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Contaminants of Emerging Concern in the Environment: Ecological and Human Health Considerations

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**Contaminants of Emerging
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Ecological and Human Health
Considerations**

Rolf U. Halden, Editor

*School of Sustainable Engineering and the Built Environment,
Arizona State University*

*Department of Environmental Health Sciences,
Johns Hopkins Bloomberg School of Public Health*

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As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previous published papers are not accepted.

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Preface

Contaminants of Emerging Concern (CECs) pose new and grave challenges to public health and the health of the ecosystem on which we depend for safe water, air, soil, and food. On the horizon looms a perfect storm. The production of new chemicals stretches and often exceeds the capacity of current safety monitoring and risk assessment technologies. Population growth and expectations for improved living standards puts more demand on finite resources. As documented by U.S. Geological Survey studies of the past decade, surface, ground, and drinking waters are already significantly contaminated.

The authors of this volume document the current science of CECs with important new data on the risks associated with a broad range of persistent organic pollutants. These include pharmaceutical and personal care products, natural and synthetic hormones, agricultural pesticides, perfluorinated compounds, and other organohalogenes. The volume is organized in three sections: 1) environmental sources, occurrences, and fates of CECs; 2) ecotoxicological and human health risks of CECs; and 3) modeling tools, research needs and policy options for managing CECs.

This organization provides a very useful terrain map of the field that will guide scientists and policy makers as they grapple with the serious problems described in each of the chapters. Recent advances in genomics and epigenomics underscore the extraordinary sensitivity of cellular structures and metabolic pathways to modification by CECs at concentrations once thought to be safe. The very qualities of stability and persistence that are desirable from a pharmacokinetic perspective, whether in drugs for human or veterinary medicine or pesticides and herbicides for agricultural use, now must be viewed as potential threats to the health of the ecosystem. In the tradition of *Silent Spring* the authors of this volume alert us to issues revealed by the application of new methods of measurement not even dreamed of in Rachel Carson's day. How successful we are in coping with these issues has profound implications for the health of the public.

Robert S. Lawrence, MD

Center for a Livable Future Professor
Professor, Environmental Health Sciences, Health Policy, and International Health
Director, Center for a Livable Future
Johns Hopkins Bloomberg School of Public Health
Baltimore, Maryland

Acknowledgments

This book is dedicated to my former colleague and mentor - the late M. Gordon ('Reds') Wolman (1924 – 2010) of Johns Hopkins University, to my parents, and to my wonderful family - my wife Barbara and our three children Lena, Maya and Marco.

My sincere thanks to all the individuals who have contributed directly and indirectly to this book. Special thanks to all contributing authors for providing the promised chapters and revisions in a timely fashion, and to the peer-reviewers, who volunteered hundreds of hours of their time to ensure the work is technically sound and fit to print (I apologize in advance for any mistakes and omissions, which may be discovered after release of this book, and which are entirely my responsibility). Many thanks to my family for allowing me to complete this work, and for their forgiveness for the many hours spent away from them physically and mentally while working on it. And finally, many thanks to the staff of the American Chemical Society who made it amazingly easy to put this book together; particular thanks go to Tim Marney and Bob Hauserman, who invited both the symposium session and the book, for guiding me through the process from book proposal to final product, and to Sherry Weisgarber, for her invaluable help in copy editing and producing this work. This book was made possible in part by the National Institute of Environmental Health Sciences (NIEHS) grant 1R01ES015445 and by the Johns Hopkins Center for a Livable Future.

Chapter 1

Introduction to Contaminants of Emerging Concern in the Environment: Ecological and Human Health Considerations

R. U. Halden^{*,1,2,3}

¹School of Sustainable Engineering and the Built Environment,
Arizona State University, Tempe, AZ 85283, USA

²Center for Environmental Biotechnology at the Biodesign Institute,
Arizona State University, Tempe, AZ 85283, USA

³Department of Environmental Health Sciences, Johns Hopkins University,
Bloomberg School of Public Health, Baltimore, MD 21205, USA.

*Corresponding author: School of Sustainable Engineering and the Built Environment, Arizona State University, 1001 South McAllister Avenue, Tempe, AZ 85287-5701. E-mail: halden@asu.edu.

In 2010, the American Chemical Society (ACS) published online and in print a new book entitled, “*Contaminants of Emerging Concern in the Environment: Ecological and Human Health Considerations*.” The present introductory chapter provides a history of key events leading up to this publication. The book contains a number of scientific studies first presented during a 2009 ACS symposium on ‘*Emerging Contaminants, Pharmaceuticals and Personal Care Products (PPCPs), and Organohalogenes in Wastewater and Municipal Biosolids*’, conducted as part of the 238th National Meeting of the ACS in Washington, D.C. However, the book’s focus is much broader, covering additional environmental media, including groundwater, raw and treated drinking water, runoff from agricultural fields, as well as terrestrial and aquatic biota exposed to contaminants of emerging concern (CECs). Scientific and regulatory information is presented in three major sections: (i) Environmental Sources, Occurrences, and Fate of CECs, (ii) Ecotoxicological and Human Health Risks of CECs, and (iii) Modeling Tools, Research Needs, and Policy

Options for Managing CECs. The book comprises some 27 chapters, many of which are written by preeminent experts from government and academia. The book adds to a rapidly growing body of literature on CECs by providing a comprehensive and authoritative, i.e., peer-reviewed, perspective of CECs in the environment and associated human and ecological health concerns. It may serve as a desk reference for the various stakeholders or as a textbook for graduate-level courses examining the connection between human society and the environment, with particular emphasis on the water cycle.

Keywords: PPCPs; CECs; emerging contaminants; perfluorinated compounds; organic wastewater compounds; OWCs

In August of 2009, the world's largest scientific society, the American Chemical Society (ACS), convened its 238th National Meeting in Washington, DC. Leading up to the event, news media across the U.S. had reported on the contamination of aqueous and terrestrial environments, including finished drinking water in several U.S. metropolitan areas, by so-called "emerging" contaminants, or more accurately stated, *contaminants of emerging concern* (CECs). Among these substances are *pharmaceuticals and personal care products* (PPCPs), natural and synthetic hormones, an array of agricultural pesticides, as well as *perfluorinated compounds* (PFCs) and other persistent and toxic *organohalogen compounds* (chemicals containing one or more carbon-halogen bonds) produced by modern society. While the presence of natural and manmade, potentially harmful chemicals in the natural and built environment certainly had been known for some time, several recent events had attracted strong media attention for this topic.

First, in 2002, the United States Geological Survey (USGS) had published in the leading U.S. journal for the environmental sciences, *Environmental Science & Technology*, a study showing the presence of pharmaceuticals, hormones, and other organic wastewater contaminants in streams across the U.S., in a national reconnaissance, representing the first of its kind (1). The agency used five newly developed analytical methods to measure concentrations of 95 *organic wastewater compounds* (OWCs) in surface water samples obtained during 1999 and 2000 from 139 streams across 30 U.S. states. Eighty percent of the streams sampled showed detectable quantities of OWCs and 82 of the 95 target compounds were found in this study (1). Although detected concentrations were generally low and rarely exceeded drinking-water guidelines, drinking-water health advisories, or aquatic-life criteria, the study struck a chord with the scientific community and the general public. Eight years later, the USGS landmark study (1) has been cited over 1,600 times in the peer-reviewed literature, and a U.S. Environmental Protection Agency (EPA) literature database now shows over 8,000 publications on PPCPs and related CECs, thus illustrating a tremendous uptick in publishing activity on this topic (2).

Second, an unusual effort by the *Associated Press* had made headlines globally. The AP had commissioned its own investigation into the occurrence of PPCPs in finished drinking water of major U.S. metropolitan areas and, in 2008, reported that the water supply of more than 41 million U.S. citizens contained traces of one or more active pharmaceutical ingredients (3).

Third, motivated in part by the extensive media coverage and uncertainty about human health implications of PPCPs in the water supply, the National Research Council of the U.S. National Academies, in December of 2008, focused its 6th Workshop of the *Standing Committee on Risk Analysis Issues and Reviews* on the topic of *Characterizing the Potential Human Toxicity from Low Doses of Pharmaceuticals in Drinking Water*. The subtitle of this workshop captured pressing concerns regarding the need for management of poorly understood associated human health risks: *Are New Risk Assessment Methods or Approaches Required?* An invited presentation at the meeting, titled *'What's in Our Water?'* pointed to existing knowledge gaps in our understanding of the occurrence and significance of CECs in the water supply (4).

Fourth, the issue of so-called “intersex fish” had emerged as a major environmental concern and was being discussed in 2009 at hearings of the U.S. Congress, following reports on the occurrence of eggs in the reproductive system of some 80 percent of male smallmouth bass caught in the Potomac River near locations where municipal sewage treatment plants discharge treated effluent into the river for water reclamation.

The 2009 ACS special symposium on “Emerging Contaminants (Pharmaceuticals and Personal Care Products) and Organohalogens in Wastewater and Municipal Biosolids” was a timely and well attended event, featuring as presenters of platform and poster presentations some of the world’s preeminent researchers in the field. At the conclusion of the symposium, the ACS Press invited the preparation of this book (5), to cover the presented science and additional aspects and contributions from authors who were unable to attend the meeting.

The book (5) features chapters organized in three main sections. After the present introduction, it discusses environmental sources, occurrences and fates of CECs. This section provides a comprehensive overview from an international perspective, of the types of CECs present in the environment and detected at various levels in diverse media, ranging from raw sewage to treated wastewater to stabilized municipal sewage sludge fit for application on land (biosolids) to raw and finished drinking water. The second part of the book examines the treatability and persistence of CECs during municipal wastewater treatment and discusses ecotoxicity issues and risk assessments for PPCPs and CECs. The book concludes with a final section, concentrating on policy options and future research needs to manage CECs in the environment and to address human health and ecotoxicological concerns.

A number of excellent books have been published previously on pharmaceuticals, personal care products and related emerging contaminants in the environment, e.g., (6–10). The present book (5) provides an update on this important topic and distinguishes itself from prior contributions by its extended aim to integrate other components of contaminant mixtures that are thought

to figure prominently into human and ecotoxicological risks posed by CECs: namely organohalogen compounds including perfluorinate, polybrominated and polychlorinated compounds [e.g., (11, 12)] as well as constituents of plastics, exposure to which as been linked to adverse human health effects (13).

Emerging contaminants in the environment is an issue that is here to stay. Whereas the rapid transition in focus from one contaminant to another sometimes cynically is being referred to as chasing the '*contaminant du jour*,' interest in the general topic of environmental trace contaminants and their effects never really has waned and its complexity ensures that it will be of relevance far into the future. It certainly has raised the public's awareness for the interconnectivity between human lifestyle choices and environmental health, and also has triggered an appreciation for the vulnerability of the water cycle that is under stress from excessive water use and a growing and demographically aging population that is becoming more and more dependent on medications that find their way into the water environment. Thus, the quest for knowledge concerning the impact of trace contaminants in drinking water continues.

Just recently, the World Health Organization initiated a working group on PPCPs in drinking water, tasked to consider the human health implications of medical drugs in the water supply and to determine the need for credible health-based guidance related to this issue. At the same time, the EPA is reexamining its current selection of Superfund priority pollutants to include newly emerging contaminants discussed in depth in this book (5); among these substances are the antimicrobial compounds triclosan and triclocarban as well as the perfluorinated compounds perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA).

Many of the lesser known CECs covered in detail in this book (5) recently were discussed at a special workshop sponsored by the National Institute of Environmental Health Sciences (NIEHS) that concentrated on underappreciated CECs of the present and the future (14). Various previously unrecognized or under-appreciated pollutants also were identified in the 2009 national sewage sludge survey conducted by the U.S. EPA (15), an important surveillance effort that is presented and analyzed in depth in this book (5).

A vexing problem that continues to engage and frustrate scientific and regulatory communities in the industrialized world is the suspected association between environmental trace contaminants and human diseases and conditions observed at the population scale, such as certain types of cancers, early onset of puberty, and various immunological, developmental and neurological disorders; this realization is illustrated in a recent statement by the Endocrine Society (16).

Since the first series of Wingspread meetings (17) on endocrine disrupting compounds in the 1990s, research has intensified on the human health impacts of industrial chemicals but many answers are still lacking. Indeed, in the summer of 2010, a follow-up meeting on endocrine disruption occurred again at the Johnson Foundation's Wingspread Conference Center in Wisconsin near Lake Michigan, to define new and more informative research strategies for better understanding endocrine disruption in humans and animals subjected to chronic, low-level exposure to environmental compounds of concern.

It is possible and maybe even likely, that some of the observed adverse outcomes in human and ecological health cannot be explained properly by looking

at a single compound or a limited set of related substances. Thus, the content of this book (5) may serve as a foundation for future research aimed to elucidate in greater detail the human health and ecological effects of chronic, low-level environmental exposures to mixtures of harmful substances and the importance of contaminant mixtures for diseases and conditions whose etiologies at present are ill defined.

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Chapter 2

Pharmaceutical Ingredients in Drinking Water: Overview of Occurrence and Significance of Human Exposure

C. G. Daughton*

National Exposure Research Laboratory, U.S. Environmental Protection
Agency, 944 East Harmon Avenue, Las Vegas, NV 89119, USA

*Chief, Environmental Chemistry Branch. Phone: (702) 798-2207.

Fax: (702) 798-2142. E-mail: daughton.christian@epa.gov.

A comprehensive examination is presented of the data published through 2009 on the active pharmaceutical ingredients (APIs) that have been reported in finished drinking water (FDW). A synoptic review reveals that quantitative occurrence data for FDW exists for 64 APIs and miscellaneous transformation products, reported in 48 publications. Significantly, however, for these 64 substances only 17 have quantitative data from more than two reports each; only 36 have corroborative data from a second study. Almost all of the available data has been published since the year 2000. The occurrence data are organized around the Anatomical Therapeutic Chemical (ATC) classification system. The top four ATC classes for which the most API data have been reported are: N, C, V, and M. APIs have been reported for 7 of the 14 main ATC classes; no API has been reported for ATC classes A, B, H, L, R, or S. Some emphasis is also placed on negative data - those APIs with either data of absence or absence of data. The six most frequently reported APIs in FDW (in descending order) are: carbamazepine, ibuprofen, sulfamethoxazole, clofibrac acid, gemfibrozil, and iopromide. The six APIs with roughly the most consistent highest reported concentrations are: ibuprofen, triclosan, carbamazepine, phenazone, clofibrac acid, and acetaminophen. With only one exception (ibuprofen and its methyl ester metabolite), no API exceeded a concentration

of 1 ppb (1 $\mu\text{g/L}$). Also covered are some of the reported transformation products and disinfection by-products unique to APIs. Some of the less-discussed aspects of the potential ramifications for human health are also included. A clearer picture is emerging as to the extent and scope of API occurrence in drinking water, some preliminary generalizations can be drawn, and a better sense is emerging of where future research should be directed.

Introduction

Publication of investigations directed at pharmaceuticals as trace environmental contaminants began in earnest in the mid-1990s (1). As of December 2009, the US EPA's citation database of publications on pharmaceuticals as environmental contaminants comprised more than 9,000 documents dealing with the many aspects of this expansive topic (2). Those with a major focus on pharmaceuticals specifically as contaminants in drinking water totaled roughly only 250. Of these, all but 20 had been published since 2000.

This chapter presents a synoptic overview of the occurrence of active pharmaceutical ingredients (APIs) in finished drinking water and some of the less-discussed aspects of the potential ramifications for human health. The discussion builds on the synopsis published in early 2008 (3); among other overviews are: (4–9) and those in (10, 11). Aspects not addressed here include treatment technologies and approaches for reducing API residues in drinking water. Assessment of risk is discussed only briefly with the intent of presenting new insights.

APIs in drinking water continue to attract attention despite a developing consensus that ecological integrity is the major concern regarding APIs as pollutants - because aquatic systems can experience continual exposures to levels of APIs one or more magnitudes higher in concentration than in drinking water. The occurrence of APIs in drinking water is a curious topic in the sense that little empirical data has hinted toward a link with human health - and yet the issue persists as one of great interest. This is because of a wealth of unanswered questions, many of which are key to understanding the more general issue of low-level chronic exposure to multiple chemical stressors.

Attention to the occurrence of APIs in drinking water is driven largely by public concerns. Public concern is disconnected from the actual concentrations of APIs in drinking waters - concentrations so low that they were not routinely measurable a decade ago and for which no toxicological risks have been documented. Rather, a major driver for public concern is the consequence of APIs as micropollutants serving to crystallize an understanding of the water cycle - highlighting the fact that "natural" waters often are derived at least in part from human sewage (and sometimes animal waste). APIs serve to demonstrate that sewage and drinking water are often closely connected hydrologically - APIs being excreted or disposed to sewage and trace amounts surviving long enough to make their way into potable waters. In the course of modern human history,

it was comparatively recent that sewage was understood as a major source of infectious disease; chlorination to reduce pathogens was widely adopted only at the beginning of the last century. Such a major determinant of public health contrasts sharply with the current focus on trace levels of chemical contaminants - concentrations that engineered treatment technologies drive ever lower.

APIs have probably long been present in drinking water (often at undetectable low levels) - ever since pharmaceuticals first came into widespread use (12). Their presence in the environment is partly a direct result of their intended use - as therapeutic use inevitably results in certain portions of all APIs to be released in excretions and during bathing. Advancements in analytical chemistry (especially the ability to measure ever-lower concentrations) have served to highlight the topic. Public interest was most recently fostered by the series of news stories published by the Associated Press beginning in 2008 (13), which also helped catalyze several Congressional Hearings in the US. The opening sentence from the first of the AP series (14) served to attract broad attention: "A vast array of pharmaceuticals including antibiotics, anti-convulsants, mood stabilizers and sex hormones have been found in the drinking water supplies of at least 41 million Americans..." But without a comprehensive understanding of the many complexities and nuances underlying the topic, determining whether APIs in drinking water pose any real concern will not be possible.

In the 2 years since the last synoptic examination of the literature (3), little new information has surfaced regarding the types and concentrations of APIs to which people might be exposed. But even so, a clearer picture is emerging as to the extent and scope of API occurrence in drinking water, some preliminary generalizations can be drawn, and a better sense is emerging of where future focus should be directed. Much less information is available concerning the scope and extent of disinfection by-products (DBPs) or transformation products unique to APIs.

Pathways to Drinking Water

Of the roughly 1,200 small-molecule APIs in common use today in the US (15), only a select few enter the environment in sufficient quantities to survive the gauntlet of hurdles and obstacles to be detectable in drinking water. A broad spectrum of environmental transformation processes and engineered treatment processes continually act to remove APIs - from the time of their release in sewage to their ultimate destination in potable water. Drinking water is the ultimate repository for many of the minute residues of APIs originally discharged primarily to sewage - after having undergone attenuation by a long series of anthropogenic and natural transformation processes.

The types and quantities of those few APIs that enter into finished drinking water are a function of the hydrologic connectivity between human (and animal) waste and the source for the drinking water - as moderated by the spectrum of upgrading treatments used to create tap water. The frequency and levels of API occurrence in drinking waters can decline dramatically as the hydrologic connectivities between waste and source waters are reduced; this includes

inputs such as from leaking sewers and landfills. Increasing the spatiotemporal distance between sewage and source water also reduces public perception of risk - regardless of the measured concentrations of chemicals unique to sewage. A balance must be found for optimizing the distance and time from treated sewage to source water.

From the moment of its intended use, the quantity of an API is reduced by absorption and metabolism in the patient, by degradation, transformation, or sorptive processes during sewage treatment followed by the natural processes occurring in the environment, and further yet by treatment for the production of polished (finished) drinking water. The quantities that survive through each of these steps are functions of the route of introduction to sewage (excretion via urine/feces, sweat, bathing), pharmacokinetics (which vary greatly among APIs as well as among individuals), natural physicochemical transformation processes (e.g., photolysis, UV irradiation, oxidation, hydrolysis, sorptive removal such as to sewage sludge or sediments), biodegradation, and the types and sophistication of the array of engineered treatment processes used in waste and water treatment (ranging from advanced oxidation and UV irradiation to reverse osmosis). Those APIs that survive this gauntlet and enter drinking water are characterized generally by their high usage rates or excretion rates (mirrored by low biodegradability), high chemical stability, high water solubility, and reduced propensity for sorption (such as to sewage sludge); the stability of an API is a characteristic important for a drug's efficacy - a half-life in the body sufficiently long to ensure biological action but short enough to avoid bioconcentration. The many factors that dictate the types and quantities of APIs that might remain in finished drinking waters have been summarized in numerous articles, including: (8, 16, 17).

A large number of drivers could be involved in future elevations or reductions in the presence of APIs in drinking water. Some prominent ones include: variables associated with the seasons or the weather (e.g., the percentage of source waters derived from STW effluents; sewage treatment efficiencies; local prescribing customs or disease patterns) and consumer disposal practices for unwanted medications (e.g., discarding to trash versus sewers).

Why the Concern?

Some perspective is important regarding the entry of APIs into drinking water sources. There is no such thing as "pure" water (18). Numerous chemicals from nature and from human activities tend to readily contaminate water at trace levels as soon as attempts are made at purification. APIs are among myriad other man-made (and naturally occurring) chemicals that can readily contaminate water - often at levels too low to detect today but undoubtedly will be detectable in the future, with the continual advancements in analytical measurement technologies.

Regardless of the scope of the existing empirical database on APIs detected or measured in drinking water, the major question is "What does it all mean?" Any discussion of APIs in drinking water inevitably segues to the topic of human exposure and risk - an aspect of the larger topic that will be touched upon at the end of this chapter.

As potential contaminants in drinking water, trace constituents in source waters will pose growing concerns as the percentage of the source water originating from either treated or raw sewage (e.g., overflow events) increases. A major driver will be the growing need to reuse water - beginning with indirect water reuse and transitioning eventually to direct reuse (18). In those locales where potable water is becoming scarce and effluent-dominated streams are common, the pressure to implement water reuse or to continually increase the percentage of recycled water will grow. The issues surrounding APIs in water will consequently attract yet more attention.

Another driver that could play a major role in the occurrence of APIs in water is climate change. Several dimensions in the environmental occurrence and distribution of APIs could be directly affected, including not just the increasing incidence of effluent-dominated source water coupled with the need to recycle water, but also the shifting of drug-use patterns and rates as a result of different geographic distribution of diseases and other health factors.

By striving to continually reduce the occurrence of APIs in the environment, the public's perception of the most visible hydrologic connections between potable water and sewage can be gradually erased. This will make the inevitable need to adopt reused water more acceptable to the public by allowing ever-shorter "loops" to be used between source and point of use - progressing from indirect to direct reuse (e.g., so-called "toilet-to-tap").

Needed - A Systems Approach

A number of currently unrelated lines of investigation intertwine in the issue of APIs in drinking water. Unfortunately, there is little cross-communication. A systems approach that integrates each of these will be required before definitive answers are possible regarding APIs in drinking water. To understand the ramifications of APIs in drinking water and how to manage the real or perceived risk involves understanding these various aspects of the larger puzzle, few of which currently intersect or inform each other. These include:

- extent and scope of API occurrence in finished drinking water (FDW), especially in point-of-use water (at the tap)
- extent and scope of API occurrence in source waters from which drinking water is derived
- effectiveness of water treatment processes
- possible role of the distribution process (e.g., biofilms in pipes; oxidation by residual chlorine)
- formation of disinfection by-products (DBPs) unique to APIs
- interactive and additive toxicological effects of trace-level APIs ("ultra-low dose" studies [concentrations orders of magnitude lower than those generally accepted to have biological effects]; so-called "micro-dosing" studies; the possible role of hormesis; and the potential role of epigenetics might each play important roles in advancing this major unknown)
- perception of risk and its role in public acceptance of recycled water

- best approach for communicating risk
- approaches for minimizing or preventing the introduction of APIs into the environment.

With regard to the scope and extent of API occurrence in drinking water, municipal utilities in the US and most other countries do not routinely perform any monitoring, as no requirements currently exist. Historically, APIs have not been regulated water pollutants (with the exception of those, such as lindane and pyrethrins, which also are - or used to be - registered as pesticides). The boundaries on the scope and magnitude of API occurrence in drinking water therefore still have much uncertainty. In the US, this may change with implementation of the third EPA Drinking Water Contaminant Candidate List - CCL3 (19). This marks the first time that the CCL has included any API. The list includes nine APIs that are endogenous or synthetic sex hormones and two miscellaneous APIs: equilenin, equilin, 17 α -estradiol, 17 β -estradiol, estriol, estrone, 17 α -ethynylestradiol (EE2), mestranol, 19-norethisterone, erythromycin, and nitroglycerin. At the same time, a joint effort of the USEPA and the U.S. Geological Survey (USGS) ("Emerging Contaminant Sampling Program") plans to monitor up to 50 drinking water treatment plants (DWTPs) for more than 60 APIs and metabolites in both source waters and finished drinking waters (20).

Objectives

The synoptic overview presented here focuses on the current state of knowledge regarding the occurrence of APIs in finished drinking water, referred to hereafter as FDW to distinguish it from source waters or raw drinking water (intake water for DWTPs). This is an important distinction and a source of confusion when examining the published literature (3). Some attention is also devoted to associated metabolites, transformation products, and DBPs. Some brief perspectives are also provided regarding risk from human exposure via FDW.

Historical Context and Perspective

To give this topic some very basic context and perspective, consider the brief history of societal norms once used in determining whether water was considered safe to drink (21). That focus is now on part-per-trillion residues of so-called micro-contaminants (such as the broad spectrum of APIs) is testament to how far sanitation has evolved. Where only 150 years ago in parts of Britain, a dominant worry was living past age 20 and having to continually ignore the stench of pervasive excrement and raw sewage, we now focus on quantities of chemicals so minute that their presence in drinking water could only have been hypothesized several decades ago.

Interest in APIs as environmental contaminants probably evolved naturally from the earlier interest in identifying and quantifying trace, unregulated chemical contaminants in water. In a landmark 1977 paper, Donaldson (22) formalized the

idea that waters contain perhaps countless chemicals; at that time, 2 million organic chemicals had been inventoried by CAS, compared with over 40 million in 2009. Donaldson argued that as analytical detection limits were lowered, the number of detectable chemicals would also increase, eventually leading to an expectation "...to find every known compound at a concentration of 10^{-12} g/L [1 pg/L] or higher in a sample of treated drinking water." This observation was followed in 1981 with a corollary by Fielding et al. (23):

"... a high proportion of the population is exposed, via drinking water (irrespective of its source), to minute quantities of a wide range of organic chemicals. The presence of these compounds at much higher concentrations would undoubtedly be grounds for grave concern in relation to possible carcinogenicity and other toxic effects. However, it is difficult to assess whether the very low levels encountered are significant."

Preceding these two reports was perhaps the first recognition that APIs can enter the environment from human use. Stumm-Zollinger and Fair (24) in a 1965 study of the biodegradation of steroid hormones noted that their paper exemplified:

"...the kind of inquiry that water engineers and water scientists conceivably will make in increasing number and rising intensity if the available water resource is allowed to become heavily contaminated with the waste products of man and with the expanding complex of chemicals synthesized by him for agricultural and industrial operations as well as for his more immediate personal use. Moreover, ... there must be an awareness of long-range and possibly synergistic effects of low-level amounts of toxic or physiologically active substances among which the steroid hormones are mentioned specifically. That population growth and population aging lie at the base of inquiries of this kind also needs to be emphasized." They went on to say that "it is our responsibility to learn in what amounts steroid hormones may occur in our drinking waters under the most unfavorable conditions,...we must find out to what extent, if any, the steroid hormones are biodegradable in the normal history of wastewaters and receiving bodies of water that may eventually supply drinking water to households."

The first actual reports of non-metabolite APIs in source or drinking waters were minor aspects of larger surveys (in the UK) for chemicals unrelated to APIs. Two of the first were Fielding et al. (23) and Waggott (25) in 1981. In non-targeted monitoring of FDW (from effluent-dominated source waters), Fielding et al. identified pentobarbital in one of 14 samples (and caffeine in another) - among 324 total trace organics. They recognized that analysis by gas chromatography limited the identification of organic matter to probably less than 20% of the compounds present. Also in 1981, Waggott published an extensive characterization of source waters (in the River Lee, UK), identifying trace levels

of a 1,4-benzodiazepine, a clofibrate metabolite, EE2, phenobarbital, and a salicylic acid metabolite, among many other organics.

Perhaps the first investigation to intentionally target an API in drinking water was Stan et al. (26), in 1994. Clofibric acid was quantified in all 64 drinking water samples taken in Berlin, Germany. Concentrations ranged from 10 to 165 ng/L. This study catalyzed a number of follow-up studies in Switzerland and Germany, all published before 2000 by Heberer, Ternes, and others. In 1998, a National Research Council workshop (27) devoted some focus to APIs as meriting attention as drinking water contaminants. One of the earliest evaluations of human risk was by Christensen in 1998 (28). One of the first targeted surveys of APIs in FDW in the US was in 2001 by Frick et al. (29), who detected acetaminophen. Questions regarding APIs in reused water were voiced as early as 2001 (30). Nearly the entire body of literature on APIs in FDW has been published since 2000.

Scope of Examination of APIs in Drinking Water

As used in this discussion, APIs will refer to the active ingredient in any over-the-counter (OTC) or prescription (Rx) medication or diagnostic intended for human or veterinary use but exclude ubiquitous natural products used in large amounts (e.g., natural stimulants such as caffeine and other xanthines, or any nicotine-related chemical or non-hormonal sterols/steroids). APIs covered in this chapter are the "small-molecule drugs" - relatively low-molecular weight, homogeneous chemicals (except for optical isomers) in contrast with the biologics, which include single molecules or multimers that are heterogeneous in composition, especially as a result of subtle amino acid sequence differences and in the degree and types of glycosylation.

Examining data from Overington et al. (31) and Wishart et al. (32) shows over 21,000 formulated drug products (various combinations of ingredients, strengths, and form) utilizing over 1,460 FDA-approved small-molecule APIs (molecularly distinct); the global pharmacopoeia comprises fewer than 1,000 frequently prescribed drugs (15). More than 800 are administered orally, over 420 parenterally, and over 270 topically; there are over 3,200 experimental drugs (only a portion of which are small molecules). Over 16% of the small-molecule drugs are prodrugs, meaning that the therapeutically active agent may not necessarily be the API itself; pro-drug active agents can be formed not just by metabolism during the course of therapy, but also by subsequent biological and abiotic transformation processes in the environment acting on excreted, unaltered prodrug.

There are three major types of FDW data available in the literature:

- (1) positive occurrence data (data of presence), which includes quantitative and qualitative data - values above limits of detection (LOD or DL) but below limits of quantitation (LOQ),
- (2) data of absence (negative data or non-detects), where an API was specifically targeted but was below its reporting limit or LOD, and
- (3) absence of occurrence data, where an API had not been targeted and therefore its presence or absence is not yet known.

The published literature contains a wealth of positive occurrence data for APIs in source waters (including ground waters and wells) and in raw (intake) waters used for generating FDW. In stark contrast, comparatively little positive data exist for actual FDWs themselves, especially waters as distributed to the final point of use (POU) - sometimes referred to in the literature as tap water (although "tap" is sometimes also used in reference to a sampling point within a DWTP). This paucity of FDW data partly reflects the analytical challenges faced by the greatly diminished API levels in FDW, which are often one or more orders of magnitude lower after the various treatment processes used for finishing/polishing. Even fewer data are available for POU drinking water, which are of the greatest relevance for assessing the potential for risk from human exposure (3); less study of POU waters is perhaps partly a result of the heightened prospects of acquiring negative monitoring data (data of absence), which is not as interesting to an investigator.

Summaries of Published Data for APIs in FDW

Various summaries of the data mined from the literature in this study are compiled in Tables I–IV. All four tables use the Anatomical Therapeutic Chemical (ATC) classification system codes (<http://www.whocc.no/atcddd/>) as a framework for organizing the data; the APIs are sorted according to their ATC codes rather than alphabetically. The in-depth list of positive occurrence data (together with some representative negative data) is shown in Table I. A distillation of just the positive occurrence data is shown in Table II, which presents the numbers of references reporting data for each API (ranked according to total number of references within and among each ATC primary group) along with the upper concentration ranges. Table III shows the top three APIs within each ATC class ranked according to frequency of quantitative data and according to the highest ranges in the published literature. Table IV summarizes the APIs reported (and examples of some not reported) in FDWs, grouped according to the primary ATC classes.

