction, growth, and avoidance behaviours. Although the digested sludge showed a decrease in earthworm biomass and reproduction at the highest amendment rate, the compost samples showed either no significant difference compared to the control, or an increase in biomass and reproduction. In addition, a clear preference of the earthworms for compost-amended soils was seen at both amendment rates, likely due to increased organic matter relative to the artificial soil. The earthworms avoided the sludge at the highest amendment rate.

Natal-da-Luz *et al.* (2009) also used *E. andrei* avoidance and reproduction bioassays to assess impact in soil amended with municipal sewage sludge at rates up to 45 t dw/ha. Similar to Moreira *et al.* (2008), Natal-da-Luz *et al.* (2009) found that earthworms preferred the amended soil, as well as an increase in biomass and juvenile production.

In contrast, Artuso *et al.* (2011) observed a negative effect on survival and reproduction with biosolids-amended soils. They used *E. fetida* to assess survival and reproduction in five different biosolids-amended soils. Three out of the five amended soils showed an inverse relationship between amendment rate and survival, with increasing rates leading to decreased survival, and the remaining two showed no significant impact. In addition, higher biosolids-amendment rates resulted in decreased post-trial worm

biomass. All five biosolids-amended soils produced significantly fewer juveniles relative to the control, with juvenile numbers significantly reduced at increasing biosolids-amendment rates.

Kinney *et al.* (2012) assessed toxicity of 2- to 8-week aged biosolids at rates of up to 4% biosolids/soil (w/w) on a dry weight basis to *E. fetida* worms. The biosolids and soil were air-dried, pulverized and sifted, likely for ease of storage and mixing; however, this does not represent an environmentally relevant situation. In addition, rates of 4% biosolids/soil are not representative of Canadian practices. Earthworm survival was decreased in the 4% mixture aged for 8 weeks. Reproduction, as measured by cocoon production, was decreased in 4%, in both 2- and 8-week aged biosolids. The authors hypothesize that the increased toxicity from aged biosolids could be due to a single or small subset of toxic components in the biosolids, such as Vernile *et al.* (2007) observed for pentachlorophenol degradation products.

More recently, Adair *et al.* (2014) used *Apporectodea caliginosa* Savigny, an endogeic species of earthworm, to assess survival and change in biomass after five months in biosolids-amended field soils at application rates of 3 and 6 g dw/L soil, corresponding to 158 and 316 kg N/ha. No significant difference was found in earthworm survival between control and biosolids treatments. Worm biomass was found to increase in biosolids treatments and decrease in control treatments. After exposure, earthworm guts were purged and the tissue analyzed for trace elements; earthworms exposed to the biosolids treatments had significantly higher levels of copper, and less cadmium, manganese, sodium, phosphorus and sulphur, relative to the control.

In another recent study, Waterhouse *et al.* (2014) assessed earthworm survival in biosolids-amended and biosolids-amended and stockpiled (aged) reclaimed mine soils, and a non-amended soil. All earthworms were absent and assumed dead in the biosolids-amended soils, compared to 42% and 25% mortality in the stockpiled and non-amended soil, respectively. Earthworm biomass declined in all treatments but was largest in the stockpiled soil.

Concurrent studies with springtails and earthworms

Some of the studies discussed above used both springtails and earthworms concurrently to assess impact (Moreira *et al.*, 2008; Natal-da-Luz *et al.*, 2009; Artuso *et al.*, 2011). In the three studies, springtails were found to be a more sensitive indicator of toxicity than earthworms, and reproduction a more sensitive endpoint than lethality. These results emphasize the need to use multiple species for a holistic toxicity assessment, because each species has different sensitivities to different stressors.

It is also important to take into consideration the environmental relevance of this kind of studies before extrapolating their conclusions to the field. The type and number of species used, and the composition of the selected soil have a big influence on the impact of the biosolids. Three out of the four laboratory-based springtail studies described above utilized an artificial reference soil made up of sand, kaolin clay and peat; only Natal-da-Luz *et al.* (2009) used a reference soil collected from the field. Similarly, half of the earthworm studies utilized artificial soils, whereas Natal-da-Luz *et*

al. (2009), Kinney *et al.* (2012), and Adair *et al.* (2014) used field-collected reference soil.

Although the use of artificial soil allows for a higher degree of standardization, it is not as environmentally relevant as field-collected soils. Moreira *et al.* (2008) suggested that avoidance tests were strongly influenced by the organic matter of the soil, and recommended the implementation of an organic matter correction factor, or that these assays simply be used as screening tools. Spurgeon and Weeks (1998) showed that soil pH and organic matter content can affect the sensitivity of earthworms to Zn through changes in the cation exchange properties of the soil. They cautioned against extrapolating laboratory-based toxicity data to the field and suggested that standardized tests, such as the OECD bioassay (1984), are "little more than a provisional benchmark exercise" that accentuates toxicity.

In addition, most studies use *Eisenia* sp., a species that, although heavily standardized, does not occur in agricultural soils (Kula, 1998). Spurgeon and Weeks (1998) pointed out that the use of *E. fetida* has been a major source of criticism of the standardized OECD protocol because it was selected on the basis of ease of culturing and robustness, rather than ecological relevance or sound taxonomy. Other species used in terrestrial toxicity testing, such as *Lumbricus* sp. and *Aporrectodea* sp., can show different sensitivity to toxicants. For example, Spurgeon and Weeks (1998) reviewed studies that showed differences in sensitivity up to a factor of 10 between *Eisenia fetida* and *Lumbricus terrestris*.

Nematodes

Nematodes (phylum Nematoda) are a ubiquitous, diverse and abundant group of organisms found both in soil and freshwater and marine environments (Banks *et al.*, 2006). Nematodes are important to many soil fertility processes and nutrient cycling, and they play an important role in the food chain as feeders of bacteria, plants, and fungi (Yeates, 1979). Some groups of nematodes are plant-parasitic, while others are vertebrate-parasitic, such as ringworm (Lee, 2002).

Their use as model organisms in soil ecotoxicology is relatively recent, with toxicity protocols for the model organism *Caenorhabditis elegans* developed in 1989 (van Kessel *et al.*, 1989). The use of nematodes in soil ecotoxicology was reviewed by Sochová *et al.* (2006).

Three studies have investigated the impact of biosolids on nematode populations, both in the field and under laboratory conditions, and overall, no adverse impact trends were observed.

Banks *et al.* (2006) used mortality of the nematode *C. elegans* as part of their invertebrate toxicity assessment. No clear trends were observed; however, some decreased survival was observed in biosolids-amended treatments. This decreased survival also corresponded to decreasing electrical conductivity, which led the authors to suggest that a nominal salt concentration was needed to sustain the organisms.

Ozores-Hampton *et al.* (2012) measured plant-parasitic nematode population densities on Class B biosolids-amended and non-amended field plots planted with peppers. No significant trends in nematode population density were observed.

Yeates *et al.* (2006) published the most comprehensive study on the impact of biosolids amendment to nematodes. Five soil types in barrel lysimeters were amended with four different biosolids treatments, plus a control. Nematode populations were sampled after 2 years. No significant trends to nematode assemblies were seen with biosolids addition, except for an increase in the population of bacterial-feeding Rhabditidae and some suppression of plant-feeding, or plant-associated nematodes that was observed with the highest biosolids-amendment rate of 800 kg N/ha.

8.1.3. Microbial communities

The soil microbial community is largely responsible for the elements cycles in the soil and it also plays a role in the decomposition of soil organic matter (Bastida *et al.*, 2008). Changes to a soil's physical and chemical properties, such as the application of biosolids, have the potential to alter the indigenous microbial community and affect soil processes as a consequence (Sastre *et al.*, 1996; Rogers and Smith, 2007).

Parameters such as microbial biomass (quantified in terms of carbon or nitrogen) are generally used to assess impact on community size, while respiration rates and enzyme activity are used to evaluate effects on elemental cycling processes (Bastida *et al.*, 2008). Additionally, molecular techniques such as phospholipid fatty acid (PLFA) profiling and automated ribosomal intergenic spacer analysis (ARISA) generate quantitative information on the microbial community structure and diversity (Bastida *et al.*, 2008; Park *et al.*, 2013; Adair *et al.*, 2014).

In general, amending the soil with sludge or biosolids has been shown to stimulate microbial communities, presumably due to the relatively large addition of organic matter.

Soil microbial biomass, respiration rate and enzymatic activity

The size and activity of the soil microbial community generally increases as the microbes decompose organic matter; microbial biomass, soil respiration rate, and enzyme activities can be assessed to quantify this change in microbial activity (Sciubba *et al.*, 2013). Other parameters that can be used to evaluate microbial activity are the ratio of microbial biomass carbon to soil organic carbon (C_{mic}/C_{org}), which reflects changes in soil organic matter (Sparling, 1992), and the metabolic quotient (qCO_2), which is the ratio of the basal respiration rate (BAS) to the soil C_{mic} (Sciubba *et al.*, 2013).

As a series of studies have shown, addition of biosolids to soils induces an increase in microbial biomass, respiration rates, and enzyme activity. A short-term study by Banks *et al.* (2006) observed increased respiration rates in biosolids-amended soils compared to the control soils, although the increase was not always statistically significant. Kao *et al.* (2006) showed a continual increase in microbial biomass and respiration in biosolids amended-soil up to an incubation period of 140 days under laboratory conditions.

A short-term laboratory-based study by Holt *et al.* (2010) that focused on nitrogen-fixing soil bacteria found an increase over time in N_2-fixation with biosolids amendment, peaking at 14 days of incubation. Carbonell *et al.* (2009) observed an increase in dehydrogenase and phosphatase enzyme activities and respiration rates, also in a laboratory-based study with biosolids-amended soil. Lakhdar *et al.* (2011) reported increased enzymatic activity 15 days after biosolids application to soil at a rate of 40 and 80 t/ha, although they also observed some decrease in enzyme activity after 70 days at the highest rate (80 t/ha).

Roig *et al.* (2012) applied biosolids to a field at various rates and frequency over a period of 16 years and found an increase in dehydrogenase activity in some applications, but no significant change in urease activity. Most recently, Mattana *et al.* (2014) observed increase in qCO_2 after biosolids application to 2 different soils, with the magnitude of the stimulatory effect dependent upon the type of post treatment received by the anaerobically-digested biosolids (thermally-dried > untreated > composted).

Besides its use in the agricultural context, land application of biosolids is also practiced for the reclamation of contaminated soils. A laboratory-based biosolids remediation study of heavy-metal contaminated soil by Pérez de Mora *et al.* (2005) found an increase in microbial biomass at 1 and 6 months after soil amendment. This study also found increased enzyme activity and increased maximum rate of glucose mineralization. Similarly, a field-based biosolids remediation study at a reclaimed mining site by Brown *et al.* (2005) found increased microbial biomass and respiration rate with application of biosolids up to 224 Mg ww/ha (approximately 38 Mg dw/ha). And a recent study by Waterhouse *et al.* (2014) found dehydrogenase activity to be higher in biosolids-amended soils compared to reference soils, with the highest levels found in amended soils that contained earthworms.

Studies have also been conducted with composted biosolids. Speir *et al.* (2004) conducted field and pot trials with compost produced with biosolids and wood and green waste, and observed a significant increase in microbial biomass carbon and basal respiration rate. Similarly, Bastida *et al.* (2008) found a two-fold increase in microbial biomass C, as well as an increase in β-galactosidase, urease, and alkaline phosphatase activities after 12 kg/m^2 application of an anaerobically-digested sludge, both in its original form and after being composted with straw.

Sciubba *et al.* (2013) investigated the differences in enzyme activity and respiration rate when aerobic and anaerobic municipal sewage sludge were applied to soil after stabilization. Aerobic sludge increased the basal respiration rate as well as multiple enzyme activities, while anaerobic sludge decreased respiration and some enzyme activities. More recently, Sciubba *et al.* (2014) examined a compost comprised of aerobic and anaerobic municipal sludge composted with rice husk and found an increase in microbial biomass carbon of up to 21% compared to untreated soil.

In field studies, Kelly *et al.* (2007) also found that biosolids addition had a significant positive effect on microbial biomass one year after application. Similarly, Andrés *et al.* (2011) examined three anaerobically-digested sewage sludges and also found an increase in microbial biomass a year after application in both dewatered and thermally-

dried sludges applied at a rate of 6 Mg organic matter/ha. They also found an increase in qCO_2 one week after application, but no change in microbial activity expressed as C_{mic}:C_{org}. Microbial activity returned to non-amended soil levels after a year. A long-term study by Sullivan *et al.* (2006b) found a change in mineralization activity 12 years after biosolids application, but only at the highest application rate, 30 t dw/ha.

Research with untreated sewage sludge has found the same increasing trend in microbial biomass. Lima *et al.* (1996) reported an increase in microbial biomass and dehydrogenase and urease activity at sewage sludge application rates of up to 160 t/ha. Sastre *et al.* (1996) also applied untreated sewage sludge at high rates (up to 100 t/ha/year over 8 years) and found an increase in both urease and β-galactosidase activity, and an increase in humic and fulvic acids, indicating organic matter degradation. García-Gil *et al.* (2004) also reported an increase in microbial biomass, basal respiration rate, metabolic quotient (qCO_2), and enzymatic activities at rates of 40 t/ha up to 9 months after application, but after 36 months the values had returned to those of non-amended soils. Fernandes *et al.* (2005) found that microbial biomass values for C and N increased with increasing sludge concentration, with the biomass concentrated in the first 10 cm of the soil. They also reported an increase in qCO_2 and amylase and urease activity that increased with increasing sludge dose.

A 4 year soil-amendment study in Denmark by Poulsen *et al.* (2013) compared the use of urine, untreated sewage sludge, municipal solid waste compost, cattle slurry and manure. All treatments showed higher microbial biomass compared to untreated plots or plots where inorganic fertilizer was used. No negative effects were observed even at the highest rates of sludge application, equivalent to > 50 years of application at normal rates.

As discussed by Fernandes *et al.* (2005), the inhibitory effects on microbial biomass observed in studies that used high sludge and biosolids application rates, can generally be attributed to existing and spiked heavy metals (Brookes and McGrath, 1984; Chander and Brookes, 1991; Khan and Scullion, 2000; Kao *et al.*, 2006). This observation is supported by the work of Charlton *et al.* (2012), who conducted a meta-analysis with data on microbial biomass changes from a long-term field experiment conducted in 9 different sites in the UK, where soils were amended with anaerobically-digested biosolids at different Zn, Cd, and Cu application rates. The analysis showed that increasing Zn and Cu concentrations in soil had an increasingly negative effect on microbial biomass starting at 211-299 mg/kg for Zn, and 140-250 mg/kg for Cu. Cd concentrations up to 3.3-4.8 mg/kg (5 to 18 times higher than the reference sites) had no effect on microbial biomass.

Only a few studies showed no effect of biosolids application on microbial biomass or respiration. Brendecke *et al.* (1993) found no significant effect on numbers of soil bacteria, fungi, or actinomycetes, as well as no effect on microbial respiration, in response to four years of annual land application of anaerobically-digested biosolids at 8 t/ha/year. Zerzghi *et al.* (2010b) observed no change in numbers of indigenous soil microbes, but found an increase in microbial enzymatic activity after 20 annual land applications of Class B biosolids.

Overall, studies applying biosolids and composted biosolids at relevant agronomic rates resulted in increases to microbial biomass, respiration rate, and enzymatic activity, unless high concentrations of heavy metals were present. This conclusion is not surprising considering the large amounts of organic matter found in biosolids that provides a readily available energy source to soil microorganisms.

Soil microbial community structure/profile

Prior to the use of molecular techniques, Dennis and Fresquez (1989) assessed the soil microbial community in a semi-arid grassland amended with anaerobically-digested sewage sludge at rates of up to 90 Mg/ha. Microbial populations, including fungal groups, were isolated by dilution and plating techniques. The authors found that bacterial, fungal, and ammonium oxidizer populations in the soil increased linearly with increasing application rates, but *Streptomyces* spp. were constant in number. At low application rates, fungal group diversity initially declined, but then rebounded within one year. At high application rates, large fungal populations with low density were found.

With the advent of molecular techniques, several studies assessing the impact of biosolids land application on the structure or profile of soil microbial communities have been conducted. In one of the earliest studies to make use of a molecular technique, Gray *et al.* (2003) used temporal temperature gradient electrophoresis (TTGE) to study the effect of additions of anaerobically-digested sludge at rates of 10-20 L/m^2 over two years. They observed changes to ammonia-oxidizing bacteria populations, with the community becoming more dynamic in amended soils; however, these changes were shown to have less of an impact than effects from natural spatial and temporal variation.

Sullivan *et al.* (2006a) used ester-linked fatty acid methyl esters (EL-FAMEs) to characterize the soil microbial community 12 years after biosolids application at rates up to 30 Mg/ha. The EL-FAME biomarkers for gram-positive bacteria increased with increasing application rate, while biomarkers for gram-negative bacteria and fungi were unaffected by biosolids application. Waterhouse *et al.* (2014) used Biolog EcoPlates™ to test for differences in carbon utilisation patterns and establish microbial community level physiological profiles (CLPP), functional diversity, and richness. They found increases in functional diversity with biosolids application, as well as a different CLPP profile; however, richness of carbon utilisation was not increased in biosolids soils relative to the control.

Kelly *et al.* (2007) used phospholipid fatty acid (PLFA) analysis to study changes to the microbial community with the addition of biosolids to land-applied river sediments. Their results showed that biosolids addition caused the community composition to shift, with relative increases in gram-negative bacteria and decreases in gram-positive bacteria, fungi and actinomycetes. Park *et al.* (2013) also used PLFA analysis to profile the microbial community changes with application of biosolids and found different results to those of Kelly *et al.* (2007). Park *et al.* (2013) not only observed an increase in PLFA biomarkers for gram-negative bacteria after biosolids-amendment, but also in biomarkers for gram-positive bacteria, fungi, actinomycetes, and eukaryotes.

Zerzghi *et al.* (2010a), Adair *et al.* (2014), and Mattana *et al.* (2014) used genetic techniques to generate DNA or RNA fingerprints of the soil microbial community. Zerzghi *et al.* (2010a) used 16S rRNA sequence analysis to study the bacterial community from soils that had been amended with biosolids for 20 years. They found increased diversity in the biosolids plots compared to the reference soils, with at least five phyla identified. Adair *et al.* (2014) used automated ribosomal intergenic spacer analysis (ARISA) to profile the bacteria and fungi community composition, and they found that the bacterial community structure was impacted by both low (158 kg N/ha) and high (316 kg N/ha) biosolids treatments, while the fungal community was only impacted by the high treatment. Mattana *et al.* (2014) used PCR-DGGE (Polymerase Chain Reaction/Denaturing Gradient Gel Electrophoresis) of bacterial and actinobacterial 16S rRNA gene-coding fragments to show community changes in response to application of different post-treatment processes of the same aerobically-digested sewage sludge. They showed that post-treatment processes as well as soil type had a key role in shaping the microbial community structure and diversity. In general, biosolids amendment increased the diversity of the microbial community, with each post-treatment changing the community structure in a different way.

Arbuscular mycorrhizal fungi communities

A recent topic of interest has been the changes to arbuscular mycorrhizal fungi (AMF) communities as a result of biosolids land application. AMF form important symbiotic relationships with the roots of plants and have been shown to influence nutrient dynamics and cycling, plant productivity, and soil aggregation (Jeffries *et al.*, 2003; Sullivan *et al.*, 2006a; Hazard *et al.*, 2014). More specifically, AMF develop inside plant roots and the surrounding soil and form a network that aids the plant in extracting nutrients and water from the soil, and they are present in almost all plant ecosystems, including agricultural settings (Jeffries *et al.*, 2003).

Recent studies have shown conflicting results regarding the impact of biosolids on plant-AMF associations. Some researchers have found a negative impact on AMF communities with biosolids-application. Sullivan *et al.* (2006a) showed a negative impact on AMF ester-linked fatty acid methyl ester (EL-FAME) biomarkers with increasing biosolids application rates. One and twelve years after biosolids application, AMF biomarkers were found to decrease by up to 22-fold, while other fungal populations decreased by less than 1.5-fold. It should be noted that the authors applied biosolids at rates up to 30 Mg/ha, which could be unrealistic depending upon local regulations.

In contrast, Hazard *et al.* (2014) found no significant effect on AMF root colonisation or community composition as a result of biosolids application at 5 Mg dry matter/ha; moreover, the authors concluded that the natural seasonal variation of the AMF community outweighed the effect of biosolids application.

Furthermore, some studies have found a positive effect on AMF communities with biosolids-application. Barbarick *et al.* (2004) found that a single biosolids application of up to 30 Mg/ha on a grassland site, and 40 Mg/ha on a shrubland site, increased AMF colonization by 23 and 33%, respectively.

8.1.4. Livestock and Wildlife

Very little research has been conducted on the effects of land application of biosolids to livestock and wildlife. Dowdy *et al.* (1983b) fed goats and lambs corn silage grown on biosolids-amended soil that had increased concentrations of Cd. No differences in goat milk production were found, and lambs that were fed biosolids-fertilized corn had increased daily weight gains compared to the control lambs. In a later study (Dowdy *et al.*, 1983a), no differences were found in Cd concentrations in the goats' milk.

In contrast, a study by Paul *et al.* (2005) showed a negative impact on livestock. Pregnant sheep fed pastures grown in biosolids-amended fields produced both male and female foetuses with reduced body weights; additionally, male foetuses presented lower levels of testosterone and inhibin A, reduced testis weight, and lower numbers of gonocytes, Leydig and Sertoli cells.

Research has also been conducted on the effect of sewage sludge as a supplemental feed to livestock; however, this research is not relevant in the Canadian context. For a review of such work, see Epstein (2002).

A single study looked at the effect of biosolids-application on wildlife. Washburn and Begier (2011) studied the long-term application (17 years) of lime-stabilized biosolids to grassland plots surrounding an airfield at an annual rate of 7.6 Mg dw/ha. The authors reported a significant change to the plant communities within the biosolids-amended plots. Plants in these plots were taller and denser in structure, with increased stands of tall fescue, leading to decreased plant diversity. As a result of this change in plant community, an increase in total birds, including grassland birds, was observed in the biosolids plots. No difference in white-tailed deer usage was observed.

8.2. Biomarkers and Omics

"Omics" refers to "a field of study in biological sciences ending in -omics, such as genomics, proteomics, or metabolomics" (Bagchi, 2012), whose general goal is "to explore the roles, relationships, and actions of the various types of molecules that make up the cells of an organism" (AltTox, 2014). The capacity to analyze large amounts of biological data rapidly and inexpensively is expected to produce better biomarkers for exposure assessment at the population level, because it allows the study of the effects of exposure to chemical or other stressors on the entire genome (or proteome, metabolome, etc.) of a large number of individuals in the population of interest (Wild, 2010; Raghavachari, 2012).

Environmental omics has trailed behind their counterparts in the biomedical sciences, where it was originally applied, and it is still in the early stages of data collection and validation (Ge *et al.*, 2013). Ge *et al.* (2013) recently published a review on the current status of environmental omics, and remarked on how most of the published studies in the field were focused on the effects of exposure to individual chemicals to the genome

or proteome, but in a purely descriptive way, without relating the observed changes to toxicity.