Table I. Compilation of Published Positive Occurrence Data for APIs in Finished Drinking Water (and select data on negative occurrence – indicated by shaded cells)

ATC name ^a	ATC code ^a	API (synonym)	CAS RN	finished drinking water, ng/L [median] ^b	distribution water (ng/L)	reference
Alimentary Tract & Metabolism	A01AB21	Chlortetracycline	57-62-5	ND (DL=5-150)		(33)
	A02BA02	Ranitidine	66357-35-5	ND (DL=0.01)		(34)
	A07AB03	Sulfaguanidine	57-67-0	ND (DL=5-75)		(33)
Cardiovascular Systems; C03 Diuretics; C04 Peripheral Vasodilators; C07 Beta Blocking Agents; C08 Calcium Channel Blockers; C10 Lipid Modifying Agents	C03AA03	Hydrochlorothiazide	58-93-5	0.8-117 [2.8] (RO DWTP)		(35)
				2.6-330 [7.1] (NF DWTP)		(35)
	C03CA01	Furosemide	54-31-9	ND (DL=4.30)		(34)
	C04AD03	Pentoxifylline	6493-05-6	ND (DL=5-60)		(33)
				ND (DL=1)		(36)
	C07AA05	Propranolol	525-66-6		ND (DL=1.9; 3 tap water samples)	(37)
	C07AB02	Metoprolol	37350-58-6	up to 13.5 [2.6] (RO/NF DWTP)		(35)
				14-26 [20] (2 of 44 samples; 22 DWTPs over 2 years)		(38)
	C07AB03	Atenolol	29122-68-7	1.2 [18] (8 of 18 samples)	0.84 [0.47] (8 of 15 samples)	(39)
				[2.8] max=26 (11 of 20 DWTPs)		(40)
ND (RL=0.25)					(41)	
ND (DL=0.05)					(34)	
	C08CA05	Nifedipine	21829-25-4		ND (DL=15.5; 3 tap water samples)	(37)
	C08CA05 metabolite	Dehydronifedipine (metabolite)	67035-22-7	2-6 (2 of 12 samples)		(42)
				4		(43)
	C08DB01	Diltiazem	42399-41-7		ND (DL=13.0; 3 tap water samples)	(37)
	C09AA02	Enalapril	75847-73-3	ND (RL=0.25)		(41)
	C10AA01	Simvastatin	79902-63-9	ND (RL=1.0)		(41)
	C10AA01 metabolite	Simvastatin hydroxy acid (Na salt)	12009-77-6	ND (RL=0.25)		(41)
	C10AA05	Atorvastatin	134523-03-8	ND (RL=0.25)		(41)
				ND (MRL=0.25) (18 samples)	<MRL (15 samples)	(39)
	C10AA05 metabolite	<i>o</i> -Hydroxy atorvastatin	214217-86-6	ND (MRL=0.50) (18 samples)	<MRL (18 samples)	(39)
	C10AA05 metabolite	<i>p</i> -Hydroxy atorvastatin	214217-88-6 (Ca-salt, acid form)	ND (RL=0.50)		(41)
				ND (MRL=0.50) (18 samples)	<MRL (18 samples)	(39)
	C10AB01	Clofibrate	637-07-0	ND (DL=55)		(44)
C10AB01 metabolite	Clofibric acid	882-09-7	up to 170 (12 of 14 DWTPs)		(45)	
			3.2-5.3 (1 of 3 DWTPs) (DL=1.50)		(34)	
			up to 70 (16 of 30 samples) (DL=1)		(46)	
			up to 70 (16 of 25 samples) (DL=1)		(47)	
			270		(45)	

Continued on next page.

Table I. (Continued). Compilation of Published Positive Occurrence Data for APIs in Finished Drinking Water (and select data on negative occurrence – indicated by shaded cells)

				32		(48)	
				0.9-1.1 (2 of 4 samples)		(49)	
				>50, <100 (2 of 22 samples)		(50)	
				13-136 [59] (3 of 44 samples; 22 DWTPs over 2 years)		(38)	
				10-165 (64 of 64 tap water samples from Berlin)		(26)	
				ND (DL=130)		(44)	
				ND (DL=3-90)		(33)	
	C10AB02	Bezafibrate	41859-67-0	27 (1 of 25 samples) (DL=25)		(47)	
				up to 14		(51)	
				[0.7] max=1.9		(52)	
				13-20 [17] (2 of 44 samples; 22 DWTPs over 2 years)		(38)	
				ND (DL=0.05)		(34)	
				ND (DL=3-90)		(33)	
	C10AB04	Gemfibrozil	25812-30-0	ND (RL=0.25)		(41)	
				1.3-6.5		(36)	
				70 (1 of 10 DWTPs) (DL=3-90)		(33)	
				2.4		(53)	
				2.1 [0.48] (7 of 18 samples)	1.2 [0.43] (4 of 15 samples)	(39)	
				0.6-10.6 (5 of 5 DWTPs)		(54)	
					up to 3.0		(51)
					[0.4] max=0.8		(52)
[1.0] max=2.0 (6 of 20 DWTPs)						(40)	
					>2.4, LOD (mean 3 tap water samples)	(37)	
					ND (LOD=0.1; 6 tap water samples)	(55)	
C10AB05					Fenofibrate	49562-28-9	14-21 [18] (2 of 44 samples; 22 DWTPs over 2 years)
C10AB05 metabolite	Fenofibric acid	42017-89-0	42 (1 of 30 samples) (DL=5)		(46)		
Dermatologics: D01 Antifungals; D06 Antibiotics & Chemotherapeutics; D08 Antiseptics & Disinfectives	D01AE12 S01BC08	Salicylic acid (also transformation product)	69-72-7	10-122 [39] (13 of 44 samples; 22 DWTPs over 2 years)		(38)	
					4.2 (mean 3 tap water samples)	(37)	
	D06AX02 (multiple)	Chloramphenicol	56-75-7	12-13 [13] (2 of 44 samples; 22 DWTPs over 2 years)		(38)	
	D08AE04	Triclosan	3380-34-5	734 (1 of 15 samples) (DL=125)		(44)	
				43 (1 of 20 locales)		(36)	
				1.2 (1 of 18 samples)	<MRL (15 samples)	(39)	
				[1.1] max=1.2 (2 of 20 DWTPs)		(40)	
				ND (DL=1)		(56)	
		ND (RL=1.0)		(41)			
				ND (DL=2.4; 3 tap water samples)	(37)		

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Table I. (Continued). Compilation of Published Positive Occurrence Data for APIs in Finished Drinking Water (and select data on negative occurrence – indicated by shaded cells)

	D08 (possible assignment)	Triclocarban (Trichlorocarbanilide)	101-20-2	ND (DL=3)		(57)
					ND (DL= 10; samples from 12 DWTPs)	(58)
Genito Urinary System & Sex Hormones: GR5 Sex Hormones & Modulators of the Genital System	G03AC01	Norethisterone (Norethindrone)	68-22-4	ND (DL=10)		(59)
	G03BA03	Testosterone	58-22-0	ND (DL=1)		(36)
				ND (MRL=0.50) (18 samples)	<MRL (15 samples)	(39)
	G03CA01	17 α -Ethinylestradiol	57-63-6	0.15-0.50		(60)
				ND (DL=5)		(59)
				ND (DL=1)		(36)
				ND (MRL=1.0) (18 samples)	<MRL (15 samples)	(39)
					<90 (below LOQ; 12 tap water samples over several months)	(61)
		ND (DL=4.8; 3 tap water samples)	(37)			
	G03CA03	17 α -Estradiol	57-91-0	0.3		(60)
	G03CA03	17 β -Estradiol	50-28-2	0.20-2.1		(60)
				>100 (below LOQ; 1 of 12 tap water samples over several months)	(61)	
ND (DL=1)					(36)	
	ND (MRL=0.50) (18 samples)	<MRL (15 samples)	(39)			
G03CA07	Estrone	53-16-7	1.1-2.3		(36)	
			0.2-0.6		(60)	
Antineoplastic Agents: J01 Antibacterials					1.7 (mean 3 tap water samples)	(37)
				1 (1 of 5 DWTPs; but not in raw water)		(62)
				ND (MRL=0.20) (18 samples)	<MRL (15 samples)	(39)
					<70 (below LOQ; 2 of 12 tap water samples over several months)	(61)
	G03CA07 metabolite	Estrone-3-sulfate	481-97-0	0.22 (post ozonation, but ND after GAC)		(63)
	G03DA04	Progesterone	57-83-0	1.1 (2 of 20 locales)		(36)
0.57 (1 of 18 samples)				<MRL (15 samples)	(39)	
G03	Estrogens: Estradiol (E2) Estrinol (E3) Estrone (E1) Estradiol-17-glucuronide Estrone-3-sulfate Estradiol-17-acetate Ethinylestradiol (EE2) Diethylstilbestrol (DES)		ND (< LOQs): -0.59 -1.02 -0.18 -1.02 -0.02 -0.23 -0.83 -0.21		(63)	
Antineoplastic Agents: J01 Antibacterials	J01AA02	Doxycycline	564-25-0	ND (DL=50-150)		(33)
	J01AA06	Oxytetracycline	79-57-2	ND (DL=50-150)		(33)
	J01AA07 (multiple)	Tetracycline	60-54-8	ND (DL=50-150)		(33)
	J01DD04	Ceftriaxone	73384-59-5	ND (DL=1.80)		(34)
	J01EA01	Trimethoprim	738-70-5	1.3 (1 of 20 locales)		(36)
ND (RL=0.25)					(41)	

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Table I. (Continued). Compilation of Published Positive Occurrence Data for APIs in Finished Drinking Water (and select data on negative occurrence – indicated by shaded cells)

				ND (DL=5-60)		(33)
				ND (MRL=0.25) (18 samples)	<MRL (15 samples)	(39)
J01EB02 (multiple)	Sulfamethizole	144-82-1	9 (1 of 8 bottled waters)			(64)
			ND (DL=5-75)			(33)
J01EB03	Sulfamethazine (Sulfadimidine)	57-68-1	ND (DL=5-75)			(33)
J01EB04	Sulfapyridine	144-83-2	ND (DL=5-75)			(33)
J01EB05 S01AB02	Sulfisoxazole (Sulfafurazole)	127-69-5	ND (DL=5-75)			(33)
J01EB07 D06BA02	Sulfathiazole	72-14-0	ND (DL=5-75)			(33)
J01EB	Sulfabenzamide	127-71-9	ND (DL=5-75)			(33)
J01EC01	Sulfamethoxazole	723-46-6	3.0-3.4 (2 of 3 DWTPs)			(65)
			13-80 (2 of 8 bottled waters)			(64)
			2 (after carbon sorption)			(66)
			14			(48)
			20 (1 of 20 locales)			(36)
			3.0 [0.39] (4 of 18 samples)	0.32 (1 of 15 samples)		(39)
			0.3-0.5 (2 of 4 samples)			(49)
			2.0-5.0 (1 of 5 DWTPs)			(54)
			[0.39] max=3.0 (3 of 20 DWTPs)			(40)
			<25 (2 of 22 samples)			(50)
			19-25 [22] (4 of 44 samples; 22 DWTPs over 2 years)			(38)
			ND (RL=0.25)			(41)
			ND (DL=5-75)			(33)
			ND (DL=10)			(42)
J01EC02	Sulfadiazine	68-35-9	ND (DL=5-75)			(33)
J01EC03	Sulfamoxole	729-99-7	ND (DL=5-75)			(33)
J01ED01	Sulfadimethoxine	122-11-2	11 (1 of 8 bottled waters)			(64)
			7.0 (1 of 5 DWTPs)			(54)
			ND (DL=5-75)			(33)
			ND (DL=10)			(42)
J01ED04	Sulfamer (Sulfametoxydiazine)	651-06-9	ND (DL=5-75)			(33)
J01ED05	Sulfamethoxy-pyridazine	80-35-3	ND (DL=5-75)			(33)
J01ED07 D06BA06	Sulfamerazine	127-79-7	ND (DL=5-75)			(33)
J01FA01 (multiple)	Erythromycin	114-07-8	1.3			(36)
			4.9 (1 of 3 DWTPs)			(65)
			ND (DL=10)			(42)
			ND (DL=0.03)			(34)
J01FA01 metabolite	Erythromycin-H2O	114-07-8?	ND (DL=10)			(42)
J01FA02	Spiramycin	8025-81-8	ND (DL=0.75)			(34)

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Table I. (Continued). Compilation of Published Positive Occurrence Data for APIs in Finished Drinking Water (and select data on negative occurrence – indicated by shaded cells)

	J01FA05	Oleandomycin	3922-90-5	ND (DL=0.02)		(34)	
	J01FA06	Roxithromycin	80214-83-1	1.4 (1 of 3 DWTPs)		(65)	
				ND (DL=10-150)		(33)	
	J01FF02	Lincomycin	154-21-2	ND (DL=0.02)		(34)	
				ND (DL=10)		(42)	
	J01MA01	Ofloxacin	82419-36-1	0.7-1.6 (2 of 4 samples)		(49)	
				ND (DL=20-100)		(33)	
	J01MA06	Norfloxacin	70458-96-7	ND (DL=20-100)		(33)	
	J01MB04	Pipemidic acid	51940-44-4	ND (DL=20-100)		(33)	
	J01MB05	Oxolinic acid	14698-29-4	2.9-4 (2 of 3 DWTPs)		(65)	
				ND (DL=20-100)		(33)	
	J01MB07	Flumequine	42835-25-6	1.2-2.5 (3 of 3 DWTPs)		(65)	
J01	Novobiocin	303-81-1	ND (DL=10-150)		(33)		
J01 (misc)	Sulfonamides (misc): <i>p</i> -Toluenesulfonamide <i>o</i> -Toluenesulfonamide Benzenesulfonamide	70-55-3 88-19-7 98-10-2	up to (95-percentile): 240 160 60		(67)		
J01 (misc)	Antibiotics		Absence of 18 of 24 in three DWTPs (with all LODs 3 ng/L or lower, except for minocycline [LOD=6ng/L]).		(65)		
			Survey of 28 antibiotics in Australia showed frequent occurrence in all waters (up to 64 µg/L in sewage influent) but absence of all 28 in drinking waters from 20 different sites (with LODs for 21 of the analytes being 20 ng/L or lower).		(68)		
L01 Antineoplastic Agents	L01AA01	Cyclophosphamide	50-18-0	ND (DL=0.02)		(34)	
				ND (DL=5-60)		(33)	
Musculo-skeletal System: MO1. Antiinflammatory & Analgesic Products	M01AB01	Indometacin (Indomethacin)	53-86-1	ND (DL=3-90)		(33)	
	M01AB05 (multiple)	Diclofenac	15307-86-5	up to 6 (8 of 30 samples) (DL=1)		(46)	
				up to 6 (8 of 25 samples) (DL=1)		(47)	
					up to 2.5 (6 tap water samples)		(55)
					14-18 [16] (2 of 44 samples; 22 DWTPs over 2 years)		(38)
					ND (RL=0.25)		(41)
					ND (MRL=0.25) (18 samples)	ND (MRL=0.25) (15 samples)	(39)
					ND (DL=1)		(36)
					ND (DL=3-90)		(33)
	M01AE01	Ibuprofen	15687-27-1	510-1,350 [930] (2 of 15 samples) (MDL=280)		(44)	
				18-23		(69)	
				up to 8.5		(70)	
up to 3 (3 of 30 samples) (DL=1)					(46)		

Continued on next page.

Table I. (Continued). Compilation of Published Positive Occurrence Data for APIs in Finished Drinking Water (and select data on negative occurrence – indicated by shaded cells)

				up to 3 (3 of 25 samples) (DL=1)		(47)
				2,7		(53)
				1-32		(36)
				up to 39		(51)
				up to 112		(56)
				2.2-3.0		(71)
					up to 0.6 (6 tap water samples)	(55)
					28 (1 of 44 samples; 22 DWTPs over 2 years)	(38)
					3.4 (mean 3 tap water samples)	(37)
					ND (DL=0.50)	(34)
					ND (DL=3-90)	(33)
	M01AE01 metabolite	Ibuprofen methyl ester	81576-55-8	4,950 (1 of 15 samples) (MDL=110)		(44)
	M01AE02 M02AA12	Naproxen	22204-53-1	up to 7.5		(70)
				8		(36)
				3.0 (1 of 5 DWTPs)		(54)
				up to 1		(56)
					up to 0.2 (6 tap water samples)	(55)
					ND (DL=0.4)	(72)
					ND (RL=0.50)	(41)
					ND (MRL=0.50) (18 samples)	<MRL (15 samples) (39)
						ND (DL=2.1; 3 tap water samples) (37)
	M01AE03	Ketoprofen	22071-15-4		up to 3.0 (6 tap water samples)	(55)
					ND (DL=3-90)	(33)
					ND (DL=8)	(70)
	M01AE04	Fenoprofen	31879-05-7		ND (DL=3-90)	(33)
	M01AG01	Mefenamic acid	61-68-7	up to 19.8 [0.9] (RO DWTP)		(35)
				up to 19.9 [1.9] (NF DWTP)		(35)
	N02BA01 A01AD05	Acetylsalicylic acid	50-78-2	>50		(48)
				>50, <100 (2 of 22 samples)		(50)
	N02BB01	Phenazone (Antipyrine)	60-80-0	50 (1 of 12 samples) (DL=10)		(46)
				400		(73)
				250		(74)
				11-29 [21] (8 of 44 samples; 22 DWTPs over 2 years)		(38)
	N02BB04	Propyphenazone (Isopropyl-antipyrine)	479-92-5	120		(73)
				80		(74)
	N02BE01	Acetaminophen (Paracetamol)	103-90-2	0.3-3 (2 of 12 samples)		(42)
				1.1-1.3 [3 of 10 brands of bottled water]		(75)

Continued on next page.

Table I. (Continued). Compilation of Published Positive Occurrence Data for APIs in Finished Drinking Water (and select data on negative occurrence – indicated by shaded cells)

				up to 210.1 (6 tap water samples)	(55)
				33 (1 of 44 samples; 22 DWTPs over 2 years)	(38)
N03AA03	Primidone (2-Deoxypheno-barbital)	125-33-7		up to 16	(76)
				0.7-1.3 (4 of 4 samples)	(77)
N03AB02	Dilantin (Phenytoin)	57-41-0		1.1-1.2	(53)
				[1.3]	(41)
				1.6-13	(36)
				19 [6.2] (10 of 18 samples)	16 [3.6] (10 of 15 samples)
				[9.4] max=32 (15 of 20 DWTPs)	(40)
				1-2 (3 of 4 samples)	(77)
N03AF01	Carbamazepine	298-46-4		0.3-2.0	(78)
				258 max	(43)
				5.3-7.5	(69)
				6.5-24 (3 of 10 DWTPs)	(33)
				up to 20 ng/L	(76)
				2 (after ozonation)	(66)
				1.1-5.7	(36)
				[29] max=140 (12 of 12 samples)	(42)
				23	(48)
				18 [6.0] (8 of 18 samples)	10 [6.8] (6 of 15 samples)
				0.8-135 (2 of 4 samples)	(49)
				2.0-7.0 (3 of 5 DWTPs)	(54)
				2.9-721	(51)
				[5.0] max=9.1	(52)
				up to 1.8 [1.2] (RO DWTP)	(35)
				0.5-5.7 [1.0] (NF DWTP)	(35)
				[5.4] max=18 (12 of 20 DWTPs)	(40)
					up to 43.2 (6 tap water samples)
				10-25 [17] (4 of 44 samples; 22 DWTPs over 2 years)	(38)
					>0.7, LOD (3 tap water samples)
				ND (RL=0.50)	(41)
				30 (1 of 12 samples) (DL=10)	(46)
				<25 (1 of 22 samples)	(50)
N03AF01 metabolite	10,11-Dihydroxy-10,11-dihydro-carbamazepine	125-28-0		up to 13	(76)
N05AX08	Risperidone	106266-06-2		ND (MRL=2.5) (18 samples)	2.9 (1 of 15 samples)
				ND (RL=0.25)	(41)

Continued on next page.

Table I. (Continued). Compilation of Published Positive Occurrence Data for APIs in Finished Drinking Water (and select data on negative occurrence – indicated by shaded cells)

	N05BA01	Diazepam	439-14-5	0.33 [0.33] (1 of 18 samples)	<MRL (15 samples)	(39)
				19.3-23.5 (1 of 3 DWTPs) (DL=0.02)		(34)
				ND (RL=0.25)		(41)
				ND (DL=1)		(36)
					ND (LOD=0.4; 6 tap water samples)	(55)
	N05BA16	Nordazepam	1088-11-5		ND (LOD=0.4; 6 tap water samples)	(55)
	N05BC01	Meprobamate (also a metabolite of the prodrug carisoprodol)	57-53-4	1.6-13		(36)
				[5.9]		(41)
				6.5-8		(53)
				42 [5.7] (14 of 18 samples)	40 [5.2] (11 of 15 samples)	(39)
[9.2] max=43 (17 of 20 DWTPs)					(40)	
			6.3-9.4 (4 of 4 samples)		(77)	
N06AA02	Imipramine	50-49-7		ND (LOD=0.7; 6 tap water samples)	(55)	
N06AA09	Amitriptyline	50-48-6		up to 1.4 (6 tap water samples)	(55)	
N06AA12	Doxepin	1668-19-5		ND (LOD=0.7; 6 tap water samples)	(55)	
N06AB03	Fluoxetine	54910-89-3	0.82 [0.71] (2 of 18 samples)	0.64 (1 of 15 samples)	(39)	
				1.0 (1 of 5 DWTPs)		(54)
				10 (1 of 44 samples; 22 DWTPs over 2 years)		(38)
				ND (DL=1)		(36)
				ND (DL=14)		(42)
				ND (DL=5-60)		(33)
				ND (RL=0.50)		(41)
	N06AB03 metabolite	Norfluoxetine	56161-73-0	ND (RL=0.50)		(41)
				ND (DL=5-60)		(33)
				ND (MRL=0.50) (18 samples)	0.77 (1 of 15 samples)	(39)
	N07BC02	Metadone	76-99-3	0.1-2.6		(79)
N07BC02 metabolite	EDDP (methadone metabolite) [2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine]	30223-73-5	1.7-4.7		(79)	
Veterinary: Q01 Antibacterials for Systemic Use; QP51 Antiprotozoals	QJ01EQ08	Sulfaphenazole	526-08-9	ND (DL=5-75)		(33)
	QJ01EQ12	Sulfachlorpyridazine	80-32-0	ND (DL=5-75)		(33)
	QJ01FA90	Tylosin	1401-69-0	0.6-1.7 (1 of 3 DWTPs) (DL=0.25)		(34)
				4.2 (1 of 3 DWTPs)		(65)
	QJ01FA91	Tilmicosin	108050-54-0	ND (DL=0.75)		(34)
	QP51AG03	Sulfaquinoxaline	59-40-5	ND (DL=5-75)		(33)
QP51AH03	Monensin	17090-79-8	0.1-2.8 (3 of 5 DWTPs)		(54)	

Continued on next page.

Table I. (Continued). Compilation of Published Positive Occurrence Data for APIs in Finished Drinking Water (and select data on negative occurrence – indicated by shaded cells)

Respiratory System: R03 Obstructive Airway Diseases	R03AC02	Albuterol (Salbutamol)	18559-94-9	ND (DL=0.02)	(34)
					ND (6 tap water samples) (55)
	R03AC03	Terbutaline	23031-25-6		ND (6 tap water samples) (55)
R03AC14 R03AC13	Clenbuterol				ND (LOD=0.6; 6 tap water samples) (55)
Sensory Organs: S01 Ophthalmol ogicals	S01AB04	Sulfacetamine (Sulfacetamide)	144-80-9	ND (DL=5-75)	(33)
Various: V08 Contrast Media	V08AA01	Diatrizoate (Diatrizoic acid; Amidotrizoic acid)	117-96-4	85 max [21] (5 of 10 samples) (DL=10)	(46)
				32	(66)
				60	(80)
				18-100 (3 of 3 DWTPs)	(62)
				129-149	(81)
	V08AA04	Iotalamic acid (Iothalamic acid)	2276-90-6	10	(80)
				ND (DL=4)	(66)
	V08AA05	Ioxitalamic acid	28179-44-4	12	(66)
				ND (4 of 4 DWTPs) (DL=25)	(62)
	V08AB02	Iohexol	66108-95-0	57 (1 of 44 samples; 22 DWTPs over 2 years)	(38)
				38-40	(81)
				Iohexol transformation product	83
	V08AB04	Iopamidol	60166-93-0 62883-00-5	244	(82)
				up to 79 (4 of 10 samples) (DL=10)	(46)
				60	(66)
				70	(80)
				180 (1 of 4 DWTPs) (DL=25)	(62)
				72-98	(81)
	V08AB05	Iopromide	73334-07-3	57	(82)
				up to 177	(69)
				86 (1 of 10 samples) (DL=10)	(46)
				1.1-31	(36)
				4.6	(53)
				40	(80)
				33-36 [35] (2 of 44 samples; 22 DWTPs over 2 years)	(38)
				29 (1 of 4 DWTPs) (DL=10)	(62)
				69-77	(81)
				ND (DL=4)	(66)
	V08AB10	Iomeprol	78649-41-9	11	(66)
				81-92	(81)
				12 (1 of 4 DWTPs) (DL=10)	(62)
				Iomeprol	18 and 289

Continued on next page.

Table I. (Continued). Compilation of Published Positive Occurrence Data for APIs in Finished Drinking Water (and select data on negative occurrence – indicated by shaded cells)

			transformation products		
Schedule I & II Controlled Substances	illicit (also: N01BC01) (multiple)	Cocaine	50-36-2	ND (after ozonation & GAC) (LOD <80 µg/L)	(83)
	illicit (also: N01BC01) (metabolite)	Benzoylcegonine (primary metabolite of cocaine)	519-09-5	[45] with max of 130 (22 of 24 samples)	(83)
	illicit	MDMA (ecstasy: 3,4-methylenedioxy-methamphetamine)	69610-10-2	2-10 (after ozonation & GAC) ND (after post-chlorination)	(83)

^a ATC/DDD Index (Anatomical Therapeutic Chemical) classification system: <http://www.whocc.no/atcddd/>; AT vet Index: <http://www.whooc.no/atcvet/database/index.php>

^b DL: detection limit; GAC: granular activated carbon; LOD: limit of quantitation; max: maximum value in range; NF: nanofiltration; ND: not detected (less than DL or RL); (M)RL: (method) reporting limit; RO: reverse osmosis.

^c Richter et al. (67) provide an overview of the work done in Germany since the 1990s on a series of sulfonamides [*p*-toluenesulfonamide (*p*-TSA), *o*-toluenesulfonamide (*o*-TSA), and benzenesulfonamide (BSA)], which have had a steady presence in certain areas such as Berlin, which rely on ground waters recharged by rivers; their concentrations in ground waters reach up into the low µg/L range. The sources of these sulfonamides in the environment are not solely from antimicrobials (e.g., *p*-TSA is the primary degradation product of the disinfectant chloramine-T), as they also have a wide variety of other non-pharmaceutical uses. Richter et al. (67) provide some of the only data on finished drinking water for *p*-TSA, *o*-TSA, and BSA, which are detected at ng/L concentrations (95th percentile) up to 240, 160, and 60, respectively.

Table II. Summary of APIs Identified in Finished Drinking Water (ranked according to total number of references providing occurrence data within and among each ATC primary group)

API	ATC code	# references ^a	High range ^b [mean range], ng/L
Nervous System: N02 Analgesics; N03 Antiepileptics; N05 Psycholeptics; N06 Psychoanalgetics; N07 Other Nervous System Drugs			
		TOTAL = 59	Grand Maximum = 400
Carbamazepine	N03AF01	20	0.7-721 [1.0-6.0]
Dilantin (Phenytoin)	N03AB02	6	1.2-32 [1.3-9.4]
Meprobamate (also a metabolite of the prodrug carisoprodol)	N05BC01	6	5.9-43 [5.7-9.2]
Acetaminophen (Paracetamol)	N02BE01	4	0.3-210
Phenazone (Antipyrine)	N02BB01	4	29-400
Fluoxetine	N06AB03	3	0.82-10
Risperidone	N05AX08	3	0.3-23.5
Acetylsalicylic acid	N02BA01; A01AD05	2	<100
Primidone (2-Deoxypheno-barbital)	N03AA03	2	1.3-16
Propyphenazone (Isopropyl-antipyrine)	N02BB04	2	80-120
Diazepam	N05BA01	2	0.33-23.5
Amitriptyline	N06AA09	1	1.4
10,11-Dihydroxy-10,11-dihydro-carbamazepine	N03AF01 metabolite	1	13
Norfluoxetine	N06AB03 metabolite	1	0.77
Methadone	N07BC02	1	0.1-2.6
EDDP (methadone metabolite)	N07BC02 metabolite	1	1.7-4.7
Cardiovascular System: C03 Diuretics; C04 Peripheral Vasodilators; C07 Beta Blocking Agents; C08 Calcium Channel Blockers; C10 Lipid Modifying Agents			
		TOTAL = 31	Grand Maximum = 330
Clofibric acid	C10AB01 metabolite	10	1.1-270 [59]
Gemfibrozil	C10AB04	9	2.1-70 [0.4-1.0]

Continued on next page.

Table II. (Continued). Summary of APIs Identified in Finished Drinking Water (ranked according to total number of references providing occurrence data within and among each ATC primary group)

Bezafibrate	C10AB02	4	1.9-27 [0.7-17]
Atenolol	C07AB03	2	18-26 [1.2-2.8]
Dehydronifedipine	C08CA05 metabolite	2	0.6-4
Fenofibrate	C10AB05	1	21 [18]
Fenofibric acid	C10AB05 metabolite	1	42
Hydrochlorothiazide	C03AA03	1	117-330 [2.8-71]
Metoprolol	C07AB02	1	13.5-26 [2.6-20]
Various: V08 Contrast Media			
		TOTAL = 26	Grand Maximum = 244
Iopromide	V08AB05	8	1-177
Iopamidol	V08AB04	6	60-244
Diatrizoate (Diatrizoic acid; Amidotrizoic acid)	V08AA01	5	32-149
Iomeprol	V08AB10	3	11-92
Iohexol	V08AB02	2	38-57
Iotalamic acid (Iothalamic acid)	V08AA04	1	10
Ioxitalamic acid	V08AA05	1	12
Musculo-skeletal system: M01 Antiinflammatory & Antirheumatic Products			
		TOTAL = 25	Grand Maximum = 4,950
Ibuprofen	M01AE01	13	1-1,350 [3.4-930]
Naproxen	M01AE02; M02AA12	5	0.2-8
Diclofenac	M01AB05 (multiple)	4	2.5-18
Ibuprofen methyl ester	M01AE01 metabolite	1	4,950
Ketoprofen	M01AE03	1	3.0
Mefenamic acid	M01AG01	1	19.9 [1.9]
Antiinfectives for Systemic Use: J01 Antibacterials			
		TOTAL = 22	Grand Maximum = 80 [22]
Sulfamethoxazole	J01EC01	11	0.3-80 [0.39-22]
Sulfadimethoxine	J01ED01	2	7.0-11
Erythromycin	J01FA01 (multiple)	2	1.3-4.9

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Table II. (Continued). Summary of APIs Identified in Finished Drinking Water (ranked according to total number of references providing occurrence data within and among each ATC primary group)

Flumequine	J01MB07	1	2.5
Ofloxacin	J01MA01	1	1.6
Oxolinic acid	J01MB05	1	4
Roxithromycin	J01FA06	1	1.4
Sulfamethizole	J01EB02 (multiple)	1	9
Trimethoprim	J01EA01	1	1.3
Sulfonamides (misc): <i>p</i> -Toluenesulfonamide <i>o</i> -Toluenesulfonamide Benzenesulfonamide	J01 (miscellaneous)	1	60-240
Genito Urinary System & Sex Hormones: G03 Sex Hormones & Modulators of the Genital System			
		TOTAL = 13	Grand Maximum = 2.3
Estrone	G03CA07	4	0.2-2.3
Progesterone	G03DA04	2	0.57-1.1
17 α -Ethinylestradiol	G03CA01	2	<5
17 β -Estradiol	G03CA03	2	<320
17 α -Estradiol	G03CA03	1	0.3
Norethisterone (Norethindrone)	G03AC01	1	<10
Estrone-3-sulfate	G03CA07 metabolite	1	0.22
Dermatologicals: D01 Antifungals; D06 Antibiotics & Chemotherapeutics; D08 Antiseptics & Disinfectives			
		TOTAL = 7	Grand Maximum = 734
Triclosan	D08AE04	4	1.2-734
Salicylic acid (also transformation product)	D01AE12; S01BC08	2	122 [39]
Chloramphenicol	D06AX02 (multiple)	1	13 [13]
Veterinary: QJ01 Antibacterials for Systemic Use; QP51 Antiprotozoals			
		TOTAL = 3	Grand Maximum = 4.2
Tylosin	QJ01FA90	2	1.7-4.2
Monensin	QP51AH03	1	2.8

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Table II. (Continued). Summary of APIs Identified in Finished Drinking Water (ranked according to total number of references providing occurrence data within and among each ATC primary group)

Schedule I & II Controlled Substances			
		TOTAL = 2	Grand Maximum = 130
Benzoylecgonine (primary metabolite of cocaine)	illicit (also: N01BC01) (metabolite)	1	130 [45]
MDMA (ecstasy: 3,4-Methylenedioxy-methamphetamine)	illicit	1	10
TOTAL APIs = 54 (plus 10 metabolites)		TOTAL distinct references presenting FDW data = 48	

^a The number of references providing quantitative data for each API. TOTAL is the sum of the numbers of individual references within an ATC class providing data.

^b For each API, the high range is the range of the maximum values [or means] reported by all references for each API. Grand Maximum is the highest value reported within an ATC class.

Table III. Top Three APIs within Each ATC Class Ranked According to Frequency of Quantitative Data and According to Highest Ranges in the Published Literature

APIs most frequently reported ^a	Number of Reports	APIs reported at highest levels within each class ^a	High Range
N: Nervous System		Total APIs = 15	
Carbamazepine*	20	Carbamazepine*	0.7-721
Dilantin	6	Phenazone*	29-400
Meprobamate	6	Acetaminophen*	0.3-210
J: Antiinfectives for Systemic Use		Total APIs = 10	
Sulfamethoxazole*	11	Sulfamethoxazole	0.5-80
Sulfadimethoxine	2	Sulfadimethoxine	7.0-11
Erythromycin	2	Erythromycin	1.3-4.9
V: Various		Total APIs = 10	
Iopromide*	8	Iopamidol	60-244
Iopamidol	6	Iopromide	1-177
Diatrizoate	5	Diatrizoate	32-149
C: Cardiovascular System		Total APIs = 9	
Clofibric acid*	10	Clofibric acid*	1.1-270
Gemfibrozil*	9	Gemfibrozil	2.1-70
Bezafibrate	4	Bezafibrate	1.9-27
G: Genito Urinary System & Sex Hormones		Total APIs = 7	
Estrone	4	Estrone	0.2-2.3
Progesterone	2	17 β -Estradiol	<320
17 α -Ethinylestradiol	2	Progesterone	0.57-1.1
M: Musculo-skeletal system		Total APIs = 6	
Ibuprofen*	13	Ibuprofen*	1-1,350
Naproxen	5	Diclofenac	2.5-18
Diclofenac	4	Naproxen	0.2-8
D: Dermatologicals		Total APIs = 3	
Triclosan	4	Triclosan*	1.2-734

Continued on next page.

Table III. (Continued). Top Three APIs within Each ATC Class Ranked According to Frequency of Quantitative Data and According to Highest Ranges in the Published Literature

Salicylic acid	2	Salicylic acid	122
Q: Veterinary		Total APIs = 2	
Tylosin	2	Tylosin	1.7-4.2

^a ATC classes arranged in descending order of number of reports per class. For each class is included only those APIs reported in more than one paper. The six APIs among all reported with the highest frequencies of reports and the six among those with the highest reported rough concentration ranges are each **bolded with asterisks**.

Table IV. Summary of APIs Reported - and Some Not Reported - in Finished Drinking Waters (grouped according to ATC code)

<i>ATC code</i>	<i>ATC main group (1st level)</i>	<i>ATC classes reported in FDW^a</i>	<i>ATC classes not reported in DW^a</i>	<i>example of API not reported^b</i>
A	Alimentary tract and metabolism	none	A01-A10	cimetidine omeprazole
B	Blood and blood forming organs	none	B01-B06	clopidogrel warfarin
C	Cardiovascular system	C03,07,08,10	C01,02,04-06,09	propranolol diltiazem
D	Dermatologicals	D01,06,08	D02-05,07,09-11	triclocarban diphenhydramine
G	Genito urinary system and sex hormones	G03	G01,02,04	testosterone ketoconazole
H	Systemic hormonal preparations (excl. sex hormones and insulins)	none	H01-H05	fludrocortisone methylthiouracil
J	Anti-infectives for systemic use	J01	J02-J07	fluconazole miconazole
L	Antineoplastic and immunomodulating agents	none	L01-L04	fluorouracil methotrexate
M	Musculo-skeletal system	M01	M02-M09	probenecid phenylbutazone
N	Nervous system	N02,03,05-07	N01,04	lidocaine selegiline

Continued on next page.

Table IV. (Continued). Summary of APIs Reported - and Some Not Reported - in Finished Drinking Waters (grouped according to ATC code)

ATC code	ATC main group (1st level)	ATC classes reported in FDW ^a	ATC classes not reported in DW ^a	example of API not reported ^b
P	Antiparasitic products, insecticides and repellents	none	P01-P03	ivermectin metronidazole
Q	<i>Veterinary (15 classes parallel to ATC)</i>	QJ01, QP51	all others	many are the same as human APIs
R	Respiratory system	none	R01-R07	albuterol terbutaline
S	Sensory organs	few APIs are specific to this class, having assignments to other classes		
V	Various	V08	V01,03,04,06-10,20	radiologicals physostigmine

^a 2nd level codes (therapeutic subgroup). Note that some APIs belong to two or more ATC 1st or 2nd level groups. ^b From 2nd level class having no APIs reported in drinking water.

Limitations of Published Data and Caveats in Data Interpretation

APIs remaining in FDW (but prior to POU) can undergo further reductions in concentrations. These reductions result from various physicochemical (including oxidation by chlorine residual) and biological (e.g., biofilm) processes occurring in the distribution system leading from the DWTP, as well from whatever treatment processes the consumer might use at the POU water fixture (such as carbon sorption or reverse osmosis). For example, Gibs et al. (84) examined the potential for APIs to persist in FDW (assuming they survived treatment) after exposure to residual chlorine in FDW distribution systems. They evaluated the persistence of roughly 30 APIs/metabolites. The majority of the initial residues for each of 14 APIs remained after 1 day, but only for five APIs (carbamazepine, dehydronifedipine, erythromycin-H₂O, gemfibrozil, and ibuprofen) did the majority of their residues remain after 10 days. Note that four of these five refractory APIs are among those reported to occur in FDW (see Table I), corroborating their resistance to removal by chlorine oxidation.

It is important to keep in mind that some of the data on FDW is from DWTPs that only use minimal finishing - usually just depth filtration. Sometimes the source waters are exceedingly contaminated with a select few APIs - as a result of spills or seepages from leaking sewer pipes, septic systems, or landfills, or because of artificial recharge or manufacturing facilities; for example, see Fick et al. (85), who documented concentrations in the mg/L range from manufacturing discharges. These data are not representative of the API content of FDW in general, especially that produced by large municipalities.

Also extremely important in evaluating the published data is the great unevenness in study scope and among the individual approaches for sampling, analysis, and application of quality assurance. Comparing data among the various published studies is fraught with a wide array of problems, including an unknown (and possibly very large) amount of quantitative and perhaps even qualitative (structural) error; the accuracy of structural identification of API unknowns in FDW is often not verified. Intercomparisons among studies such as those in this review are certainly very crude without thoroughly examining each study in much greater depth. These limitations must be kept in the foreground when trying to compare data across these disparate studies. **As such, the generalizations derived in this document should be used with at least a mild degree of skepticism. The limitations of this document point to how future overviews of APIs in FDW could be greatly improved.**

No systematic surveys have ever been conducted across countries using standardized methodologies with well-defined LOQs (preferably below 1 ng/L) for a broad range of APIs having the potential to be present (e.g., APIs with well-known occurrence in source waters or at least in wastewaters that contribute to source waters). Many published works report on detectable levels of APIs in FDW but at concentrations below the LOQs. At such low levels, many quality assurance issues arise, not the least of which is the contamination of samples from trace residues of an API (or endogenous steroid) residing on the skin of an analyst (originating from either topical application or excretion via sweat) (86).

Better-Informing the Boundaries of APIs in Finished Drinking Water

Although the focus of this document is solely on the reported levels of APIs in FDWs, data for source and raw waters (and even sewage) could serve an important role in establishing the range of APIs having the potential to occur in FDW, depending on the efficiencies of whatever treatment processes are used.

To obtain perspective on the possible boundaries for API occurrence and concentrations in FDW, the voluminous published data on influent and effluent concentrations from STPs could be evaluated. This will not be done here, but by way of example, a recent study of nine POTWs targeted 58 APIs (87). Of the 58 targeted APIs, 56 were detected in the influent for at least one POTW (34 occurring in the majority of samples) and 31 detected in the effluent from at least one POTW (10 occurring in the majority of samples: carbamazepine, clarithromycin, dehydronifedipine, erythromycin, fluoxetine, gemfibrozil, metformin, sulfamethoxazole, thiabendazole, and triclocarban). Influent levels generally ranged from low ng/L up to several $\mu\text{g/L}$, with some excursions into 101-250 $\mu\text{g/L}$ (e.g., triclocarban, triclosan, cimetidine, metformin, and ranitidine). Effluent concentrations were generally well below 1 $\mu\text{g/L}$, with some excursions up to the low $\mu\text{g/L}$ range (e.g., 4-epianhydrochlortetracycline, digoxin, metformin, and norfloxacin).

This type of data is useful for targeting APIs that might have the highest potential to occur in drinking water, as well as for delineating the upper possible

bounds for FDW concentrations. For example, of the 15 targeted hormones (87), none were detected in any STP effluent sample, greatly reducing the probability that they could occur in DWTP intakes - much less in FDW. Of the 10 APIs most often present in effluents, little evidence exists for the occurrence of four of them in FDW (clarithromycin, metformin, triclocarban, thiabendazole), while the other six have been reported (carbamazepine, dehydronifedipine, erythromycin, fluoxetine, gemfibrozil, sulfamethoxazole; see Table I). Of the four reported in the highest concentrations in STP effluents, little evidence exists for their presence (or absence) in FDW (4-epianhydrochlortetracycline, digoxin, metformin, norfloxacin; see Table I). For these seven APIs that have not been reported in FDW, the extent of published negative data is not known (that is, how frequently they have been targeted but never found).

Establishing Priority API Targets and the Role of Negative Data

The evaluation presented here focuses on positive occurrence data. The English literature was examined comprehensively (using reference (2)), with inclusion of certain major publications in other languages. While the compiled FDW data (Table I) is rather comprehensive for the English literature, it is missing an unknown portion of data published in a variety of non-English journals and reports. It would be expected that a significant body of additional data for APIs in FDW also exists but is unpublished or proprietary. For example, a wide array of APIs are monitored by Dutch DWTPs, particularly at intake points along the Rhine; see van der Aa ((88), pages 51-54) and GWRC (89). Some of the data in Dutch databases has been captured by Schriks et al. (90). Some of the additional German and Scandinavia data are compiled in Hembrock-Heger and Bergmann (91).

Only a limited amount of negative data was included in Table I. These data of absence captured in Table I were selected primarily as examples and almost always derived solely from the limited numbers of studies that also provided positive data. Compiling negative data and especially data from the third category (absence of data) would entail major efforts in mining the published literature; exhaustive data compilations from these two categories have not been published. Note that the positive occurrence data for FDW in Table I are presented without notation of the geographic locale or the type of finishing treatment used, which were not deemed necessary for the purposes of this overview.

Reducing the great uncertainty surrounding the absence of data, one of the more promising approaches for expanding the identification of APIs not yet reported but possibly occurring in FDW would be the more widespread application of nontarget analysis (e.g., via accurate mass screening); see Hogenboom et al. (92) for an example. More comprehensive characterization of those APIs not yet identified is critical for ensuring holistic assessment of risk (93).

Given the numbers of APIs that could potentially enter FDW, it is important to formulate a strategy for establishing a limited set of priority targets. Source water data can be used to inform the targeting of APIs in finished DW. Maximum concentrations in source waters or raw waters establish the upward boundaries

for concentrations in FDW (assuming that conjugates no longer persist). Such data can inform the prioritization process. An important source of complementary information to mine from the published literature is those APIs that have been targeted in either source waters or in raw or finished drinking waters and verified as not occurring above the LODs (given the specific treatment parameters). These APIs would have an extremely low probability of routine occurrence in FDW. The value of data of absence increases as the LOQs or LODs of the methods are reduced. Low detection limits are critical with respect to highly potent APIs such as hormones, which need LOQs below 1 ng/L. Without the context of the LOQs, negative data cannot be interpreted. Many challenges are faced in ultra-low-level analysis. Some are described by Briciu et al. (94).

For perspective on the types and quantities of APIs that can occur in source waters for FDW, consider the broad surveys of Barnes et al. (95) and Focazio et al. (96). The latter study found that 60% of the 36 targeted APIs were not detected in any source water sample; clearly these particular APIs might be ones that would be rarely detected in FDW in the study areas. On the other hand, this same study found carbamazepine was one of the five most frequently detected APIs in ground waters (being detected in 21% of the samples) with five other APIs being detected over 5% of the time: acetaminophen, diphenhydramine, enrofloxacin, erythromycin-H₂O, and trimethoprim. Of these six APIs, carbamazepine is the most frequently reported API in FDW (see Table III), and FDW occurrence data exists for erythromycin and acetaminophen. It is not known if enrofloxacin or diphenhydramine have been targeted in FDW.

For perspective on the types of APIs for which negative data have been reported, consider the studies of: Buseti et al. (97), providing some of the most extensive data obtained in Australia; Fawell et al. (98); Garcia-Ac et al. (99); Jux et al. (100), providing negative FDW data for seven APIs in various locales in Germany (but with LODs around 5 ng/L); Rodriguez-Mozaz et al. (63) and Stavrakakis et al. (101), applying methods with largely sub-ng/L LOQs for a number of estrogens and conjugates; Snyder, Trenholm, Snyder et al. (9); Togola and Budzinski (55), providing rare tap water data at sub-ng/L; Watkinson et al. (68), applying a method for a broad suite of 28 antibiotics across wastewaters, source waters, and FDW in South-East Queensland, Australia [showing frequent API occurrence in all waters (up to 64 µg/L in sewage influent) but absence of all 28 APIs in FDW from 20 different sites (with the LODs for 21 of the analytes being 20 ng/L or lower)]; Wenzel et al. (62), providing negative data for estrogens; and Ye et al. (65), providing a survey of 24 antibiotics with the absence of 18 of 24 in three DWTPs (but with LODs for 21 of the analytes only being 20 ng/L or lower). Petrovic et al. provide a general overview of the APIs occurring in and removed from wastewaters and surface waters (102).

Contaminants Generated from APIs during Drinking Water Treatment

Possibly as important as the APIs that might be present in FDW (a strict function of those present in respective source waters) are those substances not

necessarily present in the source water but rather created from APIs during the finishing steps used for the FDW. These chemicals include disinfection by-products (DBPs) and transformation products that are unique to APIs. Of course, a parallel issue concerns the unique transformation products that can be created from APIs by biological and abiotic processes during their transport in the environment.

Although APIs themselves have been the focus of concern for the public and legislators, a potentially large spectrum of other chemicals that can be generated from APIs during treatment or by natural processes may also be present in FDW. Monitoring studies that verify an API's absence from FDW (i.e., below the LOD) almost always fail to account for reaction products. These potential contaminants include DBPs unique to APIs as well as other unique biotransformation products (such as from bacterial metabolism). API-derived chemicals will also include many of the common lower-molecular weight DBPs that share origins from oxidation of a plethora of other organic constituents. The halogenated products (those containing chlorine, bromine, or iodine) are of particular toxicological concern. As an aside, at least one common DBP - chloral hydrate - is an API itself; chloral hydrate is still used in medicine and it is unknown what portion of the trace levels in chlorinated waters originates directly from API residues rather than the disinfection process acting on non-APIs.

There have been few surveys of API-derived chemicals in FDW; most studies have focused on laboratory model systems (103–105) or on samples from DWTPs prior to final polishing. This includes those originally present in the source water (human metabolites and environmental transformation products) as well as those created during drinking water treatment, such as DBPs or other products of conventional or advanced oxidation processes (e.g., ozonation, chlorination via chlorine or chlorine dioxide, TiO₂-oxidation, UV/peroxide irradiation, and others). Oxidation mediated by hydroxyl radicals can yield numerous intermediates and end-products from a single reactant, especially from heterocycles. These processes can create a complex array of new chemical structures, most of which are more polar and of lower molecular weight than the parent API (halogenated, hydroxylated, cleaved rings), and many of which are isomeric and more persistent. Although reaction intermediates/end products can sometimes express combined toxicity greater than the parent APIs (106), Reungoat et al. (107) found reduced toxicity after ozonation in a water reclamation plant. Narotsky et al. (108) reported no gross effects in rats fed potable disinfected waters containing hundreds of DBPs from miscellaneous chemical contaminants.

While research has been done on the types of products that could potentially be produced during treatment (e.g., from bench- and pilot-scale controlled studies or via biotransformation), very little field monitoring has been performed to identify and quantify those products that are actually present in FDW, especially POU water. General overviews and some specific studies on API transformation products and DBPs have been published by: Dodd and Huang (109), Huber et al. (110), Kormos et al. (82), Kosjek et al. (111), McDowell et al. (112), Quintana et al. (113), Radjenovic et al. (106), Zwiener (114), and Zühlke et al. (74, 115), among others.

Even less is known regarding the potential for transformation (such as by biofilms) within FDW distribution systems. Other factors adding further complexity include the potential for certain reaction products to revert back to the original API, as reported for the N-chlorinated intermediate from sulfamethoxazole when free chlorine is insufficient (109).

Of possible utility in gaining better perspective on the types of API-related chemical unknowns that might occur in FDW is the existing base of knowledge derived from the pharmaceutical industry's testing for degradation-related impurities (DRIs) and the products from "stress testing"; an overview of DRIs is available from Baertschi (116). This base of knowledge has never been evaluated for its possible relevance.

Limitations of Comparisons of Data from Different Studies

The types and quantities of APIs in FDW are intimately tied to variables that vary dramatically across studies - especially studies in different countries. Whether an API survives into FDW obviously depends first on whether it is even an ingredient in medicines used by the local populace and whether it has any potential to survive the many steps and barriers before it reaches source water. Sometimes an API is not detected in FDW because it was never present in the source water - other times because it was efficiently removed by the treatment processes or was below analytical reporting limits.

A plethora of factors dictates whether an API can establish a presence in source waters (such as whether it is excreted or disposed to sewage in sufficient amounts). But some factors depend on the specific geographic location of the DWTP. The major factors among these are the geographic prescribing practices (which dictate the types and quantities of APIs in the subset of medications most prescribed locally), the technologies used for treating raw sewage, the degree of dilution occurring before the treated sewage mixes into the source waters (effluent-dominated streams and those receiving raw sewage, such as from overflow events, will have higher API levels), weather and season (temperature, UV irradiance, and precipitation all play important roles in the efficiency of sewage treatment, the extent of natural transformation processes, and the degree of dilution), the technologies used for treating and finishing the raw drinking water (which vary from minimal to advanced), and whether the consumer employs further treatment at the point of use. The last point greatly lessens the significance of most FDW data, which is usually obtained from sampling done at the DWTP - not at the point of use.

The great number of sequential steps that serve to reduce the probability of any given API entering FDW at a detectable concentration combine to yield concentrations of APIs so low in FDW that they were not routinely and reliably detectable even 10 years ago. Of all the documents with FDW data on APIs, roughly only a dozen or so were published before 2000, and the vast majority published only since 2006.

Table I presents the data compiled from all published reports evaluated for this study. This is perhaps the most comprehensive compilation to date of data

mined from the published literature on the occurrence of APIs (and metabolites) in FDW, expanding on that first presented in 2008 (3). A total of 48 documents have reported occurrence information for APIs in FDW (Tables I and II); this represents a minimum number of studies that have examined FDW. These documents provide both quantitative and qualitative occurrence data. A limited amount of negative occurrence data (data of absence) are also included; these data of absence, however, are probably not representative of the full scope of types of APIs that have been targeted but never detected during monitoring. In many cases of data of absence, parallel positive occurrence data exist for the same API. It is critical to keep in mind, however, that these published data cannot be considered as statistically representative of API occurrence in drinking water from any particular locale. With very few exceptions, each of these studies was extremely limited in scope and employed various methods of analysis and quantitation and quality control measures; no attempt was even made in this examination to determine the veracity of actual identification of a targeted API.

Anatomical Therapeutic Chemical (ATC) Classification System

Given the thousands of distinct APIs used worldwide (formulated into tens of thousands of different medical products), it is critical to use an organizing framework around which to make sense of the reported data. This facilitates intercomparisons of data. The drug classification system of the World Health Organization (WHO) is used in this examination: the Anatomical Therapeutic Chemical (ATC) classification system. The ATC classification attempts to link APIs to their intended therapies. APIs with similar physicochemical properties often exhibit similar biological activity and will therefore group together. Some APIs have numerous trade and generic names; the ATC forces these to be grouped together. The ATC comprises more than 800 hierarchical classes that span five levels within 14 main groups; parallel ATC systems exist for human and veterinary drugs. APIs in the same 5th-level ATC class have higher probabilities of sharing common mechanisms or modes of action. APIs among the same class might therefore be expected to act via combined action; APIs in different classes have potential for interactive effects. Those sharing the same 5th-level ATC class can be readily seen in Table II. One consequence is that the individual concentrations of APIs from the same class might possibly be summed for the purposes of assessing risk. An example of its prior use for APIs as environmental contaminants is provided in Ruhoy and Daughton ((117), see Table 5 therein). Note that the challenges posed for environmental monitoring in selecting which of the thousands of molecularly distinct APIs to target are multiplied further not just by isomers composing racemates and further yet by multitudes of products from metabolism and transformation, but also by emerging aspects of drug design such as deuterated analogs and structural analogs; the latter are being increasingly synthesized as unapproved drugs, and their society-wide usage rates are unknown.

Major Findings Distilled from the Published Literature

The summary of positive occurrence data in Table II is distilled from Table I. The negative occurrence data in Table I (including qualitative data when LOQ levels were below 1 ng/L) were removed. For each API, the numbers of references that measured the API in FDW were totaled. Within the appropriate ATC group, the APIs were sorted according to the numbers of references reporting positive occurrence data. These publication numbers are then summed within each ATC group, and the groups sorted according to the summed number of publications.

The APIs reported to occur in FDW belong to the following ATC primary classes, ranked in descending order of prevalence: N (Nervous System) [59 individual reports], C (Cardiovascular System) [31], V (Various) [26], M (Musculo-skeletal system) [25], J (Antiinfectives for Systemic Use) [22], G (Genito Urinary System & Sex Hormones) [13], D (Dermatologicals) [7], Q (Veterinary) [3], and Controlled Substances [2]. **The top four classes with the most APIs (N, C, V, and M) are perhaps the classes that human-health risk assessments could focus on, especially with respect to those APIs that might share a common mechanism or mode of action.** However, since ATC V comprises exclusively iodinated X-ray contrast media, which are established as having extremely low toxicity, the next class to consider would be ATC J (the antiinfectives).

The summary of occurrence data in Table III is distilled from Table II. Published quantitative data for FDW exists for 64 APIs and transformation products (54 APIs and 10 metabolites or transformation products). Significantly, however, for these 64 substances only 17 have corroborating quantitative data from more than two reports each. The numbers of unique APIs reported in each ATC class, ranked in descending order, are: N [15], J [10], V [10], C [9], G [7], M [6], D [3], and Q [2]; illicit controlled substances comprised 2 APIs. The top three APIs within each ATC class are ranked according to frequency of quantitative data in the published literature. As in Table II, the ATC classes are arranged in descending order of number of total measurements per class for all APIs combined. For each class is shown only those APIs reported in more than one paper.

There were 54 APIs/metabolites that had been targeted in a variety of studies but not detected above reporting limits (see Table I). But of these 54, only nine had corroborating negative data from two or more studies. Since these negative data were compiled from only a portion of the studies that reported positive data for other APIs, the number of APIs for which only negative data exist is most likely higher.

From Table I, **of the studies that have surveyed the most APIs at once in FDW, none has identified more than a dozen APIs in any given sample.** The 10 studies that targeted the most APIs in FDW (ranging from 42 to 8 APIs), in decreasing order, are: Tauber (33), Benotti et al. (39), Snyder et al. (36), Vanderford and Snyder (41), Zuccato et al. (34), Togola and Budzinski (55), Ternes (46), Stackelberg et al. (42), Illinois EPA (54), and Bruchet et al. (66).

From Table III, **it is evident that the six most frequently reported APIs in FDW are: carbamazepine [20 reports], ibuprofen [13], sulfamethoxazole**

[11], clofibric acid [10], gemfibrozil [9], and iopromide [8]. The six APIs with roughly the most consistent highest reported concentrations in FDW are: ibuprofen [1,350 ng/L maximum], triclosan [734], carbamazepine [721], phenazone [400], clofibric acid [270], and acetaminophen [210]. Of the 64 APIs/metabolites, none (with only one exception - a single data point for ibuprofen) exceeded a concentration of 1 ppb (1 $\mu\text{g/L}$).

From Table II, it can also be seen that **of the 64 APIs/metabolites quantified in FDW, nearly half of them (28 APIs) have been reported individually only by a single study**. So for only 36 APIs has positive occurrence data been corroborated in at least a second study.

Of the APIs/metabolites targeted in the planned "Emerging Contaminant Sampling Program" (20), the following additional analytes are also being considered (among possibly others): 17 β -estradiol, 17 α -ethynylestradiol, clofibric acid, diclofenac, estrone, and naproxen.

For no ATC main group have APIs representing all 2nd-level classes been reported in FDW (Table IV). For six of the 14 ATC main groups, no API has been reported: A, B, H, L, R, or S. Examples for each of the ATC main classes are provided in Table IV. Notably, no antineoplastic or immunomodulating agent has been reported, nor have any radiologicals. Little data exist for any type of drinking water on the occurrence of rare earths or radionuclides used in diagnostics and treatment; one of the few examples is the positive gadolinium anomalies identified in well-water supplies for drinking water in France (118). Finally, note that certain ATC classes have APIs that can originate from other sources, such as pesticides (ATC group P); these have not been included in this survey.

General Observations and Insights

The following are some of the key observations and insights derived from Tables I, II, and III.

API levels reported in FDW rarely ever exceed 1 ppb. The vast majority are probably below 50 ng/L. Many have maximum reported concentrations of only several ng/L. To place these ppt concentrations of APIs into perspective, many halogenated DBPs in FDW occur at concentrations well above 1 $\mu\text{g/L}$ (e.g., see: (119)).

Detection limits pose a major challenge in comparing data across studies. LODs can vary by an order of magnitude or more. A positive finding in one study could easily have been a negative finding in another having a higher LOD. At the same time, as LODs are pushed inexorably lower, increasing numbers of APIs (as well as vast numbers of other ultra-trace contaminants) will continue to be revealed.

Veterinary Medicines

Two APIs used primarily in veterinary medicine (monesin, tylosin) have been identified in FDW. A number of other APIs also share human and veterinary uses, so generalizations as to their origins are not possible.

Possible Outliers

Some of the individual APIs or instances of seemingly spurious high concentrations occur only in unique and relatively rare situations not translatable to most other locales - such as those using nominal treatment or where large quantities of APIs have entered source water (e.g., from landfills, manufacturing or hospital waste streams, or groundwater recharge). For this reason, most of the data for these unusual situations was not compiled. This includes data from private wells. This was the case with some of the historic data from Berlin.

X-ray Contrast Media

X-ray contrast media are established as being among those APIs most difficult to remove in water treatment. Their presence reflects the fact that a water's origin is at least partly contributed by sewage and that other APIs therefore also have the potential to be present. A corollary is that the absence of these iodinated chemicals points to an increased probability that other APIs may not be detectable.

Bottled Water

Given that bottled water provides a significant source of drinking water for many people (120), the extremely limited data for APIs in bottled water is notable - being limited to the two studies of Perret et al. (64) and Naidenko et al. (75), with positive occurrence data presented for four APIs. A recent examination of bottled water for total estrogenic activity evaluated 20 brands of mineral water commonly available in Germany (121) and provides the first indication that contamination of bottled mineral water by estrogenic chemicals may be widespread. The bottles ranged with values from 2–40 ng/L estradiol equivalents (with a maximum of 75 ng/L estradiol equivalents). Plastic bottles typically had higher values than glass, pointing to an origin associated with the plastic rather than the water's source; APIs, therefore, might not be expected to play a significant role in terms of estrogenicity in bottled water.

Antibiotics

Antibiotics pose concerns removed from those of other API classes. The potential for selection of antibiotic-resistant pathogens from exposure to low levels in the environment is often cited as a major concern. While the low levels in the environment, which rarely ever exceed a small fraction of 1 $\mu\text{g/L}$, may pose concerns with regard to microbial community structures in native environments (122), no evidence has emerged regarding the potential for any type of effect from the ng/L concentrations that occasionally occur in FDW. Perhaps of more interest might be the possible role of biofilms in distribution pipes as a source of resistant bacteria and antibiotic resistance genes (ARGs) (123); ARGs as pollutants in their own right have received growing attention (124–126). Also of interest is that low-levels of antibiotics might affect the functioning of biofilms in drinking water distribution systems or in release of cells from biofilms; while still too high for

FDW, concentrations of 500 ng/L of phenazone, amoxicillin, or erythromycin in FDW affected the initial surface-adhesion of bacteria (sometimes enhancing it and other times inhibiting it) (127).

Reverse Osmosis

Little data has been published on the removal of APIs by full-scale reverse osmosis. Radjenovic et al. (35) published one of the only examinations of APIs handled by DWTPs using reverse osmosis (RO) and nanofiltration (NF). Of 31 APIs targeted in the source ground water, 12 (acetaminophen, carbamazepine, diclofenac, gemfibrozil, glibenclamide, hydrochlorothiazide, ketoprofen, mefenamic acid, metoprolol, propyphenazone, sotalol, sulfamethoxazole) were frequently detected at average concentrations ranging from 4.3 ng/L (sotalol) to 137 ng/L (ketoprofen), with excursions up to the hundreds of ng/L (carbamazepine, diclofenac, gemfibrozil, ketoprofen, propyphenazone) or thousands of ng/L (hydrochlorothiazide). Full-scale NF and RO DWTPs consistently eliminated all but four APIs to average concentrations below the detection limit. Average concentrations remaining in the finished drinking water (permeate) for hydrochlorothiazide, metoprolol, carbamazepine, and mefenamic acid were all less than 8 ng/L, with excursions of hydrochlorothiazide up to 117-330 ng/L. Of significance, however, were the residues of all 12 APIs that remained in the concentrate (brine stream). These ranged from averages of 0.8 ng/L (mefenamic acid) to 429 ng/L (ketoprofen), with excursions up to 520 (diclofenac), 692 (carbamazepine), 695 (ketoprofen), and 6,336 (hydrochlorothiazide). This points to the problem associated with physical removal processes (e.g., activated carbon, membrane filtration), which generate a waste stream with APIs at concentrations 3- to 5-fold higher than in the raw source waters; these brine streams are often then discharged.

Illicit Drugs

Finally, given that illicit drugs experience broad use throughout society and given their marked biological effects and potencies, surprisingly little data is available on their occurrence in FDW (or the presence of their metabolites or synthesis products). Historically, interest in the occurrence of pharmaceutical ingredients in the environment (including FDW) has focused almost exclusively on the APIs contained in medications and diagnostics dispensed legally by pharmacies and consumed for their accepted medical purposes. In parallel, however, a huge market exists for a variety of drugs that are sold illegally. Some of these drugs also have legitimate medical uses, but the remainder have no known medical uses; many of the latter are included on (or are covered by) the DEA's list of Schedule I controlled substances - those substances that have "no currently accepted medical use in treatment in the United States." This group is informally termed "illicit drugs" and includes the substances obtained illegally belonging to the general groups: anabolic steroids, narcotics (opiates), stimulants, depressants (sedatives), hallucinogens, and cannabis. More accurately, illicit

drugs are those drugs that are trafficked or consumed illegally – including those that are manufactured legally.

The striking aspect of illicit drugs is that their active ingredients clearly have marked potential for biological effects - some being quite potent - but comparatively little attention has been devoted to whether the ingredients from those drugs having major illicit markets occur in the environment. Almost no attention has focused on whether the active ingredients in illicit drugs occur in FDW. The two groups of ingredients - legal and illicit - should be considered seamlessly in characterizing and assessing risk incurred from environmental exposures.

The two major studies to date that examine illicit drugs in drinking water are Huerta-Fontela et al. (83) and Boleda et al. (79). Boleda targeted five opiates in raw drinking water in Spain: morphine, codeine, norcodeine, methadone, and EDDP (primary methadone metabolite) and found concentrations ranging from near zero (norcodeine) to 75 ng/L (codeine). Residues of four (not morphine) survived ozonation, and portions of EDDP (0.2-2.9 ng/L) and methadone (0.1-1.7 ng/L) survived carbon filtration and chlorination.

Huerta-Fontela et al. (83) presented perhaps the first data on the stepwise removal of Schedule 1 or 2 controlled substances throughout a treatment train used to generate FDW at a municipal DWTP (in Spain). A 300 MGD DWTP used water from a river and a treatment train of prechlorination, physical coagulation/filtration, ozonation, carbon sorption, and post-chlorination. Among the targeted analytes were cocaine, benzoylecgonine (BE: a primary cocaine metabolite), amphetamine, methamphetamine, MDMA (ecstasy: 3,4-methylene-dioxymethamphetamine, the methylenedioxy derivative of methamphetamine), and its N-demethylated metabolite (MDA: 3,4-methylenedioxyamphetamine).