Most of the work on environmental monitoring was related to aquatic species, such as bivalves and zebrafish (Ge *et al.*, 2013). In terms of microbial community structure in the wastewater treatment area, proteomics has been used to study activated sludge populations (Wilmes and Bond, 2006; Wilmes *et al.*, 2008), and metabolomics approaches are being developed to correlate soil contamination with microbial and earthworm metabolic profiles (Bundy *et al.*, 2007; Jones *et al.*, 2014).

Genomics has also been used in this area, as described in the section on soil microbial community structure, to produce DNA fingerprints of soil microbial communities, and to study how these communities shift with the application of biosolids to soils (Zerzghi *et al.*, 2010a; Adair *et al.*, 2014; Mattana *et al.*, 2014).

Lipidomics has also been used to study microbial and AMF communities using lipid biomarkers such as PFLA (Kelly *et al.*, 2007; Park *et al.*, 2013) and EL-FAME (Sullivan *et al.*, 2006a). However, there has been no work on the effects of biosolids on plant and invertebrate communities.

Although relatively scarce, especially for higher organisms, much of the research related to the land application of biosolids is based on the use of individual molecular markers, or biomarkers, of the biological effects of stressors on organisms (Livingstone, 1993). The most utilized biomarkers are enzymes and hormones.

In studies with fescue, corn and soy, the application of lime-stabilized and anaerobically-digested biosolids increased plant hormones indole-3-acetic acid (IAA) and trans-zeatin-riboside (ZR), as well as cytokinin content, which were associated with less leaf wilting under drought stress (Zhang *et al.*, 2009; Zhang *et al.*, 2013). Zhang *et al.* also reported an increase in proline and superoxide dismutase (SOD) activity in tall fescue and corn, also improving metabolism and leaf anti-senescence under drought conditions (Zhang *et al.*, 2009; Zhang *et al.*, 2013).

Although multiple studies have looked at the impact of individual chemicals or water samples on aquatic organisms, a sole study has looked specifically at the impact of biosolids on vertebrate biomarkers. Sullivan *et al.* (2007) studied the induction of CYP1A and DNA damage in fathead minnow (*Pimephales promelas*) exposed to anaerobically digested biosolids added to their tanks at low (0.5 g/L) and high (2.5 g/L) doses in 28-day static renewal bioassays. CYP1A was significantly induced in the low dose after 21 days, and in the high dose after 3 days. CYP1A levels peaked in both doses at day 21, with levels increasing 8- and 21-fold compared to the control for the low and high doses, respectively. In addition, significant DNA damage was seen to the hepatocytes at both doses relative to the control; however, there was no significant difference between the low and high doses, suggesting a toxicity threshold.

Langdon *et al.* (2014b) measured estrogenicity in the soil using YES after amendment with 2 anaerobically digested biosolids that were either centrifuge- or solar-dried in a lagoon. Application rates were 25 and 45 t/ha (dw) respectively. Estrogenicity was

detected in the soil immediately after application, and after 28, 56 and 112 d, ranging from below detection to 3.3 µg EEq/kg (EEq: E2 equivalents). The cause of the estrogenic response was not investigated, but the authors suggested that it could have been a number of compounds usually found in biosolids, such as NP, NPEs, and hormones.

Additionally, research has been conducted on the effects of sludge treatment on biomarkers activity, as reviewed in Chapter 6.1.2.

8.3. Summary

Impact studies add relevant information to risk assessment and other types of toxicity testing because they use whole organisms and they can better represent 'real' environmental exposure conditions, especially if conducted in the field.

The studies reviewed in this chapter show that amending the soil with biosolids at appropriate rates has a positive impact on plants, measured in terms of plant growth, biomass, and yield. This impact is attributed to the nutrient input and physical improvement of the soil resulting from the biosolids application (Olness *et al.*, 1998).

The impact studies on invertebrates (earthworms, springtails, and nematodes) showed mixed results, with the organisms in some of the studies having a preference for the amended soils over the reference—presumably due to the higher organic matter content–whereas other studies reported a negative impact, especially at high application rates. The causes for the latter were not always determined, underscoring the need for a combined approach to toxicity testing, including both biological response and chemical analysis, and the difficulty of establishing causality between observed effects and the chemicals or other conditions, even when they are evaluated.

For example, Artuso *et al.* (2011) could not relate the negative effects they observed in earthworms and springtails to the metal concentrations of the biosolids-amended soils tested, and suggested that ammonia or low oxygen could have contributed to these results. In contrast, Cole *et al.* (2001), related the decrease in collembolan populations in soils amended with metal-rich biosolids to high concentrations of Zn and Cd, but they could not rule out the presence of other chemicals. Similarly, Banks *et al.* (2006) attributed the negative impact to earthworms in some sites to Cu levels, although they related the majority of the negative effects to low pH and salinity levels.

Kinney *et al.* (2012) detected approximately 20 different ESOCs in earthworms exposed to biosolids-amended soils, but they did not establish a relationship between their presence and the toxicity observed. In the case of springtails, Moreira *et al.* (2008) hypothesized that the observed decrease in springtail reproduction might be due to unknown chemicals, presumably phenols in the case of a compost made from grape vine leaves, and included corn-cobs and grass clippings, but no biosolids.

All three studies using both springtails and earthworms (Moreira *et al.*, 2008; Natal-da-Luz *et al.*, 2009; Artuso *et al.*, 2011) concluded that the former are a more sensitive

indicator of toxicity than the latter, and reproduction a more sensitive endpoint than lethality. However, although the use of these organisms is crucial for the ecotoxicological assessment of the potential impact of biosolids to soil biota, standardization and more research are needed in order to use this data in risk assessment (Van Gestel and Weeks, 2004).

With regard to the microbial communities, studies applying biosolids and composted biosolids at relevant agronomic rates resulted in increases to microbial biomass, respiration rate, and enzymatic activity, unless high concentrations of heavy metals were present. This conclusion is not surprising considering the large amounts of organic matter found in biosolids that provides a readily available energy source to soil microorganisms.

The results for the tests of the microbial community structure after biosolids amendment also vary widely, with some studies showing no difference to reference soils, while others found changes in the community composition. However, the impacts of these changes on the health of the soil ecosystem are currently unknown.

There is very limited information on the effects of biosolids amendment on livestock or wildlife, with one study reporting developmental effects in sheep fed pastures grown in biosolids-amended soils (Paul *et al.*, 2005). However, this test was designed as proof-of-concept, with the authors trying to maximize the exposure of the test animals to the biosolids, although using application practices still within the British standards at the time of the experiment.

Although promising, biomarkers and omics have only started to be used in the soil ecotoxicology context. Because of their capacity to generate large amounts of genetic, metabolic, and protein expression data, omics has the potential to contribute in the study of the toxicity effects resulting from simultaneous exposure to multiple chemicals, the development of antibiotic resistance, and in ecosystem health assessment, but the use of these technologies in the field is still in its infancy (Ge *et al.*, 2013).

The use of biomarkers, such as estrogenicity (Langdon *et al.*, 2014a), can provide valuable information on the possible effects of biosolids to biota without the need for exhaustive chemical analysis. However, research is still necessary to relate the responses of such biomarker tests to actual impact to individuals and populations, especially in the biosolids land application context.

8.4. References

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9. PUBLIC ACCEPTANCE OF BIOSOLIDS LAND APPLICATION

9.1. Public perception of biosolids

Public awareness of biosolids tends to be low (Beecher *et al.*, 2004; CDM, 2011) and, in contrast to wastewater treatment, public perception of biosolids tends to be negative (CDM, 2011; Robinson *et al.*, 2012). Even the term 'biosolids' is controversial in some circles, due to the fact that it was created by "the sewage industry" (Beecher *et al.*, 2004; Pepper *et al.*, 2006), and despite its widespread adoption by the scientific community and regulatory bodies (s. Chapter 2.1.1).

The negative public perception of biosolids stems from a series of factors (Beecher *et al.*, 2005), including lack of knowledge (CDM, 2011; Robinson *et al.*, 2012), the presence of pathogens and industrial sewage (Apedaile, 2001), odour emissions during land application (USEPA, 2002; Pepper *et al.*, 2006; Robinson *et al.*, 2012)—which are sometimes perceived as a health risk (Robinson *et al.*, 2012)—and reports of health effects associated with biosolids land application (NRC, 2002; Viau *et al.*, 2011). A comprehensive list of these factors was compiled by Beecher *et al.* (2004) and it is reproduced in Table 9.1. Additionally, bad biosolids management practices can also be a factor affecting perception (CDM, 2011; Lowman *et al.*, 2013).

Negative public perception has been strong enough to influence public policy, including the establishment of biosolids land application bans in some American counties and the shift to more strict biosolids treatment (USEPA, 2002; CDM, 2011), and it has the potential to limit the options available to a municipality for biosolids management.

The negative perception is in stark contrast to what current scientific knowledge indicates, and points to a lack or failure of communication between the biosolids "technical community" and the general public. Although more fundamental differences in the way the two different groups perceive the risk may also play a role, because the public "bases a significant part of assessed risk on the variables that are perceived, whereas those with prior technical knowledge heavily weigh risk on the calculable hazards and fail to adequately consider the aspects that cannot be otherwise predicted", as stated by Robinson *et al.* (2012).

Beecher *et al.* (2004) published an extensive literature review on the public perception and acceptability of biosolids recycling in the United States and Canada, including a historical account of the evolution of these views, and the responses from biosolids managers and regulators. The review summarized different topics regarding biosolids public acceptance, ranging from the scientific and public health issues, and the political and legal efforts to restrict biosolids use, to the role of the media, and the public outreach efforts of biosolids managers.

Table 9.1. Factors influencing negative public perception of biosolids land application. Adapted from Beecher *et al.* (2004), and Beecher *et al.* (2005).

Biosolids land application perceived to be:	
Involuntary	Imposed on the community, and out of their control
Artificial and industrial	Distrust of artificial or industrial products/processes
Exotic and/or unfamiliar	Biosolids are not familiar to most people, unlike manure
Hard to understand	The biosolids concept is not self-explanatory
Memorable	Due to odours and other nuisances
Dreaded	The "yuck" factor of biosolids' origins creates dread
Potentially catastrophic	Issues raised about biosolids suggest potential short- or long-term negative effects at the application sites
Not reversible	Some persistent pollutants might be permanent additions to soils
Unknowable	There is a level of uncertainty in the exact content of a biosolids batch. The diverse inputs from municipal sewers make the constituents variable
Having delayed effects	Some effects from biosolids may not be evident immediately
Affecting children and mothers	Because they may happen to play around biosolids and/or consume foods grown on biosolids-amended fields
Affecting future generations	Because there is some uncertainty about long-term effects
Having identifiable victims	Reported cases of harm to cattle and people
Being controlled by "the system" or people considered untrustworthy	Social science surveys have shown that government officials, people from out of town, and those who have a financial interest are perceived as less trustworthy
Unfair	A neighbour may feel that it is unfair to put up with odours when he or she receives no apparent benefit from a biosolids program
Morally and/or ethically objectionable	If biosolids are seen as a potential threat, then it can be perceived as morally wrong for cities to foist biosolids on a rural community
Operating by a closed process	Communities around land application sites may find the process closed and difficult to understand
Receiving more media attention	Media stories about a biosolids project heighten local interest and, if they report opposition, public concern tends to increase
Having limited or no visible benefits	Land application occurs far from the wastewater facility and in communities that perceive little benefit to them

Beecher *et al.* (2004) also presented the results of a survey on public knowledge and perception of biosolids conducted in 2002 by the University of New Hampshire Survey Center. The survey consisted of over 1,000 telephone interviews to a random nationwide sample of American homeowners and house renters.

The survey results showed that the majority of the individuals interviewed (58%) had not heard of biosolids, and only a small percentage (3%) could define them accurately. Most of the surveyed people (93%) supported wastewater treatment and a majority (63%) reacted positively to biosolids defined as: "the solid matter removed from sewage that has been treated and tested so [it] can be recycled as a fertilizer." (Beecher *et al.*, 2004). However, only about one-third of the respondents would be "very likely" or "somewhat likely" to use biosolids in their own homes.

When presented with a series of statements made against the use of biosolids, the argument that "not enough is known about biosolids" was considered the strongest against biosolids use by 44% of the respondents. Adverse health impacts and bad

odours were only considered to be the strongest argument by 13 and 6% of the respondents respectively.

The survey also indicated that the majority of the respondents were in favour of biosolids recycling, but the preferred recycling options were energy generation (31%), soil reclamation (25%), and forest fertilization (16%). Only a minority considered crop (9%) or lawn/garden (7%) fertilization the best options for recycling.

Finally, the survey found that an annual certification, contacting the neighbours prior to land application, and local supervision were the most relevant factors decreasing concern about biosolids land application. In contrast, concern increased if the biosolids contained some industrial waste or if they originated in a large city (Beecher *et al.*, 2004).

More recently, Robinson *et al.* (2012) studied risk perception of biosolids land application in two American communities. Their results showed that although a majority of the people surveyed were cognisant of the biosolids nutrient contribution to the soil, most respondents also considered that "the risks to public health outweigh the benefits derived from the reuse of biosolids" (Robinson *et al.*, 2012).

In addition, most of the survey participants responded incorrectly to all other queries designed to test their general knowledge on biosolids. Thus, most respondents believed that biosolids pose a greater risk to public health than animal manure, and that they pose equal risk to both children and adults. Additionally, the survey participants did not think that the USEPA regulated pathogen levels in biosolids, or that the USEPA considered risks from biosolids exposure to be low.

Respondents also reported inadequate levels of community involvement from the biosolids applicators, lack of transparency in the decision-making process, and lack of information on the potential risks of biosolids land application.

The authors found that there was a significant gender bias in the responses, with female participants having a higher perception of biosolids risks. They also observed that the opposition to biosolids land application decreased as the distance between the application site and the respondent's residence increased (Robinson *et al.*, 2012).

The lack of information and community involvement in the decision-making progress observed by Robinson *et al.* (2012) was also noted by Lowman *et al.* (2013), after conducting a series of interviews with residents near biosolids land application sites. Additionally, Lowman *et al.* (2013) indicated that a number of the respondents reported bad biosolids management practices, such as biosolids spills from trucks on roads, lack of signage indicating biosolids application, and runoff into buffer zones and surface water bodies.

9.2. Risk communication

Traditionally, biosolids managers used a one-way communication strategy to introduce biosolids land application programs to the public (Beecher *et al.*, 2005). Technical experts made the decisions on these programs without public input, and their communication plans merely conveyed technical information on the land application programs to the public.

However, this strategy has proven to be ineffective, and the underlying assumption that experts are the appropriate group to define and manage risk has been widely challenged (Beecher *et al.*, 2005). For example, the 2002 American survey on biosolids public perception discussed in Chapter 9.1 showed that, for a third of the respondents, the level of concern regarding biosolids recycling increased when told that "most scientists say there is negligible risk associated with recycling biosolids" (Beecher *et al.*, 2004).

As early as 1996, the US National Research Council recommended early public involvement in the decision-making process of biosolids management (NRC, 1996). Additionally, biosolids managers have taken steps to improve communications and increase public participation in decision-making as the differences in perception between the general public and the technical experts were acknowledged (Beecher, 2008; CDM, 2011).

Professional organizations, such as the Water Environment Research Foundation (WERF) and the National Biosolids Partnership (NBP), have sponsored studies and the development of communication strategies to assist biosolids managers in the appropriate implementation of risk communication programs.

A 2004 report published by WERF (Beecher *et al.*, 2004) proposed a 12-step program for biosolids managers to develop public participation and earn trust. This program emphasized early stakeholder engagement, organizational commitment, transparency, independent oversight, and building long-term relationships with the community.

Additionally, this report presented 14 case studies of biosolids programs (13 in the US, 1 in Canada) chosen to exemplify a range of public acceptance issues, communication strategies, and outcomes (i.e., degree of public support).

Using this information, and the literature review and survey described in Chapter 9.1, the 2004 report concluded that biosolids programs that are successful in developing stakeholder support have the following characteristics (Beecher *et al.*, 2004):

- *They are compliant, well-run operations following best management practices*
- *Their benefits to communities and the environment are obvious*
- *They introduce themselves to stakeholders early in the siting process and employ a respectful, joint-learning process, often led in part by local farmers or other citizens*
- *They communicate well through a variety of methods with the variety of stakeholders*
- *They provide consistent, high-quality, receiver-appropriate, timely technical information to anyone interested*

- *Some incorporate significant public participation (or customer feedback) in program planning*
- *They have strong organizational commitment (staff time and funding) behind their public outreach efforts (including from the public agency producing the biosolids)*

More recently, WERF commissioned the adaptation of a risk communication process to "improve decision making on the appropriate use of biosolids by all potential users at the local, state, and national level" and to "support biosolids professionals to significantly improve communications with stakeholders on the benefits and risks of biosolids land application, and in so doing, enabling stakeholders' judgments on the beneficial use of biosolids in their communities" (Eggers *et al.*, 2011). The WERF risk communication process seeks to directly address the stakeholders' needs as part of the planning by focusing on community engagement, and it recognizes that biosolids land application cannot be considered a sustainable practice without public acceptance.

This approach has been used by different institutional entities in North America, including the Canadian Council of Ministers of the Environment in the design of a Canada-wide approach for biosolids management (CCME, 2012), and it was also used for the design of the national consultation workshop in Part B of this project.

9.3. Odour

Odour is one of the public's main complaints regarding biosolids, especially in the vicinity of land application sites (NRC, 2002; Beecher *et al.*, 2004), and it is considered to have an important influence on the public acceptance of biosolids (reviewed by Beecher *et al.* (2004)). Although odours were originally considered a nuisance, they can also have physiological and psychological effects (Schiffman *et al.*, 2000; NRC, 2002) and affect quality of life (Schiffman *et al.*, 2000; Wing *et al.*, 2014).

The factors above have resulted in the adoption of measurements intended to decrease odour emissions, mainly odour control technologies and land application practices; e.g., USEPA (2000). Although some jurisdictions have introduced regulations limiting land application of biosolids and other organic materials based on odour intensity; e.g., Ontario (Thiam and Lebeau, 2012).

In general, odour research has focused on the chemical identity and formation of odours, and on their generation and control during sludge or manure treatment. The chemical composition and generation of biosolids odour was summarized in NRC (2002), but research in this area continues to date (Erdal *et al.*, 2008; Chen *et al.*, 2011; Laor *et al.*, 2011; Liu *et al.*, 2012; Maulini-Duran *et al.*, 2013; Su and Zhao, 2013).

In contrast, research on the health effects of odours is less abundant. Schiffman *et al.* (2000) published an extensive report on the complex relationships between odour intensity and perception, and direct and indirect health effects. They also discussed the quantification of health symptoms and exposure to odours. This report was the outcome of a 1998 workshop co-organized by the USEPA, Duke University, and the US

National Institute on Deafness and Other Communication Disorders, convened to assess the health effects of odours from confined animal operations, wastewater treatment plants, and biosolids recycling.

9.4. Impact on public health

Although no causal association between biosolids land application and health impacts has been documented to date (NRC, 2002; Jenkins *et al.*, 2007; Commonwealth of Virginia, 2008), available studies targeting this association are limited in number and scope. Additionally, there have been several reports of deleterious health effects to neighbours of sites where biosolids were applied, and these reports have not always been properly investigated.

As a result, several groups have recommended different studies to better assess this issue; the proposed studies range from planned exposure assessment to a full epidemiological evaluation (NRC, 2002; Commonwealth of Virginia, 2008). Additionally, these groups acknowledge the importance of establishing a better system to investigate individual health complaints in a thorough and timely fashion. Besides addressing the affected citizen concerns, such a system could be used to gather some of the data necessary to plan a complete epidemiological assessment if necessary (Aitken *et al.*, 2007).

The USEPA and WERF funded a group at the University of North Carolina at Chapel Hill to “develop an investigation protocol to assist environmental and public health officials in responding to citizens and medical providers who report symptoms that they attribute to land application of biosolids” (Aitken *et al.*, 2007). The proposed protocol was a five-part investigation process including an initial telephone interview with the claimant, interviews to the biosolids generator and applier, and a site visit. The final step of the protocol entails reporting to enforcement agencies if necessary, and entering the information to a database, which could later be used to evaluate temporal and spatial trends. This protocol was field-tested and further refined by a different group using 33 complaints from 3 American states (Liang *et al.*, 2012).

9.5. Summary

In contrast to wastewater treatment, public perception of biosolids tends to be negative for several reasons, including lack of awareness, the presence of pathogens and industrial sewage, odour emissions during land application, and reports of health effects associated with biosolids land application. This negative perception has already influenced public policy in some jurisdictions. Insufficient information and lack of community involvement in the decision-making progress for the design of biosolids management programs have also been cited as causes for public distrust. Biosolids managers have recognized these issues and they have adopted risk communication processes to develop public participation and trust in current approaches for the design

of biosolids management programs. Additionally, WERF and USEPA commissioned the development and field-testing of a response protocol for the investigation of health symptoms attributed to biosolids land application.

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10. RISK ASSESSMENT AND SUSTAINABILITY IN THE CANADIAN CONTEXT

In general, while the specific hazard of a chemical or pathogen to a certain organism can be evaluated in one location and be applicable practically everywhere else, risk is more localized, because it is a function of exposure, which depends on a series of factors that include local environmental conditions. In the context of biosolids applied to soil, the land application practices will affect the concentrations of pathogens and ESOCs in the soil, which will in turn determine the levels of exposure.

In this chapter, a 'typical' Canadian land application scenario was identified that assumed a maximum biosolids application rate; it was then used to exemplify the quantitation of environmental risk from ESOCs using risk quotients (RQs). However, due to the lack of terrestrial toxicity data, these values are only relevant for preliminary, comparative purposes, and have little value as indicators of actual environmental risks. A discussion on the needs to develop a formal environmental risk assessment follows, and the chapter ends with recommendations to address the issue of the risk evaluation of biosolids land application in the Canadian context during the national consultation constituting Part B of this project.

10.1. Typical land application practices in Canada

The legislation and best management practices that most directly affect the impact of biosolids constituents on terrestrial environments are those governing the quantity and frequency of biosolids applications to land, and these vary significantly throughout Canada, as reviewed by CCME (2012).

Maximum allowable application rates vary among the provinces and are primarily agronomic rates based on nitrogen and phosphorous needs of the soil and crops (Table 10.1). Many provinces also specify maximum allowable additions of trace elements into the soil that further limit the quantity of biosolids that can be applied, or specify maximum application rates with minimum return periods (CCME, 2012).

Typical agronomic application rates do not exceed 8-10 t/ha (dw) for thickened biosolids slurry (3-8% solids), and 25 t/ha (dw) for dewatered biosolids (CCME, 2012). The fundamental difference between thickened and dewatered biosolids applications is the inclusion of a process (usually belt filter press or centrifuge) to actively remove liquid from the biosolids, dewatering the product to the point that it is a semi-solid, and no longer a slurry. As a result of dewatering, a significant proportion of soluble nutrients are returned to the wastewater treatment process in liquid form (known as either 'pressate' or 'centrate' depending on the dewatering process used). As a result, dewatered biosolids, with lower total and available nutrient quantities, are applied at higher agronomic rates to achieve similar initial agronomic performance, coupled with a longer-lived nutrient and organic matter benefit, due the higher quantities of organic matter and nutrients that will be slowly released to the environment through decomposition processes.