Removals after prechlorination and filtration to below detection limits occurred for the amphetamines except for MDMA; the intake concentrations for amphetamine, methamphetamine, and MDA had ranges of 5-90, 0.2-2, and 2-50 ng/L, respectively. MDMA, however, was reduced at this step by only 23% (unless its intake concentration was below 10 ng/L), and cocaine and BE were removed by only 13% and 9%, respectively (with intake concentration ranges of 3-120 and 20-1,350 ng/L, respectively). After ozonation, cocaine, BE, and MDMA had been removed by 24, 43, and 28%, respectively. After carbon sorption, more than 99% of cocaine was removed (yielding concentrations below 80 pg/L), while MDMA and benzoylecgonine were removed by 88% and 72%, respectively. After the final post-chlorination, no MDMA was detected (less than 170 pg/L), but more than 10% of the BE persisted. In 22 of 24 FDW samples, the mean concentration of BE was 45 ng/L and its maximum was 130 ng/L. Ketamine, PCP, LSD, and fentanyl were never found in the raw waters.

Clearly, some of these substances (e.g., BE) have the same potential to persist at very low levels in FDW as do many of the APIs from legal drugs.

Unapproved Drugs

A parallel issue regards unapproved drugs and "designer" drugs undeclared as active ingredients (new pharmacologic molecules) and whose existence may or may not be known to the FDA (128). These include not just new analogs of anabolic steroids, but also new (and untested) analogs of registered drugs. The latter, for example, are used not infrequently in OTC supplements (129). Whether they occur in the environment (or in FDW) is completely unknown. Adulterants in herbal supplements or OTC/Rx drugs often occur at high levels. An example is the analogs of the approved phosphodiesterase type 5 (PDE-5) inhibitors (used primarily in treating erectile dysfunction), such as sildenafil, vardenafil, and tadalafil (e.g., see: (130, 131)). Further, the emerging possibility of new APIs using isotopic substitution (deuterated analogs) could pose yet further analytical challenges for water surveys (1); olanzapine-Cd3 (an atypical antipsychotic) is but one example.

Major Unanswered Questions

A major unanswered question is the extent to which APIs have been targeted in FDW monitoring studies but have not occurred above the limits of detection (or quantitation). Such data of absence are of particular interest if they occur in the complete absence of any positive data of occurrence, as this would contribute to a preponderance of evidence for the probability of an API's occurrence in FDW as being diminishingly low. This type of valuable information would require a separate intensive examination of the literature. Comprehensive data of absence would provide justification for targeting alternative APIs for FDW monitoring and greatly reducing or eliminating any future efforts targeted at the lower-probability APIs. For example, in the literature examined here, those APIs with data of absence and lacking any data of presence (reported by more than one study) include: atorvastatin, *p*-hydroxy atorvastatin, triclocarban, testosterone, cyclophosphamide, and albuterol.

A parallel question of equal or greater importance is which APIs have never been targeted for FDW monitoring - that is, those for which neither occurrence data nor data of absence exist (namely, those with absence of data). Data of complete absence (supported by a critical number of studies) coupled with complete absence of data, would be indispensable in guiding future investigations to focus monitoring on other APIs not yet examined. Given the sheer number of APIs in use today, the scope of APIs never before examined but deserving of attention could be greatly reduced by use of published data on environmental occurrence, modeling, and potential for adverse health effects. An alternative approach is non-targeted characterization of unknowns, such as by accurate mass screening (92).

The question can now be posed as to what more can be gained by continued monitoring of FDW for the same limited set of APIs. One possible advantage, which the CCL3 (19) might have the opportunity to evaluate, is whether significant excursions in concentrations occur for the set of APIs targeted by the CCL3.

Is the body of data on APIs in FDW sufficiently comprehensive that we can be assured that ephemeral, transient, or seasonal excursions do not frequently occur significantly beyond the currently known maximum concentrations? For example, excursion could occur from seasonal fluctuations in waste dilution (e.g., effluent dominance during dry weather) or in drug use (types and quantities), sporadic disposal practices (resulting in brief transient excursions), or other special circumstances. Studies of seasonal fluctuations of APIs in FDW are rare. As one example, Kormos (51) monitored FDW from two DWTPs in Ontario for bezafibrate, carbamazepine, gemfibrozil, and ibuprofen. Reliance on grab sampling instead of time-integrative sampling increases the likelihood that spikes in concentrations will be missed. Carbamazepine concentrations varied by over two orders of magnitude over the course of 12 months (from 2.9-721 ng/L), showing the possible difficulties with obtaining grab samples that are representative over time. Buschini et al. (132) have shown the possible importance of establishing the potential for sustained exposure over longer periods of time rather than via intermittent grab samples.

While most APIs experience relatively constant usage throughout the seasons (especially maintenance medications), others undergo seasonal cycles. One example is medications associated with the flu. An extreme example would be antivirals such as oseltamivir, which could experience usage rates orders of magnitude higher than usual during epidemics. Time-averaged usage rates are not necessarily a good predictor of which APIs (or respective metabolites) have the potential to enter waterways and source waters. Also, specific or unique characteristics of individual locales can result in usage patterns completely different than indicated by overall sales data (e.g., emissions from hospitals). Worst-case modeling has predicted oseltamivir (and its carboxylate active metabolite) each in drinking water at over 100 $\mu\text{g/L}$ (133); other antivirals (such as acyclovir, nevirapine, penciclovir, stavudine, and zidovudine) are now known to persist in treated wastewater (134).

Risk and APIs in Drinking Water

Discussions regarding the presence of APIs in FDW inevitably devolve into concerns surrounding the ramifications for human health. Given the extremely low individual and combined concentrations of the very limited subset of APIs currently known to sometimes be present in FDW (sixty-some APIs among more than 1,500 in common use), the focus gravitates toward two major aspects of toxicology: (1) the unknowns surrounding the potential for biological effects at the extreme low end of dose-response curves and (2) the complexities associated with mixture effects (both additive and interactive). These unknowns are greatly exacerbated by the fact that a large array of other microconstituents unrelated to APIs - both anthropogenic and naturally occurring - also contaminate even the purest of waters. Intertwined are arguments regarding toxicity thresholds and chronic, vulnerability exposure windows, and transgenerational exposure. Teasing apart the toxicological significance of APIs from that of all the other ultra-trace contaminants is currently not possible.

Two stances bookend the extremes of the overall toxicological concern. On the one extreme, no empirical evidence has emerged indicating a known hazard of APIs in FDW - pointing to no reason for concern. On the other, an inevitable question is whether it should be acceptable at the outset to allow any detectable amounts of APIs in FDW if multiple-log removals could be achieved with best available technologies or if they can be prevented from entering the water cycle to begin with (by implementing any number of a large spectrum of pollution prevention measures). This latter extreme stance is motivated largely by the fact that APIs derive from sewage, and, as such, serve as direct measures of the length of wastewater-drinking water hydrologic connection and therefore as markers for the possible presence of other, still unidentified contaminants conveyed by sewage.

Risk Overview

Quite a number of studies of varying rigor have presented assessments of risk from trace levels of APIs in FDW. Almost without exception, these all share a similar approach. The therapeutic dose (TD), coupled with a series of safety or uncertainty factors (which are used to infer limits such as the ADI - acceptable daily intake), is almost always used as the benchmark with which to compare known or worst-case modeled API-FDW concentrations and assumptions regarding water consumption (perhaps most accurately assumed to be 20 mL/kg/day on a body-weight basis). The TD, however, may not be a relevant benchmark against which to judge risk.

Assessing the potential for risk from APIs in FDW is a topic deserving a comprehensive examination and far exceeds the scope of this review. A truly comprehensive, holistic assessment has never really been published but a recent examination by Bull et al. (135) is one of the most comprehensive. Some general points can be made, however, regarding what has been published to date.

First, for further reading, assessment of risk from general chemical exposure in drinking water (especially recycled water) has been covered in a number of publications, several of which are: Asano and Cotruvo (136), Chapman et al. (137), Falconer (138), Howd and Fan (139), and Rodriguez et al. (140).

Examinations of risk targeted specifically at APIs in drinking water began only 10 years ago and include: Bercu et al. (141), Blanset et al. (142), Bull et al. (135), Christensen (28), Collier (143), Cunningham et al. (144), Daughton (3), Dorne et al. (145), Global Water Research Coalition (146), Illinois EPA (54), Johnson et al. (147), Jones et al. (6), Kümmerer and Al-Ahmad (148), Mons et al. (149), National Water Quality Management Strategy (150), Rahman et al. (151), Randon (152), Reddersen et al. (73), Rowney et al. (153), Schulman et al. (154), Schwab et al. (155), Snyder et al. (9), Watts et al. (133), and Webb et al. (156); note, however, that some of these assessments were based on predicted rather than measured concentrations. Human exposure to APIs in drinking water was the focus of a 2008 National Academy of Sciences workshop (157).

Low-Dose Exposure

Even fewer studies have presented empirical data, especially data pointing to the potential for human effects at low API concentrations; examples include Pomati et al. (158, 159) and Vosges et al. (160). Perhaps the data of most direct relevance for human health impacts has come from epidemiological studies of worst-case human exposures. These have focused on communities using drinking water that comprised significant portions of recycled water; examples include Cook et al. (161) and Rodriguez et al. (162), and others cited in Daughton (163). The assumption is made that the concentrations of APIs in recycled water would be higher than in FDW from most municipal DWTPs using "natural" source waters. While any effects specific to APIs cannot be isolated from the effects of all other contaminants, the uniform absence of adverse health impacts from these epidemiological studies makes this concern moot. But even if adverse outcomes were to be documented, distinguishing correlation from causation would be extremely difficult.

Predicting human response to ultra-low dose (ULD) exposures currently relies on linear extrapolation from higher-dose exposures in animal test systems. Higher doses are required to have sufficient statistical power to detect responses (primarily cancers) above background. Sufficient power at ultra-low doses can only be obtained by using very large test populations. Such an approach has been used with trout as the test species. Over 40,000 trout have been used to test ultra-low doses of several known carcinogens (164, 165). Of most significance is that linear extrapolations were shown to be both overly and under conservative depending on the carcinogen, pointing to the unknowns associated with predicting responses from ULD exposures.

Therapeutic Dose as a Point of Departure

In general, assessment of risk from API exposure has been predicated on using the therapeutic dose (TD) as a point of departure (POD) (e.g., see: (135)); margins of exposure between FDW concentrations and minimum therapeutic doses can then be calculated and ranked (135). Use of the TD as the POD has been justified on the basis that (by definition) the therapeutic effect is positive (desired), rather than adverse, and therefore that doses below the TD would be without consequence. From the published data on APIs in FDW (Table I), it is hard to dispute that APIs in FDW occur at concentrations so low that daily water consumption would have to be sustained for a lifetime before a dose approaching even a small fraction of a single recommended daily dose might be reached. Many studies have emphasized this point. This appears to be true for the vast majority of all APIs (those that are not direct-acting genotoxicants) but perhaps not for a few very potent APIs such as EE2 (e.g., see: (143)). Even for APIs such as EE2, however, usage rates are so small that their routine occurrence in drinking water is doubtful - a fact supported by the published literature's data of absence (Table I).

The TD has been used as the POD for assessing risk presumably because it is readily available and is a central aspect of all medication. Its use in assessing risk from ULD exposures, however, has never been justified or cogently rationalized.

Few evaluations, however, have deviated from this general approach. Collier (143) is one example. Use of the TD as the POD probably introduces the most uncertainty in assessing the risk from APIs in FDW. Many issues point to the TD as being far too high for the POD. Therapeutic doses (and endpoints) may not be the appropriate benchmark against which to assess risk (3). Attention to doses that are known to elicit any type of effect, regardless of how subtle, may be more relevant.

Much discussion centers on the relevance to environmental exposures of high-dose testing. At ever-lower exposure levels, there is perhaps always some type of effect. These effects just may not be measurable (perhaps obscured by natural variation in homeostasis), or we may not yet know to look for them. Exposures to ever-lower levels may lead to effects from a changing variety of mechanisms or pathways. That effects can vary with dose is known as "mixed-mode dose-response" or "dose-dependent transitions in mechanisms of toxicity." This is partly a result of multiple effector sites, all having different ligand affinities and resulting in crosstalk among different signaling pathways.

The possible significance of low-dose exposures and the many issues surrounding low-dose extrapolations, dose-response thresholds, and transitions in mechanisms of action are discussed by Gore et al. (166), Holsapple and Wallace (167), Kortenkamp et al. (168), Myers et al. (169), Welshons et al. (170), and White et al. (171), among others.

Some of the questions regarding the relevance of the TD as a POD for assessing risk include:

- TD might be unrelated to other subtle endpoints or adverse effects. Is it valid to assume that the potential for adverse effects is a function of the therapeutic dose (potency)?
- Side effects that are not considered "adverse" may well still be considered unacceptable to the public if the exposure is not known, unexpected, or unwelcome. When an exposure is not expected, any type of effect that perturbs homeostasis may be deemed by the public as unacceptable. Whether the effect is normally deemed even beneficial can be irrelevant. Perceived risks need not be associated with adverse outcomes (12, 18), and adverse outcomes can result solely from negative expectations in the absence of any hazard. This is known as the nocebo response (3).
- For many APIs, the approved route of administration for therapy is not oral. Does exposure to an API by ingesting drinking water emulate the route(s) used in therapy? For example, might there be any unforeseen consequences of ingesting drugs intended solely for external administration (86), or of pulmonary exposure to APIs entrained in aerosols (such as during showering) but never intended for inhalation?
- ADIs (acceptable daily intakes) refer to exposure levels likely to not result in adverse effects. They do not, however, necessarily translate to an absence of effects. Determining ADIs assumes that effects diminish with dose until a threshold is reached. They are derived from the "no observable adverse effect level" (NOAEL). The key aspect of the NOAEL is whether an effect has been observed. Subtle effects and

latent or delayed-onset effects can be difficult to spot. A NOAEL is not a threshold. Rather, a NOAEL simply means that a particular, anticipated adverse effect has not been observed - not that one which escapes current detection or which has a delayed-onset will not occur. Are techniques available for detecting and measuring effects sufficiently sensitive for low-level exposures, or are there no effects to detect? Are the types of possible subtle effects even sufficiently understood? Thresholds also may not apply to APIs having the same mechanism of action as endogenous chemicals - sex steroids are one example - since the thresholds may often already be exceeded since they are in addition to the endogenous production (166).

- TDs are determined on comparatively extremely small, specifically targeted sub-populations before marketing an API. Often excluded from these trials are the chemically sensitive such as children, pregnant women, immune-compromised individuals, and others. Perhaps the majority of adverse effects are really only revealed post-market because longer-term exposure for a much larger test population is required to detect less frequent (and perhaps subtle) effects; the expanded population includes those receiving the API for off-label purposes. Adverse events often surface post-market partly because they can go unreported during trials as a result of the use of "human guinea pigs" in clinical trials - "professional volunteers" who are motivated to transition quickly between trials (172). Also, large segments of the population are excluded by the use of usually narrowly targeted populations that are the focus of the intended therapeutic treatments.
- Data obtained from clinical trials rarely simulate the higher frequency and duration (even transgenerational) of possible exposure via FDW.
- An argument used against the potential for low-dose effects is that the need to invoke previously unknown mechanisms of action is not plausible. This argument is not supported by the history of pharmacology, however, where new mechanisms of action are commonly revealed as new drug targets are discovered.

When evaluating the literature on ULD effects, the question needs to be "what is the evidence pointing to the potential for any type of biological response from exposure to concentrations of APIs found in FDW"? Deeming whether these responses could be "adverse" is a subjective judgment. Treatments can have biological activity but not be clinically effective. The published literature could potentially be biased by the relative under-representation of published studies finding seemingly inconsequential effects that were deemed of no clinical significance. Therefore, the concern is whether *any* type of response is possible - not just whether the targeted response is obtained.

Studies Using Ultra-Low Doses and Micro-Dosing

Instead of a focus on therapeutic doses, the issue of APIs in FDW could be more informed by the current research on sub-therapeutic doses. In the last decade,

one aspect of pharmacology that continues to develop - yielding new insights into the properties of dose-response - is the study of so-called "ultra-low" doses (ULD) and the practice of "micro-dosing." The literature surrounding these two could be mined and synthesized for its possible utility in assessing the risk of APIs in FDW. The literature in both of these areas, however, has been largely ignored in the environmental exposure arena.

While models based on minimal therapeutic doses have served to advance the assessment of risk for APIs in FDW, more relevant PODs will need to be vetted before more realistic assessments can be performed. This was alluded to in 2003 by Daughton (93) in highlighting the pioneering work of Crain and Shen with ultra-low dosing of naltrexone and its potentiation of various aspects of nociception in rats by morphine. Having shown dramatic nociceptive effects in rats from combining opiate agonist and antagonist (e.g., naltrexone) at doses approaching 6 orders of magnitude below 1 $\mu\text{g}/\text{kg}$ (e.g., minimum doses of 1 pg/kg), APIs occurring in FDW at the ng/L level clearly hold a theoretical potential for yielding effects even after consumption of a single liter of FDW; note, however, that while naltrexone serves as an example of an API with the potential for effects at ultra-low doses, it has never been reported in drinking water (although it has also perhaps never been targeted).

Increasing numbers of studies are pushing the documented range for API effects ever lower. Superficial examination of the literature quickly shows that a range of biological effects have already been demonstrated (but for a limited number of APIs) at doses much lower than the TD established during clinical trials. These lower doses can range from a couple to more than 6 orders of magnitude below TDs. Unfortunately, study of ultra-low doses is probably somewhat slowed by its mistakenly perceived entanglement with homeopathy and its Law of Infinitesimals (see debate at: (173)). Clinical studies have ignored ultra-low doses because the potential effects have traditionally been viewed as not being relevant to achieving therapeutic goals.

Microdose (MD) studies (also known as human Phase-0 studies) are performed at the early stage of drug development. MD studies can quickly and safely obtain human pharmacokinetic (PK) data on drug candidates before committing to more expensive Phase-I clinical trials. A microdose can be as low as 1% of the predicted TD. PK data from MS studies is more relevant to the exposure levels of APIs in FDW than are TDs; but even these levels far exceed those experienced with the ambient environment or FDW. Significantly, PK data from MD experiments is sometimes found to differ from the PK data obtained from TDs; PK data from even lower doses could deviate yet further.

The potential for biological responses at ultra-low doses of APIs had been little explored up until the 1990s. Numerous examples have emerged, some showing complex alternating W-shaped or multimodal dose-response curves as doses are varied over many orders of magnitude. Three examples are: (i) a single $\mu\text{g}/\text{kg}$ dose of tetrahydrocannabinol can adversely affect the cognitive ability of mice (174); (ii) different combined doses of naltrexone and a cyclic AMP-phosphodiesterase inhibitor such as rolipram (down into the pg/kg range) induce varying analgesia or hyperalgesia (175); (iii) femtomolar concentrations of dextromethorphan afford neuroprotection from inflammatory damage,

reported by the authors as demonstrating for the first time that a small molecule (dextromethorphan) can exert neuroprotection at such low concentrations (176).

Threshold of Toxicological Concern

In the absence of empirical dose-response data at extremely low concentrations, an alternative to using the TD as a POD is the threshold of toxicological concern (TTC). Rodriguez et al. (140) proposed the TTC for assessing the risk of individual microconstituents in recycled water used for drinking. Daughton (3) suggested the TTC be used for assessing the potential toxicological significance of exposure to multiple APIs. With a worst-case assumption that all APIs were genotoxicants (and therefore requiring a TTC of 1 µg/L), upwards of 50 APIs could be perpetually present in drinking water at individual levels of 10 ng/L while maintaining a lifetime excess cancer risk of less than 10^{-6} (3). If the simultaneous exposures were only intermittent, then the total number of APIs that could be present would be upwards of 6,000 - clearly far exceeding any possible exposure scenario.

With the studies on APIs in FDW surveyed here (Table I), of those identified as co-occurring in individual studies of FDW, no individual study has identified more than a dozen APIs in any given sample. These include the studies of: Benotti et al. (39), Snyder et al. (36), Snyder (40), Stackelberg et al. (42), Ternes (46), and Vanderford and Snyder (41). The reported individual concentrations for all of these APIs were well below 1 µg/L.

Sensitive Subpopulations

A major concern regarding exposure to APIs via FDW is not just exposure via routes never intended for the API but also for populations inappropriate for the API. Unique and vulnerable subpopulations are perhaps the major focus of concern regarding inappropriate API exposure via FDW. Among these, children and the fetus possess many unique and complex aspects of physiology and API pharmacokinetics that make low-dose exposure a particular concern; this is especially true given that few relevant empirical data exist for many API classes (particularly for in utero exposure and for antineoplastics). These complexities are covered by: Aksglaede et al. (177), Genuis (178, 179), Houlihan et al. (180), and the WHO (181), among many others.

A particularly important variable in the vulnerability of a subpopulation is critical windows of vulnerability, which involve the timing of exposure relative to key events in biological development or intercellular communication. Dose timing is already established as a key determinant in certain drug therapies - where knowledge of chronobiology can reveal how the actual time of dose administration can alter therapeutic outcomes (182). Perhaps more significantly, with respect to low-dose exposure, are windows of vulnerability for fetal development. Better established in animal models, studies are just emerging regarding the timing of fetal exposure to ambient levels of xenobiotics. The first study investigating prenatal exposure to bisphenol A and childhood behavior found correlations

between certain behaviors in 2-year old girls and maternal exposure (as measured by urine concentration), especially as measured at 16 weeks of gestation (183).

Epigenetics

A weakness cited regarding hypotheses involving low-dose effects is that even if effects were to occur, they would be transient. Absent direct-acting genotoxicants, no other mechanism has been advanced for the possibility of lasting effects from brief low-dose exposures. Other than for direct-acting carcinogens (for which a single mutation might theoretically be sufficient), the assumption has been that certain minimum thresholds exist for indirect-acting carcinogens and non-carcinogens simply because no mechanism has seemed possible whereby a single molecular event could persist.

Not mentioned in these discussions, however, is a possible role of epigenetic alterations - a non-genetic pathway that began attracting attention in the early 2000's as an explanation for low-level effects from endocrine disruptors (e.g., (170)).

Epigenetics involves heritable change in gene expression in the absence of alteration to the underlying DNA sequence itself. The most common mechanism for epigenetic alteration is via DNA methylation - specifically, cytosine methylation in CpG dinucleotides within promoter regions known as CpG islands. Methylation by methyltransferases at the cytosine C-5 position forms 5-methylcytosine - sometimes termed the "5th base," in recognition of its profound importance; more recently discovered variants such as 5-(hydroxymethyl)cytosine also might play roles. Other types of epigenetic change include modification of histones, such as by acetylation, phosphorylation, and ubiquitination/SUMOylation.

Unlike the genome, the epigenome is plastic, dynamic, extraordinarily complex, and varies across tissues and individuals; it is also sensitive to a wide array of non-chemical environmental influences. Of most significance, epigenetic alterations can accumulate, resulting in delayed-onset outcomes that can persist long after exposure has ceased - even across several generations.

Given the thousands of publications devoted to APIs as environmental pollutants, few address the possible role of epigenetics in human (or even aquatic) health. Epigenetics has been mentioned only in passing in perhaps a dozen or so of the thousands of published works; most of these have been published since 2006. In a forward-looking examination by the National Research Council (184) of the future of the life sciences and areas of focus and collaboration ("A New Biology for the 21st Century") and in the US EPA's "Strategic Plan for Evaluating the Toxicity of Chemicals" (185), epigenetics is mentioned only briefly.

Epigenetics provides a route by which very small numbers of discrete, isolated events (e.g., post-replication cytosine methylation) could persist. They could then accumulate from chronic low-level exposure, eventually reaching levels sufficient for measurable change (via alteration in gene expression). The concept of "thresholds of minimum exposure" would no longer need to be based on discrete exposure events, but rather on cumulative exposure - that point in the trajectory of accumulated epigenetic alterations at which phenotypic change

emerges. Needed instead would be a way to evaluate the threshold of cumulative epigenetic alterations (each alteration perhaps being inconsequential by itself) leading to dysfunction or disruption of homeostasis. **Exposure would then not be considered in terms of discrete molecular events (each required to meet a threshold), but rather could be viewed as a continuum of accumulated events, whose combined, sustained accretion could eventually reach a threshold.**

Indeed, the accumulation of seemingly innocuous, individual methylation events ("stochastic methylation events" leading to "methylation spreading") has been hypothesized as a major determinant of aging (186). Surprisingly little is known, however, regarding epigenetics and pharmaceuticals. Epigenetics research with regard to environmental contaminants has been limited to a select few chemicals, such as bisphenol A, vinclozolin, and estrogenic hormones.

Epigenetics is a mechanism being explored, however, as a source of side effects (and possible explanation of mechanisms of action) for many medications. A comprehensive overview of the possible direct and indirect epigenetic actions of APIs is provided by Csoka and Szyf (187). The major unknown is whether there are thresholds for discrete epigenetic alterations to occur. Epigenetic pathways are also being used as targets for new drugs (e.g., histone deacetylase inhibitors – valproic acid being one existing example). Csoka and Szyf (187) provide a list of drugs/classes known or postulated to effect epigenetic alterations; they maintain that any API-induced side effect caused by epigenetic alterations might persist after cessation of drug treatment. Many of these same APIs have been identified in FDW. General epigenetic effects such as hyper- or hypo-methylation of DNA shared by many different drugs would be a mechanism for additive effects across disparate drug classes.

But while mechanisms of epigenetic modification by APIs are becoming clear, low-dose epigenetic alterations are another question. It is still unknown whether the required minimum levels of epigenetic modifiers can be as low as those in the environment. The fundamental question persists - is there a threshold level below which an epigenetic modifier cannot result in a discrete alteration? In the final analysis, the debate regarding effects at vanishingly low levels is tethered to the fundamental question of toxicological thresholds. What level of receptor interaction is required for an effect - regardless of how subtle it might be? A mechanism around this requirement would be one where infinitesimally small numbers of receptor interactions can accumulate over time, eventually reaching the threshold. Epigenetics may provide a means for this to occur.

Conclusions

Quantitative data exists for over 60 active pharmaceutical ingredients (APIs) and metabolites in finished drinking water (FDW). These derive from roughly 50 publications. An unknown but possibly large number of publications report negative data for a wide spectrum of additional APIs. For roughly half of the APIs having positive occurrence data, corroborating occurrence data from more than a single isolated study do not exist. No more than a dozen APIs have been reported in any single FDW sample. Only one API has been reported in any FDW sample at

a concentration exceeding 1 µg/L (1 ppb) – and it was for a single sample. The vast majority of APIs when present in FDW are probably at concentrations below 50 ng/L. Many have maximum reported concentrations of only several ng/L. Those APIs most frequently reported and in the highest concentrations are carbamazepine, ibuprofen, and clofibric acid. For six Anatomical Therapeutic Chemical (ATC) classification system main groups (A, B, H, L, R, and S), no API has been reported. No antineoplastic or immunomodulating agent has been reported in FDW, nor have any radiologicals. The relative lack of data for commercial bottled water is notable. Only two APIs used primarily in veterinary medicine have been reported in FDW: monesin and tylosin. Surveys of FDW for illicit and unapproved drugs seem comparatively under-represented, even though some are known to have the potential to persist.

That APIs can occur in drinking water certainly poses complex questions regarding the significance of long-term human exposure. Although the minute concentrations when compared with therapeutic doses appear to be far below those that might pose any health concerns, the possibility of delayed-onset health effects cannot yet be ruled out. The possibility of cumulative epigenetic alterations as a possible mechanism of ultra-low-dose effects deserves attention. Even if sufficient knowledge eventually exists for setting scientifically defensible FDW standards for APIs, APIs will perhaps always exist in water - albeit at ever-lower levels, given anticipated advancements in treatment technology and as detection limits in analytical chemistry improve.

API occurrence in drinking water also poses challenges in communicating the risk regarding the inevitable implementation of widespread water recycling. Is it acceptable to have active pharmaceuticals in drinking water even at subtherapeutic levels regardless of the absence of predicted risks? Perhaps the issue is really the knowledge that the minute levels of APIs one might drink originated from others (unplanned potable reuse) or from oneself (planned potable reuse). Drugs in drinking water essentially serve as road signs for the water cycle - as billboards that say "this water used to be sewage." They serve as the chemical equivalent of garden weeds - not necessarily harmful but certainly unwelcome or undesired. APIs in FDW can serve as a major barrier to public acceptance of reused water, especially for drinking. Summaries of risk perception and risk communication regarding APIs in FDW can be found in Daughton (3, 18), Ragain (188), and Randon (152).

With these concerns aside, however, perhaps the most important aspect of APIs in drinking water is that it serves to highlight for the public the intimate, direct, and complex interconnections between human activities, the environment, and human health. While state-of-the-art engineering end-of-pipe controls can reduce APIs and other water micropollutants to ever-diminishing concentrations, a sustainable approach will need to be holistic - with a focus on reducing the numerous routes and mechanisms by which APIs gain entry to the environment to begin with. A bewildering array of modifications and reengineering of consumer behavior, medical practices, and healthcare administration holds the potential to greatly reduce the entry of APIs to the environment. Progress in this direction is already underway; see: Bengtsson et al. (189), Daughton and Ruhoy (190), Hempel and Kümmerer (191), Keil et al. (192), and Kümmerer (193). Perhaps

most significantly is the possibility that pollution prevention efforts targeted at API release also holds the collateral potential for reducing healthcare cost and improving healthcare outcomes (190), making such an approach not just sustainable - but, more importantly, the optimal solution.

Postscript

After completion of this review, a new DWTP monitoring study was released by the Ontario Ministry of the Environment's Drinking Water Surveillance Program: Ontario MOE. "Survey of the Occurrence of Pharmaceuticals and Other Emerging Contaminants in Untreated Source and Finished Drinking Water in Ontario." Ontario Ministry of the Environment (MOE), Canada, 2010 (January), 31 pp; <http://www.ene.gov.on.ca/publications/7269e.pdf>.

The Ontario MOE study represents one of the largest yet completed. It targeted 47 APIs (more than half being antibiotics) in 123 samples from 17 DWTPs in Ontario, Canada. Among all 123 samples, 22 APIs were detected. Of these APIs, 16 had been previously reported (according to Table I). The three most frequently detected were **carbamazepine** (25% of samples from 8 of 17 sites; median 0.21 ng/L, max 601), **gemfibrozil** (15% of samples from 6 of 17 sites; median 0.5 ng/L, max 4), and **ibuprofen** (15% of samples from 9 of 17 sites; median 0.33 ng/L, max 25). These data comport with the data compiled in Table I. The frequency of occurrence for only two APIs seemed to be higher than indicated by previous work: **monensin** (7% of samples from 4 of 17 sites) and **tylosin** (6% of samples from 4 of 17 sites). The frequency of detection for the remaining APIs was less than 4%. Of note were six APIs that were reported in FDW for the first time (all but one being an antibiotic), but all occurring infrequently: **enrofloxacin** (3% of samples from 4 of 17 sites), **norfloxacin** (1% of samples from 1 of 17 sites), **meclocycline** (1% of samples from 1 of 17 sites), **tetracycline** (4% of samples from 5 of 17 sites), **sulfachloropyridazine** (2% of samples from 2 of 17 sites), and **equilin** (1% of samples from 2 of 17 sites). Four APIs were detected in FDW samples but not in any untreated source water (clofibric acid, diclofenac, equilin, and sulfachloropyridazine).

The findings from the Ontario MOE study do not alter any of the conclusions or trends developed in the review compiled here. The data from the study largely comport with what is currently known.

U.S. EPA Notice

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Chapter 3

Occurrence of Pharmaceuticals in U.S. Drinking Water

Shane A. Snyder^{*,1,2}

¹Applied R&D Center, Southern Nevada Water Authority,
Las Vegas, NV 89193

²College of Engineering, University of Arizona, Tucson, AZ 85721

*snyders2@email.arizona.edu

Steroid hormones and certain pharmaceuticals have been reported in U.S. waters for over 40 years. Early reports demonstrating that certain hormones and pharmaceuticals were not completely eliminated by wastewater treatment did not gain significant attention until the 1990s when reports of reproductive disorders in fish were linked to trace contaminants in wastewater outfalls. Treatment efficacy for these emerging contaminants varies widely depending on unit processes, operational parameters, and compound structure. A study was undertaken in 2002 to evaluate the occurrence and fate of pharmaceuticals in U.S. drinking water systems. From the 20 drinking water systems evaluated, the five most prevalent pharmaceuticals detected in raw water were carbamazepine, dilantin, sulfamethoxazole, ibuprofen, and meprobamate. However, in finished water the occurrence pattern shifted based on treatment processes and operational conditions. A similar study was launched in 2006 with the investigation of 19 U.S. drinking water facilities. This study unveiled similar findings for pharmaceutical occurrence. The most prevalent pharmaceuticals detected in U.S. drinking water were the anti-anxiety pharmaceutical meprobamate, the anti-epileptic drug dilantin, and the anticonvulsant carbamazepine. Despite the use of ultra-trace analytical methods, steroid hormones used as pharmaceuticals were not detected in U.S. drinking water. Pharmaceuticals have more robust human health data than essentially all other environmental contaminants due to

extensive clinical testing. Thus, research to date suggests that concentrations of pharmaceuticals in U.S. drinking water are far below any human health relevance.

As early as the 1940s, scientists had begun to unravel the relationship between molecular structure and ability to mimic endogenous steroid hormones in animals (1). By the 1950s, published manuscripts demonstrated that both natural products isolated from plants (2) and synthetic chemicals such as pesticides (3) were capable of mimicking or blocking the action of steroid hormones in the endocrine system. These compounds would later be referred to as endocrine disrupting compounds (EDCs). In 1965, researchers at Harvard University demonstrated that both endogenous steroid hormones and synthetic hormones used in pharmaceuticals were not completely eliminated during wastewater treatment (4). This report is likely the first in U.S. history, and perhaps globally, that specifically demonstrated the potential for pharmaceutical compounds and endogenous hormones to contaminate surface waters. In this pioneering manuscript, the authors state that despite the relatively low concentrations observed, “it is our responsibility to learn in what amounts steroid hormones may occur in drinking waters under the most unfavorable conditions” (4). These “unfavorable conditions” refer to the discharge of wastewater into water that later serves as the source for drinking water for a “downstream” community. The U.S. Department of the Interior, through the Federal Water Pollution Control Administration (which would become part of the U.S. Environmental Protection Agency created in 1970), further investigated the biodegradability of natural and synthetic estrogen and androgen steroid hormones (5). These authors concluded that “The synthetic estrogen and progestin components of oral contraceptives exhibited greater overall resistance to microbial degradation than the natural hormones.” The authors predicted that concentrations of hormones in wastewater would not likely exceed “a few tenths of a milligram per liter”, but cautioned that “since they are physiologically active in very small amounts, it is important to determine to what extent the steroids are biologically degraded in the normal history of wastewater and receiving bodies of water that may eventually be used for water supplies.” Thus, these early investigations into the biological degradation and subsequent discharge of natural and synthetic (pharmaceutical) hormones began to pave the way for future studies that would link occurrence with impacts to aquatic life.

In 1971, the newly created U.S. Environmental Protection Agency (EPA) began a program to identify extractable organic compounds in wastewater (6). Among the organic constituents identified, the EPA scientists discovered the steroids cholesterol and coprostanol as well as the pharmaceuticals 2-(4-chlorophenoxy)-2-methyl propanoic acid (clofibric acid) and salicylic acid in wastewater originating from Cincinnati, Ohio; Athens, Georgia; and Washington D.C. Clofibrate is a lipid-lowering drug that is administered in relatively high amounts (grams/day) and salicylic acid is the primary hydrolysis product from aspirin used primarily as a pain relief medication. A publication from 1977 further

demonstrated that the pharmaceuticals initially detected by the EPA were also found in wastewater effluent entering the Mississippi River from a wastewater treatment facility serving a population of approximately 600,000 from the Kansas City, Missouri area (7). The researchers collected 24-hour composite samples monthly from August through December of 1975 as well as samples in March and May of 1976. Concentrations of clofibric acid ranged from 2.54 to 9.74 $\mu\text{g/L}$ with an average concentration of 7.09 $\mu\text{g/L}$, while salicylic acid ranged from 1.83 to 95.62 $\mu\text{g/L}$ with an average concentration of 28.79 $\mu\text{g/L}$. While these values initially appear to be quite high, the treatment plant investigated employed only primary treatment (essentially solids removal) and thus substantial removal would not be expected (8). However, the researchers did demonstrate that while the concentration of clofibric acid was not substantially changed during primary treatment, salicylic acid concentration decreased by “approximately 90%.” The researchers also analyzed raw and treated sewage before and after acid hydrolysis and concluded that conjugated forms of clofibric acid were negligible, while conjugates of salicylic acid accounted for less than 25% of the total. While the public and media interest did not seem stirred by this report, the authors did state that “Drugs, therefore, represent another potentially serious source of biologically active environmental contamination since these compounds may eventually enter public water supplies.” While concentrations of clofibric acid and salicylic acid were non-detectable in the drinking water from two downstream cities, the authors concluded by stating “It may be well to consider further evaluation of public drinking water supplies for the presence of drugs consumed in large quantities.” These early manuscripts had unambiguously documented that pharmaceuticals were being released into the environment from municipal wastewater treatment facilities; however, further investigations were relatively sparse for the next 20 years (9–13).

In the early 1990s, reports surfaced from Europe indicating that wastewater effluents contained estrogenic substances that were capable of inducing reproductive abnormalities in fish (14, 15). The EPA and the U.S. Geological Survey (USGS) both published manuscripts in 1996 documenting similar impacts to fish exposed to wastewater effluents in the Mississippi and Colorado River watersheds, respectively (16, 17). By coupling bioassays and analytical measurements, researchers in the U.K. and in the U.S. demonstrated that while estrogenic chemicals such as alkylphenols were present in far greater concentrations, natural and synthetic steroid hormones were the most potent estrogenic compounds within the complex wastewater effluent matrix (18–22). However, substantial evidence regarding the presence of other xenobiotics (19, 20, 23) and the bioaccumulation potential for certain alkylphenols (24, 25) clearly suggests that mixtures of various estrogen agonists are likely contributing to observed impacts in fish.

Pharmaceuticals in U.S. Drinking Water

By the end of the 1990s, two major reviews regarding pharmaceuticals in the environment had been published (26, 27). As of 2010, each of these

ground breaking reviews had been cited more than 800 times in peer-reviewed literature. While some reports from Europe documented the occurrence of pharmaceuticals in drinking water in the late 1990s (28, 29), there were essentially no peer-reviewed reports demonstrating pharmaceutical occurrence in U.S. drinking water until the mid-2000s. The drinking water from the Jefferson Parish East Bank Water Treatment Plant in Louisiana, U.S., was analyzed in 2002 for five pharmaceuticals, none of which were detected (30). In 2004, researchers from the USGS and the U.S. Centers for Disease Control and Prevention (CDC) published a manuscript describing the occurrence and removal of pharmaceuticals at a drinking water treatment plant in the U.S. (31). In this study, the researchers investigated 106 organic wastewater contaminants, including 25 antibiotics (which oddly included the anticancer drug methotrexate) and 22 other pharmaceuticals (which included caffeine, a caffeine metabolite, and cotinine). Of the 47 pharmaceuticals tested, only four were detected in the finished drinking water: caffeine, carbamazepine, cotinine, and dehydronifedipine. Of these, carbamazepine is a prescription pharmaceutical and dehydronifedipine is a metabolite of a pharmaceutical, while caffeine and cotinine (a tobacco metabolite) are not commonly considered pharmaceuticals. In 2006, occurrence of pharmaceuticals in a large western U.S. watershed, including the drinking water for a large metropolitan city, was investigated (32). Samples of drinking water were analyzed for a diversity of pharmaceuticals, yet only meprobamate and dilantin were detectable. An investigation of four southern California drinking water plants in 2006 for clofibrate, clofibric acid, ibuprofen, and ibuprofen methy ester determined that only ibuprofen and the corresponding ester metabolite were detectable (33). A study in 2007 investigated the occurrence of six antibiotics at three U.S. drinking water treatment plants (34). Of the three drinking waters tested, one contained none of the six antibiotics above the limit of quantitation, one contained only flumequine at 2.5 ng/L, and the other detected five of the six antibiotics with concentrations of each less than 5.0 ng/L. Research reports published in 2007 and 2008 from the Water Research Foundation (formerly American water works association Research Foundation – AwwaRF) investigated the occurrence and fate of pharmaceuticals at several U.S. drinking water plants (35, 36), which will be discussed in greater detail in the following sections..

Initial Survey of Pharmaceuticals in U.S. Drinking Waters

In 2002, the AwwaRF awarded a grant to investigate the impact of conventional and advanced water treatment processes on pharmaceuticals and suspected endocrine disrupting compounds (35). As part of this investigation, 20 drinking water facilities in the U.S., all of which utilized source water with known wastewater impact, were evaluated for 18 pharmaceuticals (not including caffeine, endogenous hormones, and other contaminants). Of the pharmaceuticals analyzed, several were detected in raw source water (Table I) and in finished drinking water (Table II). Solid-phase extraction followed by analysis using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) was utilized for this study and is explained in detail elsewhere (37, 38).

Source Water

Source water samples were collected at the intake of the drinking water plants before treatment. In the source water samples evaluated, 11 of the 18 target pharmaceuticals were detected. Carbamazepine and dilantin (phenytoin) were detected in 18 of the 20 waters evaluated with maximum concentrations of 39 and 13 ng/L, respectively. Sulfamethoxazole, ibuprofen, and meprobamate were the next three most frequently detected pharmaceuticals (over 80% detection frequency) in the raw waters investigated with maximum concentrations of 44, 24, and 16 ng/L, respectively. Of the pharmaceuticals surveyed, iopromide (an intravenously administered x-ray contrast agent) had the greatest concentration in source water at 46 ng/L, while the antibiotic sulfamethoxazole exhibited the greatest average concentration at 14 ng/L. Pentoxifylline, ethynylestradiol, diclofenac, hydrocodone, diazepam, and fluoxetine were not detected in any of the source waters analyzed (35). It should again be noted that the source waters evaluated in this study were all known (or highly suspected) to have a portion of their flow originating from wastewater effluent discharges.

Table I. Initial survey of pharmaceuticals in source waters: 2002-2005 (35)

	<i>Raw Drinking Water (n=20)</i>					
	<i>Detects</i>	<i>% Freq.</i>	<i>Min (ng/L)</i>	<i>Max (ng/L)</i>	<i>Median (ng/L)</i>	<i>Ave (ng/L)</i>
Carbamazepine	18	90	1.2	39	3.1	6.2
Dilantin	18	90	1.1	13	3.2	3.5
Sulfamethoxazole	17	85	1.2	44	8.1	14
Ibuprofen	16	80	1.1	24	4.2	6.1
Meprobamate	16	80	1.4	16	5.9	7.0
Iopromide	14	70	2.2	46	7.6	12
Gemfibrozil	13	65	1.2	11	4.8	5.2
Naproxen	10	50	1.1	16	2.2	5.7
Erythromycin	8	40	1.0	3.5	2.2	2.2
Acetaminophen	7	35	1.1	9.5	1.6	2.7
Trimethoprim	3	15	1.0	2.3	2.2	1.8

Table II. Initial survey of pharmaceuticals in finished drinking water: 2002-2005 (35)

<i>Finished Drinking Water (n=20)</i>						
	<i>Hits</i>	<i>% Freq</i>	<i>Min (ng/L)</i>	<i>Max (ng/L)</i>	<i>Median (ng/L)</i>	<i>Ave (ng/L)</i>
Meprobamate	15	75	1.6	13	3.8	6.1
Dilantin	14	70	1.1	6.7	2.3	2.7
Ibuprofen	13	65	1.0	32	3.8	7.9
Iopromide	13	65	1.1	31	6.5	8.5
Carbamazepine	11	55	1.1	5.7	2.8	2.8
Gemfibrozil	5	25	1.3	6.5	4.2	3.9
Erythromycin	1	5	1.3	1.3	1.3	1.3
Naproxen	1	5	8.0	8.0	8.0	8.0
Sulfamethoxazole	1	5	20	20	20	20
Trimethoprim	1	5	1.3	1.3	1.3	1.3

Finished Drinking Water

The results from pharmaceutical monitoring in the finished drinking waters evaluated in this study are shown in Table II. From the drinking waters tested, 10 of the 18 pharmaceuticals analyzed were detectable. The only pharmaceutical detected in source waters, but not detected in any finished water was acetaminophen. This is likely due to rapid reaction with chlorine and other oxidants (39). While the detected compounds were fairly conserved from raw to finished drinking water, the frequency and concentrations of many pharmaceuticals changed significantly. For instance, sulfamethoxazole was detected in 17 source waters but only once in finished water. The finished water where sulfamethoxazole was detected originated from a water treatment plant that utilized chloramination as the primary and secondary disinfectant, thus the oxidation efficiency was much lower than the other treatment plants that utilized free chlorine or ozone as the primary disinfectant. In the finished drinking water, meprobamate, dilantin, ibuprofen, iopromide, and carbamazepine were the five most frequently detected compounds at 75, 70, 65, 65, and 55% occurrence, respectively. These compounds were also determined to be highly resistant to chlorine oxidation which explains their persistence in many drinking water facilities. Those facilities where these compounds were detected in raw water but not in finished water generally utilized ozonation or activated carbon in the treatment scheme. Coagulation, flocculation, and conventional media filtration utilized in these plants was determined to have no appreciable attenuation of the compounds evaluated.

Conclusions from the Initial Survey

This drinking water survey conducted from 2002-2005 demonstrated that pharmaceuticals do occur in the intake water (raw water) entering U.S. drinking water plants and that most of the pharmaceuticals that were detected in the raw water also were detectable in the finished drinking water. However, the relative frequency of occurrence was compound and treatment specific. From the treatment facilities evaluated, the use of chlorine was the dominant mechanism for apparent compound removal (transformation). Further, it should be noted that the concentrations of most pharmaceuticals were generally near the method reporting limits. The follow-on question of relevance to human health would be addressed in a subsequent AwwaRF report to be discussed in the following sections.

Occurrence and Health Relevance of Pharmaceuticals in U.S. Drinking Water

The AwwaRF, WaterReuse Foundation, the California Urban Water Agencies, the Southern Nevada Water Authority, and 16 other public water agencies across the U.S. joined forces to fund an evaluation of the occurrence and public health relevance of pharmaceuticals in U.S. drinking water. Since it was unambiguous that pharmaceuticals could and did occur to some degree in most drinking waters, generally as the result of discharges of wastewater effluents to source waters (also known as unplanned potable water reuse). This project sought to develop a list of compounds based upon toxicological relevance and potential for occurrence (36). New compounds were added in this study (atenolol, atorvastatin and two metabolites, enalapril, a metabolite of fluoxetine, risperidone, simvastatin and one metabolite), while six pharmaceuticals were dropped from the initial survey (ibuprofen, iopromide, erythromycin, acetaminophen, pentoxifylline, and hydrocodone). Compounds were eliminated from consideration due to lack of occurrence in drinking water, lack of exhibited toxicity, and/or lack of suitable isotopic standards. All parent pharmaceuticals and metabolites in this survey were analyzed using LC-MS/MS with isotope-dilution (32). Complete occurrence results can be found elsewhere (36, 40).

Source Waters

Of the compounds analyzed in both studies, the frequency of occurrence and relative ranking were amazingly similar (Table III). The top five most commonly detected pharmaceuticals were sulfamethoxazole, meprobamate, carbamazepine, dilantin, and atenolol. However, concentrations determined in each study were at times less consistent. For instance, sulfamethoxazole had a maximum concentration of 110 ng/L in the 2006 study, while exhibiting a maximum concentration of 44 in the initial study. Similarly, meprobamate had a maximum concentration of 73 ng/L in the 2006 study, but only 16 ng/L in the initial study. Regardless, the general patterns and median concentration values remained remarkably similar, especially considering the differences in analytical methods

applied and some diversity in sampling locations. Atorvastatin and its two major metabolites were included in the 2006 study and were detected at three of the sites evaluated at 2.0 ng/L or less. It is noteworthy that atorvastatin is one of the most widely prescribed pharmaceuticals in the U.S., yet detection was relatively infrequent. Conversely, meprobamate is rarely prescribed in the U.S. (rxlist.com), yet is one of the most frequently detected pharmaceuticals in the studies described here. Interestingly, meprobamate is the primary CYP2C19 metabolite of the more widely used pharmaceutical carisoprodol and is excreted at relatively percentages of the oral dose of carisoprodol (41). Additionally, carisoprodol was listed as the 75th most prescribed pharmaceutical by Mosby's Drug Consult in 2003 (http://www3.us.elsevierhealth.com/DrugConsult/Top_200/) and reports oral daily doses of carisoprodol as 1400 mg/day (http://www3.us.elsevierhealth.com/DrugConsult/Top_200/Drugs/e0664.html). Considering the relatively large dosage rates for carisoprodol, the pervasive prescription rate, and the high degree of cytochrome P450 mixed-function oxidase metabolism of carisoprodol resulting in the formation of the excreted meprobamate, it is not surprising that meprobamate is frequently detected in wastewater outfalls and receiving waters.

Finished Drinking Water

Just as observed in the source water, finished drinking water occurrence of pharmaceuticals was also quite similar between the initial study (Table II) and the 2006 study (Table IV). Considering those compounds common to both studies, the frequency rankings are identical with meprobamate as the most frequently detected and sulfamethoxazole as the least frequently detected (excluding non-detectable compounds). Concentrations quantified were also very similar among the two studies. Sulfamethoxazole was an exception, as it was detected at one site in the initial study (discussed previously) at 20 ng/L, yet it was detected at three sites in the 2006 study at a maximum value of 3.0 ng/L. Moreover, it is clear that sulfamethoxazole concentrations are greatly attenuated, as the maximum concentration in source waters was 110 ng/L. It should also be noted that method reporting limits were generally lower in the 2006 study. Fluoxetine (ProzacTM) and diazepam were both detected in one finished drinking water at sub-part per trillion concentrations of 0.82 and 0.33 ng/L, respectively. While fluoxetine was detected in three source waters, diazepam was not. Diazepam was not detected in the initial study either. Although not detected in blanks, the single detection of diazepam in one finished water at less than one-ng/L is suspect.

Table III. Occurrence of pharmaceuticals in source waters: 2006-2008 (36)

<i>Compound</i>	<i>Raw Drinking Water (n=19)</i>			
	<i>Max</i> <i>ng/L</i>	<i>Median</i> <i>ng/L</i>	<i>Frequency</i>	
			<i>#</i>	<i>%</i>
Sulfamethoxazole	110	12	17	89
Meprobamate	73	8.2	16	84
Carbamazepine	51	4.1	15	79
Dilantin	29	5.0	14	74
Atenolol	36	2.2	12	63
Gemfibrozil	24	2.2	11	58
Naproxen	32	0.93	11	58
Trimethoprim	11	0.75	11	58
Diclofenac	1.2	1.1	4	21
Atorvastatin	1.4	0.83	3	16
Fluoxetine	3.0	0.8	3	16
o-Hydroxy atorvastatin	1.2	0.66	3	16
p-Hydroxy atorvastatin	2.0	1.0	3	16
Ethinylestradiol	1.4	1.4	1	5

Summary and Discussion

The frequency of occurrence and concentrations of analytes analyzed in both studies were remarkably similar. Based on relative prescription volumes of the pharmaceuticals analyzed, the frequency of occurrence does not seem to correlate. However, there were limited numbers of pharmaceuticals evaluated and more research would be necessary to better correlate the link between usage or prescription volume to occurrence in drinking water. Although not examined in this manuscript, the relationship between pharmaceutical occurrence and drinking water treatment processes is obvious. Chlorine is used at the vast majority of U.S. drinking water facilities for primary and/or secondary disinfection and is the primary reason for attenuation at the majority of facilities evaluated. Ozone is a far more efficient oxidation, but is used less frequently in the U.S. Activated carbon can be extremely effective for organic contaminant removal; however, it is relatively rare in the U.S. and efficacy depends on water quality and operational parameters (42). While water treatment processes can and do reduce the concentration of some pharmaceuticals, substances such as meprobamate are quite resistant to oxidation and thus are generally not well removed (43). From the studies summarized in Tables III-IV, it is obvious that pharmaceuticals are relatively common contaminants in U.S. surface waters. However, the sites

selected and the ultra-trace analytical methods applied must be considered in order to better appreciate the minute levels of pharmaceuticals detected.

Pure water, containing only molecules of water with no other substances/elements dissolved, does not naturally occur on Earth's surface. Due to mankind's influence on the environment and in consideration of the hydrologic cycle, it is not surprising that pharmaceuticals are detected at extremely minute concentrations in drinking water. Moreover, as human population continues to grow and urbanize, more anthropogenic substances will certainly be detected in drinking water. The wastewater discharged from one community often becomes part of the source water for another community. Furthermore, in consideration of ecological impacts demonstrated from wastewater effluents containing trace contaminants it makes far greater sense to concentrate treatment efforts in wastewater as opposed to drinking water. Thereby, both ecological and public health benefits can be realized. In consideration of both water and energy sustainability, water quality goals should be established that are protective of public and environmental health. They should not be based solely on analytical detection limits that will continue to be pushed lower as analytical technology develops. If public perception from detection alone becomes the sole driver for increasing the purity of drinking water, it is likely that the only sustainable solution will be point of use/point of entry devices as the vast majority of municipal water (often >99%) is used for non-potable applications.

Table IV. Occurrence of pharmaceuticals in U.S. drinking waters: 2006-2008 (36)

<i>Compound</i>	<i>Max</i> <i>ng/L</i>	<i>Median</i> <i>ng/L</i>	<i>Frequency</i>	
			<i>#</i>	<i>%</i>
Meprobamate	42	5.7	14	78
Dilantin	19	6.2	10	56
Atenolol	18	1.2	8	44
Carbamazepine	18	6.0	8	44
Gemfibrozil	2.1	0.48	7	39
Sulfamethoxazole	3.0	0.39	4	22
Fluoxetine	0.82	0.71	2	11
Diazepam	0.33	0.33	1	6

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Chapter 4

Trace Organics in Arizona Surface and Wastewaters

C. Chiu¹ and P. K. Westerhoff*,¹

¹School of Sustainable Engineering and the Built Environment,
Arizona State University, Engineering Center, G-Wing Room ECG252,
P.O. Box 5306, Tempe, AZ 85287

*Corresponding author e-mail: p.westerhoff@asu.edu.

To investigate the occurrence of trace organic compounds (TrOCs) in waters throughout Arizona, 26 compounds (pharmaceuticals and personal care products (PPCPs), endocrine disrupting compounds (EDCs), and artificial sweetener) were analyzed in samples from surface waters, drinking water treatment plants (WTPs), wastewater treatment plants (WWTPs), and a groundwater recharge site during September 2007 to July 2009. Samples were analyzed using liquid chromatography/tandem mass spectrometry (LC/MS/MS). TrOCs were prevalent during this study, as they were found in 95% of the samples collected ($n > 100$). The most frequently detected compounds in surface waters were oxybenzone, caffeine, and sucralose. The total concentration of TrOCs varied seasonally with the highest concentration detected during summer. For WTPs, the majority of TrOCs detected in surface water were also identified in WTP raw waters and sedimentation effluent. High TrOC concentrations were detected in raw wastewater, and certain compounds (e.g. oxybenzone, ibuprofen, DEET, etc.) exhibited an increasing trend during summer. WWTP processes were shown to remove 11 of 26 compounds with up to 98% efficiency. Sucralose and sulfamethoxazole were dominant compounds (>60%) in WWTP effluent. Pharmaceuticals were also present in the ground water system of the Phoenix water supply area (<5 ng/L). Overall, the top six compounds detected were a) by frequency: oxybenzone, caffeine, sucralose, DEET,

sulfamethoxazole, and acetaminophen; and b) by concentration: oxybenzone, caffeine, sucralose, DEET, sulfamethoxazole, and dilantin. Results of this study demonstrate the occurrence of TrOCs in the Phoenix drinking water system. Other sources of TrOCs that might impact drinking water, such as lakes, WWTP recharging sites, and landfill sites, need to be investigated for overall water resource management in Arizona.

Introduction

Trace organic compounds (TrOCs) like endocrine disrupting compounds (EDCs), pharmaceuticals and personal care products (PPCPs), and artificial sweetener (e.g., sucralose) have been detected in water supplies and wastewater effluents around the world (1–4). Some TrOCs exhibit adverse ecological impacts that have raised concern among public and regulatory groups about the fate of such compounds during potable water treatment and human exposure from drinking water (3, 5–13). The impacts of EDCs on the environment are not entirely known, but undesirable effects on non-target aquatic organisms and damage to sensitive ecosystems are possible. Although pharmaceuticals are not considered to pose an appreciable risk to human health through drinking water and the food chain (14), a previous study that applied a life cycle impact assessment to a Spanish wastewater treatment plant located on the Mediterranean coast revealed that PPCPs, rather than the priority pollutants, contributed most to water toxicity (15). However, not only the persistence in the environment but also the biological activity of degradation by-products will exert the impact of these chemicals on the environment. Despite all the drawbacks related to the presence of TrOCs in the environment, most people are not aware of the importance of this growing issue. A survey conducted in the United States showed that unused or expired medicines are generally disposed of through trash, toilet, and sink rather than by return to pharmacies (16). Recent studies reported the occurrence of numerous TrOCs in both groundwater and surface waters throughout the world. The greatest amount of data was available for Italy, Spain, Sweden, Switzerland, Netherlands, UK, Israel, USA, China, Canada, and Germany (17–24). According to the United States Geological Survey (USGS), the most frequently detected TrOCs in water streams are nonprescription and prescription drugs, insect repellents, detergent metabolites, plasticizers, fire retardants, antibiotics, herbicides, hormones, and solvents (25, 26).

TrOCs enter in the environment from several sources such as: (i) effluents from wastewater treatment plants (WWTPs), (ii) leakage from septic tanks or landfill sites, (iii) surface water run-off, and (iv) direct discharge into waters. The major sources of such chemicals are WWTP effluents and secondary terrestrial run-offs. Run-off and leaching from livestock are the main paths for transport of veterinary drugs to groundwater. Human pharmaceuticals enter the aquatic system by ingestion followed by excretion in the form of non-metabolized parent compounds or as metabolites. Personal care products are discharged in particular through shower waste and water activities. All these contaminants

can pass through WWTPs and enter into water streams because they may not be completely degraded (27). A study conducted in Taiwan showed that the highest drug concentrations were found in waste streams (37.5%), followed by animal husbandries (27.9%) and drug production facilities (23.4%) (28).

The majority of TrOCs are polar and hydrophilic, They have low octanol/water partition coefficients ($\log K_{ow}$), which result in less binding to organic fractions of sludge or suspended sediments compared to other persistent organic compounds that are capable of bioaccumulation (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, or organochlorine pesticides). The low volatility of TrOCs suggests that their distribution through the environment will mainly occur through aqueous transport and food chain dispersal (4). Moreover, pharmaceuticals have shown a greater resistance to microbial degradation in estuarine and coastal waters than many other similarly sized naturally occurring biogenic molecules, which are abundant in seawater (29). Batch experiments also indicated that the removal of these soluble pharmaceuticals from water columns to sediments was insignificant (29). Although the scientific literature about the fate of many of these substances is extensive, limited knowledge is available on the effects of these TrOCs on the environment, especially in the case of exposure to mixtures of these compounds.

Some EDCs/PPCPs are more polar than current USEPA regulated polyaromatic contaminants. This, coupled with occurrence at trace levels (parts per trillion), creates unique challenges for analytical detection and assessment of removal performance by drinking water treatment plant (WTP) processes (3, 12). Drinking water treatment relies primarily upon adsorptive and oxidative processes to remove or transform organic materials. Recent studies on selected groups of EDCs/PPCPs, pesticides, and herbicides indicate that coagulation, sedimentation, and filtration achieve minimal levels of removal (13, 30–32). However, addition of common disinfectants (e.g., chlorine or ozone) can result in reaction and transformation of these compounds (33–42). Chemical coagulation and softening aid in removing suspended solids (i.e., turbidity) from the water and aid in removing dissolved organic carbon (DOC). Coagulation alone is generally not effective at removing trace-level organic pollutants (43, 44).

Activated carbon adsorbs many organic pollutants (45). The USEPA identifies packed-bed granular activated carbon (GAC) as a “Best Available Technology” for treating numerous regulated organic pollutants. Powdered activated carbon (PAC) effectively removes many problematic organic pollutants (e.g., taste and odor compounds, some pesticides and herbicides) (46). For some organic compounds, adsorptive removal by PAC may not be effective, but the compounds may react with oxidants (47, 48). The formation, fate, detection, and toxicity of oxidative by-products from pesticides and EDCs/PPCPs is of potential concern (33, 49).

Several studies have investigated EDC or PPCP removal by ozone or chlorine (30, 37, 40, 49, 50), but direct comparisons are lacking between these two oxidants and a broad range of EDCs/PPCPs under the typical conditions of drinking water treatment facilities. Molecular ozone is a selective electrophile that reacts with amines, phenols, and double bonds, whereas HO^{\bullet} reacts less selectively with organic compounds (41, 51, 52). Due to the selective nature of

ozone, micropollutant transformation may require the use of advanced oxidation processes (AOPs), such as O_3/H_2O_2 (1, 48).

Many municipalities are concerned about the potential for EDCs/PPCPs to occur in their water supplies, and the presence of these compounds may affect future treatment decisions. Collecting data over a watershed scale will provide useful information to address public questions or concern from advocacy groups, and this locally funded investigation demonstrates the proactive nature of local government for monitoring water quality. The key benefit will be providing a set of baseline data for trace organic occurrence from which future decisions regarding the need for additional monitoring can be rationally developed. The objectives of this article are to provide baseline data for EDCs/PPCPs in the Salt River watershed, including canals and recharge systems, as a state-wide view of these trace organics present in Arizona waters. Arizona is known for its limited water sources, and the water is used for many purposes such as irrigating and drinking. These data provide information on the occurrence of TrOCs as well as their natural attenuation in the environment. To accomplish the objectives, samples were collected from multiple water sources (such as watersheds, canals, groundwater, drinking water treatment facilities, etc.), as will be described in the next section.

Methods

Site Selection and Sampling

To provide a metro-Phoenix-wide view of TrOCs present in Arizona waters, samples were collected from three main water sources and from a selected drinking water treatment plant, wastewater treatment plants, and groundwater near a recharging site. Surface waters were collected from three different water sources for the Phoenix area water supply: Verde River (Verde River at Beeline Highway), Waddell Canal (near Lake Pleasant Road), and Salt River (Blue Point Bridge); these samples were collected bi-monthly (see Appendix A). Samples were also collected bi-monthly from one drinking water treatment plant (WTP A, see Appendix A) to represent drinking water samples including raw water, sedimentation effluent, and finished waters (after chlorination). Wastewater samples were collected once during this investigation from eight wastewater treatment plant (see Appendix A and Appendix B) effluents; a full investigation (samples from raw wastewater, tertiary effluent, and effluent) was conducted for WWTP A. Three measuring wells of the GRUSP (Granite Reef Underground Storage Project) recharging project (see Appendix C) were selected as groundwater sampling sites that were visited three times during this investigation. In addition, two water recreation sites (see Appendix D) were selected to testify to the occurrence of TrOCs in surface waters as a result of human activities. Moreover, an investigation of natural attenuation of TrOCs was conducted by collecting samples from the Santa Cruz River, which receives treated wastewater from Nogales WWTP directly. All these samples were collected during September 2007 to July 2009.

All samples were collected by ASU using 1-L ashed amber bottles and stored on ice. One hundred mg/L of sodium azide and 50 mg/L of ascorbic acid were added right after return to the laboratory to prevent biodegradation. Samples were filtered using 0.7 μm filter paper (GF/F, Whatman) before analysis. For the purpose of recovery correction for analyte loss during solid phase extraction (based on EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS), all standard and field samples were spiked with the same amount (50 ng/L) of internal standard (IS) (Acetaminophen- D_4 , Caffeine- $^{13}\text{C}_3$, ^{13}C -Naproxen- D_3 , Carboamazepine- D_{10} , Estradiol- $^{13}\text{C}_2$) after filtration. However, the filtration might lead to underestimated total concentrations, particularly for more hydrophobic compounds in raw wastewater. All the data that reported in this study are dissolved concentrations detected in filtered water samples.

Analytical Methods

Solid phase extraction was performed for each of the filtered water samples taken from the WWTPs and surface water samples using a Caliper Life Sciences Auto Trace extraction manifold. Methanol (HPLC grade, Fisher Chemical), MTBE (HPLC grade, Fisher Chemical), toluene (HPLC grade, Fisher Chemical), and water (HPLC grade, Honeywell, B&J) were used as solvents with Waters Oasis HLB extraction cartridges. PPCP analysis was performed with cooperation from the Arizona Department of Health Services using an Applied Biosystems API4000 triple quadrupole mass spectrometer and an Agilent 1100 Series HPLC system. A Phenomenex Synergi 4 micron Max RP 80A column was used for analyte separation. An LC gradient of water with 0.01% formic acid (A) and methanol with 0.01% formic acid (B) at a flow rate of 700 $\mu\text{L}/\text{min}$ was used. The gradient was as follows: 5% (B) held for 3.5 min, increased linearly to 80% (B) at 10 min. and held for 3 min., at 13.5 min ramped to 100% (B) and held until 21.0 min., at 21.5 min ramped back down to 5% (B) and held until 30.0 min.