As a result of the ability to apply a higher quantity of biosolids to land through dewatered processes, a higher concomitant load of the additional chemical components in the biosolids is also applied. This includes the potential for higher quantities of ESOCs. For the purposes of this review, an application rate of 25 t/ha (dw) has been used for reference.

10.2. Risk characterization of ESOCs

A common way to evaluate the environmental risk of a chemical is to use risk quotients (RQ), that are defined as the ratio of the predicted concentration of the chemical (PEC) in the specific environmental compartment being evaluated, to the predicted no effect concentration (PNEC) to the species of interest. The chemicals are then sometimes assigned a relative "low risk" if RQ values are < 0.1, "medium risk" if the values are between 0.1 and 1, and "high risk" if RQ is > 1 (Hernando *et al.*, 2006).

Clearly, the choice of PEC and PNEC is crucial for the risk evaluation; they must be selected carefully, and in accordance with the intended use for the RQs. However, these values are not always available, especially PNECs for soil organisms.

PECs can be measured or estimated, and local or regional. The use of local or regional data, and the choice of measured or estimated values, depend on the scale and intended use of the assessment; e.g., the European Commission guidelines for environmental risk assessment recommend the use of modelling data for the initial "reasonable worst-case" exposure assessment of a substance at the continental (EU) level (EC, 2003). (For a detailed discussion on the calculation of PECs in soils at the local and regional levels, see sections 2.3.8.5 and 2.3.8.7 in EC (2003).)

PNEC estimation is even more complex, and it should be chosen to represent the concentration of the chemical under which undesired effects on the organism(s) of concern will most likely not occur. Ideally, a PNEC should incorporate short- and long-term toxicity values for several species in different trophic levels (from bacteria to vertebrates), but because these data are difficult to generate, PNEC calculations are generally derived from the toxicity data for the most sensitive species in the ecosystem and an assessment factor. The assessment factor typically ranges between 10 and 1,000 depending on the type of toxicity data available. Higher factors are applied to short-term acute toxicity values, and lower factors are used when long-term NOECs from different species or species sensitivity distribution data are available (EC, 2003).

Because toxicity data for soil organisms is very limited, PNECs for soil are calculated from aquatic PNECs as illustrated in the example below. However, these soil PNEC values should only be used at a general screening level, because aquatic toxicity is obviously not necessarily representative of toxicity in soil ecosystems (EC, 2003). A detailed discussion on the estimation of PNECs in soils can be found in section 3.6 of EC (2003).

To illustrate their use, RQs were calculated for a selected number of ESOCs (see Table 10.2). The PECs used for this example were calculated using the maximum application

rate of 25 t/ha (dw) derived in the preceding section in conjunction with concentrations of ESOCs in biosolids reported in the literature (CCME, 2010; WEAO, 2010), using Equation 1 and assuming that:

- Biosolids are applied at a maximum rate of 25 t/ha (dw) and incorporated into the top 10 cm of soil within 24 hours of application, which is typical practice for agricultural biosolids application;
- A typical soil bulk density of 1600 kg/m^3 was used to determine the final quantity of biosolids constituents in the total bulk of the soil and biosolids mixture;
- There is no background concentration of any ESOC prior to biosolids application;
- Typically there is a minimum 30-metre buffer to waterways or wellheads from biosolids land application areas; and,
- Typically there is a minimum 10-metre buffer to property boundaries.

$$PEC_{soil} = \frac{C \times App}{H \times r} \qquad \text{(Eq. 1)}$$

where PEC_{soil} is the predicted environmental concentration of the chemical in soil in µg/kg; C is the concentration of the chemical in biosolids obtained from CCME (2010) or WEAO (2010) in µg/kg; App the application rate of biosolids to the soil in kg/m^2; H the biosolids mixing depth in m; and ρ the bulk density of the soil in kg/m^3.

In order to estimate the PNECs, toxicity data were gathered from the literature (see Annex A.3). Because very few toxicity values are available for soil species, PNECs were estimated from aquatic toxicity data using Equation 2:

$$PNEC_{soil} = EP \times \frac{1}{f} \times K_d \qquad \text{(Eq. 2)}$$

where $PNEC_{soil}$ is the predicted no effect concentration of the chemical in soil in µg/kg; EP is the aquatic toxicity endpoint chosen as explained below (in µg/L); f the assessment factor; and K_d the soil/water partition coefficient in L/kg.

Finally, risk quotients (RQ) were calculated with Equation 3:

$$RQ = \frac{PEC_{soil}}{PNEC_{soil}} \qquad \text{(Eq. 3)}$$

The RQ values obtained for the selected chemicals ranged from 0.005 to 450 (Table 10.2), suggesting that the risk to soil organisms exposed to some of these chemicals is high. However, as mentioned above, these values were mainly derived from aquatic toxicity and they should only be considered for screening purposes. It is essential to generate soil toxicity data if the RQ approach is to be used for further evaluation; e.g., the 2 RQ values calculated in Table 10.2 for AHTN vary by one order of magnitude, changing the risk evaluation from “medium” when using an RQ (0.4) calculated from an aquatic toxicity endpoint, to “low” when using the RQ (0.06) generated with data for *F. candida*, a soil-dwelling organism.

Furthermore, if an RQ-based environmental risk assessment is to be used to evaluate the risk of ESOCs in the biosolids land application context, a protocol needs to be established to define the data and procedures to be used, such as those followed in the EU (EC, 2003).

These protocols need to establish, amongst other parameters, the type of toxicity data to use. For example, is the use of modelling data acceptable? What weight do they have relative to experimental data? (e.g., s. ATN in Table 10.2); is the use of data generated for bacteria adequate to estimate risk for soil species (e.g., s. AZM in Table 10.2), and are *in vitro* data acceptable (e.g., MCZ in Table 10.2), and if so, which kinds?

Examples of other parameters that need to be established for an RQ-based risk-assessment:

- PECs: The type and number of chemical concentrations in biosolids that are necessary; e.g., are regional or national occurrence data needed? Should these be actual or modelling measurements? What volume of data is necessary? What type of statistics is to be used for the PNECs, averages, medians, maxima, or ranges?
- Soil data: If aquatic toxicity data are to be used, at least for a first round of screening, a better assessment of K_d values needs to be considered; i.e., are region-representative K_d values necessary or is a generic value for the whole country enough?
- Assessment factors: A protocol for the use of these factors needs to be agreed upon; currently, different organizations use different values (CoCAP, 2011).
- Quality of the data: The quality of both toxicity and analytical data needs to be standardized.

Additionally, the approach above only considers toxicity to soil organisms, but an additional evaluation has to be included to assess aquatic toxicity in water bodies adjacent to biosolids amended-fields after runoff events. Langdon *et al.* (2010) assessed the risk posed by several ESOCs to aquatic biota after a hypothetical runoff event using a conservative scenario, assuming no degradation and no dilution. They concluded that 10 out of the 45 studied compounds would present “a high hazard to aquatic ecosystems”, but also considered that the risk was most likely overestimated.

As noted in Chapter 7.1.1, ESOCs transport in soils is a complex and site-specific phenomenon, which still requires better understanding, but that can be modelled; however, the priority of this type of event has to be established in the general context of ESOCs risk evaluation, especially considering that management practices aim to minimize runoff from agricultural fields.

Even more relevant in the context of Part B of this project (i.e., the national consultation) would be to define the steps to take after ranking the risks of ESOCs with adequate RQ values or by any other methodology, as discussed in Chapter 3.4.

10.3. Risk characterization of emerging pathogens

As discussed in Chapter 4, overall risks to the general population from exposure to pathogens from biosolids amended soils are considered low. In the specific case of the possibility of infection from ingestion of crops grown in biosolids amended soils, the risks are considered to be even lower, especially if the waiting periods between application and harvest are observed (Gale, 2003, 2005; Brooks *et al.*, 2012). Higher risks are usually associated to occupational exposure in unprotected scenarios (Tanner *et al.*, 2008).

Historically, health complaints or specific public health issues resulting from biosolids land application have not been well documented in Canada. But according to Gerba and Smith (2005), who cited data from the US Centers for Disease Control and Prevention, the cause of water diseases outbreaks in the US between 1986 and 1998 with an identified origin was related to animal manure.

Nonetheless, there has been very limited epidemiology work on the possible health effects of biosolids land application, and most of it was based on self-reporting questionnaires (Jenkins *et al.*, 2007; Viau *et al.*, 2011). As stated in Chapter 4.3, conducting scientifically based inquiries following reports of adverse health effects, especially by residents neighbouring biosolids-amended fields, would be a significant contribution to elucidate the possible links of these health effects to biosolids exposure.

Also, data is scarce on emerging pathogens of concern such as *Clostridium difficile*, H1N1, H1N5 and H5N5 influenza virus, and *Cryptosporidium*, in the context of biosolids land application (see Annex A.2). Furthermore, currently used indicator organisms might underestimate risks by not considering pathogens that might have greater infectivity or be more resistant to inactivation during treatment or in the environment, as it is the case for some of the emerging organisms, (Harwood *et al.*, 2005; Viau *et al.*, 2011).

Additionally, although risk estimates always have a certain degree of uncertainty, the estimates for pathogen infection in the biosolids land application context can be relatively large (several orders of magnitude) because some of the factors contributing to the magnitude of the risk are not well understood. Examples of factors requiring better characterization are pathogen concentrations in biosolids, effects of treatment, inactivation in the environment, frequency and duration of exposure, dose-response relationships, and differences in human sensitivity to pathogens. These factors should be evaluated within a risk assessment framework, such as those developed by Eisenberg *et al.* (2008) and Gurian *et al.* (2012).

10.4. Designing a Canadian risk evaluation and sustainability strategy for ESOCs and pathogens in biosolids

The stated purpose of Part B of this project, whose central part is a national consultation workshop and dialogue, is to “elevate the discussion on practice and policy options,

risks, and opportunities for the application of municipal biosolids on agricultural lands in Canada." This discussion can only be fruitful if the potential risks of biosolids land application are well understood, and most stakeholders consider the management of the risks protective of both human and environmental health.

The current state of the knowledge of such potential risks was summarized in this review. In general, currently available evidence suggests that the risk posed by ESOCs and pathogens in the biosolids land application context can be considered low for the general public, especially compared to the risks posed by both in different contexts; e.g., human exposure of PBDEs is more likely to occur from a domestic source than from agricultural products grown in biosolids-amended soil containing PBDEs. Although the lack of research on health symptoms attributed to biosolids, especially in the case of residents living in the proximity of land application sites, still needs to be addressed.

In contrast, the risks to biota, especially soil biota, have not been well characterized, mainly because of the lack of toxicity data for terrestrial organisms. Additionally, as noted in Chapter 3.4, the reactive, chemical-by-chemical approach currently used does not consider the effects of simultaneous exposure to mixtures of chemicals or their transformation products, and it does not address the need to prevent the introduction of potential toxic chemicals before it actually occurs.

The preceding chapters have summarized the multiple needs for additional information and research to develop full risk assessments for ESOCs and pathogenic organisms, and they have also been discussed elsewhere (Higgins *et al.*, 2010), including in the Canadian context (Kleywegt *et al.*, 2007). However, the opportunity afforded by the national consultation workshop and dialogue to assemble a multidisciplinary group of experts on these topics, along with representatives of different stakeholder groups, should be used to evaluate if the current approach is sufficient to determine whether ESOCs are having an effect on human or environmental health. Several researchers have already indicated that it is indeed not enough.

In a comprehensive review of the state of the science of ESOCs in biosolids-amended soils, Higgins *et al.* (2010) recommended the "evaluation of current risk models for their ability to protect the environment and human health." Similarly, the extensive review commissioned by WEAO (2010) concluded by recommending that "costs to continue to address single substances is prohibitive and efforts should be made to address mixtures, their fate and significance to the environment and human health."

Brown (2014) considered that it is necessary to take into account more than just the effects of chemicals to single organisms; the consequences at the population level also need to be established. Similarly, Focks *et al.* (2014) concluded that the current toxicity approach, centred on individual organisms, needs to be expanded to the landscape level.

Burton *et al.* (2012) proposed an alternative approach based on the evaluation of ecosystem health, which would combine chemical, biological, and physical assessment data in a holistic, iterative evaluation process. An approach such as this, which looks at

the state of an ecosystem, is ultimately the only way to really demonstrate whether the introduction of ESOCs to an environmental compartment has a negative effect.

As discussed in Chapter 3.4, the national consultation would be an appropriate venue to discuss the development of a new Canadian risk evaluation strategy for ESOCs in the biosolids land application context. Although open for debate by the stakeholders, the strategy should incorporate the evaluation of ecosystem health, such as Burton *et al.* (2012) proposed, and the following elements, already discussed in Chapter 3.4:

- Definition of the environmental protection goals of the strategy.
- Establishment of a prioritization process for ESOCs.
- Development of an endpoint-monitoring plan.
- Identify and address research gaps.

Ideally, the strategy to evaluate the risks, and especially the strategy for risk management, should take into account ESOCs in contexts other than biosolids land application in order to provide a more holistic perspective that helps to optimize resources; e.g., if a chemical is banned or its used restricted, its concentrations in biosolids will eventually decrease and investment in additional treatment to eliminate such chemical might be unnecessary. The creation of a centralized data 'clearinghouse' for ESOCs-related issues, as suggested by Kleywegt *et al.* (2007), could contribute to improve the exchange of information.

10.4.1. Sustainability

Traditionally, sustainability of biosolids land application has focused exclusively on the potential environmental and public health risks and the long-term preservation of the soil ecosystem's quality (O'Connor *et al.*, 2005). However, as discussed in previous sections, public acceptance has also become an important part of biosolids management. Currently, this social aspect is also considered an essential part of the sustainability of the practice (O'Connor *et al.*, 2005).

Other factors influencing the sustainability of biosolids land application should also be evaluated. Especially when comparing this practice to others being re-examined, and implemented (CDM, 2011), such as gas co-generation and recovery from anaerobic digestion, and power generation from incineration in so-called waste-to-energy (WtE) schemes. Examples of these factors include energy demand, the contribution to global warming (in the form of green house gas emissions), and ozone depletion potential (Yoshida *et al.*, 2013).

Sustainability of the different biosolids management options can be evaluated through methodologies such as life cycle impact assessments (Muñoz *et al.*, 2009; Sablayrolles *et al.*, 2010; Yoshida *et al.*, 2013). However, rather than restricting the analysis to the management practices by themselves, sustainability evaluation should ideally include a more comprehensive environmental assessment at a larger scale; e.g., at the watershed level (Hester and Little, 2013). This approach would allow the evaluation of biosolids land application role as an overall waste treatment system (Bastian, 2005) and its contribution to ecosystem sustainability.

Biosolids have also been proposed as predictors for the sustainability of some chemicals. Venkatesan and Halden (2014) suggest that measuring the concentrations of organic ESOCs during wastewater treatment in the different effluents, sludge, and biosolids can be used to predict their biodegradability and bioaccumulation potential. The chemical's behaviour during secondary treatment would function as an aerobic biodegradability test, and its behaviour in sludge treatment would be a surrogate for bioaccumulation and anaerobic biodegradability. Their findings also suggest that ESOCs concentrations in biosolids could be used as an indicator of chemical exposure and body burden in humans (Venkatesan and Halden, 2014).

10.5. Summary

A 'typical' Canadian land application scenario was used to exemplify the quantitation of environmental risk from selected ESOCs using risk quotients. However, due to the lack of terrestrial toxicity data, these values are only relevant for preliminary, comparative purposes, and have little value as indicators of actual environmental risks. The elements needed to develop complete environmental risk assessments for ESOCs are discussed.

Additionally, it is necessary to develop a different strategy to address the ultimate goal of assessing the risk of the application of biosolids to agricultural land in Canada, which is to determine whether ESOCs as a group affect human and/or environmental health. The design of such Canadian risk evaluation strategy for ESOCs in biosolids requires the participation of representatives from the different stakeholder groups, and it should be part of the national consultation constituting Part B of this project.

Although open for debate by the stakeholders, the strategy should be centered on the evaluation of ecosystem health, such as Burton *et al.* (2012) proposed, and the following elements should be incorporated:

- Definition of the environmental protection goals of the strategy.
- Establishment of a prioritization process for ESOCs.
- Development of an endpoint-monitoring plan.
- Identify and address research gaps.

Ideally, the strategy to evaluate the risks, and especially the strategy for risk management, should take into account ESOCs in contexts other than biosolids land application in order to provide a more holistic perspective that helps to optimize resources; e.g., if a chemical is banned or its use restricted, its concentrations in biosolids will eventually decrease and investment in additional treatment to eliminate such chemical might be unnecessary. The creation of a centralized data 'clearinghouse' for ESOCs-related issues, as suggested by Kleywegt *et al.* (2007), could contribute to improve the exchange of information.

From a public health perspective, conducting scientifically based inquiries following reports of adverse health effects, especially by residents neighbouring biosolids-

amended fields, would be a significant contribution to elucidate the possible links of these health effects to biosolids exposure.

Finally, the strategy should also consider that the sustainability of biosolids land application involves factors other than the potential environmental and public health risks. Examples of these factors include energy demand, the contribution to global warming (in the form of green house gas emissions), and ozone depletion potential. Evaluation of all sustainability factors is especially important when comparing land application to other beneficial uses for biosolids, such as gas co-generation and recovery from anaerobic digestion, and power generation from incineration in so-called waste-to-energy (WtE) schemes.

Table 10.1. Limits on biosolids application rates across Canada (CCME, 2012)

Criteria	Basis	Maximum	Other Considerations	Potential to Mitigate Risks from ESOCs by limiting application amount
Organic Matter Recycling Regulation (BC)	Agronomic (N-based)	None specified	P-based rates recommended in high P soils or near sensitive surface water bodies; soil TE content must not exceed maximum allowable limits for site.	Moderate - agronomic application rate could potentially result in very high application rates depending on site and crop needs. Only restriction on repeated application rates are TE limits in the soil.
Guidelines for the Application of Municipal Wastewater Sludges to Agricultural Land (AB)	Based on maximum allowable N additions (dependent on treatment and site class)	25 t/ha (dw)	Dependent on biosolids type and site classification. Application restricted to once per 3 years, must not exceed cumulative loading of TE for site; soil available N must not exceed 250 kg/ha	High - application maximum not as restrictive as Ontario and Québec, but also includes other restrictions based on maximum nutrient and metals additions.
Land Application of Municipal Sewage Sludge Guidelines (SK)	Agronomic (N-based)	None specified	Soil TE must not exceed maximum allowable limits.	Moderate - agronomic application rate could potentially result in very high application rates depending on site and crop needs. Only restriction on repeated application rates are TE limits in the soil.
Nutrient Management Regulation (ON)	Agronomic with maximum allowable nutrient additions	22 t/ha (dw) every 5 years	Soil TE, boron, sodium, and fats, oil and grease must not exceed maximum allowable limits.	Highest - Most restrictive application maximum as well maximum allowable limits for various parameters
Guidelines for the beneficial use of fertilizing residuals (QC)	Agronomic (N and P)	22 t/ha (dw) every 5 years	Maximum is for C2 residuals only.	High - most restrictive application maximum, but no further restrictions based on soil content
Atlantic Canada Wastewater Guidelines (PEI)	Agronomic (N-based)	None specified	Soil TE must not exceed maximum allowable limits.	Moderate - agronomic application rate could potentially result in very high application rates depending on site and crop needs. Only restriction on repeated application rates are TE limits in the soil.

NS Guidelines	Agronomic	None specified	Soil TE must not exceed maximum allowable limits.	Moderate - agronomic application rate could potentially result in very high application rates depending on site and crop needs. Only restriction on repeated application rates are TE limits in the soil.
Organic Matter Recycling Regulation (BC)	Agronomic (N-based)	None specified	P-based rates recommended in high P soils or near sensitive surface water bodies; soil TE content must not exceed maximum allowable limits for site.	Moderate - agronomic application rate could potentially result in very high application rates depending on site and crop needs. Only restriction on repeated application rates are TE limits in the soil.

Table 10.2. Example of risk quotient calculations for selected ESOCs.

Chemical	Conc. biosolids, µg/kg [1]	PEC soil, µg/kg	Kd	Endpoint, µg/L	Factor	PNEC soil, µg/kg	RQ	Endpoint
AHTN	4,015	63	481 [3]	33	100	160	0.4	NOEC, *D. rerio* [4]
AHTN	4,015	63	481 [3]	105 mg/kg (soil)	100	1,050	0.06	LOEC (soil), *F. candida* [5]
ATN	90 [2]	1.4	15 [6]	1,800	100	272	0.005	NOEC, *D. magna* [7]
ATN	90 [2]	1.4	15 [6]	78	10	120	0.01	PNEC, green algae [8]
AZM	205	3.2	316 [9]	5.2	1,000	1.6	**2**	NOEC, *P. subcapitata* [10]
AZM	205	3.2	316 [9]	0.15	1,000	0.047	**67**	PNEC, bacteria [11]
CBZ	67	1	25 [12]	25	100	6.3	0.2	NOEC, *C. dubia* [13]
CIP	3,610	56	20,000 [6]	5	1,000	100	0.6	EC50, *M. aeruginosa* [14]
EE2	25 [2]	0.4	56 [12]	0.00031	10	0.0017	**220**	NOEC, *D. rerio* [15]
FRS	543	8.5	40 [9]	10	1,000	0.4	**21**	LOEC, riverine biofilm [16]
GFB	56	0.88	1.3 [12]	1.5	100	0.0019	**450**	LOEC, *C. auratus* [17]
HHCB	8,975	140	618 [3]	140	100	865	0.2	LOEC, *P. promelas* [18]
HHCB	8,975	140	618 [3]	105 mg/kg (soil)	100	1,050	0.1	LOEC (soil), *E. fetida* [18]
IBP	522	8.1	28 [12]	1	10	2.8	**3**	LOEC, *O. latipes* [19]
MCZ	441	6.9	957 [3]	27	1,000	26	0.3	IC50, aromatase (*in vitro*) [20]
NOR	558	8.7	3,200 [9]	38	1,000	120	0.07	EC50, *M. wesenbergii* [21]
OFX	276	4.3	300 [6]	21	1,000	6.3	0.7	EC50, *M. aeruginosa* [22]
PRP	30	0.47 [2]	58 [12]	9	100	5.2	0.09	NOEC, *C. dubia* [23]
SMZ	5.2	0.08	7.9 [12]	81	1,000	0.64	0.1	EC50, *L. minor* [24]

1: Median value in WEAO (2010); 2: High end of range in CCME (2010); 3: Langdon *et al.* (2010); 4: Carlsson and Norrgren (2004); 5: Balk and Ford (1999); 6: Martín *et al.* (2015); 7: Küster *et al.* (2010); 8: Jones *et al.* (2002); 9: Narumiya *et al.* (2013); 10: Harada *et al.* (2008); 11: Kümmerer and Henninger (2003); 12: Martín *et al.* (2012); 13: Ferrari *et al.* (2003); 14: Halling-Sørensen *et al.* (2000); 15: Schäfers *et al.* (2007); 16: Lawrence *et al.* (2005); 17: Mimeault *et al.* (2005); 18: EC (2008); 19: Flippin *et al.* (2007); 20: Trösken *et al.* (2004); 21: Ando *et al.* (2007); 22: Robinson *et al.* (2005); 23: Ferrari *et al.* (2004); 24: Brain *et al.* (2004).