Twenty-two prescription drugs, artificial sweetener, and personal care products as well as four steroids were selected as target compounds because of their high frequency of household application and identification in previous research (*I*) as being prevalent in the environment. None of the 26 TrOC compounds have the regulated criteria of guidelines in the form of maximum contaminant level (MCL). The effects of short term, high dose exposure conducted on aquatic life are summarized in Table 1, and the detected concentrations of these compounds in the surface waters were much lower than the aquatic life criteria. However, chronic effects from long term, low level environmental exposure to select TrOCs appear to be of much greater concern, and sufficient data collection will be required for further study. Table 1 also lists the K_{ow} (octanol-water partition coefficient) values and functions of these target compounds. Standards for these compounds were supplied by ADHS, and the LC/MS/MS operating conditions are shown in Appendix E. Internal standards including Acetaminophen- D_4 (Cerilliant), ^{13}C -Naproxen- D_3 (Cambridge Isotope), Estradiol- $^{13}\text{C}_2$ (Cambridge Isotope), Caffeine- $^{13}\text{C}_3$ (Cambridge Isotope), and Carboamazepine- D_{10} (Cambridge Isotope) were spiked before solid phase extraction for recovery correction.

Table 1. Summary of TrOCs studied and their properties

<i>Compound name</i>	<i>CAS #</i>	<i>Use</i>	<i>Log K_{ow}^a</i>	<i>LC50 or EC50^b</i>	<i>Ion-ization mode^c</i>
Acetaminophen*	103-90-2	NSAID ^d	0.27	41.5(D) ^e	ESI+
Caffeine*	58-08-2	stimulant	0.16	46.9(D)	ESI+
Carbamazepine*	298-46-4	anticonvulsant	2.25	6.4(D)	ESI+
Cotinine	486-56-6	metabolite of nicotine	0.34	112.5(D)	ESI+
DEET ^f	134-62-3	insect repellent	2.26	5.1(D)	ESI+
Diazepam	439-14-5	anxiolytic	2.7	2.3(D)	ESI+
Diclofenac	15307-86-5	NSAID ^d	4.02	4.2(D)	ESI-
Dilantin	57-41-0	antiepileptic	2.16	103.8(F)	ESI-
Erythromycin	114-07-8	antibiotic	2.48	7.8(D)	ESI+
Fluoxetine	54910-89-3	antidepressant	4.65	0.2(D)	ESI+
Hydrocodone	125-29-1	narcotic analgesic	2.16	4.9(D)	ESI+
Ibuprofen	15687-27-1	NSAID ^d	3.79	4.3(D)	ESI-
Meprobamate	57-53-4	anxiolytic	0.98	8.9(GA)	ESI+
Naproxen*	22204-53-1	NSAID ^d	3.1	15.1(D)	ESI-
Oxybenzone	131-57-7	sunscreens	3.52	2.9(D)	ESI+
Pentoxifylline	6493-05-6	antiplatelet drug	0.56	39.2(D)	ESI+
Primidone	125-33-7	anticonvulsant	0.73	73.0(D)	ESI+
Sucralose	56038-13-2	artificial sweetener	-1	2341.5(D)	ESI-
Sulfamethoxazole	723-46-6	antibiotic	0.48	4.5(D)	ESI+
TBBA ^g	79-94-7	flame retardant	7.2	0.007(GA)	ESI-
Triclosan	3380-34-5	antibiotic	4.66	0.6(GA)	ESI-
Trimethoprim	738-70-5	antibiotic	0.73	2.6(GA)	ESI+
Estradiol*	50-28-2	steroid hormone	3.94	2.4(D)	APCI
Ethinyl Estradiol	57-63-6	estrogen	4.12	2.0(GA)	APCI

Continued on next page.

Table 1. (Continued). Summary of TrOCs studied and their properties

<i>Compound name</i>	<i>CAS #</i>	<i>Use</i>	<i>Log K_{ow}^a</i>	<i>LC50 or EC50^b</i>	<i>Ion-ization mode^c</i>
Progesterone	57-83-0	steroid hormone	3.67	3.3(GA)	APCI
Testosterone	58-22-0	steroid hormone	3.27	6.1(GA)	APCI

^a estimated data were acquired from ECOSAR. ^b lowest predicted LC50 (lethal concentration, concentration in water having 50% chance of causing death to aquatic life) or EC50 (half maximal effective concentration) for the most sensitive indicator aquatic species (ppm). ^c ionization mode for compounds during LC/MS/MS analysis (ESI+: electrospray ionization positive mode; ESI-: electrospray ionization negative mode; APCI: atmospheric pressure chemical positive mode). ^d nonsteroidal anti-inflammatory drugs. ^e indicator organism for LC50 or EC50 determination, D:daphid, F:fish, GA:green algae. ^f N,N-Diethyl-meta-toluamide. ^g Tetrabromobisphenol A. ^{*} isotope labeled analog of these compounds were spiked before solid phase extraction for recovery correction.

Quality Assurance Protocol

Laboratory blanks were used to assess potential sample contamination. These blanks were prepared using nanopure water in the laboratory. Field blanks were also used to determine the effect, if any, of field equipment and procedures on the concentrations of TrOCs during water sampling. These field blanks were also prepared using nanopure water and transferred into another clean, ashed bottle during water sampling. All blanks were subject to the same sample processing, handling, and equipment as the real samples. Concentrations obtained from blanks were not subtracted from environmental results. Environmental concentrations within the values observed in the set of blanks plus two times the standard deviation were reported as insignificant concentration or not detected. The measured concentrations in all blank samples and the statistical report are shown in the loss might be occurred during solid phase extraction and the recovery varied between different water matrices. Fifty µg/L of 5 internal standards were spiked into calibration standards as well as all field samples and blanks (before solid phase extraction) for recovery calculation of the loss due to matrix effect. All data shown in this research are compared with blank results (average+2SD) for significance of detection and corrected with recovery efficiency (Appendix G). In addition, no recovery test was performed for sucralose and steroids, and the results for these compounds shown in this article represent the minimum contamination level.

Results

Overview of Trace Organics Occurrence in Arizona Waters

The trace organics concentrations present in different water sources are varied. Table 2 summarizes the concentrations of TrOCs present in different waters during the investigation period.

The TrOC levels were varied in different waters from less than 10 ng/L (most compounds in groundwater) to 100 $\mu\text{g/L}$ (in raw wastewaters). In groundwater and surface waters (Salt River, Verde River, and CAP canal), about 13 compounds (including 4 steroids) were measured to be 2 ng/L or less than in blank samples. Some compounds were present in groundwater and surface waters consistently, like acetaminophen, caffeine, cotinine, dilantin, meprobamate, triclosan, DEET, and etc., with levels below 20 ng/L. Oxybenzone and sucralose concentrations in surface waters were higher (20 ng/L to 1 $\mu\text{g/L}$) but varied with seasons, which will be discussed later. CAP water is from the Colorado River which is located downstream of Lake Mead, which receives approximately 1% of its inflow from wastewater (53), and the observed sucralose concentrations are roughly 100x lower than observed in wastewater effluents. Sulfamethoxazole and sucralose were consistently present in one of the groundwater measuring well samples with a concentration of approximately 100 to 300 ng/L.

Most of the target compounds analyzed in WWTP effluents had concentrations in the range of 20 ng/L to 1 $\mu\text{g/L}$ (WWTP adopted activated sludge and nitrification processes). The concentrations of acetaminophen, ibuprofen, and steroids (shown in Table 2) are relatively low, which means the treatment processes removed these compounds better than others. This result will be compared below with TrOC concentrations in effluents from other treatment techniques. High TrOC concentrations of 20 ng/L to 100 $\mu\text{g/L}$ were detected in raw wastewaters. These high concentrations of emerging contaminants might accumulate in sludge or be transformed by microorganisms. The disposal of the wastewater sludge as well as the discharge of treated wastewater into natural water systems must be carefully managed.

Table 2. Summary of TrOC levels detected in different waters sampled across the state of Arizona

<i>Source</i>	<i>Non-detected or < Blank</i>	<i>2 to 10 ng/L</i>	<i>10 to 20 ng/L</i>	<i>20 ng/L to 1 ug/L</i>	<i>> 1 ug/L</i>
Groundwater at recharge site	Testosterone progesterone, ethinyl estradiol, estradiol and others	Acetaminophen, caffeine, DEET, erythromycin, meprobamate, oxybenzone, pentoxifylline	None	Sulfamethoxazole, sucralose (from one site)	None
Salt River Project waters (Verde River & Salt River)	Testosterone progesterone, ethinyl estradiol, estradiol and others	Sucralose, sulfamethoxazole, acetaminophen, cotinine, dilantin,	Caffeine, DEET	Oxybenzone	None
Central Arizona Project Canal from Colorado River	Testosterone progesterone, ethinyl estradiol, and others	Sulfamethoxazole, oxybenzone, meprobamate, DEET, cotinine, dilantin, carbamazepine, acetaminophen, primidone, estradiol	Caffeine, triclosan	Sucralose	None

Continued on next page.

Table 2. (Continued). Summary of TrOC levels detected in different waters sampled across the state of Arizona

<i>Source</i>	<i>Non-detected or < Blank</i>	<i>2 to 10 ng/L</i>	<i>10 to 20 ng/L</i>	<i>20 ng/L to 1 ug/L</i>	<i>> 1 ug/L</i>
Activated sludge WWTP with nitrification	Testosterone progesterone, ethinyl estradiol, estradiol	Acetaminophen, ibuprofen, diazepam, pentoxifylline	Cotinine	Caffeine, naproxen, oxybenzone, carbamazepine, hydrocodone, meprobamate, sulfamethoxazole, TBBA, DEET, erythromycin, fluoxetine, trimethoprim, primidone, triclosan, sucralose, dilantin, diclofenac	None
Raw wastewater	None	None	Diazepam, Progesterone, Ethinyl Estradiol, Estradiol	Testosterone, hydrocodone, pentoxifylline, erythromycin, trimethoprim, primidone, fluoxetine carbamazepine, dilantin, diclofenac	Ibuprofen, naproxen, triclosan, sucralose, acetaminophen, caffeine, cotinine, oxybenzone, meprobamate, DEET, TBBA, sulfamethoxazole

Occurrences of TrOCs in Surface Waters

Three surface waters in the Phoenix metropolitan area serving as drinking water sources were investigated in this study: Waddell Canal, Salt River, and Verde River. From September 2007 to July 2009, 24 samples were collected from these three surface waters. One or more TrOCs were found in more than 95% of the 24 surface water samples during the investigation. The TrOC results were compared with lab-blank and field-blank samples to make sure the measurements were significant and not due to artificial error.

Table 3 shows the TrOCs detected in the three surface waters during this study. Progesterone was not detected in any surface water samples. Measured concentrations were generally low (less than 50 ng/L); two compounds (oxybenzone and sucralose) exceeded 300 ng/L in some samples during the investigation. Fifteen of 26 target compounds were identified as prevalent anthropogenic contaminants in the surface waters (>50% occurrence); caffeine, DEET, sucralose, and oxybenzone were detected in most samples (>90% occurrence). The occurrence of estradiol was detected near and slightly above our reporting level of 2 ng/L in Arizona surface waters (not shown in Table 3). Although these values are lower than the suggested EC50 value (25 ng/L, conducted with fish) in previous study (54), the occurrence of estradiol is higher in this study than many surface waters reported in the United States (55, 56). Our atypical estradiol detected in surface waters probably results from the detection method which is not suggested for drinking waters and which can show false positives in the analysis of steroid hormones in water. To obtain a broader view of the long-term variation for individual surface waters, the monthly results were divided into two groups, summer and other seasons. Figure 1 shows the monthly total TrOC concentration for different surface waters and the diversified TrOC content between summer and other seasons. Overall, the occurrence of TrOCs in the three surface waters was higher during summer than in other seasons. Salt River water was found to have the highest TrOC concentration during summer (>350 ng/L, on average) but decreased sharply during other seasons (to 120 ng/L). CAP canal and Verde River samples exhibit less variance (30% difference) between summer and other seasons; the CAP canal had the highest total concentration during other seasons. From the point of view of content percentage, sucralose had the highest concentration in the CAP canal at any season (60% to 75%). For the Salt River, oxybenzone accounted for more than 80% of the total TrOCs measured during summer but decreased to 16% during other seasons. The Verde River had the lowest concentration of total TrOCs present, and oxybenzone had the highest concentration among the 26 compounds during summer.

Table 3. Summary of TrOCs detected in Arizona surface waters^{a, b}

Concentration: (ng/L)*	<i>CAP canal</i>		<i>Salt River</i>		<i>Verde River</i>	
	<i>mean detected</i>	<i>mean total</i>	<i>mean detected</i>	<i>mean total</i>	<i>mean detected</i>	<i>mean total</i>
Acetaminophen	4	2	10	2	5	2
Caffeine	17	10	19	11	14	7
Carbamazepine	3	2	2	<MRL	2	<MRL
Cotinine	5	3	4	2	2	<2
DEET	6	4	17	8	10	5
Diazepam	<2	<MRL	2	<MRL	<2	<MRL
Diclofenac	2	<MRL	3	<MRL	3	<MRL
Dilantin	7	3	10	2	6	2
Erythromycin	2	<2	3	<2	2	<2
Fluoxetine	3	<2	6	<2	3	<2
Hydrocodone	2	<2	3	<2	2	<2
Ibuprofen	5	<2	3	<2	<MRL	<MRL
Meprobamate	11	6	5	<2	3	<2
Naproxen	3	<2	5	2	2	<2
Oxybenzone	10	6	230	130	36	21
Pentoxifylline	3	<2	3	<2	2	<2
Primidone	7	3	3	<MRL	2	<2
Sucralose	180	100	5	2	13	9
Sulfamethoxazole	17	8	6	2	5	2
TBBA	4	<MRL	6	<2	4	<MRL
Triclosan	13	<2	8	<2	7	<2
Trimethoprim	3	<2	6	<2	3	<2
Estradiol	-	-	-	-	-	-
Ethinyl Estradiol	<2	<MRL	<2	<MRL	<2	<MRL
Progesterone	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Testosterone	<2	<MRL	<MRL	<MRL	<MRL	<MRL

^a <2: concentration lower than 2 ng/L. ^b <MRL: concentration lower than method reporting limit which is indistinguishable from the blank sample (blank results are summarized in Appendix F). * the data reported are dissolved concentrations detected in filtered water samples.

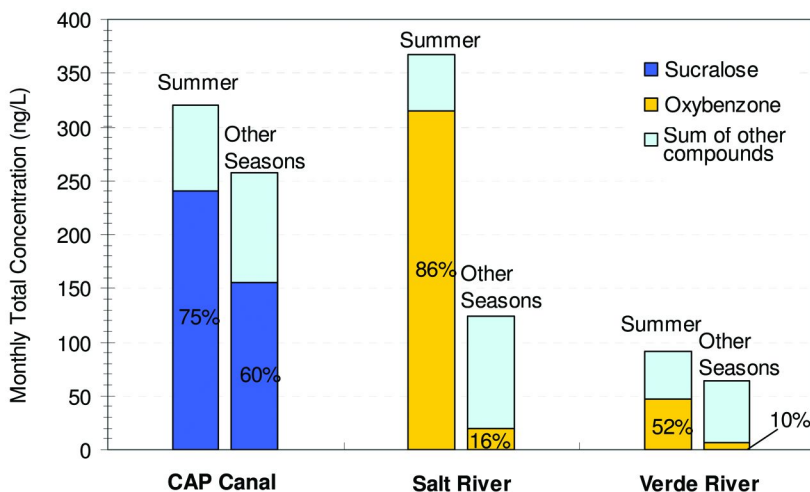


Figure 1. Seasonal variation of TrOCs detected in surface waters

These dramatic increases in oxybenzone, an ingredient in sunscreens, during summer suggest that water-based recreation (e.g., swimming and tubing) is popular upstream in the Salt River and Verde River. For example, the sampling site for Salt River was close to water recreational area which might be the direct contribution of oxybenzone during summer. The high proportion of sucralose present in the CAP canal might result from the discharge of treated wastewater into Colorado River. Artificial sweetener has been suggested as an ideal marker of domestic wastewater in drinking water sources (57), but further investigation is needed for verification. The total concentration of compounds other than oxybenzone and sucralose in surface waters was constant, which indicates a constant discharge into water column upstream of these three rivers/canal. The different patterns of TrOC content between different water sources during different seasons could be important information for drinking water treatment plants planning to switch source waters throughout the year.

Occurrence and Fate of TrOCs in a Drinking Water Treatment Plant

Samples collected from drinking water treatment plant A (WTP A) were used for TrOC analysis and examination of fate during drinking water treatment processes. WTP A is a utility on a Salt River Project canal with conventional coagulation/ sedimentation/ filtration treatment processes. Raw water, sedimentation effluent, and finished water from this drinking water treatment plant were collected for analysis. Finished water samples were collected after disinfection but before entering the distribution system. Thirty-one samples were collected from September 2007 to July 2009. Four out of 11 raw water samples were found to contain >100 ng/L of total TrOCs present. Eight out of nine finished water samples had a TrOC residual (27 ng/L on average).

Table 4 shows the TrOCs detected in WTP A during the investigation. The measured concentrations for each compound were generally lower than 5 ng/L,

and hydrocodone was not detected in any sample. For raw waters, five compounds (caffeine, cotinine, DEET, oxybenzone, and sucralose) were defined as prevalent emerging contaminants (>50% occurrences); 90% of raw waters contained DEET. Caffeine, DEET, oxybenzone, and sucralose concentrations greater than 10 ng/L were detected in some raw water samples; in one, oxybenzone was greater than 100 ng/L. The drinking water treatment processes achieved > 50% removal when concentrations of these compounds higher than 5 ng/L were present in raw waters. The remaining oxybenzone could be completely oxidized by chlorination, with no oxybenzone residual detected in the finished water. Nonetheless, the TrOC residual remaining in the finished water are constant and significant. For long-term drinking water management, monitoring of source waters and the fate of TrOCs in drinking water systems is recommended. In addition, it is important to be addressed that the value of ethinyl estradiol in drinking water at 4 ng/L is high compared with values ever reported in literature (55, 56, 58). Considering the suggested EC50 value of 0.9 ng/L in previous study (54), the concentration of ethinyl estradiol would cause serious concern for the public health. However, the kinetics of reaction between chlorine and ethinyl estradiol are extremely efficient (~100% removal) according to the study of Westerhoff et al. (59). The atypical value of ethinyl estradiol detected in drinking water was probably resulted from the detection method which is not suggested for drinking waters and shows great errors in the analysis of steroid hormones in water.

Seasonal variations were observed for TrOCs in WTP waters. Figure 2 shows the summer and other seasons total TrOC content. The highest concentration of oxybenzone (78 ng/L) was detected in raw waters in the summer, which is consistent to the result from surface water investigation. During the other three seasons, the concentration of oxybenzone decreased and sucralose increased in raw water, and the total concentration of TrOCs detected declined to only half of the summer level. This is because the Salt River is the major source (~66%) of raw water during summer; SRP (Salt River Project) switched its raw water source to the Verde River (>80%) mixed with CAP water (~10%) during winter. WTP processes prior to chlorination (coagulation, sedimentation, and filtration) exhibit similar capacity to remove oxybenzone during different seasons (16 ng/L for summer and 12 ng/L for the other seasons). However, >99% of the oxybenzone residual will be oxidized by chlorination in all seasons. The similar total TrOC concentration in finished water in all seasons indicated that TrOC residues remaining in the water phase were relatively stable, that is, they could resist most chemical, biological, and physical degradation throughout the environmental system and drinking water treatment processes. This 30 ng/L of TrOC residual in the distribution system can serve as the baseline and worth of monitoring in the aspect of public health.

Table 4. Summary of TrOCs detected in WTP samples^{a, b}

<i>Concentration: (ng/L)*</i>	<i>WTP influent</i>		<i>Sedimentation effluent</i>		<i>Finished water</i>	
	<i>mean detected</i>	<i>mean total</i>	<i>mean detected</i>	<i>mean total</i>	<i>mean detected</i>	<i>mean total</i>
Acetaminophen	6	2	6	2	8	2
Caffeine	14	10	13	7	11	5
Carbamazepine	<2	<MRL	<2	<MRL	2	<MRL
Cotinine	2	<2	2	<2	2	<2
DEET	6	6	5	3	6	3
Diazepam	<2	<MRL	<2	<MRL	<2	<MRL
Diclofenac	2	<MRL	2	<MRL	2	<MRL
Dilantin	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Erythromycin	2	<2	2	<2	2	<2
Fluoxetine	5	<MRL	6	<2	6	<2
Hydrocodone	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Ibuprofen	<MRL	<MRL	<MRL	<MRL	5	<2
Meprobamate	3	<2	3	<2	4	<2
Naproxen	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Oxybenzone	78	43	93	25	<MRL	<MRL
Pentoxifylline	3	<2	3	<2	3	<2
Primidone	2	<MRL	2	<MRL	2	<2
Sucralose	15	10	8	4	11	4
Sulfamethoxazole	4	<2	4	<2	3	<MRL
TBBA	4	<2	4	<2	4	<MRL
Triclosan	<MRL	<MRL	<MRL	<MRL	9	<2
Trimethoprim	3	<2	4	<MRL	4	<MRL
Estradiol	<MRL	<MRL	3**	<MRL	<MRL	<MRL
Ethinyl Estradiol	2**	<MRL	<2	<MRL	4**	<2
Progesterone	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Testosterone	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL

^a <2: concentration lower than 2 ng/L. ^b <MRL: concentration lower than method reporting limit which is indistinguishable from the blank sample (blank results are summarized in Appendix F). * the data reported are dissolved concentrations detected in filtered water samples. ** steroid concentrations were at or near reporting levels

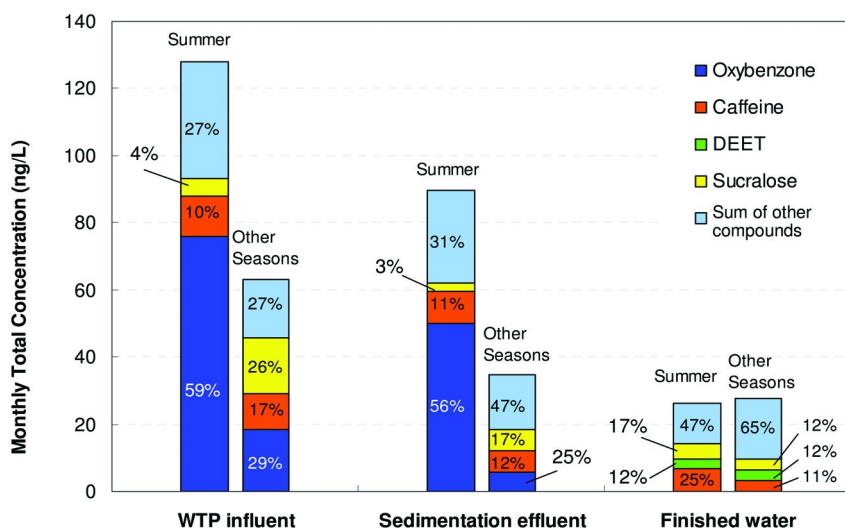


Figure 2. Seasonal variations in TrOC levels detected at three sampling locations of an Phoenix WTP

Occurrence and Fate of TrOCs in a Wastewater Treatment Plant

Wastewater samples were collected from wastewater treatment plant A. Raw wastewaters, tertiary effluent, and effluent after UV treatment were collected for investigation. Thirty samples were collected from September 2007 to July 2009. All 26 compounds were detected in more than one raw wastewater sample, and the highest measured concentration was that of acetaminophen (250 $\mu\text{g/L}$) and lowest that of diazepam (2 ng/L).

Table 5 shows the detected TrOCs in wastewater treatment plant A samples. In raw wastewaters, most compounds (16/26) were detected in the range of 0.1 to 10 $\mu\text{g/L}$, and acetaminophen, caffeine, ibuprofen, and naproxen concentrations higher than 10 $\mu\text{g/L}$ were detected. Steroid concentrations were generally from 20 to 80 ng/L . In tertiary effluent and effluent after UV treatment, the total concentration of TrOCs declined significantly from 250 $\mu\text{g/L}$ (in raw wastewaters) to 9 $\mu\text{g/L}$. The highest detected concentrations in treated wastewaters were those of sucralose and sulfamethoxazole (about 2.4 $\mu\text{g/L}$). Hormones and diazepam concentrations were found to be less than 5 ng/L . However, the removal efficiencies of the compounds by wastewater treatment processes are varied. Several compounds (acetaminophen, caffeine, naproxen, ibuprofen, oxybenzone, cotinine, triclosan, TBBA, testosterone, pentoxifylline, estradiol, ethinyl estradiol, and progesterone) were >90% removed in wastewater treatment units. For other compounds (sulfamethoxazole, carbamazepine, dilantin, and erythromycin) the removal efficiency was much lower (<20%). As observed by other researchers (60), K_{ow} (in Table 1) is not the only factor in the TrOC removal for a wastewater treatment plant adopted activated sludge and nitrification techniques because the poor correlations were found (not shown here). However, the filtration process before analysis might lead to removal of hydrophobic compounds and

underestimate the total concentration in wastewaters. Overall, the low removal sucralose and sulfamethoxazole suggested that these two compounds might serve as indicators of wastewater intrusion.

There also appears to be a seasonal effect on raw wastewaters. Of the 26 compounds detected in raw wastewaters, 73% were found at higher concentrations during the summer (May to September), and 46% had more than a 50% difference between summer and other seasons. Apparently, oxybenzone (sunscreen), DEET (insect repellent), and triclosan (sanitizer) were applied more frequently during summer. The average concentrations of hormones and fluoxetine (antidepressant) in summer samples were also higher than in the other seasons. These data on TrOCs in raw wastewaters reflect the Arizona household pharmaceutical application frequency during different seasons.

To provide a state-wide view of TrOCs in wastewater effluent, which could be potential source of trace organics in surface water, wastewater effluent samples were collected once during May 2009 from eight wastewater treatment plants (Table 6) (see Appendix A and Appendix B for WWTP locations). Overall, three hormones (ethinyl estradiol, progesterone, and testosterone), diazepam, and TBBA were reported to be not detectable or in concentrations of less than 5 ng/L in treated wastewaters. Sucralose was found in all eight wastewater effluents (3 ± 1.74 $\mu\text{g/L}$). Sulfamethoxazole was also present in all eight effluents with high concentration (2.3 ± 0.95 $\mu\text{g/L}$) except for one sample with 12 ng/L of sulfamethoxazole detected (WWTP C using wetland with treated water ponds). This might result from different wastewater processes in different WWTPs. Generally, the total TrOC concentration remaining in treated wastewaters varied from 3.5 $\mu\text{g/L}$ to 1.2 $\mu\text{g/L}$ depending on treatment technique (Figure 3). Wetland and membrane bioreactor WWTPs exhibited the lowest TrOC residuals remaining in treated wastewaters (< 5 $\mu\text{g/L}$), but wetland-based WWTPs had the highest caffeine and DEET concentrations remaining among the eight treated wastewaters. Compared with the other techniques, trickling filter led to the highest TrOC residual in wastewater effluent. WWTP E with aerated lagoon treatment process shown highest estradiol concentration remaining in effluent (61 ng/L). Membrane bioreactor and wetland had the lowest trimethoprim (0 to 30 ng/L) concentration in effluent compared with other treatment techniques (0.6 to 2.5 $\mu\text{g/L}$). In addition to sucralose and sulfamethoxazole, caffeine (0.02 to 4 $\mu\text{g/L}$), carbamazepine (0.15 to 0.3 $\mu\text{g/L}$), DEET (0.03 to 0.5 $\mu\text{g/L}$), oxybenzone (0.04 to 0.3 $\mu\text{g/L}$), and primidone (0.03 to 0.2 $\mu\text{g/L}$) were also detected in all wastewater effluents. The efficiency comparison between different wastewater treatment techniques requires the inclusion of other parameters (such as removal percentage) to yield a more solid conclusion.

Table 5. Summary of TrOCs detected in WWTP A^{a,b}

<i>Concentration: (ng/L)*</i>	<i>Raw wastewater</i>		<i>Tertiary effluent</i>		<i>Effluent after UV</i>	
	<i>mean detected</i>	<i>mean total</i>	<i>mean detected</i>	<i>mean total</i>	<i>mean detected</i>	<i>mean total</i>
Acetaminophen	140,000	140,000	13	4	20	8
Caffeine	51,000	51,000	32	26	47	37
Carbamazepine	480	480	440	440	450	450
Cotinine	2,200	2,200	11	11	14	14
DEET	1,200	1,200	210	210	200	200
Diazepam	11	6	5	3	4	3
Diclofenac	200	160	99	89	48	43
Dilantin	520	470	640	580	430	390
Erythromycin	160	160	180	160	190	170
Fluoxetine	120	72	63	63	48	48
Hydrocodone	99	89	61	61	53	53
Ibuprofen	10,000	9,200	17	8	14	8
Meprobamate	1,200	1,200	680	680	690	690
Naproxen	22,000	19,000	130	120	88	80
Oxybenzone	8,100	8,100	93	65	99	49
Pentoxifylline	46	32	7	3	8	3
Primidone	660	660	250	250	230	230
Sucralose	5,400	4,800	2,800	2,600	2,700	2,500
Sulfamethoxazole	2,500	2,500	3,000	3,000	2,300	2,300
TBBA	1,100	640	140	69	88	44
Triclosan	1,500	1,400	150	120	90	81
Trimethoprim	840	840	270	240	280	260
Estradiol	68	30	6	2	5	<2
Ethinyl Estradiol	25	17	2	<2	<2	<MRL
Progesterone	22	15	2	<2	<MRL	<MRL
Testosterone	75	67	3	<2	<MRL	<MRL

^a <2: concentration lower than 2 ng/L. ^b <MRL: concentration lower than method reporting limit which is indistinguishable from the blank sample (blank results are summarized in Appendix F). * the data reported are dissolved concentrations detected in filtered water samples.

Table 6. Summary of TrOCs in effluent from eight WWTPs (one time sampling in May 2009)^{a,b}

	<i>WWTP A</i>	<i>WWTP B</i>	<i>WWTP C</i>	<i>WWTP D</i>
<i>Concentration: (ng/L)*</i>	<i>Activated sludge</i>	<i>Membrane bioreactor</i>	<i>Wetland with treated water ponds</i>	<i>Activated sludge</i>
Acetaminophen	<MRL	<MRL	<MRL	170
Caffeine	44	37	1,000	49
Carbamazepine	290	250	150	290
Cotinine	4	11	27	22
DEET	190	31	490	230
Diazepam	<MRL	<MRL	<MRL	2
Diclofenac	41	17	<MRL	20
Dilantin	470	150	<MRL	220
Erythromycin	<MRL	<MRL	<MRL	70
Estradiol	14	<MRL	10	<MRL
Ethinyl Estradiol	<MRL	<MRL	<MRL	<MRL
Fluoxetine	25	<MRL	<MRL	51
Hydrocodone	51	37	<MRL	38
Ibuprofen	<MRL	6	<MRL	39
Meprobamate	430	280	11	670
Naproxen	<MRL	<MRL	<MRL	270
Oxybenzone	35	64	59	130
Pentoxifylline	5	<MRL	<MRL	<MRL
Primidone	180	122	78	89
Progesterone	<MRL	<MRL	<MRL	<MRL
Sucralose	3,200	1,700	2,500	4,000
Sulfamethoxazole	3,200	780	12	2138
TBBA	<MRL	<MRL	<MRL	<MRL
Testosterone	<MRL	<MRL	<MRL	<MRL
Triclosan	<MRL	<MRL	<MRL	41
Trimethoprim	63	27	0	1,800
Acetaminophen	53	52	280	27
Caffeine	14	250	4,000	55
Carbamazepine	160	250	210	230

Continued on next page.

Table 6. (Continued). Summary of TrOCs in effluent from eight WWTPs (one time sampling in May 2009)^{a,b}

	<i>WWTP A</i>	<i>WWTP B</i>	<i>WWTP C</i>	<i>WWTP D</i>
<i>Concentration: (ng/L)*</i>	<i>Activated sludge</i>	<i>Membrane bioreactor</i>	<i>Wetland with treated water ponds</i>	<i>Activated sludge</i>
Cotinine	13	410	180	20
DEET	140	95	82	180
Diazepam	2	3	<2	3
Diclofenac	320	11	24	18
Dilantin	120	240	180	820
Erythromycin	64	60	<MRL	4
Estradiol	61	10	16	9
Ethinyl Estradiol	<MRL	<MRL	<MRL	2
Fluoxetine	<MRL	130	36	49
Hydrocodone	10	70	47	45
Ibuprofen	23	37	97	<MRL
Meprobamate	63	490	430	610
Naproxen	47	440	1,000	30
Oxybenzone	45	60	340	55
Pentoxifylline	27	21	33	4
Primidone	30	110	54	230
Progesterone	<MRL	<MRL	<MRL	<MRL
Sucralose	1,400	2,900	2,000	7,000
Sulfamethoxazole	3,100	1,600	1,500	2,800
TBBA	<MRL	<MRL	<MRL	<MRL
Testosterone	<MRL	<MRL	<MRL	<MRL
Triclosan	13	32	42	13
Trimethoprim	2,500	2,400	950	600

^a <2: concentration lower than 2 ng/L. ^b <MRL: concentration lower than method reporting limit which is indistinguishable from the blank sample (blank results are summarized in Appendix F). * the data reported are dissolved concentrations detected in filtered water samples.