10.6. References

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11. EPILOGUE

After the Literature Review was finalized, it was submitted to the Canadian Municipal Water Consortium (CMWC) of the Canadian Water Network (CWN) for evaluation. Additionally, it was distributed to a group of experts in the biosolids field, who convened at a one-day workshop to discuss the Literature Review and to put together the elements for a National Biosolids Research Agenda for Canada. The revisions and comments of the anonymous reviewers appointed by CWN and the experts present at the workshop were incorporated into the final version of the Literature Review.

This section summarizes the principal outcomes of the workshop and the elements for the National Biosolids Research Agenda determined by the workshop participants.

11.1. Expert validation workshop

The expert validation workshop, *Biosolids Risks in Context*, was organized by CWN's CWMC and it took place on January 22, 2015 in Ottawa, ON.

The overall purpose of the workshop was to validate the state of the knowledge presented in the literature review through a structured dialogue among key biosolids expert stakeholders.

More specifically, the workshop participants were asked to:

- Provide expert input on the current state of knowledge on ESOCs and pathogens – what is and is not known
- Discuss the risks and uncertainties and the impact these have on the acceptable use of biosolids for agricultural purposes
- Define the research needs to address the unknowns and uncertainties identified above, and to reach consensus on the sustainability of biosolids land application
- Evaluate the practicality of developing a National Biosolids Management Consortium to coordinate the research agenda, to foster the interaction of researchers and regulators, and to develop public information and risk communication strategies

11.1.1 Workshop participants

- Erik Apedaile – Director, Apedaile Environmental, Ottawa, ON
- Susheel Arora – Director, Wastewater and Stormwater Services, Halifax Water, Halifax, NS
- Robert Bastian – Senior Environmental Scientist, US Environmental Protection Agency (USEPA), Office of Wastewater Management, Washington, DC
- Ned Beecher – Executive Director, North East Biosolids and Residuals Association (NEBRA), Tamworth, NH
- Tammy Bellamy – Project Engineer, Engineering and Wastewater Programs, Region of Waterloo, Kitchener, ON

- Shelly Bonte-Gelok – Engineer, Ontario Ministry of the Environment and Climate Change (MOECC), Standards Development Branch, Toronto, ON
- Trevor Brown – Senior Project Engineer (Water Services), Region of Waterloo, Kitchener, ON
- Danaëlle Delâge – Head of the Exposure Unit, Ecological Assessment Division, Environment Canada, Gatineau, QC
- Jennifer Dingman – Program Coordinator, Canadian Municipal Water Consortium, Canadian Water Network, Waterloo, ON
- Gordon Dinwoodie – Land Reclamation Specialist, Government of Alberta, Edmonton, AB
- Nancy Fleming – Senior Engineer, Wastewater Treatment, City of Toronto, Toronto, ON
- Natasha Harckham – Senior Regulatory Analyst (Biosolids), The City of Calgary, Calgary, AB
- Cailin Hillier – Project Officer, Canadian Municipal Water Consortium, Canadian Water Network, Waterloo, ON
- Dean Iamarino – Biosolids Operations Coordinator, Region of Halton, Oakville, ON
- John Lavery – Business Development and Special Projects Manager, Sylvis Environmental, New Westminster, BC
- Benoit Lebeau – Nutrient Management Engineer, Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), Kemptville, ON
- Jorge Loyo – Postdoctoral Fellow, Department of Chemistry and Biology, Ryerson University, Toronto, ON
- Lynda McCarthy – Professor, Department of Chemistry and Biology, Ryerson University, Toronto, ON
- Hugh Monteith – Senior Consultant, Hydromantis, Hamilton, ON
- Jan Oleszkiewicz – Professor, Department of Civil Engineering, University of Manitoba, Winnipeg, MB
- Michael Payne – Residuals and Biosolids Utilization Specialist, Black Lake Environmental, Perth, ON
- Ian Pepper – Professor, College of Public Health, and Department of Agricultural and Biosystems Engineering; Director, Environmental Research Laboratory, and Water and Environmental Technology Center, The University of Arizona, Tucson, AZ
- Jesse Shen – Environment Canada, Gatineau, QC
- Warren Wishart – Manager, Canadian Municipal Water Consortium, Canadian Water Network, Waterloo, ON

11.1.2 Overall research needs and suggestions for future work

- Additional evidence-based information on ESOCs and pathogens in biosolids to improve risk assessment
- New approach to address biosolids issues as an ongoing process; e.g. worries about chemicals are cyclical, and there is always going to be one chemical that draws public attention. This approach should include mechanisms of (rapid) response to these risk perception crises
- Long-term studies to evaluate impact and sustainability

- Connect CWN-sponsored biosolids projects, such as this literature review, the ecotoxicology impact assessment, and the nutrient management project, to assemble an overall picture of the sustainability of biosolids land application
- Address the issues of nutrient availability in chemical sludge, N/P ratio adjustment to land/crop requirements, and the risk of phosphorus leaching in fragile lake drainage areas
- Investigate ESOCs removal during wastewater treatment as a whole (effluent and solids), including the use of biosolids as a mechanism to remove undesirable chemicals from effluent, and promote their degradation during sludge treatment and subsequent land application

11.1.3 Main factors preventing public acceptance

- Odour is the main issue for the public, more important than concerns about ESOCs or pathogens
- Lack of information on biosolids. Educational materials would be helpful for people who do not know about biosolids and are interested in the science, even if the views of a small proportion of the opposing public cannot be changed
- The public does not usually inquire about the science, although the majority trusts scientists

11.1.4 Suggestions to improve communication and public acceptance

- Establish risk communication programs
- Explain the risks in context; e.g., the exposure to Triclosan from personal care products at home versus the minimal potential exposure from land applied biosolids
- Address odour issues
- A more positive, proactive approach to biosolids management; promote rather than defend beneficial use of biosolids
- Emphasize the beneficial uses and benefits; e.g., use in reclamation to improve soil quality, such as in mining sites; metal sequestration
- Produce a PowerPoint presentation on biosolids that municipalities could use with the public

11.1.5 Main characteristics of a good biosolids program

- Sustainable (socially, economically, and environmentally)
- Climate-friendly
- Beneficial use of the biosolids
- Publicly supported

11.2 Elements for a Canadian National Biosolids Research Agenda

The workshop participants agreed that a National Biosolids Research Agenda for Canada should address the following topics:

- ESOCs and pathogens risk assessment process
- Phosphorus availability, removal, and release
- National communication strategy
- Odour and other nuisances
- Long-term impact studies
- Promotion of beneficial uses of biosolids
- Climate change mitigation

Relevant themes that should be considered in the refinement of the agenda topics included:

- Establish appropriate metrics for quantitation where relevant; e.g., what is meant by safe or acceptable.
- Collaborate with organizations such as the National Biosolids Partnership (NBP, part of the Water Environment Federation, WEF), the Water Environment Association of Ontario (WEAO), BC Water & Waste Association (BCWWA), and the Ontario Clean Water Agency (OCWA), both to refine the National Biosolids Research Agenda, and to discuss the creation of a National Biosolids Consortium.
- Involve managers of other residuals, such as municipal organic composting programs, in the collaboration discussed above.
- Economics did not surface as an important issue during the workshop. In particular, it did not seem to factor in the lack of public acceptance. It was mainly brought up in the context of comparing land application to energy recovery.

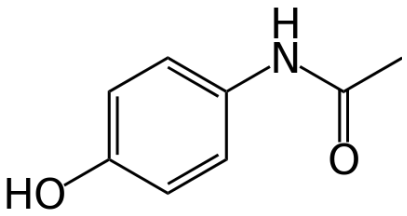
Finally, CMWC will use the literature review findings, the report of Part B of this project, and the outcomes of the expert validation workshop to plan a national consultation involving a larger number of stakeholders.

APPENDICES

A.1. ESOCs

A.1.1. Acetaminophen

Acetaminophen (ACM), known internationally as paracetamol (both names derived from *para*-acetylaminophenol), is the common name of *N*-(4-hydroxyphenyl)acetamide, an analgesic and antipyretic sold as Tylenol® in North America.



Property	Value	References
Formula	$C_8H_9NO_2$	HSDB (2007a)
CAS Registry Number	103-90-2	HSDB (2007a)
MW, g/mol	151.16	HSDB (2007a)
Solubility, mg/L (25°C)	14,000	HSDB (2007a)
pK_a	9.38	HSDB (2007a)
log K_{ow} (25°C)	0.46	HSDB (2007a)
V_p, Pa (25°C)	8.4×10^{-3}	HSDB (2007a)
Henry's law constant, Pa m^3 mol^{-1} (25°C)	6.5×10^{-8}	HSDB (2007a)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2007a)
- Risk assessment in biosolids: Eriksen *et al.* (2009) did not consider ACM for the risk assessment due to its high biodegradability.
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

- Concentrations in sludge or biosolids reported by: Edwards *et al.* (2009); CCME (2010); Higgins *et al.* (2010); WEAO (2010); Gao *et al.* (2012); Jensen *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014); Martín *et al.* (2015); Yan *et al.* (2014)
- Concentrations in WWTPs also reported by: Miège *et al.* (2009); Gao *et al.* (2012); Jensen *et al.* (2012); Verlicchi *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014); Luo *et al.* (2014); Yan *et al.* (2014)

Fate in wastewater and sludge treatment

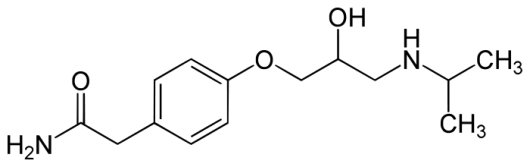
- Wastewater: High removal due to biodegradation. Removal percentages reported by Eriksen *et al.* (2009); Miège *et al.* (2009); Gao *et al.* (2012); Verlicchi *et al.* (2012); Yan *et al.* (2014)
- Salveson *et al.* (2012) used it as an indicator compound in wastewater treatment for rapidly biodegradable chemicals with low sorption to solids

Fate and transport in agricultural soils after biosolids application

- Detected in tile drainage (Gottschall *et al.*, 2012) and runoff (Sabourin *et al.*, 2009)
- Considered mobile in runoff by Langdon *et al.* (2010)

A.1.2. Atenolol

Atenolol (ATN) is a β-adrenergic receptor blocker (β-blocker) used for the treatment of cardiovascular diseases.



Property	Value	References
Formula	$C_{14}H_{22}N_2O_3$	HSDB (2004b)
CAS Registry Number	29122-68-7	HSDB (2004b)
MW, g/mol	266.34	HSDB (2004b)
Solubility, mg/L (25°C)	13,300 mg/L	HSDB (2004b)
pK_a	9.6	HSDB (2004b)
log K_{ow} (25°C)	0.16	HSDB (2004b)
V_p, Pa (25°C)	1.025×10^{-7}	Küster *et al.* (2010)
Henry's law constant, Pa m^3 mol^{-1} (25°C)	1.396×10^{-13}	Küster *et al.* (2010)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2004b)
- Risk assessment in biosolids: Eriksen *et al.* (2009)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)
- European environmental risk assessment: Küster *et al.* (2010)

Occurrence in biosolids and WWTPs

- Concentrations in sludge or biosolids reported by: Edwards *et al.* (2009); CCME (2010); Higgins *et al.* (2010); WEAO (2010); Jensen *et al.* (2012); Hernandez-Raquet (2013); Martín *et al.* (2015)
- Concentrations in WWTPs reported by: Miège *et al.* (2009); Verlicchi *et al.* (2012); Hernandez-Raquet (2013); Luo *et al.* (2014)

Fate in wastewater and sludge treatment

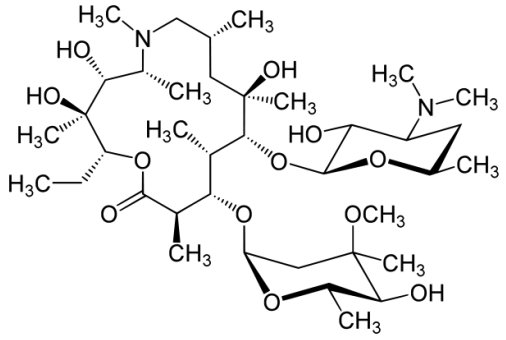
- Wastewater: Low to moderate removal (Eriksen *et al.*, 2009; Miège *et al.*, 2009; Verlicchi *et al.*, 2012; Hernandez-Raquet, 2013; Luo *et al.*, 2014)
- Selected by Salveson *et al.* (2012) as an indicator in wastewater treatment for highly biodegradable compounds with low sorption capacity.

Fate and transport in agricultural soils after biosolids application

- Transported in runoff (Sabourin *et al.*, 2009)

A.1.3. Azithromycin

Azithromycin (AZM) is an azalide antibiotic, a macrolide derived from erythromycin.



Property	Value	References
Formula	$C_{38}H_{72}N_2O_{12}$	HSDB (2013)
CAS Registry Number	83905-01-5	HSDB (2013)
MW, g/mol	748.98	HSDB (2013)
Solubility, mg/L (25°C)	2.37 mg/L (estimated)	HSDB (2013)
pK_a	8.74	HSDB (2013)
log K_{ow} (25°C)	4.02	HSDB (2013)
V_p, Pa (25°C)	3.53×10^{-22} (estimated)	HSDB (2013)
Henry's law constant, Pa m^3 mol^{-1} (25°C)	5.37×10^{-24} (estimated)	HSDB (2013)

The most frequently prescribed antibiotic in the US from 2009 to 2012; in 2013 it was surpassed by amoxicillin (Hicks *et al.*, 2013; IMS, 2014). In Canada, however, prescriptions for amoxicillin, clarithromycin, and ciprofloxacin have consistently been higher than for AZM from 2007 to 2011 (PHAC, 2014).

AZM is mainly used in Canada to treat respiratory ailments (e.g., acute bronchitis), diseases of the genitourinary system and the ear. However, AZM use has decreased since 2007, as it has for other macrolides (PHAC, 2014).

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, www.wikipharma.org, and Kümmerer and Henninger (2003); Harada *et al.* (2008)
- Human toxicity: Summarized in HSDB (2013)
- Risk assessment in biosolids: Eriksen *et al.* (2009)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

- As for most antibiotics and pharmaceuticals, the main route of entry to the environment is assumed to be WWTP effluents (Clarke and Smith, 2011).
- Concentrations in biosolids reported by: Higgins *et al.* (2010); WEAO (2010); Clarke and Smith (2011); Jensen *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014)
- Concentrations in WWTPs reported by: Hernandez-Raquet (2013); Miège *et al.* (2009); Guerra *et al.* (2014); Yan *et al.* (2014)

Fate in wastewater and sludge treatment

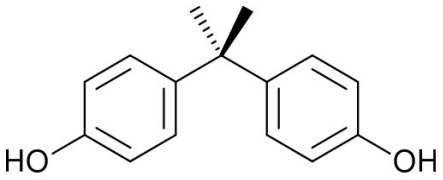
- Wastewater: Removal percentages very widespread, tend to be low, higher in general for activated sludge than membrane biological reactors, s. Miège *et al.* (2009); Verlicchi *et al.* (2012); Guerra *et al.* (2014); Yan *et al.* (2014)
- Biosolids: Data sparse according to WEAO (2010); in Canada: Guerra *et al.* (2014)
- UV degradation in WWTP: De la Cruz *et al.* (2012)

Fate and transport in agricultural soils after biosolids application

- Fate in soils: Half-life: 408-990 d (Walters *et al.*, 2010); 71 d (Gottschall *et al.*, 2012)

A.1.4. Bisphenol A

Bisphenol A (BPA) is a plasticizer. Because of its ubiquity and the increasing evidence of toxic effects (see next section), BPA was included in the List of Toxic Substances (Schedule 1) of the CEPA in 2010 (Environment Canada, 2013). As a consequence, polycarbonate baby bottles containing BPA were banned in Canada (Hazardous Products Act, 2010), and Environment Canada required the implementation of pollution prevention plans by some manufacturers and users of BPA to limit the concentration in their effluents to a maximum of 1.75 µg/L (CEPA, 2012), among other measures.



Property	Value	References
Formula	$C_{15}H_{16}O_2$	
CAS Registry Number	80-05-7	
MW, g/mol	228.3	
Solubility, mg/L (pH 7.0)	120 – 300	Staples *et al.* (1998)
pK_a	9.6 – 11.3	Staples *et al.* (1998)
log K_{ow} (25°C)	3.40	Staples *et al.* (1998)
V_p, Pa (25°C)	5.3×10^{-6}	Environment Canada and Health Canada (2008)
Henry's law constant, Pa m^3 mol^{-1} (25°C)	1.0×10^{-6}	Environment Canada and Health Canada (2008)

Toxicity concerns and biological endpoints

- Biological endpoints: ECB (2003)
- Human toxicity: ECB (2003)
- Canadian risk assessment: Environment Canada and Health Canada (2008)
- European risk assessment: ECB (2003); IHCP (2008a); (IHCP, 2008b)

Occurrence in biosolids and WWTPs (see risk assessments above)

- Concentrations in sludge or biosolids reported by: CCME (2010); Higgins *et al.* (2010); WEAO (2010); Clarke and Smith (2011)
- Concentrations in WWTPs reported by: Luo *et al.* (2014)
- Detected in groundwater (Lapworth *et al.*, 2012)

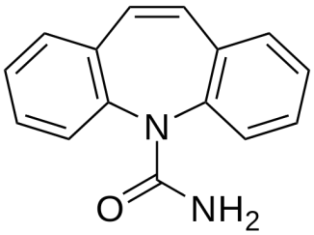
Fate in wastewater and sludge treatment

- Wastewater: High removal (Luo *et al.*, 2014)

- Sludge: Recalcitrant (CCME, 2010)

A.1.5. Carbamazepine

Carbamazepine (CBZ) is an antiepileptic (anticonvulsant) recalcitrant to treatment.



Property	Value	References
Formula	$C_{15}H_{12}N_2O$	HSDB (2007b)
CAS Registry Number	298-46-4	HSDB (2007b)
MW, g/mol	236.27	HSDB (2007b)
Solubility, mg/L (25°C)	18 mg/L (estimated)	HSDB (2007b)
pK_a	13.9	HSDB (2007b)
log K_{ow} (25°C)	2.45	HSDB (2007b)
V_p, Pa (25°C)	2.45 x 10^{-5} (estimated)	HSDB (2007b)
Henry's law constant, Pa m^3 mol^{-1} (25°C)	0.0186 (estimated)	HSDB (2007b)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2007b)

Occurrence in biosolids and WWTPs

- Concentrations in sludge or biosolids reported by: Edwards *et al.* (2009); CCME (2010); Higgins *et al.* (2010); WEAO (2010); Clarke and Smith (2011); Gao *et al.* (2012); Jensen *et al.* (2012); Lajeunesse *et al.* (2012); Hernandez-Raquet (2013); Martín *et al.* (2015); Yan *et al.* (2014)
- Concentrations in WWTPs reported by: Miège *et al.* (2009); Gao *et al.* (2012); Lajeunesse *et al.* (2012); Hernandez-Raquet (2013); Golovko *et al.* (2014); Luo *et al.* (2014); Yan *et al.* (2014)
- Detected in groundwater (Lapworth *et al.*, 2012)

Fate in wastewater and sludge treatment

- Wastewater: Recalcitrant (Miège *et al.*, 2009; Lajeunesse *et al.*, 2012; Golovko *et al.*, 2014; Luo *et al.*, 2014; Yan *et al.*, 2014)
- Sludge treatment: Recalcitrant (CCME, 2010; Stasinakis, 2012)

Fate and transport in agricultural soils after biosolids application

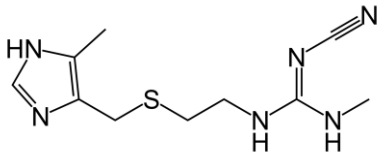
- Fate in soils: Half-life: 495 d (Walters *et al.*, 2010)

- Detected in tile drainage (Gottschall *et al.*, 2012) and runoff (Sabourin *et al.*, 2009)

A.1.6. Cimetidine

Cimetidine (CIM), also known as Tagamet®, is a stomach-acid inhibitor.

is a fluoroquinolone antibiotic. It is commonly found in raw sewage and biosolids in relatively high concentrations.



Property	Value	References
Formula	$C_{10}H_{16}N_6S$	HSDB (2003d)
CAS Registry Number	51481-61-9	HSDB (2003d)
MW, g/mol	252.38	HSDB (2003d)
pK_a	6.8	HSDB (2003d)
log K_{ow} (25°C)	0.40	HSDB (2003d)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2003d)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

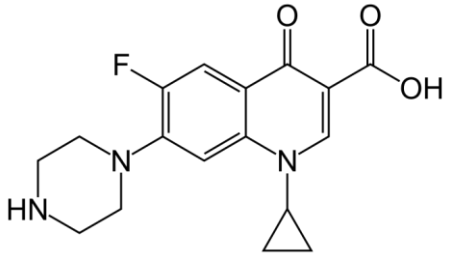
- Concentrations in sludge or biosolids reported by: (CCME, 2010); Higgins *et al.* (2010); WEAO (2010); Clarke and Smith (2011); Jensen *et al.* (2012); Hernandez-Raquet (2013)
- Concentrations in WWTPs reported by: Jensen *et al.* (2012); Verlicchi *et al.* (2012); Hernandez-Raquet (2013)

Fate in wastewater and sludge treatment

- Wastewater: Intermediate removal (Verlicchi *et al.*, 2012)
- Use by Salveson *et al.* (2012) as an indicator for compound with medium sorption and biodegradation capability in wastewater treatment.

A.1.7. Ciprofloxacin

Ciprofloxacin (CIP) is a fluoroquinolone antibiotic. It is commonly found in raw sewage and biosolids in relatively high concentrations.



Property	Value	References
Formula	$C_{17}H_{18}FN_3O_3$	HSDB (2012e)
CAS Registry Number	85721-33-1	HSDB (2012e)
MW, g/mol	331.34	HSDB (2012e)
Solubility, mg/L (20°C)	30,000 mg/L	HSDB (2012e)
pK_a	6.09 (carboxyl) 8.74 (nitrogen on piperazinyl ring)	HSDB (2012e)
log K_{ow} (25°C)	0.28 (non-ionized)	HSDB (2012e)
V_p, Pa (25°C)	3.8×10^{-11} (estimated)	HSDB (2012e)

Ciprofloxacin was one of the five most frequently prescribed antibiotics in the US in 2010 (Hicks *et al.*, 2013). It is the most commonly recommended fluoroquinolone in Canada (PHAC, 2014).

CIP is mainly used in Canada to treat diseases of the gastrointestinal and genitourinary systems and the ear (PHAC, 2014).

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, www.wikipharma.org, Higgins *et al.* (2010)
- Human toxicity: Summarized in HSDB (2012e) and by Higgins *et al.* (2010)
- Risk assessment in biosolids: Eriksen *et al.* (2009)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and the environment

- As for most antibiotics and pharmaceuticals, the main route of entry to the environment is assumed to be WWTP effluents (Clarke and Smith, 2011).
- Commonly found in biosolids (Clarke and Smith, 2011), usually in the highest concentrations (low ppb) (WEAO, 2010). Concentrations in sludge or biosolids also reported by: Eriksen *et al.* (2009); Miège *et al.* (2009); CCME (2010); Higgins *et al.* (2010); Jensen *et al.* (2012); Martín *et al.* (2015); Guerra *et al.* (2014), and Gottschall *et al.* (2012) in Canada
- One of the antibiotics in the highest concentrations (low ppb) in influents and secondary effluents (Verlicchi *et al.*, 2012). Concentrations in WWTPs also reported by: Hernandez-Raquet (2013); Guerra *et al.* (2014) in Canada.

- Concentrations in soils reported by: Du and Liu (2012)

Fate in wastewater and WWTPs

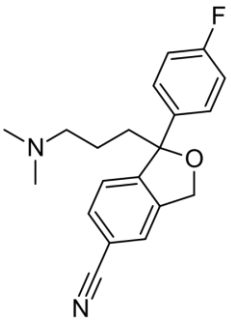
- Wastewater: Relatively high removal (~70%), driven by sorption (Verlicchi *et al.*, 2012). Removal percentages also reported by Eriksen *et al.* (2009); Miège *et al.* (2009); Guerra *et al.* (2014)
- Biosolids: High concentrations (low mg/kg) (Eriksen *et al.*, 2009)

Fate and transport in agricultural soils after biosolids application

- Fate in soils: Persistent, limited mobility (Eriksen *et al.*, 2009; Higgins *et al.*, 2010; WEAO, 2010). Half-life: 1155-3466 d (Walters *et al.*, 2010). Strong sorption to soil, no degradation (Wu *et al.*, 2009)
- Might promote antibacterial resistance in soils because of persistence (Eriksen *et al.*, 2009)

A.1.8. Citalopram

Citalopram (CTP), used as an antidepressant, is a phthalane derivative normally commercialized as the racemic mixture of the hydrochloride form.



Property	Value	References
Formula	$C_{20}H_{21}FN_2O$	HSDB (2003a)
CAS Registry Number	59729-33-8	HSDB (2003a)
MW, g/mol	324.40	Christensen *et al.* (2007)
Solubility, mg/L	31.1 (estimate)	Christensen *et al.* (2007)
pK_a	9.1 (estimate)	Christensen *et al.* (2007)
log K_{ow} (25°C)	3.7	Christensen *et al.* (2007)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2003a)

Occurrence in biosolids and WWTPs

- Concentrations in sludge or biosolids reported by: Lajeunesse *et al.* (2012); Hernandez-Raquet (2013); Niemi *et al.* (2013)

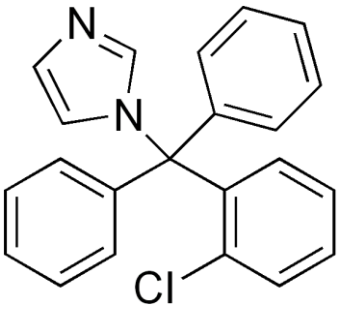
- Concentrations in WWTPs also reported by: Golovko *et al.* (2014)

Fate in wastewater and sludge treatment

- Wastewater: Low removal (Lajeunesse *et al.*, 2012; Golovko *et al.*, 2014)

A.1.9. Clotrimazole

Clotrimazole (CTZ) is an antimycotic, generally used in the treatment of dermal and vaginal infections.



Property	Value	References
Formula	$C_{22}H_{17}ClN_2$	HSDB (2003c)
CAS Registry Number	23593-75-1	HSDB (2003c)
MW, g/mol	344.84	HSDB (2003c)
Solubility, mg/L	0.49 mg/L	OSPAR (2005)
pK_a	6.12	OSPAR (2005)
log K_{ow} (25°C)	4.1	OSPAR (2005)
V_p, Pa (25°C)	2.84 - 3.31 x 10^{-7} (estimate)	OSPAR (2005)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, OSPAR (2013), and www.wikipharma.org
- Human toxicity: Summarized in OSPAR (2013)
- Risk assessment in biosolids: Eriksen *et al.* (2009)
- Environmental risk assessment: OSPAR (2013)

Occurrence in biosolids and WWTPs

- Concentrations in sludge or biosolids reported by: Huang *et al.* (2010); Lindberg *et al.* (2010)
- Concentrations in WWTPs reported by: Kahle *et al.* (2008); Miège *et al.* (2009); Lindberg *et al.* (2010)

Fate in wastewater and sludge treatment

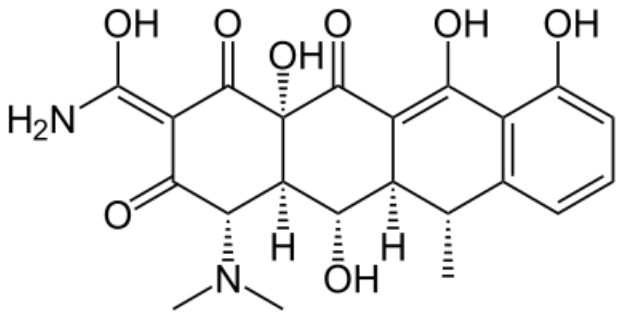
- Wastewater: High removal through sorption to solids (Kahle *et al.*, 2008)

Fate and transport in agricultural soils after biosolids application

- Dissipates in soils (Sabourin *et al.*, 2011)
- Persistent in biosolids-amended soils (Chen *et al.*, 2013a; Chen *et al.*, 2013b)

A.1.10. Doxycycline

Doxycycline (DTC) is a tetracycline antibiotic.



Property	Value	References
Formula	$C_{22}H_{24}N_2O_8$	HSDB (2002b)
CAS Registry Number	564-25-0	HSDB (2002b)
MW, g/mol	462.46	HSDB (2002b)

DTC is mainly used in Canada to treat diseases of the gastrointestinal and genitourinary systems (PHAC, 2014).

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, www.wikipharma.org, Higgins *et al.* (2010)
- Human toxicity: Summarized in HSDB (2002b), Higgins *et al.* (2010)
- Risk assessment in biosolids: Eriksen *et al.* (2009); Jensen *et al.* (2012)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

- As for most antibiotics and pharmaceuticals, the main route of entry to the environment is assumed to be WWTP effluents (Clarke and Smith, 2011).
- Concentrations in sludge or biosolids in the high ppb range; reported by: Eriksen *et al.* (2009); (CCME, 2010); Higgins *et al.* (2010); WEAO (2010); Clarke and Smith (2011); Hernandez-Raquet (2013); Guerra *et al.* (2014) in Canada
- Concentrations in WWTPs reported by: Verlicchi *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014) in Canada

Fate in wastewater and sludge treatment

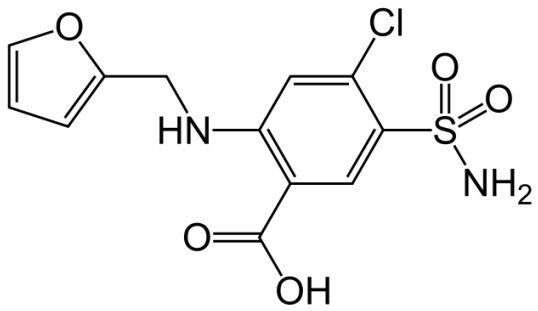
- Wastewater: Relatively high removal (~70%), but high variability (Verlicchi *et al.*, 2012). Removal percentages also reported by Gao *et al.* (2012), low
- Biosolids: Slight removal during storage (Hernandez-Raquet, 2013)

Fate and transport in agricultural soils after biosolids application

- Fate in soils: Half-life: 533-578 d (Walters *et al.*, 2010). Sorbed strongly; showed degradation before reaching plateau (Wu *et al.*, 2009)
- Classified as possible high risk to aquatic organisms in runoff modelling (Langdon *et al.*, 2010)

A.1.11. Furosemide

Furosemide (FRS) is a diuretic usually prescribed during the treatment of cardiovascular and liver disease.



Property	Value	References
Formula	$C_{12}H_{11}ClN_2O_5S$	HSDB (2005b)
CAS Registry Number	54-31-9	HSDB (2005b)
MW, g/mol	330.75	HSDB (2005b)
Solubility, mg/L (30°C)	73.1 mg/L	HSDB (2005b)
pK_a	3.8; 7.5	HSDB (2005b)
log K_{ow}	2.03	HSDB (2005b)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2005b)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

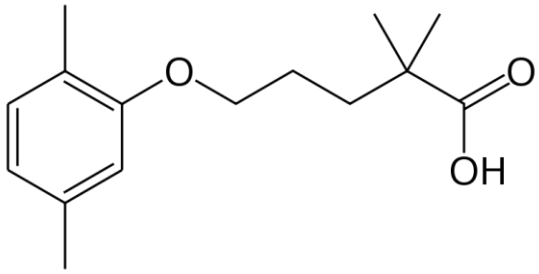
- Concentrations in sludge or biosolids reported by: Jensen *et al.* (2012); Narumiya *et al.* (2013)
- Concentrations in WWTPs reported by: Verlicchi *et al.* (2012); Hernandez-Raquet (2013)

Fate in wastewater and sludge treatment

- Wastewater: Moderate removal (Verlicchi *et al.*, 2012; Hernandez-Raquet, 2013)

A.1.12. Gemfibrozil

Gemfibrozil (GFB) is a hypolipidemic drug.



Property	Value	Reference
Formula	$C_{15}H_{22}O_3$	HSDB (2009a)
CAS Registry Number	25812-30-0	HSDB (2009a)
MW, g/mol	250.33	HSDB (2009a)
Solubility, mg/L (25°C)	11	HSDB (2009a)
pK_a	4.75	HSDB (2009a)
log K_{ow} (25°C)	4.77	HSDB (2009a)
V_p, Pa (25°C)	4.1×10^{-3}	HSDB (2009a)
Henry's law constant, Pa m^3 mol^{-1} (25°C)	1.2×10^{-3}	HSDB (2009a)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2009a)
- Risk assessment in biosolids: Eriksen *et al.* (2009)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

- Concentrations in sludge or biosolids reported by: Edwards *et al.* (2009); CCME (2010); Higgins *et al.* (2010); WEAO (2010); Jensen *et al.* (2012); Martín *et al.* (2012); Hernandez-Raquet (2013); Martín *et al.* (2015)
- Concentrations in WWTPs reported by: Metcalfe *et al.* (2003); Miège *et al.* (2009); Skolness *et al.* (2012); Verlicchi *et al.* (2012); Hernandez-Raquet (2013); Luo *et al.* (2014)
- Detected in groundwater (Fang *et al.*, 2012)

Fate in wastewater and sludge treatment

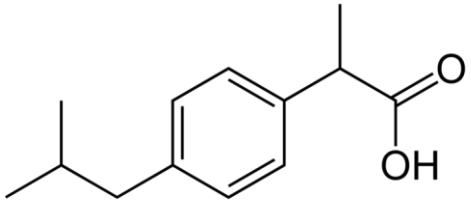
- Wastewater: Moderate to high removal (Eriksen *et al.*, 2009; Miège *et al.*, 2009; Verlicchi *et al.*, 2012; Luo *et al.*, 2014)

Fate and transport in agricultural soils after biosolids application

- Fate in soils: Half-life: 231 d Walters *et al.* (2010)
- Not detected in tile drainage (Gottschall *et al.*, 2012)
- Low transport potential in runoff (Sabourin *et al.*, 2009; Langdon *et al.*, 2010)

A.1.13. Ibuprofen

Ibuprofen (IBP) is a non-steroidal anti-inflammatory drug (NSAID).



Property	Value	References
Formula	$C_{13}H_{18}O_2$	HSDB (2005a)
CAS Registry Number	15687-27-1	HSDB (2005a)
MW, g/mol	206.28	HSDB (2005a)
Solubility, mg/L (25°C)	21 mg/L	HSDB (2005a)
pK_a	4.91, 5.2	HSDB (2005a)
log K_{ow}	3.97	HSDB (2005a)
V_p, Pa (25°C)	6.3×10^{-3}	HSDB (2005a)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2005a)
- Risk assessment in biosolids: Eriksen *et al.* (2009) did not consider IBP for risk assessment because of its high degradability
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

- Concentrations in sludge or biosolids reported by: Edwards *et al.* (2009); Eriksen *et al.* (2009); CCME (2010); WEAO (2010); Jensen *et al.* (2012); Martín *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014); Martín *et al.* (2015); Yan *et al.* (2014)
- Concentrations in WWTPs also reported by: Miège *et al.* (2009); Verlicchi *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014); Luo *et al.* (2014); Yan *et al.* (2014)
- Detected in groundwater by Lapworth *et al.* (2012)

Fate in wastewater and sludge treatment

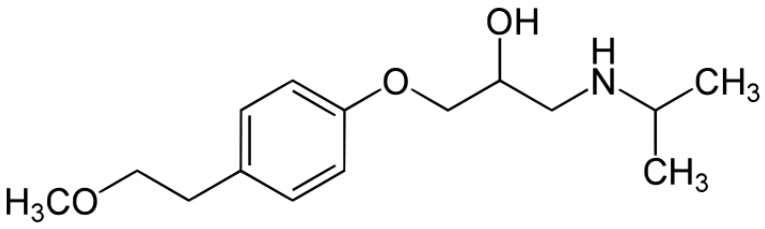
- Wastewater: High removal reported by Eriksen *et al.* (2009); Miège *et al.* (2009); Luo *et al.* (2014); Yan *et al.* (2014)
- Sludge: Moderate removal reported by Stasinakis (2012);
- Selected by Salveson *et al.* (2012) as indicator for removal in wastewater treatment of highly biodegradable compounds with low sorption capacity.

Fate and transport in agricultural soils after biosolids application

- Detected in tile drainage by Gottschall *et al.* (2012)
- Low potential for transport in runoff Sabourin *et al.* (2009)

A.1.14. Metoprolol

Metoprolol (MTP) is a β-adrenergic receptor blocker (β-blocker) used for the treatment of cardiovascular diseases.



Property	Value	References
Formula	$C_{15}H_{25}NO_3$	HSDB (2004c)
CAS Registry Number	37350-58-6	HSDB (2004c)
MW, g/mol	267.36	HSDB (2004c)
Solubility, mg/mL (25°C)	> 1,000	HSDB (2004c)
pK_a	9.68	HSDB (2004c)
log K_{ow}	1.88	HSDB (2004c)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2004c)
- Risk assessment in biosolids: Eriksen *et al.* (2009)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

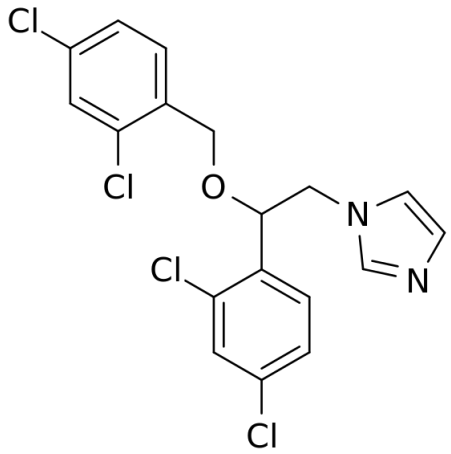
- Concentrations in sludge or biosolids reported by: Jensen *et al.* (2012); Hernandez-Raquet (2013); Yan *et al.* (2014)
- Concentrations in WWTPs reported by: Miège *et al.* (2009); Verlicchi *et al.* (2012); Hernandez-Raquet (2013); Luo *et al.* (2014); Yan *et al.* (2014)

Fate in wastewater and sludge treatment

- Wastewater: Low removal (Miège *et al.*, 2009; Verlicchi *et al.*, 2012; Luo *et al.*, 2014)

A.1.15. Miconazole

Miconazole (MCZ) is an antimycotic.



Property	Value	References
Formula	$C_{18}H_{14}Cl_4N_2O$	DrugBank (2013)
CAS Registry Number	22916-47-8	DrugBank (2013)
MW, g/mol	416.13	DrugBank (2013)
Solubility, mg/L	0.763 (estimated)	DrugBank (2013)
pK_a	6.77 (estimated)	DrugBank (2013)
log K_{ow}	6.25 (estimated)	Chen *et al.* (2013a)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, Higgins *et al.* (2010)
- Human toxicity: Summarized in Higgins *et al.* (2010)

Occurrence in biosolids and WWTPs

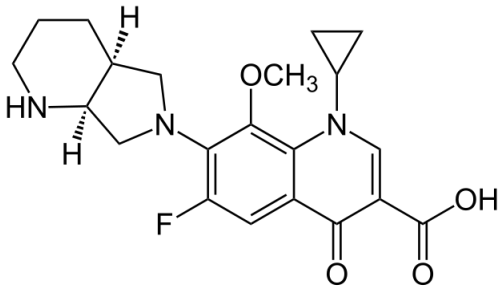
- Concentrations in sludge or biosolids reported by: CCME (2010); Higgins *et al.* (2010); Huang *et al.* (2010); Lindberg *et al.* (2010); WEAO (2010); Clarke and Smith (2011); Jensen *et al.* (2012); Guerra *et al.* (2014)
- Concentrations in WWTPs reported by: Lindberg *et al.* (2010); Guerra *et al.* (2014)

Fate and transport in agricultural soils after biosolids application

- Fate in soils: Half-life: 1,386 d (Walters *et al.*, 2010)
- Not detected in tile drainage (Gottschall *et al.*, 2012)
- Considered immobile in soil runoff by Langdon *et al.* (2010)

A.1.16. Moxifloxacin

Moxifloxacin (MOX) is a 4th generation fluoroquinolone antibiotic. Little information on the biosolids land application context. Mainly used in Canada for respiratory system ailments (PHAC, 2014)



Property	Value	References
Formula	$C_{21}H_{24}FN_3O_4$	HSDB (2012a)
CAS Registry Number	151096-09-2	HSDB (2012a)
MW, g/mol	401.43	HSDB (2012a)
Solubility, mg/L (25°C)	1,146 (estimated)	HSDB (2012a)
pK_a		
log K_{ow}	0.95 (estimated)	HSDB (2012a)
V_p, Pa (25°C)	9.68×10^{-13} (estimated)	HSDB (2012a)
Henry's law constant, Pa m^3 mol^{-1} (25°C)	3.15×10^{-15} (estimated)	HSDB (2012a)

Toxicity concerns and biological endpoints

- Biological endpoints: N/A
- Human toxicity: Summarized in HSDB (2012a)

Occurrence in biosolids and WWTPs

- As for most antibiotics and pharmaceuticals, the main route of entry to the environment is assumed to be WWTP effluents (Clarke and Smith, 2011).
- Concentrations in sludge or biosolids reported by Yan *et al.* (2014)
- Concentrations in WWTPs (ppt) reported by Yan *et al.* (2014)

Fate in wastewater and sludge treatment

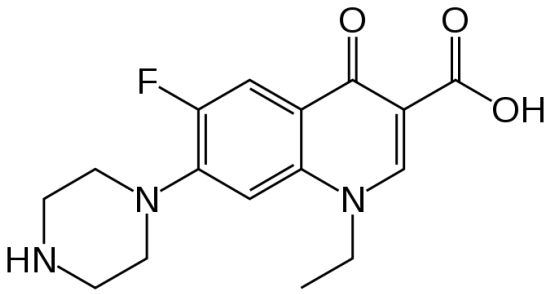
- Wastewater: Relatively high removal (~70%), driven by both sorption and biodegradation reported by Yan *et al.* (2014)
- Sludge: N/A

Fate and transport in agricultural soils after biosolids application

- Fate in soils: N/A
- Higher incidence of resistant Enterococci from pig manure vs sewage sludge (Hölzel *et al.*, 2010)

A.1.17. Norfloxacin

Norfloxacin (NOR) is a fluoroquinolone antibiotic. Present in biosolids and sludge in low ppm concentrations.



Property	Value	References
Formula	$C_{16}H_{18}FN_3O_3$	HSDB (2012b)
CAS Registry Number	70458-96-7	HSDB (2012b)
MW, g/mol	319.33	HSDB (2012b)
Solubility, mg/mL (25°C)	0.28	HSDB (2012b)
pK_a	$pK_a1 = 6.34$; $pK_a2 = 8.75$	HSDB (2012b)
log K_{ow}	0.46	HSDB (2012b)

NOR is mainly used in Canada for urinary tract infections, but consumption has decreased in the last few years (PHAC, 2014).

Decrease in use related to decrease in sludge concentrations in Sweden (Olofsson *et al.*, 2012)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2012b)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

- As for most antibiotics and pharmaceuticals, the main route of entry to the environment is assumed to be WWTP effluents (Clarke and Smith, 2011).
- Concentrations in sludge or biosolids reported by: CCME (2010); Higgins *et al.* (2010); WEAO (2010); Clarke and Smith (2011); Jensen *et al.* (2012); Hernandez-Raquet (2013); (Guerra *et al.*, 2014); Martín *et al.* (2015); Yan *et al.* (2014)
- Concentrations in WWTPs reported by: Miège *et al.* (2009); Verlicchi *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014); Yan *et al.* (2014)
- Concentrations in soil reported by Du and Liu (2012)

Fate in wastewater and sludge treatment

- Wastewater: High removal reported by Yan *et al.* (2014)

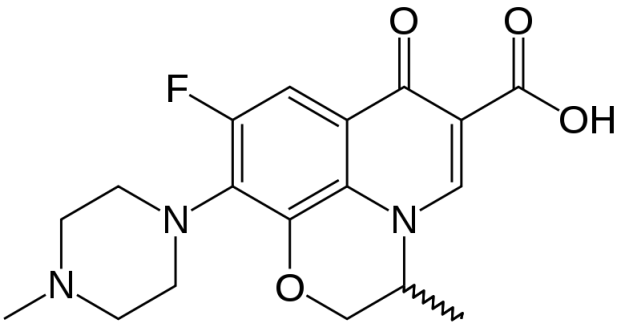
Fate and transport in agricultural soils after biosolids application

- Fate in soils: half-life: 1,155 d (Walters *et al.*, 2010)

- Highly mobile in soil runoff (Langdon *et al.*, 2010)
- Not detected in tile drainage (Gottschall *et al.*, 2012)

A.1.18. Ofloxacin

Ofloxacin (OFX) is a fluoroquinolone antibiotic. It is commonly found raw sludge and biosolids in relatively high concentrations (low ppm).



Property	Value	References
Formula	$C_{18}H_{20}FN_3O_4$	
CAS Registry Number	82419-36-1	HSDB (2012d)
MW, g/mol	371.37	HSDB (2012d)
Solubility, mg/L		
pK_a	$pK_a1 = 5.97$, $pK_a2 = 9.28$	HSDB (2012d)
log K_{ow}	-0.39	HSDB (2012d)
V_p, Pa	1.3×10^{-10} (estimated)	HSDB (2012d)

OFX is only sparsely used in Canada (PHAC, 2014).