To understand the natural attenuation of trace organics, samples were collected from the Santa Cruz River, which receives treated wastewater (from WWTP E with aerated lagoon treatment) as its only water source during winter (shown in Table 7). Most of the TrOCs value were at the same magnitude as in Table 6, WWTP E. The extremely high value of trimethoprim might result from analytical error and could be referred to the value of 2.5 $\mu\text{g/L}$ in Table 6. Concentrations of several compounds (e.g., acetaminophen, cotinine, diclofenac) declined along the stream, whereas others remained constant (e.g., caffeine, sucralose, sulfamethoxazole) in all samples. The results shown might not be able to represent real scenario of natural attenuation since the sampling time didn't match the stream flow rate. However, the trend of decline could serve as important information for understanding the fate of trace organics in the environment. Further research is necessary to clarify whether partitioning or biotransformation dominates the distribution and the potential impact on ecosystems.

Table 7. TrOCs detected in the Santa Cruz River^{a,b}

	<i>WWTP E effluent</i>	<i>Santa Cruz River A</i>	<i>Santa Cruz River B</i>	<i>Santa Cruz River C</i>	<i>Santa Cruz River D</i>
<i>Concentration: (ng/L)*</i>	<i>River start 0 mile</i>	<i>Down- stream 2.5 mile</i>	<i>Down- stream 5.4 mile</i>	<i>Down- stream 7.5 mile</i>	<i>Down- stream 10.5 mile</i>
Acetaminophen	310	120	130	120	110
Caffeine	210	250	220	350	370
Carbamazepine	88	110	100	100	110
Cotinine	1,200	1,400	1,400	990	860
DEET	150	160	160	160	150
Diazepam	3	4	4	4	3
Diclofenac	510	490	500	320	300
Dilantin	270	92	92	98	97
Erythromycin	220	130	260	250	340
Fluoxetine	17	12	10	<MRL	<MRL
Hydrocodone	<MRL	4	<MRL	<MRL	5
Ibuprofen	280	270	320	400	330
Meprobamate	40	58	55	57	70

Continued on next page.

Table 7. (Continued). TrOCs detected in the Santa Cruz River^{a,b}

	<i>WWTP E effluent</i>	<i>Santa Cruz River A</i>	<i>Santa Cruz River B</i>	<i>Santa Cruz River C</i>	<i>Santa Cruz River D</i>
<i>Concentration: (ng/L)*</i>	<i>River start 0 mile</i>	<i>Down- stream 2.5 mile</i>	<i>Down- stream 5.4 mile</i>	<i>Down- stream 7.5 mile</i>	<i>Down- stream 10.5 mile</i>
Naproxen	1,900	550	500	490	480
Oxybenzone	40	33	36	41	41
Pentoxifylline	110	67	63	80	92
Primidone	39	55	55	61	63
Sucralose	1,500	1,700	1,400	1,300	1,100
Sulfamethox- azole	4,900	2,900	2,600	2,700	2,700
TBBA	<MRL	<MRL	<MRL	<MRL	<MRL
Triclosan	210	98	99	88	78
Trimethoprim	(11,000)**	230	270	330	290
Estradiol	110	89	48	32	67
Ethinyl Estradiol	<MRL	<MRL	<MRL	<MRL	<MRL
Progesterone	<MRL	5	5	3	3
Testosterone	<MRL	<MRL	<MRL	<MRL	<MRL

^a <2: concentration lower than 2 ng/L. ^b <MRL: concentration lower than method reporting limit which is indistinguishable from the blank sample (blank results are summarized in Appendix F). * the data reported are dissolved concentrations detected in filtered water samples. ** this value might be wrong due to analytical error and could be referred to the value of 2.5 µg/L shown in Table 6, WWTP E.

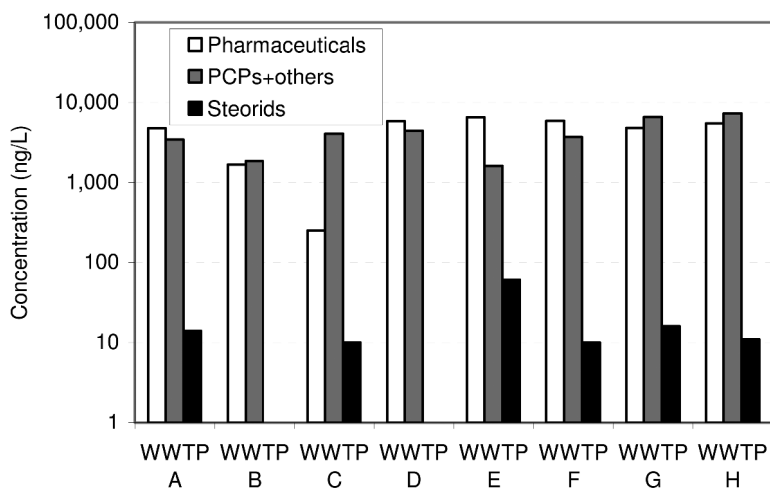


Figure 3. Concentrations of different groups of TrOCs in each WWTP effluent.

Occurrence of TrOC in Groundwater near Recharge Site

Groundwater samples were collected from three monitoring wells at a GRUSP recharge site during August 2008 and January and July 2009. Nine samples were collected, and three well volumes of groundwater were pumped out until pH and conductance were stable before sampling.

Table 8 shows the TrOC results from the groundwater samples. Most of the compounds had concentrations lower than 5 ng/L in these three samplings. Seven out of 26 compounds were not detected in significant concentrations in any of these samples. Erthromycin, meprobamate, and pentoxifylline were the most prevalent compounds in all nine samples but had low concentrations (<10 ng/L). Dilantin, oxybenzone, sucralose, and sulfamethoxzole concentrations higher than 10 ng/L were detected in some samples, whereas sucralose and sulfamethoxzole concentrations greater than 100 ng/L were found in samples from measuring well No. 3. As discussed in section on wastewaters, sucralose and sulfamethoxzole had the highest TrOC residuals in wastewater effluent, and their high concentrations in groundwater might be correlated with Arizona household habits. However, these high-concentration compounds in groundwater need further investigation to clarify the possible sources, such as surface water discharge, wastewater discharge, or intrusion from landfill sewage. Groundwater hydrology is worth investigation for understanding the occurrence of TrOCs in groundwater systems as part of drinking water sources.

Table 8. Summary of TrOCs detected in groundwater^{a,b}

<i>Concentration: (ng/L)*</i>	<i>GW MW1</i>		<i>GW MW2</i>		<i>GW MW3</i>	
	<i>mean detected</i>	<i>mean total</i>	<i>mean detected</i>	<i>mean total</i>	<i>mean detected</i>	<i>mean total</i>
Acetaminophen	4	2	4	2	4	2
Caffeine	8	3	5	2	6	2
Carbamazepine	<2	<2	<2	<2	<2	<2
Cotinine	2	<2	2	<2	2	<2
DEET	4	<2	<MRL	<MRL	5	2
Diazepam	<2	<2	<2	<2	<2	<2
Diclofenac	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Dilantin	<MRL	<MRL	<MRL	2	<MRL	8
Erythromycin	2	2	2	2	2	2
Fluoxetine	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Hydrocodone	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Ibuprofen	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Meprobamate	3	3	3	3	3	3
Naproxen	7	2	<MRL	<MRL	<MRL	<MRL
Oxybenzone	17	6	24	8	<MRL	<MRL
Pentoxifylline	2	2	2	2	2	2
Primidone	<2	<MRL	<2	<MRL	2	2
Sucralose	3	2	3	2	92	92
Sulfamethoxazole	5	3	17	11	200	200
TBBA	<MRL	<MRL	<MRL	<2	3	<2
Triclosan	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Trimethoprim	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Estradiol	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Ethinyl Estradiol	<MRL	<MRL	<MRL	<MRL	<2	<MRL
Progesterone	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL
Testosterone	<MRL	<MRL	<MRL	<MRL	<MRL	<MRL

^a <2: concentration lower than 2 ng/L. ^b <MRL: concentration lower than method reporting limit which is indistinguishable from the blank sample (blank results are summarized in Appendix F). * the data reported are dissolved concentrations detected in filtered water samples.

Conclusions

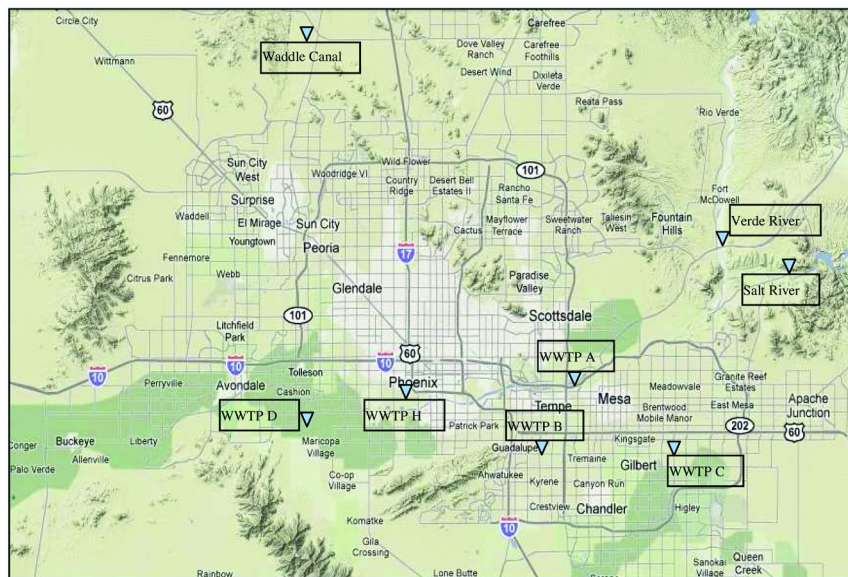
Results of this study demonstrate that low levels of TrOCs are present throughout the surface water system in Arizona. Public recreation in rivers and lakes could be major sources of skin-applied PCPs present in the canal system of the Phoenix area, especially during summers. Seasonal effects on surface waters might influence raw water quality in WTPs during source water switches by SRP. The seasonal effects of PCP occurrence from different sources might cause pulses of higher concentration in the drinking water system. Overall, the top six TrOCs detected are a) by frequency: oxybenzone, caffeine, sucralose, DEET, sulfamethoxazole, and acetaminophen; and b) by concentration: oxybenzone, caffeine, sucralose, DEET, sulfamethoxazole, and dilantin. Tracking the occurrence and fate of these TrOCs in drinking water systems and establishing a database is important for water management of metropolitan Phoenix to control these unregulated organic compounds in case of emergent issue.

For future monitoring, other possible sources of TrOCs that might impact drinking water, such as lakes, WWTP recharging sites, and landfill sites, need to be investigated for overall water resource management and potential ecosystem impact in Arizona. Long term monitoring of TrOCs in drinking water systems is recommended in case of abrupt contamination from wastewater discharge or landfill. The monitoring should at least focus on the 10 compounds detected most frequently and with highest concentration, including oxybenzone, caffeine, sucralose, DEET, sulfamethoxazole, acetaminophen, and dilantin.

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Appendix A. Sampling sites for surface waters and WWTPs in metropolitan Phoenix



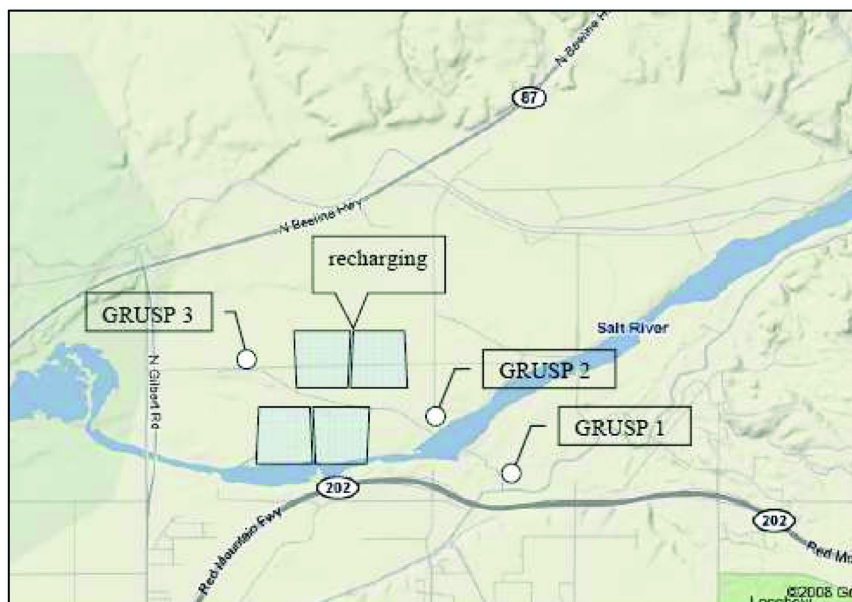
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Appendix B. More sampling sites for WWTPs in Tucson, Arizona

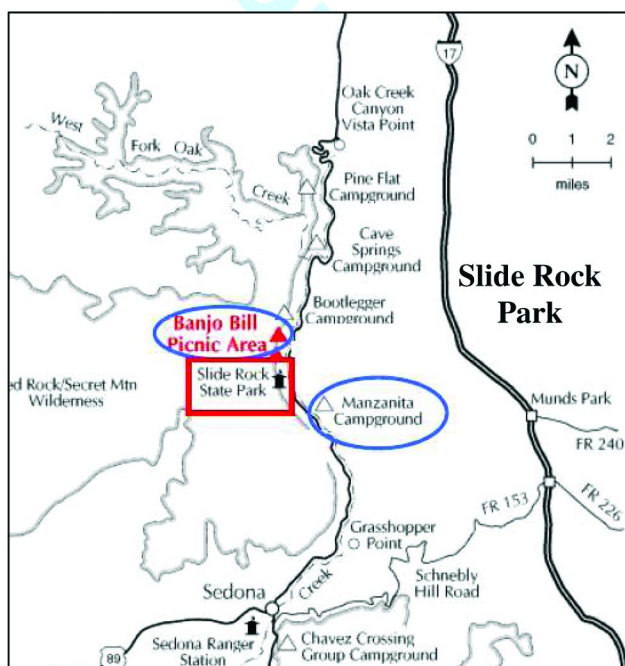


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Publication Date (Web): November 2, 2010 | doi: 10.1021/bk-2010-1048.ch004

Appendix C. Sampling sites for groundwater recharging project near Phoenix, Arizona



Appendix D. Two water recreational sites near metropolitan Phoenix



Appendix E

Ionization Source	Compound	Class/Use	Quantifier Ion Q1/Q3	Qualifier Ion(s) Q1/Q3	
APCI Positive	Estradiol	Steroid/Estrogen	255.3/159.2	255.30/133.1	
	Ethinyl Estradiol	Steroid/Synthetic Estrogen	279.2/133.0	279.2/159.1	
	Progesterone	Steroid/Estrogen	315.3/97.3	315.3/109.0, 315.3/109.2	
	Testosterone	Steroid/Androgen	289.3/97.3	289.3/109.2, 289.3/123.3	
ESI Negative	Cotinine	Personal Care Product/Nicotine metabolite	177.2/80.2	177.2/98.3	
	Diclofenac	Pharmaceutical/Anti-arthritis	294.3/250.0	294.3/214.0	
	Dilantin (Phenytoin sodium)	Pharmaceutical/Anti-convulsant	251.4/102.0	251.4/180.0	
	Ibuprofen	Pharmaceutical/Analgesic	205.1/159.0	205.1/161.0	
	Naproxen	Pharmaceutical/Analgesic	229.0/169.0	229.0/185.1, 229.0/140.9	
	Sucralose	Personal Care Product/Sweetener	395.3/359.0	397.2/361.1	
	Tetrabromobisphenol A	Personal Care Product/Flame retardant	442.9/239.0	442.9/102.9	
	Triclosan (Ingasol)	Personal Care Product/Antibiotic	287.2/34.9	287.2/241.1	
	Warfarin	Pharmaceutical/Anti-coagulant	307.3/161.0	307.3/250.0, 307.3/117.0	
	ESI Positive	Acetaminophen	Pharmaceutical/Analgesic	152.1/110.2	152.1/65.3
		Atrazine	Pesticide	216.1/174.2	216.1/104.2
Caffeine		Personal Care Product/Stimulant	195.2/138.3	195.2/110.0	
Carbamazepine		Pharmaceutical/Anti-seizure	237.3/194.0	237.3/179.3	
DEET		Personal Care Product/Insect Repellent	192.1/119.3	192.1/91.3	
Diazepam		Pharmaceutical/Muscle relaxant	285.2/193.3	285.2/154.0, 285.2/222.0	
Dluron		Pesticide	233.3/72.3	233.3/159.9	
Erythromycin-H2O		Pharmaceutical/Antibiotic	716.5/158.3	716.5/558.6	
Fluoxetine		Pharmaceutical/Anti-depressant	310.3/44.2	310.3/148.3	
Hydrocodone		Pharmaceutical/Analgesic	300.3/199.2	300.3/171.3, 300.3/128.3	
Imazamox		Pesticide	306.4/261.2	306.4/245.2	
Imazthapyr		Pesticide	290.3/245.2	290.3/177.2	
Meprobamate		Pharmaceutical/Anti-anxiety	219.2/158.3	219.2/97.3	
Oxybenzone		Personal Care Product/Sunscreen	229.3/151.2	229.3/105.1	
Pentoxifylline		Pharmaceutical/Blood thinner	279.4/138.2	279.4/99.2	
Primidone		Pharmaceutical/Anti-convulsant	219.2/162.1	219.2/91.3	
Prometryne		Pesticide	242.2/157.9	242.2/200.3	
Sulfamethoxazole		Pharmaceutical/Antibiotic	254.3/156.2	254.3/108.1	
Trimethoprim		Pharmaceutical/Antibiotic	291.3/123.3	291.3/230.4, 291.3/261.2	
INST/Surrogate	Acetaminophen-D4		156.2/114.1	156.2/69.1	
	Cotinine-D3		180.3/80.10	180.3/101.2	
	Diazepam-D5		290.3/198.4	290.3/154.0, 290.3/227.4	
	Estradiol-D5		260.3/161.10	260.3/135.10	
	Fluoxetine-D6		316.2/44.2	316.2/154.2	
	Hydrocodone-D6		306.3/202.3	306.3/174.3, 306.3/128.3	

Note: both quantifier ion and qualifier ion are shown with the unit of m/z (mass-to-charge ratio)

Appendix F. Concentration detected in blank sample^a

Table F.

	<i>Blank samples</i>		
	<i>Average (ave)</i>	<i>Standard Deviation (SD)</i>	<i>ave+2SD</i>
Acetaminophen	0.2	0.3	0.9
Caffeine	1.0	1.0	3.0
Carbamazepine	0.1	0.3	0.7
Cotinine	0.1	0.1	0.3
DEET	1.1	1.0	3.0
Diazepam	0.1	0.2	0.5
Diclofenac	0.4	0.5	1.3
Dilantin	0.7	1.1	2.8
Erythromycin	0.1	0.3	0.7
Fluoxetine	0.3	0.4	1.1
Hydrocodone	0.5	0.8	2.0
Ibuprofen	0.6	1.0	2.6
Meprobamate	0.2	0.3	0.8
Naproxen	0.2	0.5	1.3
Oxybenzone	2.0	1.3	4.6
Pentoxifylline	0.2	0.5	1.1
Primidone	0.1	0.2	0.6
Sucralose	0.4	0.7	1.7
Sulfamethoxazole	0.3	0.7	1.7
TBBA	0.5	0.9	2.4
Triclosan	1.0	1.0	3.0
Trimethoprim	0.1	0.4	0.8

^a Note: this table shows the averaged concentration and standard deviation from all blank samples. The value of “average+2SD” is the criteria to obtain statistical significant concentration from field samples.

Appendix G. Recovery correction of compounds in different water matrices after solid phase extraction^a

Table G.

	<i>Surface water</i>	<i>WTP sedimentation effluent</i>	<i>WTP disinfection effluent</i>	<i>Raw wastewater</i>	<i>WWTP tertiary effluent</i>
Acetaminophen	32%	31%	27%	14%	16%
Caffeine	61%	62%	61%	59%	33%
Carbamazepine	65%	79%	75%	54%	49%
Cotinine	65%	79%	75%	54%	49%
DEET	65%	79%	75%	54%	49%
Diazepam	65%	79%	75%	54%	49%
Diclofenac	65%	79%	75%	54%	49%
Dilantin	28%	27%	29%	23%	14%
Erythromycin	61%	62%	61%	59%	33%
Fluoxetine	32%	31%	27%	14%	16%
Hydrocodone	65%	79%	75%	54%	49%
Ibuprofen	65%	79%	75%	54%	49%
Meprobamate	65%	79%	75%	54%	49%
Naproxen	28%	27%	29%	23%	14%
Oxybenzone	32%	31%	27%	14%	16%
Pentoxifylline	61%	62%	61%	59%	33%
Primidone	65%	79%	75%	54%	49%

^a Note: the recovery correcting factor were obtain from the isotopes spiking in different water matrices. The loss of mass due to matrix effect during solid phase extraction were corrected by this recovery factor.

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Chapter 5

Illicit Drugs as Emerging Contaminants

Sara Castiglioni* and Ettore Zuccato

Department of Environmental Health Sciences, Mario Negri Institute for
Pharmacological Research, Via La Masa 19, 20156 Milan

*Italysara.castiglioni@marionegri.it

Illicit drugs were recently indicated as emerging contaminants since they have been detected in waste, surface and drinking water and in the airborne particulates in several European countries and USA. In analogy with pharmaceuticals, the main source of contamination for illicit drugs is human consumption. The residues of drugs of abuse persisting in consumers' urine can reach sewage treatment plants (STPs) in detectable amounts, escaping degradation, and can be released into surface water. The first investigations of illicit drugs in the environment were carried out in U.S.A. in 2004 for amphetamines, and in Italy in 2005 for cocaine and its main urinary metabolite (benzoylecgonine, BE). Several other substances were later measured in water and air, including cannabinoids, cocaine and its metabolites, opioids, amphetamines, ephedrine, ketamine, lysergic acid diethylamide (LSD), and some related opioid pharmaceuticals. The first step to measure illicit drugs in the environment consists in the preconcentration of analytes operated mainly by solid phase extraction (SPE). Considering the complexity of the environmental matrices and the low concentrations of the analytes, mass spectrometry is the most powerful technique to detect illicit drugs simultaneously with high specificity and accuracy. Therefore, the technique used most frequently is high-pressure liquid chromatography tandem mass spectrometry (HPLC-MS-MS). Illicit drugs were frequently detected at concentrations up to the $\mu\text{g/L}$ range in STP influents (untreated wastewater) in Europe and U.S.A. Cocaine and its major metabolite BE were investigated in the largest number of countries (Spain, Italy, Switzerland,

UK, Belgium, Germany, Ireland and U.S.A.), and were the most abundant compounds. Other substances measured at high concentrations were several stimulatory drugs, including amphetamines and ephedrine, opioids, and the main metabolite of cannabis, 11-nor-9-carboxy- Δ^9 -THC (THC-COOH). Despite the fact that illicit drugs are generally well removed in STPs (removal higher than 60%), several substances were still detected at concentrations up to the hundreds of ng/L in STPs effluents. Treated wastewater is generally discharged into surface water (rivers, lakes, sea) or undergoes further treatment to produce drinking water. Substantial amounts of illicit drugs therefore directly enter surface water or drinking water treatment plants. These substances were still detectable in rivers and lakes up to tenths of ng/L in several countries, and trace amounts of BE, methadone and its main metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), were still present in finished drinking water in a Spanish drinking water treatment plant. Traces of illicit drugs were also detected in airborne particulate in several of the world's cities, indicating the possible distribution of these substances in the air compartment, despite their polarity and high water solubility. The amounts of illicit drugs detected in wastewater could roughly reflect the amounts consumed. Our group recently proposed a novel approach (*sewage epidemiology*) for estimating drug consumption in a community by the direct measurement of the residues of the illicit drugs in urban wastewater. This method can give evidence-based estimates of drug use in a defined area with the unique ability to monitor local consumption in real time and promptly identify changes.

Introduction

Illicit drugs were recently listed as emerging contaminants in a review covering all the latest developments in water analysis (1). This class of substances has characteristics very similar to pharmaceuticals, already known as environmental contaminants (2), such as the source of contamination, the polar chemical structure and the similar behaviour in the environment. Like pharmaceuticals, illicit drugs are a heterogeneous group of compounds with different structures and physico-chemical properties, and are biologically active. Since about 200 million individuals worldwide are current users of cocaine, heroin, amphetamine-like stimulants, marijuana and other drugs (3), these substances are consumed worldwide in quantities comparable to therapeutic drugs (thousands of tons per year), and can be detected in the environment at the same levels.

In analogy with pharmaceuticals, the main source of contamination for illicit drugs is human consumption, while other minor sources are uncontrolled

discharges related to the handling of these substances. The residues of drugs of abuse persisting in consumers' urine enter the sewage networks with the wastewater and are only partially removed by sewage treatment plants (STPs). As a result these substances are still detectable in treated water and contaminate the receiving surface waters (4). On account of their polarity or moderate lipophilic properties, illicit drugs can be expected to be distributed mostly in water, or adsorbed on suspended solids in the water phase, sludge in the STPs and sediment and soils in the environment. Despite their low volatility, these substances have been also detected in airborne particles in several of the world's cities.

The first investigations of illicit drugs in the environment were carried out in the U.S.A. (2004) for methamphetamine and methylenedioxyamphetamine (MDMA, or Ecstasy) in treated wastewaters (5), and in Italy (2005) for cocaine and its main urinary metabolite (benzoylecgonine) in waste and surface water (6). Successively, our research group started working systematically on this topic, extending the investigation to other common drugs of abuse, i.e. opioids, amphetamines, and cannabis derivatives, and to some related opioid pharmaceuticals, such as codeine and methadone, which have been detected in wastewater (7) and surface water (8) in Italy, Switzerland and the United Kingdom. The occurrence, behaviour and fate of illicit drugs in waste, surface and even drinking water were subsequently investigated in several European countries and the U.S.A. (4), where these substances were found at concentrations up to the $\mu\text{g/L}$ range. Two groups also measured illicit drugs in the airborne particulate in Italy, Portugal, Serbia, Algeria, Chile and Brazil (9) and in Spain (10), reporting cocaine as the most abundant, with concentrations in the pg/m^3 range.

Illicit drugs are therefore considered ubiquitous environmental contaminants in populated areas and, with their often high pharmacologic activity, could have potential toxic implications for wildlife, like therapeutic pharmaceuticals (11). Moreover, the amounts of illicit drugs detected in wastewater could roughly reflect the amounts consumed. Our group recently proposed a novel approach (*sewage epidemiology*) for estimating drug consumption in a community, by wastewater analysis (12). The method is based on direct measurement of the residues of the illicit drugs excreted in consumers' urine into urban wastewater, and back-calculation of the local drug consumption from the measured levels. This method can give evidence-based estimates of drug use in a defined area with the unique ability to monitor local consumption in real time and promptly identify changes.

In view of the widespread interest and growing knowledge on this issue, the present paper summarizes current information on the occurrence and behaviour of illicit drugs in the environment, outlining and discussing the main features of these contaminants, and including an interesting application of the measurement of illicit drugs in wastewater.

Illicit Drugs Investigated

The illicit drugs that have been measured in environmental media are generally those most widely used worldwide, including cannabinoids, amphetamines, opioids, and cocaine (3). Some studies included other illegal substances such as ephedrine, ketamine, lysergic acid diethylamide (LSD), and some related opioid pharmaceuticals, such as codeine and methadone. The molecules selected for analysis were generally the main urinary excretion products, either the unchanged parent drugs, or their most abundant metabolites. The typical structures of some of the illicit drugs and metabolites often detected in the environment are shown in Figure 1.

Cocaine and its main urinary metabolite, benzoylecgonine (BE), were among the first compounds detected in the environment (6). Other metabolites such as ecgonine methyl ester, ecgonine, norbenzoylecgonine, norcocaine, cocaethylene (formed in the presence of ethanol by cocaine trans-esterification), anhydroecgonine and anhydroecgonine methyl ester (the main pyrolytic products formed when cocaine is smoked as crack) were later included in several studies (4).

Cannabis is the most widely produced and trafficked illicit drug worldwide (3). Δ^9 -Tetrahydrocannabinol (THC), its active metabolite 11-hydroxy-THC (OH-THC) and its main human metabolite 11-nor-9-carboxy- Δ^9 -THC (THC-COOH) have been measured in the environment (4).

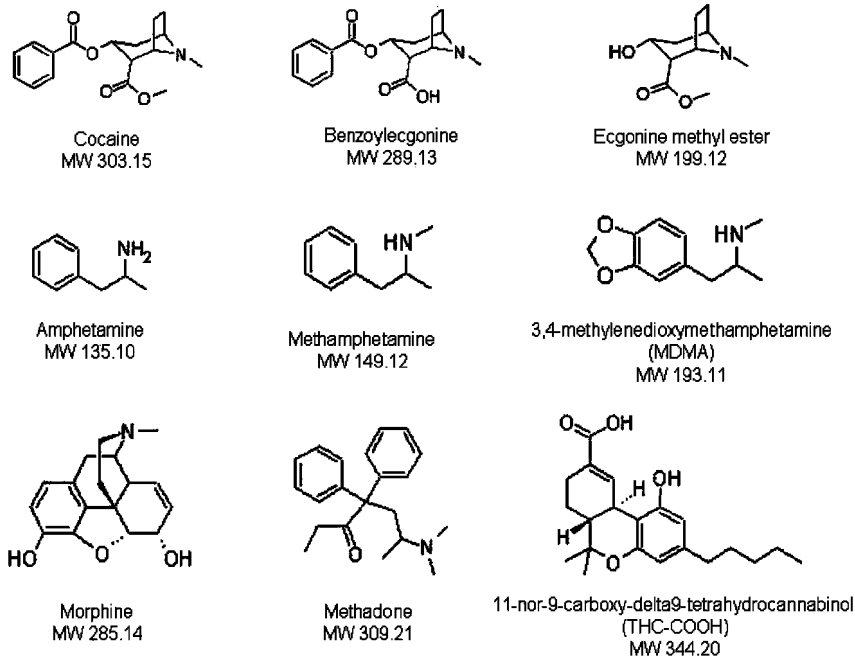


Figure 1. Structure and molecular weight of some of the illicit drugs and metabolites detected in environmental media.

Amphetamines are the most used synthetic drugs among young adults and were among the first to be detected in the environment (5). Amphetamine and several related compounds with similar central stimulant activity, such as methamphetamine, methylenedioxyamphetamine (MDMA or Ecstasy), methylenedioxyamphetamine (MDA) and methylenedioxyamphetamine (MDEA) were mainly considered (4). Other stimulatory drugs such as ephedrine (13), ketamine, fentanyl, LSD and phencyclidine (PCP) (14) have also been investigated in wastewater.

Among opioids, the analyses focused on heroin, its main metabolite morphine and some minor metabolites such as 6-acetylmorphine, and the glucuronide conjugates of morphine. Other opioids were oxycodone, hydrocodone, codeine, dihydrocodeine, 6-acetylcodeine, norcodeine, methadone and its main metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP).

Analysis of Illicit Drugs in the Environment

Sampling Protocols

The collection of samples for analyses must ensure realistic samples representing the environmental levels of the compounds investigated. Normally the best option is to collect composite samples, pooling water sampled at fixed times by automatic sampling devices. Illicit drugs have mostly been investigated in aqueous matrices (waste, surface and drinking water), employing some different sampling protocols (Table 1). Wastewater was collected in STPs mostly as composite influent and/or effluent samples over 24 h (6, 7, 12–23), but also as grab samples (24, 25). Surface water was collected as 2-h composite samples (8), or as grab samples (15, 17, 20, 23, 24), sometimes in two replicates (26, 27). Polar organic chemicals integrative samplers (POCIS), consisting of a solid sequestration phase for integrative sampling of hydrophilic organic chemicals, were also used for sampling sewage effluents and rivers (5, 28), allowing up to 30 days exposure of the sorbent phases.

Water samples were generally frozen (-20°C) or stored at 4°C in the dark until analysis within 1-3 days after collection, depending on the drugs' stability in wastewater (7). In some cases, the acidification of water samples to pH 2 has been adopted to prevent drug's degradation (17).

Air was sampled by collecting airborne particles on quartz microfiber filters with high-volume samplers (Table 1) (9, 10). The filters were then stored at -20°C until analysis.