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2012d)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

- As for most antibiotics and pharmaceuticals, the main route of entry to the environment is assumed to be WWTP effluents (Clarke and Smith, 2011).
- Concentrations in sludge or biosolids reported by: CCME (2010); Higgins *et al.* (2010); WEAO (2010); Clarke and Smith (2011); Jensen *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014); Martín *et al.* (2015); Yan *et al.* (2014)
- Concentrations in WWTPs also reported by: Verlicchi *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014); Yan *et al.* (2014)

Fate in wastewater and sludge treatment

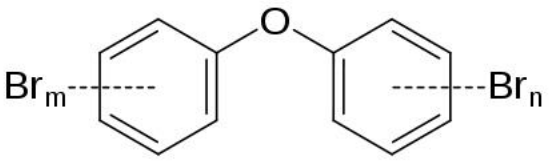
- Wastewater: Wide range in removal from wastewater (Guerra *et al.*, 2014; Yan *et al.*, 2014)

Fate and transport in agricultural soils after biosolids application

- Fate in soils: Half-life: 1,386 d (Walters *et al.*, 2010)
- High mobility reported in soil runoff (Langdon *et al.*, 2010)
- Not detected in tile drainage (Gottschall *et al.*, 2012)

A.1.19. Polybrominated diphenyl ethers (PBDEs)

The polybrominated diphenyl ethers (PBDEs) are a class of flame retardants used mainly as additives in polymer resins and plastics (Environment Canada, 2006). In use since the 1970s, they are currently banned or in the process to be prohibited in Canada and the rest of the world. They were added in 2009 to Annex A of the Stockholm Convention, which will result in the global prohibition of their production, use, import, and export (Stockholm Convention, 2009). Due to their hydrophobicity, they accumulate in soil and sediment after their release into the environment. For the same reason, PBDEs partition into sewage sludge when present in wastewater.



Name	Formula	CAS Registry Number	MW, g/mol	log K_{ow} (25°C)	V_p, Pa (25°C)	Henry's law constant, Pa m^3 mol^{-1} (25°C)	References
BDE-47	$C_{12}H_6Br_4O$	5436-43-1	485.8	6.81	2.5×10^{-4}	0.85	Morrissey Donohue *et al.* (2008a)
BDE-99	$C_{12}H_5Br_5O$	60348-60-9	564.7	6.5-8.4	5×10^{-5}	0.60	Morrissey Donohue *et al.* (2008b)
BDE-209	$C_{12}Br_{10}O$	1163-19-5	959.17	6.3-12.6	4.63×10^{-6}	1.93×10^{-8}; 0.04	Morrissey Donohue *et al.* (2008c)

PBDEs consists of two phenyl groups linked by an ether bond. One to all ten of the hydrogen atoms on the phenyl rings are substituted with bromine, giving rise to a series of 10 homologue groups based on the total number of bromine atoms in the molecule, and 209 possible individual congeners (Health Canada, 2006). The three most consistently found congeners in Canadian biosolids (Environment Canada, 2011) are BDE-47: 2,2',4,4'-tetrabromodiphenyl ether, BDE-99: 2,2',3,4,4'-pentabromodiphenyl ether, and BDE-209: decabromodiphenyl ether.

Toxicity concerns and biological endpoints

- Multiple reviews available: Morrissey Donohue *et al.* (2008a, 2008b); Morrissey Donohue *et al.* (2008c); Wiseman *et al.* (2011)
- Biological endpoints: www.wikipharma.org
- Human toxicity: Health Canada (2006, 2012)

Occurrence in the environment

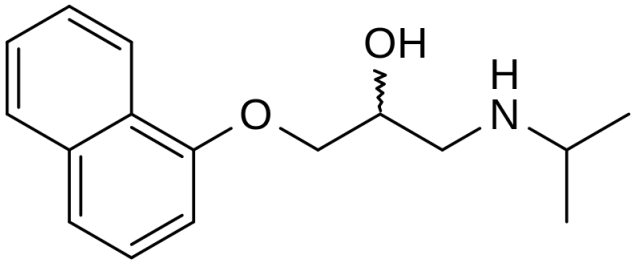
- Multiple sources available: Environment Canada (2010, 2011)
- For concentrations in Canadian biosolids, see Environment Canada (2011)

Fate and transport in agricultural soils after biosolids application

- Persistent in soils (Andrade *et al.*, 2010)

A.1.20. Propranolol

Propranolol (PRP) Metoprolol (MTP) is a β-adrenergic receptor blocker (β-blocker) used for the treatment of cardiovascular diseases.



Property	Value	References
Formula	$C_{16}H_{21}NO_2$	HSDB (2004a)
CAS Registry Number	525-66-6 318-98-9 (Hydrochloride)	HSDB (2004a)
MW, g/mol	259.34 295.81 (Hydrochloride)	HSDB (2004a)
Solubility, mg/L	281.8 (estimated)	Liu *et al.* (2009)
pK_a	9.45	HSDB (2004a)
log K_{ow}	2.6 (estimated); -0.45 (HCl at pH 2.0)	Liu *et al.* (2009); HSDB (2004a)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2004a)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

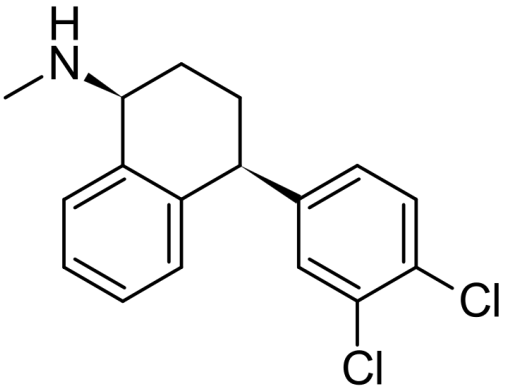
- Concentrations in sludge or biosolids reported by: CCME (2010); Higgins *et al.* (2010); WEAO (2010); Jensen *et al.* (2012); Martín *et al.* (2012); Hernandez-Raquet (2013); Martín *et al.* (2015)
- Concentrations in WWTPs reported by: Miège *et al.* (2009); Verlicchi *et al.* (2012); Hernandez-Raquet (2013)

Fate in wastewater and sludge treatment

- Wastewater: Variable removal (Miège *et al.*, 2009; Verlicchi *et al.*, 2012)

A.1.21. Sertraline

Sertraline (SER) is a selective serotonin reuptake inhibitor (SSRI) antidepressant.



Property	Value	References
Formula	$C_{17}H_{17}Cl_2N$	HSDB (2002c)
CAS Registry Number	79617-96-2	HSDB (2002c)
MW, g/mol	306.24	HSDB (2002c)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2002c)

Occurrence in biosolids and WWTPs

- Concentrations in sludge or biosolids reported by: Chari and Halden (2012); Lajeunesse *et al.* (2012); Niemi *et al.* (2013)
- Concentrations in WWTPs also reported by: Lajeunesse *et al.* (2012); Golovko *et al.* (2014)

Fate in wastewater and sludge treatment

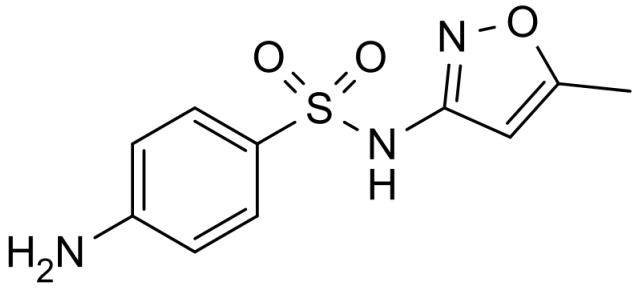
- Wastewater: Medium to high removal (Lajeunesse *et al.*, 2012; Golovko *et al.*, 2014)

Fate and transport in agricultural soils after biosolids application

- Fate in soils: Half-life: 50-86 d (Li *et al.*, 2013)

A.1.22. Sulfamethoxazole

Sulfamethoxazole (SMZ) is a sulfonamide antibiotic.



Property	Value	References
Formula	$C_{10}H_{11}N_3O_3S$	HSDB (2009b)
CAS Registry Number	723-46-6	HSDB (2009b)
MW, g/mol	253.28	HSDB (2009b)
Solubility, mg/L (37°C)	610	HSDB (2009b)
pK_a	pK_{a1} = 1.6; pK_{a2} = 5.7	HSDB (2009b)
log K_{ow}	0.89	HSDB (2009b)
V_p, Pa (25°C)	9.2 x 10^{-6} (estimated)	HSDB (2009b)
Henry's law constant, Pa m^3 mol^{-1} (25°C)	6.5 x 10^{-8} (estimated)	HSDB (2009b)

SMZ is mainly used in Canada in combination with trimethoprim for urinary tract infections, but use is declining due to high levels of resistance (PHAC, 2014).

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2009b)
- Risk assessment in biosolids: Eriksen *et al.* (2009)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)

Occurrence in biosolids and WWTPs

- As for most antibiotics and pharmaceuticals, the main route of entry to the environment is assumed to be WWTP effluents (Clarke and Smith, 2011).
- Concentrations in sludge or biosolids in low ppb; reported by: Edwards *et al.* (2009); CCME (2010); Higgins *et al.* (2010); WEAO (2010); Jensen *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014); Martín *et al.* (2015)
- Concentrations in WWTPs also reported by: Miège *et al.* (2009); Jensen *et al.* (2012); Verlicchi *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014); Luo *et al.* (2014); Yan *et al.* (2014)
- Detected in groundwater (Lapworth *et al.*, 2012)

Fate in wastewater and sludge treatment

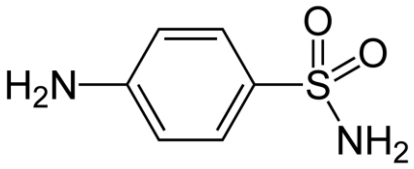
- Wastewater: Variable removal. Reported by Eriksen *et al.* (2009); Miège *et al.* (2009); CCME (2010); Luo *et al.* (2014); (Yan *et al.*, 2014)
- Considered to have low sorption and moderate biotransformation potentials respectively by Salveson *et al.* (2012)

Fate and transport in agricultural soils after biosolids application

- Fate in soils: Considered to have medium mobility in runoff by Langdon *et al.* (2010)

A.1.23. Sulphanilamide

Sulphanilamide (SUL) is a sulfonamide antibiotic.



Property	Value	References
Formula	$C_6H_8N_2O_2S$	HSDB (2003b)
CAS Registry Number	63-74-1	HSDB (2003b)
MW, g/mol	172.21	HSDB (2003b)
Solubility, mg/L (25°C)	8,360	HSDB (2003b)
pK_a	10.43	HSDB (2003b)
log K_{ow}	-0.62	HSDB (2003b)

Toxicity concerns and biological endpoints

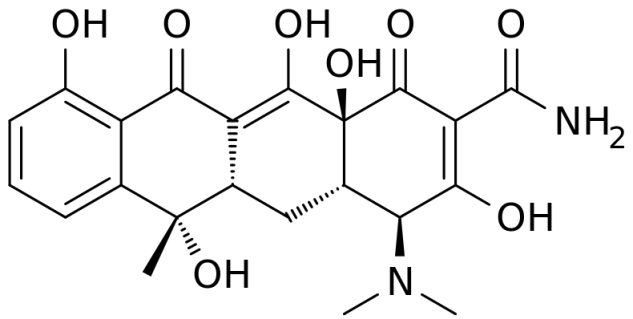
- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2003b)

Occurrence in biosolids and WWTPs

- As for most antibiotics and pharmaceuticals, the main route of entry to the environment is assumed to be WWTP effluents (Clarke and Smith, 2011).
- Concentrations in sludge or biosolids reported by: CCME (2010); Higgins *et al.* (2010); WEAO (2010); Guerra *et al.* (2014)
- Concentrations in WWTPs also reported by: Guerra *et al.* (2014)
- Measured in Chinese rivers; origin assumed to be pork and poultry farms (Jia *et al.*, 2011)

A.1.24. Tetracycline

Tetracycline (TC) is an antibiotic, and 4-epitetracycline (4-ETC) is a metabolite of TC (Zurhelle *et al.*, 2000) that is found in high concentrations in sludge (both in low ppm)



Property	TC	References
Formula	$C_{22}H_{24}N_2O_8$	HSDB (2002a)
CAS Registry Number	60-54-8	HSDB (2002a)
MW, g/mol	444.43	HSDB (2002a)
Solubility, mg/L (25°C)	231	HSDB (2002a)
pK_a	3.30; 7.8; 9.6	HSDB (2002a); Wollenberger *et al.* (2000)
log K_{ow}	-1.37	HSDB (2002a)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, Higgins *et al.* (2010), and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2002a)
- Risk assessment in biosolids: Eriksen *et al.* (2009)
- Risk assessment in secondary WWTP effluent: Verlicchi *et al.* (2012)
- Changes to microbial community reported by Granados-Chinchilla *et al.* (2013)

Occurrence in biosolids and WWTPs

- As for most antibiotics and pharmaceuticals, the main route of entry to the environment is assumed to be WWTP effluents (Clarke and Smith, 2011).
- Concentrations in sludge or biosolids reported by: Eriksen *et al.* (2009); CCME (2010); Higgins *et al.* (2010); WEAO (2010); Clarke and Smith (2011); Du and Liu (2012); Gao *et al.* (2012); Jensen *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014)
- Concentrations in WWTPs also reported by: Miège *et al.* (2009); Gao *et al.* (2012); Jensen *et al.* (2012); Verlicchi *et al.* (2012); Hernandez-Raquet (2013); Guerra *et al.* (2014)

Fate in wastewater and sludge treatment

- Wastewater: Variable levels of removal reported (Eriksen *et al.*, 2009; Miège *et al.*, 2009; Gao *et al.*, 2012; Verlicchi *et al.*, 2012; Guerra *et al.*, 2014)
- Biosolids: XXX concentrations (low XX)

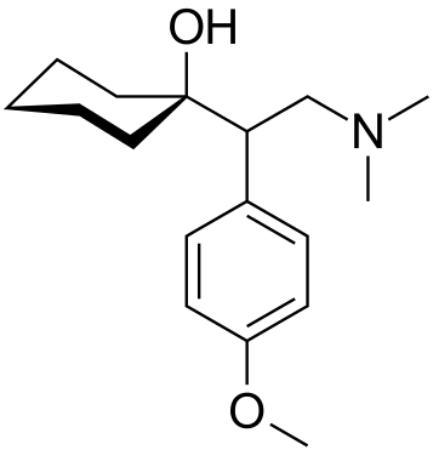
Fate and transport in agricultural soils after biosolids application

- Fate in soils: Half-life for 4-ETC: 630 d (Walters *et al.*, 2010)
- Not detected in tile drainage (Gottschall *et al.*, 2012)

- Sorbed strongly to biosolids and degraded, but reaching a plateau in lab experiment by Wu *et al.* (2009)

A.1.25. Venlafaxine

Venlafaxine (VEN) is a serotonin-norepinephrine reuptake inhibitor (SNRI) antidepressant.



Property	Value	References
Formula	$C_{17}H_{27}NO_2$	HSDB (2012c)
CAS Registry Number	93413-69-5	HSDB (2012c)
MW, g/mol	277.40	HSDB (2012c)
Solubility, mg/L (25°C)	267 (estimated)	HSDB (2012c)
pK_a	10.09 (estimated)	HSDB (2012c)
log K_{ow}	3.20	HSDB (2012c)
V_p, Pa (25°C)	3.28×10^{-5} (estimated)	HSDB (2012c)
Henry's law constant, Pa m^3 mol^{-1} (25°C)	2.07×10^{-6} (estimated)	HSDB (2012c)

Toxicity concerns and biological endpoints

- Biological endpoints: See Appendix A.3, and www.wikipharma.org
- Human toxicity: Summarized in HSDB (2012c)

Occurrence in biosolids and WWTPs

- Concentrations in sludge or biosolids reported by: Lajeunesse *et al.* (2012); Niemi *et al.* (2013)
- Concentrations in WWTPs also reported by: Lajeunesse *et al.* (2012); Golovko *et al.* (2014)

Fate in wastewater and sludge treatment

- Wastewater: Recalcitrant (Lajeunesse *et al.*, 2012; Golovko *et al.*, 2014)

A.1.26. References

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A.2. PATHOGENS

A.2.1. Bacteria

Some bacteria are naturally present in the gastrointestinal tract and are excreted in human feces, and as a result they are present in raw sewage and biosolids. Bacteria are the most commonly regulated pathogen, with standards in both Canada and the United States for fecal coliforms and *Salmonella*.

Other bacterial species are naturally present in the environment and have also been found in raw sludge and biosolids. Recent outbreaks of some of these bacteria, such as *Listeria* and *Legionella*, have made them pathogens of concern. Reviews of the general characteristics and health concerns of these common bacteria can be found in Epstein (2002), Pepper *et al.* (2006), and Sidhu and Toze (2009).

A.2.1.1. Mycobacterium

Mycobacterium is a genus of Gram-positive bacteria including *M. tuberculosis* and *M. bovis* which are responsible for tuberculosis in humans and animals, and *M. leprae*, which is the cause of leprosy (USEPA, 1999a). Mycobacterial species have been isolated from natural waters such as ponds, streams, and estuaries, and from municipal waters including piped water supplies (USEPA, 1999a).

Mycobacterium was detected in raw sewage and digested sludge from the early studies of the bacterial content of these residues. In 1954, Jensen (1954) detected tubercle bacilli, or *M. tuberculosi*s, in raw sewage from towns with tuberculosis hospitals in Denmark, and he found no tubercle bacilli in the raw sewage from towns without such hospitals. In 1980, Dudley *et al.* (1980) reported 4 x 10^7 CFU/g *Mycobacterium* sp. in digested sludge, and 2 x 10^9 in lagooned sludge. In 1994, Slosarek *et al.* (1994) detected *Mycobacterium* sp. in 8.2% (102/1244) of wastewater samples from the Czech Republic.

More recently, Bibby *et al.* (2010) detected *Mycobacterium* in all biosolids tested (produced by mesophilic and thermophilic anaerobic digestion, and composting) in addition to agricultural soil. The lowest detection was in composted and thermophilic-digested biosolids, and the highest was in mesophilic-digested biosolids and agricultural soil. Bibby *et al.* (2010) analyzed and quantified 16S rRNA sequences; therefore, numbers of *Mycobacterium* were relative rather than absolute.

A.2.1.2. Enterohaemorrhagic E. coli

E. coli is a Gram-negative bacterium found in the gastrointestinal tract of all warm-blooded animals, including humans, and is shed in fecal matter. Infection typically occurs by fecal-oral transmission Pepper *et al.* (2006). Only some strains are pathogenic, including the O157:H7 strain, which has been of recent concern due to a number of highly-publicized outbreaks of contaminated drinking water and food (Pepper *et al.*, 2006), including the outbreak in Walkerton, Ontario in 2000 (Gerba and Smith, 2005). *E. coli* O157:H7 causes haemorrhagic colitis (bloody diarrheal syndrome) and haemolytic uremic syndrome (Griffin and Tauxe, 1991).

Enterohaemorrhagic *E. coli* has been isolated from sewage sludge, but in low numbers. For example, two strains of *E. coli* O157:H7 were detected in raw sewage in Japan (Awais *et al.*, 2007), and one strain from the inflow of a sewage treatment plant in France (Vernozy-Rozand *et al.*, 2002). Sahlström *et al.* (2004) surveyed 8 WWTPs in Sweden and found *E. coli* O157:H7 in 2% of raw sewage sludge samples, but not in anaerobically digested and composted biosolids. No information was found for Canadian biosolids. Biosolids should not be discounted as potential sources, however, although land applied manure is likely to be a more significant source (Strachan *et al.*, 2002). Avery *et al.* (2004) found that *E. coli* from livestock feces could survive on grass for 5-6 months, depending upon environmental conditions.

A.2.1.3. Legionella

Legionella is a Gram-negative, non-enteric pathogenic bacterium that can cause legionellosis, a potentially life-threatening respiratory illness, or a milder flulike illness called Pontiac Fever (Pepper *et al.*, 2006; Sidhu and Toze, 2009). *Legionella* can grow in the cooling towers of buildings or in thermally treated water, but outbreaks have also been associated with composted potting mixes (Steele *et al.*, 1990; Cramp *et al.*, 2010) (Steele *et al.*, 1990; Cramp *et al.*, 2010). *Legionella* has also been detected in the air above activated sludge aeration tanks (USEPA, 1999b).

Viau and Peccia (2009) reported detectable levels of *Legionella pneumophila* in 31% (N=16) of Class B mesophilic anaerobic digested (MAD) biosolids, and in 25% and 50% of temperature-phased anaerobic digested (N=8) and MAD plus composted (N=10) Class A biosolids. *L. pneumophila* was not detected in two samples of heat-pelletized biosolids. Viau and Peccia (2009) suggested that this increase in *L. pneumophila* detection in composted biosolids could be due to the potential proliferation of the bacteria in the warm, aerobic conditions of the compost heap. Similar conditions exist in piles of potting mixes, where *Legionella* has been detected.

A.2.1.4. Listeria

Listeria, and more specifically its most common species, *L. monocytogenes,* is a Gram-positive bacterium that causes listeriosis, a food-borne disease that usually infects immunocompromised people (Pepper *et al.*, 2006). *Listeria* is ubiquitous, and is found in decaying vegetation, soils, and the feces of healthy individuals (Al-Ghazali and Al-Azawi, 1990; Sahlström *et al.*, 2004).

Listeria has been detected in raw and treated sewage sludges. Al-Ghazali and Al-Azawi (1990) detected *L. monocytogenes* in incoming raw sewage and all treatment steps at a WWTP in Iraq. Incoming raw sewage contained 7-210 counts/g; levels were reduced to <3-15 counts/g in final sewage sludge cake. In France, Garrec *et al.* (2003) found *L. monocytogenes* in 73% of dewatered sewage sludges, at levels of 0.15 to 20 MPN/g dry matter. More recently, Horan *et al.* (2004) reported a 2 $\log_{10}$ reduction in *Listeria* numbers during anaerobic digestion. Shannon *et al.* (2007) used TaqMan® real-time PCR to track bacterial pathogens through wastewater treatment, *L. monocytogenes* could only be detected in the raw wastewater, and was undetectable in the primary and subsequent effluents.

A.2.1.5. Helicobacter

Helicobacter pylori is a Gram-negative bacterium associated with several types of gastric disease, including gastric cancer and peptic/duodenal ulcer disease (Friis *et al.*, 1996; Moreno and Ferrús, 2012). *H. pylori* has been detected in surface water and wastewater (Moreno and Ferrús, 2012); however, it rapidly loses its cultivability in aqueous environments and is therefore difficult to detect. Recently, new culture-independent methods such as fluorescent in-situ hybridization (FISH) have detected viable and non-viable cells, but these methods do not indicate if the bacteria are infective (Moreno and Ferrús, 2012). Moreno and Ferrús (2012) detected viable *H. pylori* using culturing plus FISH in wastewater effluent after secondary treatment and tertiary UV disinfection, although the concentration of cells was unknown.

A.2.1.6. Aeromonas

Aeromonas is a genus of Gram-negative bacteria that are ubiquitous in aquatic environments (Ashbolt *et al.*, 1995; Wu *et al.*, 2007; Igbinosa and Okoh, 2013). It is not a true enteric bacterium, but it is present in the feces of healthy humans and animals presumably from ingestion, because it is naturally found in surface waters, and it has been proposed as a possible indicator of sewage contamination (Poffé and Op de Beeck, 1991; USEPA, 2006; Chen *et al.*, 2011). Some of the 13 named species are opportunistic pathogens, and are particularly infectious to immunocompromised people, causing gastrointestinal illness as well as soft-tissue and systemic infections (Ashbolt *et al.*, 1995; Wu *et al.*, 2007; Igbinosa and Okoh, 2013).

Aeromonas has been detected in surface water (Burke *et al.*, 1984) and wastewater samples (Stecchini and Domenis, 1994; Igbinosa and Okoh, 2013), but very little data exists with regards to *Aeromonas* in biosolids. *Aeromonas* has been isolated from activated sludge (Neilson, 1978; Kämpfer *et al.*, 1996). Poffé and Op de Beeck (1991) reported concentrations of up to 10^7 g^{-1} in raw sewage sludge, and removal rates of 99.975% and 98.25% by activated sludge and trickling filters, respectively. More recently, Shannon *et al.* (2007) used TaqMan® real-time PCR to detect pathogens at various stages of wastewater treatment. *Aeromonas hydrophila* was detected in the raw wastewater, but was undetectable in the primary effluents and later treatment stages.

For a comprehensive review on *Aeromonas*, see USEPA (2006).

A.2.1.7. Clostridium difficile

Clostridium difficile is a Gram-positive, commensal, spore forming, anaerobic bacterium found in the GI tract of healthy individuals (Romano *et al.*, 2012). However, highly virulent strains are emerging and it is increasingly becoming a pathogen of concern, particularly associated with hospital-acquired infections and deaths among compromised patients. *C. difficile* infection can cause mild to severe diarrhea and pseudomembranous colitis, an inflammation of the colon (PHAC, 2014). Community-acquired infections are also becoming common.

The information on *C. difficile* in WWTP products is limited. Romano *et al.* (2012) examined *C. difficile* from nine WWTP in Switzerland and found it present in all of the samples analyzed; ribotype O78, which is associated with the most frequently isolated community acquired infections, was the most frequently isolated in 6 of the treatment plants. Hospital associated strains were also isolated. Viau and Peccia (2009) detected *C. difficile* in 25% (N=16) of Class B mesophilic anaerobic digested biosolids, and in 38% (N=8) of temperature-phased

anaerobic digested biosolids. *C. difficile* was not detected in composted or heat-pelletized biosolids.

In Canada, Xu *et al.* (2014) recently demonstrated the recovery of *C. difficile* from 92% (108/117) of raw sludge samples, 96% (106/110) of anaerobic digested sludge samples, and 73% (43/59) of dewatered biosolids from two Ontario WWTPs. A higher prevalence of the toxigenic ribotype O78 was found in biosolids (35%, 15/43) compared to 19% (21/108) and 7.5% (8/106) in primary sludge and digested sludge, respectively. The authors hypothesized that the O78 ribotype may survive better in biosolids treatment processes than other ribotypes.

A.2.1.8. Burkholderia

Burkholderia is a group of Gram-negative bacteria that are ubiquitous in the environment and are found mainly in soils (Coenye and Vandamme, 2003; Sidhu and Toze, 2009). The genus contains over 30 species, most of which are associated with plants (Coenye and Vandamme, 2003). *B. cepacia* is primary a plant pathogen, but it has emerged as a human pathogen in the last 20 years, specifically amongst cystic fibrosis patients (Govan and Deretic, 1996; Coenye and Vandamme, 2003).

Limited data has been published on *Burkholderia* in biosolids. Bahlaoui *et al.* (1997) reported 10^1 mL^{-1} cells in wastewater. Bibby *et al.* (2010) reported *Burkholderia* sp. detected using 16S rDNA analysis in mesophilic anaerobic digestion residuals and agricultural soil samples.

A.2.2. Virus

Reviews of common and emerging viruses found in biosolids have been published by (Gerba *et al.*, 2002; Sidhu and Toze, 2009). Similar to bacteria, some viruses are found in the gastrointestinal tract of humans and are excreted in the feces at concentrations of up to 10^{11} viral particles per gram of feces (Pepper *et al.*, 2006). A sub-type of enteric viruses, called enteroviruses, which include poliovirus, coxsackievirus and echovirus, has been well characterized and encompasses nearly all the existing data on viruses in biosolids (Sidhu and Toze, 2009). Less is known regarding other viruses, such as norovirus, rotavirus and adenovirus.

Many viruses, including norovirus, cannot be cultured, so detection and quantification of viruses in biosolids is difficult (Sidhu and Toze, 2009). Recently, molecular and genetic methods have been used to detect viral genes in biosolids. However, the detection of viral genes does not necessarily mean the virus is active and infectious. For a review of molecular techniques for detection of pathogens in water, see Girones *et al.* (2010).

In 2010, Wong *et al.* (2010) used qPCR to detect genes of enteric viruses in 12 samples of mesophilic anaerobic digested (MAD) biosolids and 3 samples of dewatered biosolids. Adenovirus genes were detected in 100% (3/3) of the dewatered biosolids, and 83% (10/12) of the MAD biosolids. Polyomavirus genes were also detected in 100% (3/3) of the dewatered biosolids, and in 58% (7/12) of the MAD biosolids. Norovirus genes were detected in 67% (2/3) of the dewatered biosolids, and 50-75% (6-9/12), depending upon the norovirus sub-type, in the MAD biosolids. Genes from the Hepatitis A virus were not detected in any samples.

The most thorough study of viruses in biosolids to date was conducted by Bibby and Peccia (2013). They employed a shotgun metagenome analysis to identify the viral pathogen diversity in 10 biosolids samples (mesophilic anaerobic digested) from 5 WWTPs in the United States. In total, 43 different types of human viruses were detected in the biosolids. The most abundant human pathogens found were *Herpesvirus* and *Papillomavirus*, which were found in 100% of the samples. *Adenovirus* occurred in 92% of the samples. RNA viruses including *Klassevirus* (occurrence 92%), *Rotavirus* (occurrence 83%), and *Astrovirus* (occurrence 75%) were also detected. One drawback to this type of analysis is that metagenomic analysis measures only a relative abundance of the genome, not the absolute abundance. In addition, the detection level is unknown (Bibby and Peccia, 2013). Parallel comparison of samples with PCR showed metagenome sequencing under-represented viruses compared to PCR (Bibby and Peccia, 2013).

A.2.2.1. Norovirus/Norwalk-like viruses

Norowalk viruses, or noroviruses, are the most common cause of acute gastroenteritis (Sidhu and Toze, 2009). They are shed in the feces of infected humans and are transmitted through the fecal-oral route (Wei *et al.*, 2010). van den Berg *et al.* (2005) reported norovirus levels of 10^5/L in raw sludge, with limited reduction (10^3/L) after wastewater treatment. Wei *et al.* (2010) studied the infectivity of norovirus over time in biosolids and found that the virus can maintain some infectivity after 60 days in both 4 and 20°C conditions in some biosolids; however, the virus was inactivated most rapidly in lime-stabilized biosolids that had an alkaline pH. Similar results were found for the Hepatitis A virus.

A.2.2.2. Adenovirus

Adenoviruses are amongst the most common and persistent viruses detected in wastewater effluent (Enriquez *et al.*, 1995). Adenoviruses cause gastroenteritis and respiratory infections, and they can also cause serious infections in immunosuppressed cancer patients (Pepper *et al.*, 2006).

Adenoviruses have been quantified in wastewater effluent and raw sewage, but rarely in biosolids. He and Jiang (2005) used both culture assay methods and real-time PCR to quantify adenoviruses in sewage effluent. They reported 10^4 viral genomic copies/100 mL using PCR techniques, and 67-80 plaque forming units (PFU)/100 mL using cell culture techniques. However, the relationship between viral copies and PFU was not defined in this study, and therefore the values cannot be compared.

Bofill-Mas *et al.* (2006) used PCR to report 10^2-10^5 copy numbers of adenoviruses/g biosolids. Viau and Peccia (2009) detected *Adenovirus* spp. in 70-100% of biosolids samples with various treatments, including composting. It is thought that adenoviruses are more stable and thermally-resistant than other viruses (Gerba *et al.*, 2002; Bofill-Mas *et al.*, 2006). Similar to the noroviruses, adenoviruses have been shown to lose infectivity in biosolids with alkaline pH (Wei *et al.*, 2009).

Adenoviruses have been shown to be stable in soil conditions. Schwarz *et al.* (2014) Schwarz *et al.* (2014) reported the time for one $\log_{10}$ reduction (T_{90}) of *Adenovirus* to occur to be >180 days, whether in biosolids-amended soil or un-amended soil.

A.2.2.3. Astrovirus

Astroviruses are a leading cause of gastroenteritis in children worldwide (Chapron *et al.*, 2000; Pepper *et al.*, 2006), and they are transmitted by the fecal-oral route (Chapron *et al.*, 2000). Astroviruses are structurally different from enteroviruses and therefore react differently to wastewater treatment processes (Chapron *et al.*, 2000).

Astroviruses have been detected and quantified in both raw and treated wastewater and sewage sludge. A study by Egglestone *et al.* (1999) detected no astroviruses in any sewage samples from a WWTP in England; however, Chapron *et al.* (2000) suggested that the samples may not have been incubated correctly prior to analysis. Chapron *et al.* (2000) used a nested PCR technique and reported 94% (15/16) occurrence in raw and treated sludges. Le Cann *et al.* (2004) used real-time PCR to quantify astroviruses before and after wastewater treatment, and they reported a 2 $\log_{10}$ reduction, with influent concentrations at 4 x 10^6 genomes/100 mL, and effluent concentrations at 1 x 10^4 genomes/100 mL.

A.2.2.4. Rotavirus

Like norovirus and astrovirus, rotavirus causes gastroenteritis in children and immunocompromised individuals (Pepper *et al.*, 2006). Rotaviruses are shed in human feces (Casas and Suñén, 2002). Little data exists for rotavirus in biosolids, but they have been detected in raw sewage (Casas and Suñén, 2002; Villena *et al.*, 2003). Hansen *et al.* (2007) reported inactivation of rotavirus in limed, rotavirus-spiked biosolids after 24 hours at 4 and 22°C.

A.2.2.5. Polyomavirus

Human polyomaviruses such as BK virus and JC virus infect immunocompetent individuals who show no symptoms, but polyomaviruses have also been linked to serious diseases such as polyomavirus-associated nephropathy, haemorrhagic cystitis and multifocal leukoencephalopathy (Egli *et al.*, 2009). The virus has been detected in urine but its transmission is not yet fully understood (Egli *et al.*, 2009). Transmission by the fecal-oral route has been suggested (Bofill-Mas *et al.*, 2001).

Very little data exists regarding polyomaviruses in sewage or biosolids. Bofill-Mas *et al.* (2000) detected polyomaviruses in 93% of raw sewage samples. In a later study, the same group quantified polyomaviruses in sewage at 6 x 10^3 genome copies/mL, and in biosolids at 8 x 10^2 genome copies/g dry weight (Bofill-Mas *et al.*, 2006). It is thought that the virus' supercoiled double-stranded DNA structure makes it more heat stable and therefore persistent throughout the wastewater treatment process (Nwachcuku and Gerba, 2004).

A.2.2.6. Hepatitis A, E

Hepatitis viruses A and E are shed in fecal matter and are transmitted via the fecal-oral route (Wei *et al.*, 2010). The virus primarily affects the liver and causes hepatitis, with the E virus responsible for the serious acute viral hepatitis disease (Gerba *et al.*, 2002). Hepatitis A has been detected in raw sewage (Casas and Suñén, 2002), and Hepatitis E has been detected in wastewater and biosolids (Clemente-Casares *et al.*, 2009). Wei *et al.* (2010) reported significant loss of Hepatitis A RNA after 60 days in biosolids at 20°C, with the most rapid loss in lime-stabilized biosolids. Similarly, Katz and Margolin (2007) also reported significant inactivation of Hepatitis A with alkaline stabilization treatment.

A.2.2.7. H5N1, H5N2 Avian Influenza; H1N1 Swine Influenza;

H5N1 and H5N2 avian influenzas emerged in the late 1990s to early 2000s in China and spread to Europe and Africa (WHO, 2014). The H5N1 strain is highly pathogenic, and is shed in large quantities in the feces and respiratory secretions of infected birds (Rice *et al.*, 2007) and humans (de Jong *et al.*, 2005; WHO, 2006). The majority of human infection has been associated with contact with infected poultry (WHO, 2014). Human-to-human transmission is not yet fully understood, but it may occur through nasal secretions (WHO, 2006).

Very little research has been conducted regarding H5 viruses in wastewater or biosolids. H5N1 virus has been shown to persist in simulated water environments (Stallknecht *et al.*, 1990; Brown *et al.*, 2007), and is readily inactivated by chlorination (Rice *et al.*, 2007). In the only study found related to biosolids, Lucio-Forster *et al.* (2007) reported inactivation of H5N2, used as a surrogate for H5N1, by anaerobic digestion of wastewater sludge to undetectable levels.

H1N1 swine influenza emerged in the late 2000s and was declared a pandemic in the summer of 2009 by the World Health Organization (CDC, 2009). The pandemic title was declared due to the spread of the virus and not the severity of the cases (CDC, 2009). Transmission occurs through respiratory secretions of infected persons (CDC, 2009). Very little scientific data exists regarding the detection, quantification, or inactivation of H1N1 swine influenza in wastewater or biosolids with only one study to date. Heijnen and Medema (2011) reported no detected H1N1 virus in sewage and surface water in the Netherlands.

A.2.3. Other Emerging Pathogens

A.2.3.1. Prions

Prions are pathogens that lack nucleic acids and are primarily composed of an abnormally folded isoform of a protein (Hinckley *et al.*, 2008). The pathogenic prion mainly causes transmissible spongiform encephalopathies (TSEs), a class of fatal neurodegenerative disease that affect mammals, including humans (Hinckley *et al.*, 2008). Well-known TSEs include Creutzfeldt-Jakob disease and kuru in humans, and bovine spongiform encephalopathy (BSE), or “mad cow” disease in cattle (Hinckley *et al.*, 2008; Miles *et al.*, 2011). Prions can migrate to WWTPs from slaughterhouses, laboratories, or landfill leachate containing infected carcasses and other materials (Hinckley *et al.*, 2008; Miles *et al.*, 2011). The pathogenic prion is insoluble in water and many detergents, it can form aggregates, and it is resistant to chemical and thermal degradation (Taylor, 2000). The infectivity of prions is not eliminated by boiling or standard autoclaving conditions; exposure to UV and ionizing radiation, treatment with proteases, and use of many chemical disinfectants is ineffective (Taylor, 2000). To degrade prions, the most effective method is incineration above 1,000°C, although this may not always be practical (Saunders *et al.*, 2008).

A limited number of studies have investigated prions in biosolids; the following were reviewed by Miles *et al.* (2011). Kirchmayr *et al.* (2006) incubated a surrogate prion under mesophilic and thermophilic conditions and analyzed survival with a luminescent western blot. They found no reduction of prion survival under mesophilic conditions, but they did see a reduction of 20-40% luminescence (related to survival) after 302 hours (12.6 days) of incubation under

thermophilic conditions. Hinckley *et al.* (2008) also used a surrogate prion, but coupled with an oral infectivity assay to study the persistence of prions through the wastewater treatment process. The results showed that incubation of the prion with activated sludge did not result in significant degradation, and the prion partitioned strongly to the activated sludge and would therefore be expected to enter the biosolids treatment process. Furthermore, they found a large fraction of the prion survived mesophilic anaerobic sludge digestion. Based on these results, Hinckley *et al.* (2008) suggested that if prions were to enter the wastewater treatment system, they would be present in treated biosolids.

The studies above evaluated survival, rather than infectivity, of prions in the wastewater treatment process. Miles *et al.* (2011) evaluated the infectivity of a pathogenic prion surrogate in Class B alkaline biosolids after incubation under mesophilic and thermophilic conditions using a western blot assay. They observed a 2.43 $\log_{10}$ reduction in prion infectivity after 15 days at mesophilic conditions, and a 3.41 $\log_{10}$ reduction after 10 days at thermophilic conditions.

Several studies have looked at the transport and persistence of prions in soil, such as the reviews by Saunders *et al.* (2008) and Smith *et al.* (2011). In general, these reviews summarized studies regarding the attachment of prions to soil particles, and concluded that this attachment likely influences the conformation of the protein, and therefore the persistence and infectivity. In addition, Smith *et al.* (2011) stated that soil was a plausible environmental reservoir of prions, and that they could persist in the environment for years.

One study has assessed the risk of prion-diseases from land application of biosolids and found it to be very low. Gale and Stanfield (2001) used a Source-Pathway-Receptor approach to quantify the risk to humans through consumption of vegetable crops grown in biosolids-amended soil and found it to be acceptably low, at 1.32×10^{-9} persons infected/year. They found a greater risk to cattle, of 7.1×10^{-5} cows infected/year, due to their long-term grazing behaviour.

A.2.3.2. Microsporidia

Microsporidia are a group of unicellular, spore-forming parasites recently reclassified as fungi that contain over 1200 species, of which only 14 have been associated with human infections (Nwachcuku and Gerba, 2004; Mathis *et al.*, 2005; Pepper *et al.*, 2006). Human infection is usually caused by two species, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, which affect mainly HIV-infected and other immunocompromised patients with acute and chronic diarrhea (Mathis *et al.*, 2005).

Very little data exists regarding microsporidia in biosolids or wastewater. Dowd *et al.* (1998) detected human-pathogenic microsporidia *E. bieneusi*, *E. intestinalis*, and *Vittaforma corneae* in tertiary sewage effluent. More recently, Cheng *et al.* (2011) reported up to 19 000 spores/kg *E. bieneusi*, and up to 32 000 spores/kg *E. hellem*, and up to 16 000 spores/kg *E. intestinalis* in biosolids originating from four Irish WWTPs, with seasonal variation favouring higher concentrations in the summer months. Removal efficiencies varied from negative removal (-90%) to 100%, with some microsporidia loads in treated effluents higher than the influent loads. Microsporidia are of particular concern in biosolids due to possible aerosolization of their spores; however, it should be noted that very few (14/approximately 1200) species are known pathogens to humans.

A.2.4. References

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A.3. ECOTOXICITY ENDPOINTS FOR SELECTED ESOCS

These selected ecotoxicity endpoints were compiled from the scientific literature with the help of the excellent databases maintained by MistraPharma in Sweden (http://www.wikipharma.org/welcome.asp), and the US EPA database of publications on PPCPs (Daughton CG and Scuderi MST, 2014. "Pharmaceuticals and Personal Care Products (PPCPs): Relevant Literature," U.S. Environmental Protection Agency, Las Vegas, NV (a comprehensive database of literature references; first implemented 19 February 2008); available: http://www.epa.gov/ppcp/lit.html"

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
Acetaminophen	$C_8H_9NO_2$	103-90-2	814 mg/L	96-h LC50, *Pimephales promelas*	HSDB (2007)
			577 mg/L	24-h LC50, *Artemia salina*	Calleja *et al.* (1994)
			378 mg/L	48-h LC50, *Brachydanio rario* embryos	Henschel *et al.* (1997)
			> 32 mg/L	48-h LC50, *Daphnia magna*	Brun *et al.* (2006)
			26.6 mg/L	96-h EC50 (immobilization), *D. magna*	Kim *et al.* (2007)
			20.1 mg/L	48-h LC50, *Daphnia magna*	Han *et al.* (2006)
			1,724 mg/L	21-d EC50 (shoot inhibition), *Triticum aevestium* L. [no soil]	An *et al.* (2009a)
			669 mg/L	21-d EC50 (root elongation inhibition), *Triticum aevestium* L. [no soil]	An *et al.* (2009a)
			1 mg/L	EC50 (ECOSAR), fish	Sanderson *et al.* (2003)
			1 ug/L	PNEC, derived from EC50 (ECOSAR), fish	Verlicchi *et al.* (2012)
Atenolol	$C_{14}H_{22}N_2O_3$	29122-68-7	2,000 mg/kg	LD50 (oral), mouse	HSDB (2004)
			200 mg/L	48-h EC50 (immobilization), *D. magna*	Hernando *et al.* (2004)
			313 mg/L	48-h EC50 (immobilization), *D. magna*	Cleuvers (2005)
			3.2 mg/L	NOEC (wet weight 28-d post-hatch), *Pimephales promelas*	Winter *et al.* (2008)
			3.2 mg/L	NOEC (length 28-d post-hatch), *Pimephales promelas*	Winter *et al.* (2008)
			1.8 mg/L	NOEC (second generation reproduction test), *D. magna*	Küster *et al.* (2010)
			77.7 ug/L	PNEC, derived from ECOSAR, green algae	Jones *et al.* (2002)
Azithromycin	$C_{38}H_{72}N_2O_{12}$	83905-01-5	0.019 mg/L	96-h EC50 (growth rate inhibition), *Pseudokirchneriella subcapitata*	Harada *et al.* (2008)
			5.2 ug/L	NOEC (96-h growth rate inhibition), *Pseudokirchneriella subcapitata*	Harada *et al.* (2008)

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
			0.15 ug/L	PNEC[2], pathogenic bacteria	Kümmerer and Henninger (2003)
Bisphenol A	$C_{15}H_{16}O_2$	80-05-7	0.175 ug/L	Aquatic (marine) PNEC in Canadian Government Risk Assessment	Environment Canada and Health Canada (2008)
			1.0 mg/kg-soil (dw)	Soil PNEC in Canadian Government Risk Assessment	Environment Canada and Health Canada (2008)
			1.5 ug/L	Freshwater PNEC from the European Union Risk Assessment	IHCP (2008)
			3.7 mg/kg-soil (dw)	Soil PNEC from the European Union Risk Assessment	IHCP (2008)
Carbamazepine	$C_{15}H_{12}N_2O$	298-46-4	105 mg/L	72-h EC50 (root growth), *Allium cepa* [no soil]	Jos *et al.* (2003)
			0.754 mg/L	LOEC (reproduction inhibition, 48-h), *Brachionus calyciflorus*	Ferrari *et al.* (2003)
			0.377 mg/L	NOEC (reproduction inhibition, 48-h), *Brachionus calyciflorus*	Ferrari *et al.* (2003)
			77.7 mg/L	48-h EC50 (mobility inhibition), *Ceriodaphnia dubia*	Ferrari *et al.* (2003)
			100 ug/L	LOEC (reproduction inhibition, 7-d), *Ceriodaphnia dubia*	Ferrari *et al.* (2003)
			25 ug/L	NOEC (reproduction inhibition, 7-d), *Ceriodaphnia dubia*	Ferrari *et al.* (2003)
			37 mg/L	48-h EC50 (growth inhibition), *Chlorella vulgaris*	Jos *et al.* (2003)
			50 mg/L	LOEC (mortality, 10-d), *Danio rerio*	Ferrari *et al.* (2003)
			25 mg/L	NOEC (mortality, 10-d), *Danio rerio*	Ferrari *et al.* (2003)
			111 mg/L	48-h LC50, *Daphnia magna*	Han *et al.* (2006)
			112 mg/L	24-h EC50 (immobilization), *D. magna*	Jos *et al.* (2003)
			0.11 mg/L	28-d EC50 (spore production), *Glomus intraradices*	Hillis *et al.* (2008)
			0.39 mg/L	28-d EC50 (hyphal length growth), *Glomus intraradices*	Hillis *et al.* (2008)
			9.9 mg/L	10-d LC50, *Hyalella azteca*	Dussault *et al.* (2008)
			25.5 mg/L	7-d EC50 (average growth rate	Cleuvers (2003)

[2] PNEC was defined as MIC_{50}/100, where MIC = minimum inhibitory concentration for susceptible pathogenic bacteria

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
				inhibition), *Lemna minor*	
			35.4 mg/L	48-h LC50, *Oryzias latipes*	Kim *et al.* (2007)
Cimetidine	$C_{10}H_{16}N_6S$	51481-61-9	271 mg/L	96-h EC50 (immobilization), *D. magna*	Kim *et al.* (2007)
			> 100 mg/L	96-h LC50, *Oryzias latipes*	Kim *et al.* (2007)
			35 mg/L	EC50 (ECOSAR), daphnid	Sanderson *et al.* (2003)
			35 ug/L	PNEC, derived from EC50 (ECOSAR), daphnid	Verlicchi *et al.* (2012)
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$	85721-33-1	65.3 mg/L	48-h EC50 (immobilization), *D. magna*	Martins *et al.* (2012)
			4.83 mg/L	96-h EC50 (growth inhibition), *Pseudokirchneriella subcapitata*	Martins *et al.* (2012)
			3.75 mg/L	7-d EC50 (growth inhibition), *Lemna minor*	Martins *et al.* (2012)
			203 ug/L	7-d EC50 (reproduction), *Lemna minor*	Robinson *et al.* (2005)
			17 ug/L	5-d EC50 (growth and reproduction), *Microcystis aeruginosa*	Robinson *et al.* (2005)
			18.7 mg/L	3-d EC50 (growth inhibition), *Pseudokirchneriella subcapitata*	Robinson *et al.* (2005)
			0.005 mg/L	72-h EC50 (growth inhibition), *Microcystis aeruginosa*	Halling-Sørensen *et al.* (2000)
Citalopram	$C_{20}H_{21}FN_2O$	59729-33-8	3.9 mg/L	48-h LC50, *Ceriodaphnia dubia*	Henry *et al.* (2004)
			4.0 mg/L	LOEC (reproduction inhibition, 7-8-d), *Ceriodaphnia dubia*	Henry *et al.* (2004)
			0.8 mg/L	NOEC (reproduction inhibition, 7-8-d), *Ceriodaphnia dubia*	Henry *et al.* (2004)
			20 mg/L	48-h EC50 (immobilization), *D. magna*	Christensen *et al.* (2007)
			1.6 mg/L	48-h EC50 (growth inhibition), *Pseudokirchneriella subcapitata*	Christensen *et al.* (2007)
Clotrimazole	$C_{22}H_{17}ClN_2$	23593-75-1	0.098 mg/L	72-h EC50 (growth), *Desmodesmus subspicatus*	OSPAR (2005)
			0.010 mg/L	NOEC (reproduction, 21-d), *D. magna*	OSPAR (2005)
			0.032 mg/L	LOEC (reproduction, 21-d), *D. magna*	OSPAR (2005)
			0.025 mg/L	NOEC (EC10, mortality, behaviour, juveniles weight, 28-d), *Oncorhynchus mykiss*	OSPAR (2005)
			0.034 mg/L	NOEC (chlorophyll a content, 4-d), periphyton	Porsbring *et al.* (2009)
4-Cumylphenol	$C_{15}H_{16}O$	599-64-4	0.35 ug/L	Australian drinking water guideline	NWQMS (2008)

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
			0.64 mg/L	1-h EC50 (settlement and metamorphosis), *Capitella* larvae	Biggers and Laufer (2004)
Doxycycline	$C_{22}H_{24}N_2O_8$	564-25-0	316 ug/L	7-d EC50 (growth inhibition), *Lemna gibba*	Brain *et al.* (2004)
			37 ug/L	28-d EC50 (spore production), *Glomus intraradices*	Hillis *et al.* (2008)
			0.3 ug/L	PNEC[3], pathogenic bacteria	Kümmerer and Henninger (2003)
Ethinylestradiol	$C_{20}H_{24}O_2$	57-63-6	1.23 mg/L	72-h EC50 (population growth rate), *Brachionus calyciflorus*	Radix *et al.* (2002)
			7.26 ng/L	14-d EC50 (vitellogenin induction), *Carassius auratus*	Zhang *et al.* (2009)
			4 ng/L	LOEC (vitellogenin induction, 14-d), *Carassius auratus*	Zhang *et al.* (2009)
			1 ng/L	NOEC (vitellogenin induction, 14-d), *Carassius auratus*	Zhang *et al.* (2009)
			200 ug/L	LOEC (survival, 48-h), *Ceriodaphnia reticulata*	Jaser *et al.* (2003)
			8.83 mg/L	240-h LC50, *Chironomus riparius*	Segner *et al.* (2003)
			4.1 mg/L	240-h LC50, *Chironomus tentans*	Dussault *et al.* (2008)
			1.1 mg/L	240-h LC50, *Hyalella azteca*	Dussault *et al.* (2008)
			0.31 ng/L	NOEC (length increase from 42 to 75 dpf[4]), *Danio rerio*	Schäfers *et al.* (2007)
			1.1 ng/L	LOEC (length increase from 42 to 75 dpf[5]), *Danio rerio*	Schäfers *et al.* (2007)
			1.48 ug/L	Different developmental parameters and male-to-female ratio, *Rana pipiens*	Hogan *et al.* (2008)
Furosemide	$C_{12}H_{11}ClN_2O_5S$	54-31-9	61 mg/L	24-h EC50 (immobilization), *D. magna*	Isidori *et al.* (2006)
			2.3 mg/L	7-d EC50 (population growth inhibition), *Ceriodaphnia dubia*	Isidori *et al.* (2006)
			2.5 mg/L	48-h EC50 (population growth inhibition), *Brachionus calyciflorus*	Isidori *et al.* (2006)
			10 ug/l	LOEC, different effects on a riverine	Lawrence *et al.*

[3] PNEC was defined as MIC_{50}/100, where MIC = minimum inhibitory concentration for susceptible pathogenic bacteria

[4] dpf: days post-fertilization

[5] dpf: days post-fertilization

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
				biofilm community	(2005)
Gemfibrozil	$C_{15}H_{22}O_3$	25812-30-0	74 mg/L	24-h EC50 (immobilization), *D. magna*	Isidori *et al.* (2007)
			0.44 mg/L	48-h EC50 (population growth inhibition), *Brachionus calyciflorus*	Isidori *et al.* (2007)
			0.53 mg/L	7-d EC50 (population growth inhibition), *Ceriodaphnia dubia*	Isidori *et al.* (2007)
			15.2 mg/L	72-h EC50 (growth inhibition), *Pseudokirchneriella subcapitata*	Isidori *et al.* (2007)
			1.5 ug/L	LOEC (plasma testosterone decrease, 14-d), *Carassius auratus*	Mimeault *et al.* (2005)
			10.4 mg/L	48-h LC50, *Daphnia magna*	Han *et al.* (2006)
			0.9 mg/L	EC50 (ECOSAR), fish	Sanderson *et al.* (2003)
Ibuprofen	$C_{13}H_{18}O_2$	15687-27-1	108 mg/L	48-h EC50 (immobilization), *D. magna*	Cleuvers (2003)
			22 mg/L	7-d EC50 (average growth rate inhibition), *Lemna minor*	Cleuvers (2003)
			133 mg/L	48-h LC50, *Daphnia magna*	Han *et al.* (2006)
			13.4 mg/L	14-d (after 24hr exposure) EC50 (reproduction), *Daphnia magna*	Heckmann *et al.* (2007)
			22.36 mg/L	96-h LC50, *Hydra attenuata*	Quinn *et al.* (2008)
			1.65 mg/L	96-h LC50 (morphology), *Hydra attenuata*	Quinn *et al.* (2008)
			4.01 mg/L	7-d EC50 (growth inhibition), *Lemna minor*	Pomati *et al.* (2004)
			1 ug/L	LOEC (reproduction, 6-wk), *Oryzias latipes*	Flippin *et al.* (2007)
			10 ug/L	LOEC, different effects on a riverine biofilm community	Lawrence *et al.* (2005)
			39.9 mg/L	96-h EC50 (malformations), *Xenopus laevis*	Richards and Cole (2006)
Mestranol	$C_{21}H_{26}O_2$	72-33-3	0.68 mg/kg	Produced a larger vitellogenin concentration and similar HSI than 0.6 mg/kg E2, *Ictalurus punctatus*	Nimrod and Benson (1996)
			0.64 mg/L	30-min EC50 (*in vitro* lysosomal membrane stability assay), *Mytilus*	Canesi *et al.* (2007)

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
Metoprolol	$C_{15}H_{25}NO_3$	37350-58-6	63.9 mg/L	48-h LC50, *Daphnia magna*	Huggett *et al.* (2002)
			8.8 mg/L	48-h LC50, *Ceriodaphnia dubia*	Huggett *et al.* (2002)
			6.15 mg/L	NOEC (growth, 9-d), *Daphnia magna*	Dzialowski *et al.* (2006)
			12.5 mg/L	LOEC (growth, 9-d), *Daphnia magna*	Dzialowski *et al.* (2006)
			3.2 mg/L	LOEC (growth, 9-d), *Daphnia magna* (F1 generation)	Dzialowski *et al.* (2006)
			7.9 mg/L	48-h EC50 (growth inhibition), *Desmodesmus subspicatus*	Cleuvers (2005)
			1 ug/L	LOEC (ultrastructural liver damage, 28-d), *Oncorhynchus mykiss*	Triebskorn *et al.* (2007)
Miconazole	$C_{18}H_{14}Cl_4N_2O$	22916-47-8	27 ug/L	IC50, human aromatase (CYP19) inhibition (*in vitro*)	Trösken *et al.* (2004)
Moxifloxacin	$C_{21}H_{24}FN_3O_4$	151096-09-2	N/A		
Galaxolide	$C_{18}H_{26}O$	1222-05-5	0.723 mg/L	72-h EC50 (growth inhibition), *Pseudokirchneriella subcapitata*	European Commission (2008)
			0.282 mg/L	21-d EC50 (reproduction), *Daphnia magna*	European Commission (2008)
			0.140 mg/L	LOEC (survival, 32-d post hatch), *Pimephales promelas*	European Commission (2008)
			105 mg/kg-soil	LOEC (reproduction, 8-wk), *Eisenia fetida*	European Commission (2008)
			105 mg/kg-soil	LOEC (mortality and reproduction, 4-wk), *Folsomia candida*	European Commission (2008)
			143.4 mg/L	EC50 (shoot elongation), *Triticum aestivum* L. [no soil]	An *et al.* (2009b)
Tonalide	$C_{18}H_{26}O$	21145-77-7	0.468 mg/L	72-h EC50 (growth inhibition), *Pseudokirchneriella subcapitata*	HERA (2004)
			0.244 mg/L	21-d EC50 (reproduction), *Daphnia magna*	HERA (2004)
			0.140 mg/L	LOEC (survival, 32-d post hatch), *Pimephales promelas*	Balk and Ford (1999)
			250 mg/kg-soil	LOEC (reproduction, 8-wk), *Eisenia fetida*	Balk and Ford (1999)
			105 mg/kg-soil	LOEC (mortality and reproduction, 4-wk), *Folsomia candida*	Balk and Ford (1999)
			33 ug/L	NOEC (heart rate, 48-h), *Danio rerio*	Carlsson and

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
					Norrgren (2004)
Norfloxacin	$C_{16}H_{18}FN_3O_3$	70458-96-7	0.038 mg/L	144-h EC50 (growth inhibition), *Microcystis wesenbergii*	Ando *et al.* (2007)
			913 ug/L	7-d EC50 (growth inhibition), *Lemna gibba*	Brain *et al.* (2004)
			10.4 mg/L	7-d EC50 (growth inhibition), *Chlorella vulgaris*	Eguchi *et al.* (2004)
Nonylphenol, octylphenol and derivatives[6]	$C_{15}H_{24}O$	84852-15-3	5.7 mg-TEQ/kg-soil[7]	Canadian Soil Quality Guideline for Agricultural Soil	Environment Canada (2002)
			1.0 ug-TEQ/L[2]	Canadian Water Quality Guideline for Freshwater	Environment Canada (2002)
Ofloxacin	$C_{18}H_{20}FN_3O_4$	82419-36-1	0.53 mg/L	48-h EC50 (growth inhibition), *Brachionus calyciflorus*	Isidori *et al.* (2005)
			3.13 mg/L	7-d EC50 (growth inhibition), *Ceriodaphnia dubia*	Isidori *et al.* (2005)
			1.44 mg/L	72-h EC50 (growth inhibition), *Pseudokirchneriella subcapitata*	Isidori *et al.* (2005)
			16 ug/L	96-h EC50 (growth), *Synechococcus leopolensis*	Ferrari *et al.* (2004)
			21 ug/L	5-d EC50 (growth and reproduction), *Microcystis aeruginosa*	Robinson *et al.* (2005)
			126 ug/L	7-d EC50 (reproduction), *Lemna minor*	Robinson *et al.* (2005)
Monomethyltins (trichloromethyl-stannane)	CH_3Cl_3Sn	993-16-8	178 ug/L	96-h EC50 (growth), *Scenedesmus obliquus*	Environment Canada (2009)
Dimethyltins (dichlorodimethyl-stannane)	$C_2H_6Cl_2Sn$	753-73-1	756 ug/L	96-h EC50 (growth), *Scenedesmus obliquus*	Environment Canada (2009)
Monobutyltins (butyltin tris(2-ethylhexylmercapto-acetate)	$C_{34}H_{66}O_6S_3Sn$	26864-37-9	16 ug/L	MATC, *Daphnia magna*	Environment Canada (2009)
Dibutyltins (dibutyltin	$C_{28}H_{56}O_4S_2Sn$	69536-57-8	13 ug/L	48-h EC50, *Daphnia magna*	Environment

[6] Formula and CAS number both refer exclusively to NP, not the derivatives.

[7] TEQ = $\Sigma(c_i \times TEF_i)$, where c_i = concentration of compound i; TEF_i = toxic equivalent factors. TEF values: NP & OP = 1; NPnEO & OPnEO ($1 \leq n \leq 8$) = 0.5; NPnEO & OPnEO ($n \geq 9$) = 0.005; NPnEC & OPnEc ($1 \leq n \leq 2$) = 0.005

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
bis(2-ethylhexylmercapto-acetate)					Canada (2009)
Dibutyltins (dibutyltin dichloride)	$C_8H_{18}Cl_2Sn$	683-18-1	8 ug/L	NOEC (21-d), *Daphnia magna*	Environment Canada (2009)
Tributyltins (hexabutyldistannoxane)	$C_{24}H_{54}OSn_2$	56-35-9	0.01 ug/L	NOEC (90-d), *Poecilia reiculata*	Environment Canada (2009)
Tributyltins (tributyltin oxide)	$C_{24}H_{54}OSn_2$	56-35-9	0.008 ug/L	Canadian Environmental Freshwater Quality Guideline	CCME (1999)
Tetrabutyltin	$C_{16}H_{36}Sn$	1461-25-2	45 ug/L	96-h LC50, *Pimephales promelas*	Environment Canada (2009)
Dioctyltin dichloride	$C_{16}H_{34}Cl_2Sn$	3542-36-7	4.1 ug/L	48-h LC50, *Daphnia magna*	Environment Canada (2009)
Triphenyltins (triphenyltin hydroxide)	$C_{18}H_{16}OSn$	76-87-9	0.022 ug/L	Canadian Environmental Freshwater Quality Guideline	CCME (1999)
Triphenyltins (triphenyl chloride)	$C_{18}H_{15}ClSn$	639-58-7	0.209 ug/L	LOEC (increased mortality, liver glycogen depletion, decreased resistance to *Aeromonas*, 110-d), *Oncorhynchus mykiss* yolk sac fry	Environment Canada (2009)
Tetraphenyltin	$C_{24}H_{20}Sn$	595-90-4	398 ug/L	48-h LC50, *Oryzias latipes*	Environment Canada (2009)
Propranolol HCl	$C_{16}H_{21}NO_2HCl$	318-98-9	2.22 mg/L	48-h EC50 (reproduction inhibition), *Brachionus calyciflorus*	Liu *et al.* (2009)
			0.77 mg/L	72-h EC50 (growth inhibition), *Pseudokirchneriella subcapitata*	Liu *et al.* (2009)
			0.8 mg/L	48-h LC50, *Ceriodaphnia dubia*	Huggett *et al.* (2002)
			9 ug/L	NOEC (reproduction, 7-d), *Ceriodaphnia dubia*	Ferrari *et al.* (2004)
			2 mg/L	NOEC (mortality, 10-d), *Danio rerio*	Ferrari *et al.* (2004)
			1.6 mg/L	48-h LC50, *Daphnia magna*	Huggett *et al.* (2002)
			50 ug/L	LOEC (increased fecundity, 21-d), *Daphnia magna*	Stanley *et al.* (2006)
			869 ug/L	LOEC (decreased fecundity, 21-d), *Daphnia magna*	Dzialowski *et al.* (2006)
			0.22 mg/L	NOEC (body mass, 9-d), *Daphnia magna*	Dzialowski *et al.* (2006)
			0.055 mg/L	NOEC (fecundity, 9-d), *Daphnia magna*	Dzialowski *et al.* (2006)

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
			0.7 mg/L	48-h EC50 (growth), *Desmodesmus subspicatus*	Cleuvers (2005)
			0.001 mg/L	NOEC (reproduction, 7-d), *Hyalella azteca*	Huggett *et al.* (2002)
			29.8 mg/L	48-h LC50, *Hyalella azteca*	Huggett *et al.* (2002)
			21.2 mg/L	7-d EC50 (growth inhibition), *Lemna minor*	Kaza *et al.* (2007)
			10 mg/L	LOEC (growth rate decrease, 40-d), *Oncorhynchus mykiss*	Owen *et al.* (2009)
			0.5 ug/L	LOEC (egg production, 4-wk), *Oryzias latipes*	Huggett *et al.* (2002)
			0.5 mg/L	LOEC (growth inhibition, 14-d), *Oryzias latipes*	Huggett *et al.* (2002)
			0.1 mg/L	LOEC (hatchability, 21-d), *Pimephales promelas*	Giltrow *et al.* (2009)
			128 ug/L	LOEC (survival, 7-d), *Pimephales promelas*	Stanley *et al.* (2006)
Sertraline	$C_{17}H_{17}Cl_2N$	79617-96-2	0.12 mg/L	48-h LC50, *Ceriodaphnia dubia*	Henry *et al.* (2004)
			0.045 mg/L	LOEC (reproduction inhibition, 7-8-d), *Ceriodaphnia dubia*	Henry *et al.* (2004)
			0.009 mg/L	NOEC (reproduction inhibition, 7-8-d), *Ceriodaphnia dubia*	Henry *et al.* (2004)
			764 ug/L	96-h IC50 (growth inhibition, 96-h), *Chlorella vulgaris*	Johnson *et al.* (2007)
			12.1 ug/L	96-h IC50 (growth inhibition, 96-h), *Pseudokirchneriella subcapitata*	Johnson *et al.* (2007)
			98.9 ug/L	96-h IC50 (growth inhibition, 96-h), *Scenedesmus acutus*	Johnson *et al.* (2007)
			1.3 mg/L	48-h EC50 (immobilization), *Daphnia magna*	Minagh *et al.* (2009)
			0.066 mg/L	21-d EC50 (reproduction), *Daphnia magna*	Minagh *et al.* (2009)
			0.14 mg/L	72-h EC50 (growth inhibition), *Pseudokirchneriella subcapitata*	Minagh *et al.* (2009)
			0.38 mg/L	96-h LC50, *Oncorhynchus mykiss*	Minagh *et al.* (2009)
			131.4 ug/L	7-d EC50 (growth), *Pimephales promelas*	Valenti *et al.* (2009)
			149.5 ug/L	7-d EC50 (feeding rate), *Pimephales promelas*	Valenti *et al.* (2009)

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
			3.3 mg/L	96-h EC50 (malformations), *Xenopus laevis*	Richards and Cole (2006)
			3.9 mg/L	96-h LC50, *Xenopus laevis*	Richards and Cole (2006)
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$	723-46-6	0.21 mg/L	7-d EC50 (growth inhibition), *Ceriodaphnia dubia*	Isidori *et al.* (2005)
			0.52 mg/L	72-h EC50 (growth inhibition), *Pseudokirchneriella subcapitata*	Isidori *et al.* (2005)
			15.51 mg/L	48-h EC50 (immobilization), *Ceriodaphnia dubia*	Isidori *et al.* (2005)
			45.4 ug/L	28-d EC50 (root length), *Daucus carota* [cell culture w/*Glomus intraradices*]	Hillis *et al.* (2008)
			10 ug/L	LOEC (root length, 28-d), *Daucus carota* [cell culture w/*Glomus intraradices*]	Hillis *et al.* (2008)
			81 ug/L	7-d EC50 (weight), *Lemna minor*	Brain *et al.* (2004)
			27 mg/L	24-h EC50 (EROD activity inhibition), *Oncorhynchus mykiss* [cell culture]	Laville *et al.* (2004)
			26.8 ug/L	96-h EC50 (growth), *Synechococcus leopolensis*	Ferrari *et al.* (2004)
Sulphanilamide	$C_6H_8N_2O_2S$	63-74-1	23 mg/L	72-h EC50 (growth rate inhibition), *Pseudokirchneriella subcapitata*	MOE (2014)
			13 mg/L	48-h EC50 (immobilization), *Daphnia magna*	MOE (2014)
			> 100 mg/L	96-h LC50, *Oryzias latipes*	MOE (2014)
Tetracycline	$C_{22}H_{24}N_2O_8$	60-54-8	340 mg/L	NOEC (immobilization, 48-h), *Daphnia magna*	Wollenberger *et al.* (2000)
			44.8 mg/L	21-d EC50 (reproduction), *Daphnia magna*	Wollenberger *et al.* (2000)
			1 mg/L	LOEC (root length, 28-d), *Daucus carota* [cell culture w/*Glomus intraradices*]	Hillis *et al.* (2008)
			300 ug/L	LOEC (hyphal length growth, 28-d), *Glomus intraradices* [cell culture]	Hillis *et al.* (2008)
			723 ug/L	7-d EC50 (weight), *Lemna minor*	Brain *et al.* (2004)
			0.09 mg/L	7-d EC50 (growth inhibition), *Microcystis aeruginosa*	Halling-Sørensen (2000)
			2.2 mg/L	3-d EC50 (growth inhibition), *Selenastrum capricornutum*	Halling-Sørensen (2000)
			1.0 mg/L	72-h IC50 (growth inhibition), *Pseudokirchneriella subcapitata*	Yang *et al.* (2008)
			0.0241 mg/L	24-h EC50 (bioluminescence inhibition),	Backhaus *et al.*

Chemical name	Formula	CAS RN	Endpoint value	Endpoint description	Reference
				Vibrio fischeri	(1997)
Venlafaxine	$C_{17}H_{27}NO_2$	93413-69-5	157 ug/L	LOEC (foot detachment from substrate, 4-h), *Chlorostoma funebralis* (black turban marine snail)	Fong and Molnar (2013)
			313 pg/L	LOEC (foot detachment from substrate, 4-h), *Leptoxis carinata* (crested mudalia, freshwater snail)	Fong and Hoy (2012)
			141 mg/L	48-h EC50 (immobilization), *Daphnia magna*	Minguez *et al.* (2014)
			305 ng/L	21-d exposure caused 40% mortality at this concentration, *Pimephales promelas*	Schultz *et al.* (2011)

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