Table 1. Summary of the analytical techniques used to measure illicit drugs in the environment

<i>Aqueous samples (waste, surface and drinking water)</i>	
Sampling	References
Composite samples (24 h)	(6, 7, 12–23)
Composite samples (2 h)	(8)
Grab samples	(15, 17, 20, 23–27)
Polar organic chemicals integrative samplers (POCIS)	(5, 28)
Extraction	
Solid Phase Extraction (SPE)	
Oasis HLB	(5, 14, 16–18, 20, 24, 25, 28–30)
Oasis MCX	(6–8, 12, 21, 22, 26, 27, 29)
Strata-XC	(15)
Bond-Elut Certify LRC	(31)
SupelMIP-Amphetamines	(32)
On-line-SPE	
PLRP-s and Oasis HLB	(13, 23)
Large volume injection (LVI)	(19)
Chromatography	
Liquid Chromatography (LC)	
Reverse phase high pressure LC (HPLC) - C18	(5–8, 12, 13, 15, 19, 23–25, 28, 29, 32)
Ultra-performance LC (UPLC)	(14, 16, 18, 21, 26, 27)
Hydrophilic interaction LC (HILIC)	(17, 20, 22)
Gas-chromatography (GC)	
HP-5MS capillary column	(30)
Phenylmethylsilicone 5% capillary column	(31)
Mass Spectrometry (MS)	
Triple quadrupole (QQQ)	(6–8, 12, 14, 16, 18, 19, 21, 22, 24, 26–28, 32)
Ion trap MS (ITMS)	(5, 15, 17, 20, 25, 30, 31)
Hybrid Triple Quadrupole-Linear Ion Trap (QLIT)	(13, 23)
Hybrid Linear Ion Trap- Orbitrap (LIT-FT Orbitrap)	(29)

Continued on next page.

Table 1. (Continued). Summary of the analytical techniques used to measure illicit drugs in the environment

<i>Aqueous samples (waste, surface and drinking water)</i>	
Air samples (airborne particulate)	
Sampling	
High-volume samplers	(9, 10)
Extraction	
Soxhlet extraction	(9)
Pressurized liquid extraction (PLE)	(10)
Chromatography	
Reverse phase high pressure LC (HPLC) - C18	(10)
Gas-chromatography (GC)-Restek RTX-MS	(9)
Mass Spectrometry	
Single quadrupole	(9)
Hybrid Triple Quadrupole-Linear Ion Trap (QLIT)	(10)

Extraction Procedures

The common practice is to filter aqueous samples to remove solid particles before processing. The analysis of both filtrate and filter cake should also be performed to avoid misleading estimates of drug quantities arriving at the plant. Since illicit drugs are present at concentrations in the low ng/L range, the next phase is preconcentration of analytes before analysis.

The extraction procedures used for illicit drugs are summarized in Table 1. The most common technique is solid phase extraction (SPE) which is generally done using 3 or 6 mL disposable cartridges equipped with different phases, ranging from 60 to 500 mg. The cartridges used most frequently are copolymers with hydrophilic and lipophilic properties, Oasis HLB (Waters) (14, 16–18, 20, 24, 25, 29, 30), and mixed reverse-phase cation exchange properties, Oasis MCX (Waters) (6–8, 12, 21, 22, 26, 27, 29) that retain a wide range of compounds. Other cartridges are Strata-XC (Phenomenex) (15) or Bond-Elut Certify LRC (31).

The pH of samples is adjusted immediately before the SPE procedure. Molecularly imprinted polymers (MIPs) sorbents are also employed now for amphetamine drugs extraction from wastewater (32), giving better performance in terms of selectivity, sensitivity, accuracy and precision compared to Oasis HLB and MCX cartridges.

Other preconcentration techniques for aqueous samples involve on-line SPE employing polymeric phases PLRP-s and Oasis HLB cartridges (13, 23), or the direct injection of large volumes (LVI) (1800 μ L) (19). These methods speed up the extraction phases, but can produce higher matrix effects in wastewater, reducing the sensitivity of the methods. POCIS samplers are equipped with

disposable cartridges, giving the advantage of longer exposure of the solid phases to the matrix (5, 28), but the limitation of time-consuming calibration studies necessary to calculate the uptake rates.

Illicit drugs in drinking water are evaluated only in two studies in Spain (33, 34) and samples were processed following the methods previously published by the same authors (14, 16).

Microfiber filters used to collect airborne particulate containing illicit drugs are extracted using Soxhlet extraction (9) or pressurized liquid extraction (PLE) in two cycles (10).

Analysis and Quantification

Considering the complexity of the matrix and the low concentrations of the analytes, mass spectrometry is the most powerful technique to detect illicit drugs simultaneously with high specificity and accuracy. Some recent reviews summarize all the analytical techniques used for measuring illicit drugs in the environment (35–37). The technique used most frequently is high-pressure liquid chromatography tandem mass spectrometry (HPLC-MS-MS), while gas chromatography-mass spectrometry (GC-MS and GC-MS/MS), widely employed in forensic sciences in the past, was used only in three environmental applications (9, 30, 31). Table 1 summarizes the analytical techniques for illicit drug analyses.

Chromatographic separation is operated mainly using C18 or polar reverse phase columns (5–8, 12, 13, 15, 19, 23–25, 28, 29, 32), or Hydrophilic Interaction Liquid Chromatography (HILIC columns) (17–22). Other groups used ultra-performance liquid chromatography (UPLC) with reverse-phase columns to increase the sensitivity and rapidity (14, 16, 18, 21, 26, 27).

Illicit drugs have been ionized using atmospheric pressure ionisation (API) with electrospray (ESI) or turbo ion spray, which are the most suitable techniques for analysis of a wide range of polar compounds (35–37). However, this source can cause substantial signal suppression, which must be considered carefully. To minimize matrix effects, deuterated analogs of the substances to be analyzed are frequently used as internal standards. The ions of the substances and their corresponding deuterated analogs are chemically equivalent and behave similarly towards signal suppression. All the investigations were conducted in the positive ionization mode, although the negative ionization mode was used to detect cannabinoids in some investigations (7, 8, 12, 13, 23, 29).

Mass spectrometric analysis was done using different mass spectrometers such as triple quadrupole (QqQ), ion trap (ITMS), hybrid triple quadrupole-linear ion trap (QLIT), and hybrid linear ion trap (LTQ) FT Orbitrap (Table 1). The most common acquisition mode was selected reaction monitoring (SRM), allowing the detection of at least two precursor/product ion transitions for each compound. The analytes were generally quantified by isotope dilution after spiking samples with deuterated internal standards before processing.

Most of these analytical methods have been validated in waste and surface water, and the specific papers provide data about recoveries of the compounds, method repeatability, limits of detection and quantification, and linearity of the response (35–37). Generally, recoveries in wastewater varied widely (20 to

100%) because of the complexity of the matrix and the wide range of substances analyzed, while recoveries in surface water ranged from 70 to 100%. The limits of quantification (LOQs) were in the low ng/L range.

Illicit Drugs in Wastewater

Untreated Wastewater (STP Influent)

Several studies were recently conducted in Europe and U.S.A. to investigate illicit drugs in the environment. Residues have often been documented in untreated wastewater of municipal STPs. The median concentrations of the most abundant substances found in untreated wastewater are shown in Figure 2.

Cocaine and its major metabolite BE were investigated in the largest number of countries (Spain, Italy, Switzerland, UK, Belgium, Germany, Ireland and U.S.A.), sometimes by different groups in the same country (four groups in Spain and two in the U.S.A.). Cocaine and BE were the most abundant compounds in untreated wastewater (Figure 2), with concentrations up to the $\mu\text{g/L}$ range (median values). The highest levels of BE, originating exclusively from cocaine consumption, were in Spain, Italy, UK, and U.S.A. Another abundant cocaine metabolite in untreated wastewater was ecgonine methyl ester with median concentrations of 75 ng/L in Belgium (22) and 175 ng/L in Italy (data not published). Other minor metabolites of cocaine such as norbenzoylecgonine, norcocaine and cocaethylene were detected at median concentrations usually lower than 30 ng/L (4), except cocaethylene in Spain (83 ng/L) (13).

Other substances measured in STP influents at high concentrations were stimulatory drugs, including amphetamine, ephedrine, methamphetamine, and MDMA (Figure 1). Amphetamine was detected in Spain, Italy, Belgium and U.S.A. at concentrations up to 170 ng/L, and in UK at higher levels (2500 ng/L). Ephedrine was investigated only in Spain and U.S.A., where the concentrations were 350 ng/L and 1.4 $\mu\text{g/L}$, respectively. Concentrations were generally lower (10-20 ng/L) for methamphetamine and MDMA, except in U.S.A. (85 ng/L). Trace amounts of MDA, MDEA, and ketamine are reported in Spain (13, 14, 32) and U.S.A. (19). LSD and its main metabolites were measured in trace amounts in Spain (13, 23), but were not found in U.S.A. (19).

Morphine, heroin, 6-acetylmorphine (a specific metabolite of heroin) and other opioids such as codeine, oxycodone, hydrocodone, methadone and its main metabolite EDDP have also been measured in several STP influents throughout Europe and the U.S.A. Morphine median concentrations were lower than 100 ng/L in Italy and Spain, but higher in Switzerland, Germany and England (respectively about 200, 300 and 600 ng/L), probably reflecting higher consumption of therapeutic morphine in these countries (Figure 1). Heroin was detected in untreated wastewater only in Spain (4, 13), and 6-acetylmorphine was detected in traces in Spain (13), Italy and Switzerland (7).

STP influents (median, ng/L)

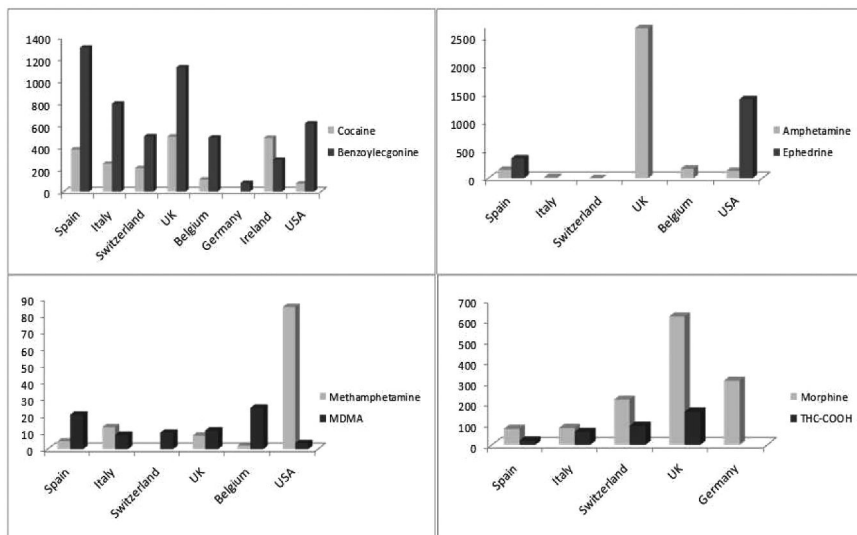


Figure 2. Levels of the most abundant illicit drugs in untreated wastewater in Europe and U.S.A. Data were from the following references: **Spain** (13) 4 STPs; (16) 5 STPs; (18) 42 STPs; (21) 1 STP; (23) 7 STPs; (32) 3 STPs; (34) 15 STPs. **Italy** (7, 12) 5 STPs. **Switzerland** (7, 12) 1 STP. **UK** (12, 26) 2 STPs. **Belgium** (17) 10 STPs; (20) 30 STPs; (22) 11 STPs. **Germany** (24) 12 STPs. **Ireland** (15) 1 STP. **U.S.A.** (19) 7 STPs; (25) 1 STP.

Opioid pharmaceuticals such as codeine, methadone and its major metabolite EDDP were detected in Italy and Switzerland (7), Spain (13, 23, 34), Belgium (22), and Germany (codeine only) (24) at concentrations lower than 200 ng/L. Oxycodone and hydrocodone were not detected in Europe (24), but up to 80 ng/L was found in the U.S.A. (19).

The principal active ingredient of cannabis, THC, and its metabolites, THC-COOH and OH-THC, were investigated in several countries, and THC-COOH was found in Spain, Italy, Switzerland and UK with median concentrations up to 160 ng/L. THC and OH-THC median values were lower than 30 ng/L and were found only in Spain (13, 16).

Treated Wastewater (STP Effluents)

Illicit drugs are detected in treated wastewater too indicating that STPs often only partially remove these substances, which therefore still persist in the effluents. The median concentrations of the most abundant substances in STP effluents are shown in Figure 3.

STP effluents (median, ng/L)

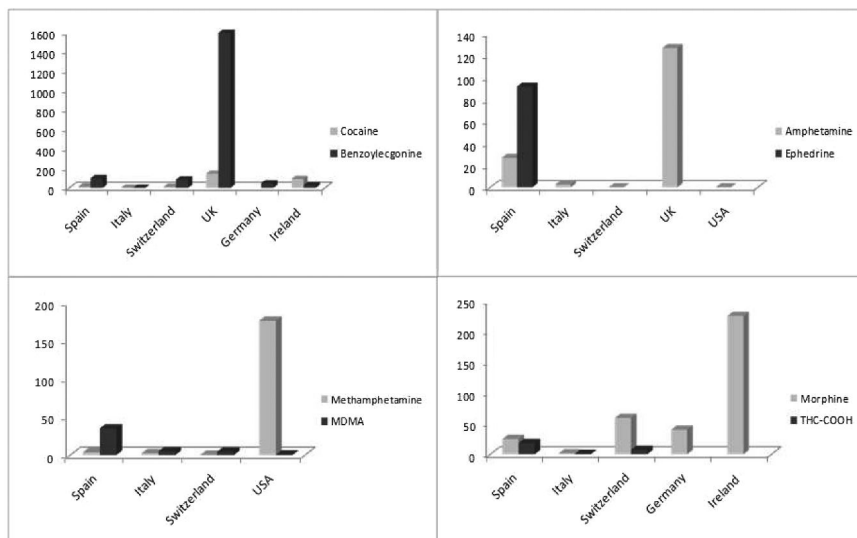


Figure 3. Levels of the most abundant illicit drugs in treated wastewater in Europe and U.S.A. Data were from the following references: **Spain** (13) 4 STPs; (16) 5 STPs; (18) 42 STPs; (21) 1 STP; (23) 7 STPs; (32) 4 STPs; (34) 15 STPs. **Italy** (7) 1 STP. **Switzerland** (7) 1 STP. **UK** (26) 1 STP. **Germany** (24) 12 STPs. **Ireland** (15) 5 STPs. **U.S.A.** (4) 4 STPs; (25) 1 STP; (28) 1 STP.

Concentrations in effluents were generally lower than in influents. Cocaine and BE, which showed the highest levels in STP influents, were generally detected at concentrations lower than 100 ng/L in effluents, except in UK where the concentrations were comparable. The median concentrations of amphetamine, methamphetamine, MDMA, morphine and THC-COOH in treated wastewater were generally lower than 50 ng/L, except for amphetamine in UK (140 ng/L), methamphetamine in U.S.A. (170 ng/L), and morphine in Ireland (220 ng/L). Ephedrine was still detected in STP effluents in Spain (13, 23) at concentrations up to 150 ng/L. Ketamine, LSD, and the minor metabolites of cocaine, heroin, amphetamines and THC were found in trace amounts in STP effluents (4, 23, 34).

Treated wastewater is generally discharged into surface water (rivers, lakes, sea) or undergoes further treatment to produce drinking water. Substantial amounts of illicit drugs therefore directly enter surface water or drinking water treatment plants.

Removal of Illicit Drugs in STPs

The rate of removal of illicit drugs in STPs is potentially affected by several factors, such as the molecular structure, the treatment process, the age of the activated sludge, environmental conditions such as the temperature and light intensity, and the characteristics of the influents. Removal of illicit drugs during wastewater treatment was investigated in several countries.

Cocaine, BE, norbenzoyllecgonine, norcocaine and cocaethylene were extensively removed in STPs, with removal rates higher than 80% in Spain (13, 18, 23), Italy and Switzerland (7), and Ireland (15), while no removal was observed for BE in UK (26). Removal of amphetamine and methamphetamine was almost complete (85-99% and 60-98%) in several studies (7, 13, 18, 21, 25, 26), while the removal of MDMA was about 50% (7, 13, 18) or lower (0-35%) (23, 32). Ephedrine removal was investigated only in Spain (13, 23) and ranged between 64 and 74%. Morphine removal ranged from 50% (23) to 70% (7, 18) or 90% (7, 34), indicating wide variability probably due to the specific characteristics of the treatment plants.

Codeine and THC-COOH were also variably removed in different STPs (removal ranges were 12-100% and 11-99%, respectively), resulting in variable levels in effluents. THC was never found in effluents, but its lipophilic character suggests it is probably adsorbed on particulate and sludge more than removed in STPs. In contrast, methadone and its metabolite EDDP were resistant to degradation in STPs, with low removal rates (9-48% and 8-27%, respectively), and their concentrations in effluents were still close to those in influents (7, 16, 34).

Several substances were found at higher concentrations in effluents than in influents (16, 23, 34). Some of these compounds were excreted mainly as glucuronide metabolites, that can be deconjugated by the β -glucuronidase enzymes of the fecal bacteria in wastewater (7, 12); for other compounds the processes involved in the formation during sewage water treatment have not yet been elucidated (23). The transformation of morphine-3 β -D-glucuronide to free morphine was also observed during a three-day stability test in wastewater (7).

Illicit Drugs in Surface Water

STPs are the main source of illicit drug contamination in the environment, discharging the residues of illicit drugs not completely removed during wastewater treatment. These substances are therefore detectable in rivers and lakes, as reported in the literature (Table 2). Several monitoring campaigns were conducted in Italy, Spain, UK, Belgium, Germany, Ireland and U.S.A. Table 2 shows the median concentrations of the illicit drugs detected most frequently in surface water. Median levels were up to tenths of ng/L for cocaine and BE, and a few ng/L for amphetamine, methamphetamine, MDMA, and morphine. THC-COOH levels reached 24 ng/L in Spain, and were lower in the other countries (Table 2). THC (23, 34), heroin and ketamine were not detected in surface water (4, 14), while traces of ephedrine, LSD and some of its metabolites were found in Spain (23). Median levels were trace amounts or below the limit of quantification (LOQ) for the other substances and metabolites (4). On the contrary, codeine, methadone and EDDP, which are quite stable in STPs, reached concentrations up to tenths of ng/L in several countries (4).

Table 2. Levels (ng/L) of the illicit drugs investigated most frequently in surface water. BE, benzoylecgonine; MDMA, methylenedioxyamphetamine; THC-COOH, 11-nor-9-carboxy- Δ^9 -THC^a

<i>Surface water (median concentrations, ng/L)</i>								
<i>Illicit drugs</i>	<i>Spain Llobregat river and 3 tributaries (14, 16, 33) Ebro river and 6 tributaries (23)</i>	<i>Italy Rivers Po, Lam- bro, Olona and Arno (8)</i>	<i>Italy Lakes Mag- giore, Varese and Lugano (8)</i>	<i>UK Rivers Thames, Taff and Ely (8, 26, 27)</i>	<i>Bel- gium Three rivers (17) Six rivers (20)</i>	<i>Ger- many Eleven rivers (24)</i>	<i>Ire- land Three rivers (15)</i>	<i>U.S.A. Four rivers (28)</i>
Cocaine	5.7	1	<LOQ	3.5	13	na	29	na
BE	30.7	14	9.7	18	48	3	<LOQ	na
Amphet- amine	7.9	<LOQ	<LOQ	1.75	na	na	na	<LOQ
Metham- phetamine	0.7	1	<LOQ	<LOQ	na	na	na	2.3
MDMA	2	1.1	<LOQ	4	na	na	na	na
Mor- phine	5.2	3.4	3.5	8	na	10	<LOQ	na
THC- COOH	24	0.7	<LOQ	1	na	na	na	na

^a LOQ = limit of quantification, na=not analysed.

Daily loads of the drug residues were determined in filtered samples of surface water from the Italian rivers Po, Arno, Lambro and Olona (8). The Po carried up to 390 g of BE daily, equivalent to about 1 kg of cocaine, 60 g of pure cocaine, 30 g of amphetamines, 38 g of the cannabis metabolite THC-COOH and 196 g of methadone and its metabolite EDDP. Loads were lower in the other rivers (8). The daily loads of cocaine and BE were also calculated in Belgium in six rivers and brooks (20). The median loads were 14 g/d of BE, equivalent to about 32 g/d of cocaine, and 3 g/d of cocaine.

Illicit Drugs in Drinking Water

Illicit drugs were also evaluated in raw waters used for drinking water production and in finished drinking water in a Spanish drinking water treatment plant (DWTP) (33, 34). The Lobregat river basin supplies raw water to this DWTP and was therefore monitored. In surface waters, cocaine, BE, amphetamine, methamphetamine, MDMA (ecstasy), and MDA were detected at mean concentrations from 4 to 350 ng/L (33). Opioids, cannabinoids and their metabolites were measured at the intake of the DWTP at concentrations up to 76 ng/L for codeine, 31 ng/L for EDDP, 12 ng/L for morphine and 9 ng/L for methadone (34).

The elimination of these compounds during drinking water treatment was investigated. Amphetamine-type substances (except MDMA) were completely removed during pre-chlorination, flocculation, and sand filtration. Subsequent granulated activated carbon (GAC) filtration removed cocaine (100%), MDMA (88%), and BE (72%), and post-chlorination removed 90% BE which was therefore still detectable in drinking water despite the high percentage of removal. This metabolite was found in 22 out of 24 drinking water samples at a mean concentration of 45 ng/L, maximum 130 ng/L. Removal of opioids, cannabinoids and metabolites was complete during the treatment, except for methadone and EDDP (91 and 87% removal). These substances were therefore found in finished drinking water in trace amounts (below 3 ng/L).

Illicit Drugs in Airborne Particulate

The presence of illicit drugs was assessed in airborne particulate in two recent investigations (9, 10). Cocaine was detected in the air of several of the world's cities, and cocaine, BE, amphetamine, methamphetamine, MDMA, heroin, 6-acetylmorphine and THC were measurable in Barcelona and Madrid in Spain (Table 3). Cocaine was found in all the sites investigated, except Pančevo and Algiers, and the average concentrations ranged from 55 to 2800 pg/m³. Heroin and its metabolite 6-acetylmorphine were measurable only in Madrid at concentrations up to 80 pg/m³, while THC was found in both the Spanish cities (27 pg/m³ in Barcelona and 44 pg/m³ in Madrid). Amphetamines were the least abundant compounds, with only traces in airborne particulate.

Despite the wide variability of concentrations in airborne particulate, that can be influenced by multiple factors, the presence of illicit drugs in air might theoretically be related to activities such as handling and dealing drugs of abuse, indicating also illicit drug consumption in a population.

Table 3. Levels of illicit drugs (pg/m³) investigated in airborne particulate. BE, benzoylcgonine; MDMA, methylenedioxyamphetamine; THC-COOH, 11-nor-9-carboxy- Δ 9-THC^a

<i>Airborne particulate (average concentrations, pg/m³)</i>									
<i>Illicit drugs</i>	<i>Spain (10)</i>		<i>Italy (9)</i>		<i>Serbia (9)</i>	<i>Portugal (9)</i>	<i>Algeria (9)</i>	<i>Chile (9)</i>	<i>Brazil (9)</i>
	<i>Barcelona</i>	<i>Madrid</i>	<i>Milan</i>	<i>Rome</i>	<i>Pančevo</i>	<i>Oporto</i>	<i>Algiers</i>	<i>Santiago</i>	<i>Sao Paulo, Piracicaba, Araraquara, Ouro Preto</i>
<i>Cocaine</i>	204	480	187	53	<LOD	148	<LOD	2800	179
<i>BE</i>	29	14	-	-	-	-	-	-	-
<i>Amphetamine</i>	2.3	1.4	-	-	-	-	-	-	-
<i>Methamphetamine</i>	<LOD	3.5	-	-	-	-	-	-	-
<i>MDMA</i>	2.9	<LOD	-	-	-	-	-	-	-
<i>Heroin</i>	<LOD	84	-	-	-	-	-	-	-
<i>6-acetylmorphine</i>	<LOD	23	-	-	-	-	-	-	-
<i>THC</i>	27	44	-	-	-	-	-	-	-

^a LOD = limit of detection

Sewage Epidemiology

A novel approach (*sewage epidemiology*) has been proposed to estimate drug consumption in a community by wastewater analysis (6, 12). The idea that wastewater analysis could be employed to estimate drug consumption in a community was tested in several case studies, with encouraging results (12, 37). The approach was first applied in several cities in Italy and Switzerland to calculate the total amounts of illicit drugs entering urban STPs (12). For example, in Milan the amounts of cannabis, cocaine, heroin and amphetamines entering an urban STP daily were respectively 4 kg, 1 kg, 100 g and 40 g. Weekly monitoring of the influent wastewater in the same STP in Milan showed, as

expected, increases in cocaine and amphetamine consumption over the weekends, and steady consumption of cannabis and heroin all days of the week. Other investigations were conducted in Belgium (38), where cocaine consumption was estimated to be 1.88 tons per year, Spain (23), where the total consumption of illicit drugs was estimated as 36 tons per year, and U.S.A. (39).

The drug consumption profiles for Milan, Lugano and London calculated by the present method were similar to the national profiles of drug use based on the annual prevalence data in the countries under study (12). This has encouraged us to propose this method to complement and extend existing epidemiological strategies for studying drug abuse. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) recently considered the present approach as a new tool for checking the use of illicit drugs in a population (40). Wastewater analysis to track local habits in a population (sewage epidemiology) was proposed as an objective, non-invasive approach to estimate drug consumption in a community, with the potential for use with other classes of contaminants in the future.

Conclusions

Illicit drugs have become a class of emerging contaminants on account of their ubiquitous presence throughout the environment in populated areas. Like for therapeutic drugs, this contamination appears to be common, consumers being the main source. Illicit drugs are frequently detected at concentrations up to the $\mu\text{g/L}$ range in STP influents in Europe and U.S.A.

Recent studies indicate that some molecules are poorly removed in STPs, which might therefore be considered an important point source of contamination. Consequently, illicit drugs are still present in treated wastewater and are discharged directly into surface waters. The drinking water treatment processes generally removed all the illicit drugs present in raw water, except BE, methadone and EDDP. These substances were also the most stable during wastewater treatment and their concentrations in surface water were higher than the other compounds. Traces of illicit drugs were also detected in airborne particulate in several of the world's cities, indicating the possible distribution of these substances in the air compartment, despite their polarity and high water solubility.

Most of these residues still have potent pharmacological activity and their presence in the aquatic environment may have implications for human health and wildlife. Even if environmental concentrations are low, risks for human health and the environment cannot be excluded. Morphine, cocaine, methamphetamine, MDA and ecstasy all have strong activity, and their presence as complex mixtures in surface waters – together with residues of many therapeutic drugs – may lead to unforeseeable pharmacological interactions, with toxic effects on aquatic organisms.

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Chapter 6

Persistent Organic Pollutants in Sewage Sludge: Levels, Sources, and Trends

Bradley O. Clarke* and Nichola A. Porter

School of Applied Sciences, College of Science, Engineering and Health,
RMIT University, Melbourne, Victoria, Australia 3001

*Corresponding author. Phone: +61 3 9925 1787. E-mail:
brad.clarke.aus@gmail.com. Address: RMIT University School of Applied
Sciences (Bld 3 Level 1), LaTrobe St., Melbourne, Victoria, Australia 3001

All sewage sludges in developed countries contain Persistent Organic Pollutants (POPs) and are a source of these chemicals to the environment when recycling this material to land. Therefore, it is important to understand the risks to human health and the environment from this practice. Over the past thirty years a significant amount of research has focused on this subject and this chapter presents an overview of that research. The chemicals that have been reviewed are polychlorinated dioxins/furans, polychlorinated biphenyls, organochlorine pesticides, polybrominated diphenyl ethers, polybrominated biphenyls and perfluorochemicals. A discussion of levels, sources (if known) and trends of each of the chemical groups is presented. The chapter finishes with a brief review of risk assessment work conducted in the area. Most risk assessments have concluded that there is negligible risk to human health from non-ionic POPs when applying this material to land at concentrations typically observed in contemporary sewage sludges. However, assessment of the ecological consequences of sewage sludge land application still remains to be completed. Finally, studies indicate that the concentrations of most POPs in sewage sludge are declining globally, demonstrating the effectiveness of source control in reducing environmental contamination.

Introduction

The land application of treated sewage sludge (biosolids) is a management option that is favoured in most countries throughout the world (1–3), as it takes advantage of the positive fertilizer and soil ameliorating properties of the material (4, 5). However, sewage sludge can be contaminated with Persistent Organic Pollutants (POPs) and it is important to understand the risks to human health and the environment when applying biosolids to land. This topic has been the subject of considerable scientific and regulatory attention over the past thirty years (6–10) and this chapter presents an overview of that research.

POPs refer to organic compounds that are environmentally persistent, accumulate in tissues of humans and wildlife, are toxic, and capable of long-range atmospheric transport. Therefore, they pose a significant threat to human health and the environment (11). To address this problem, the United Nations Environment Programme (UNEP) Stockholm Convention on Persistent Organic Pollutants was developed in 2001 to restrict and ultimately eliminate the production, use, release and storage of POPs (11). There were twelve original POPs that included ten intentionally produced compounds (aldrin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, hexachlorobenzene (HCB), mirex, polychlorinated biphenyls (PCBs), toxaphene) and two un-intentionally produced classes of compounds [polychlorinated dioxins (PCDD) and polychlorinated furans (PCDFs)]. This has since been augmented to include specific polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), perfluorooctane sulfonic acid (PFOS), pentachlorobenzene, lindane (including α,β hexachlorocyclohexane (HCH) isomers) and chlordecone (12–15).

Regulatory authorities in many countries, including Australia, Austria, Czechoslovakia, Denmark, France, Germany and Sweden, consider that land applying sewage sludge contaminated with POPs poses a risk to human health and/or the environment. As a result, they have instigated contaminants limits for various chemicals such as organochlorine pesticides (OCPs), PCBs and PCDD/Fs when recycling sewage sludge to land (1, 2). However, there is not an internationally consistent approach taken to determine how contaminant limits are derived or if indeed they are warranted to protect human health or the environment when applying sludge to land (2, 3).

A common consideration when assessing historical scientific studies is the improvement in analytical techniques. The analysis of organic pollutants in sewage sludge is challenging and requires an appropriate extraction, clean-up and quantification technique, each of which has evolved and improved through the years. The early studies utilized packed column chromatography (with quite low resolution by today's standards) and were later replaced with capillary column chromatography, referred to as High Resolution Gas Chromatography (HRGC). Similarly, the non-specific Electron Capture Detector (ECD) was replaced in later studies with Mass Spectrometry (MS), which provided the analyst with greater confidence in the identity of the analyte and reduced problems associated with interfering compounds. These advances have aided researchers in achieving lower detection levels and greater levels of precision. It is possible that the

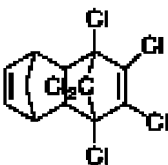
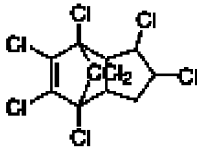
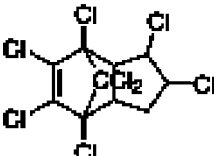
concentrations reported in earlier studies are not accurate, as quite significant variation in the measurement of the same samples can be found even in modern inter-laboratory studies (16).

The aims of this review are to summarise international research conducted into POPs in sewage sludge, to assess the significance for use as a fertilizer and soil ameliorant in agriculture and provide a resource for the professionals in the international water industry. The chemicals that will be discussed in the review are:

- Polychlorinated dibenzo dioxins and furans
- Polychlorinated biphenyls
- Organochlorine pesticides
- Polybrominated diphenyl ethers
- Polybrominated biphenyls
- Perfluorochemicals

A review of literature on the research focusing on each of these classes of compounds is presented in this chapter. Each section discusses levels, trends and sources of these compounds to sewage sludge. Finally, a brief overview of risk assessment studies when land applying sewage sludge is provided.

Table I. Chemical structure and properties of UNEP Persistent Organic Pollutants

<i>Compound</i>	<i>Chemical Structure</i>	<i>Chemical Properties</i>
Aldrin		CAS No: 309-00-2 Formula: C ₁₂ H ₈ Cl ₁₅ Mw: 365 g mol ⁻¹ Log K _{ow} : 5.30 Vp: 0.003 Pa (20 °C) Reference: (73, 179)
Chlordane		CAS No: 57-74-9 Formula: C ₁₀ H ₆ Cl ₁₈ Mw: 409.83 g mol ⁻¹ Log K _{ow} : 4.58 – 5.57 Vp: 0.001 Pa (20 °C) Reference: (73, 179)
Dieldrin		CAS No: 60-57-1 Formula: C ₁₂ H ₈ Cl ₁₆ O Mw: 381 g mol ⁻¹ Log K _{ow} : 3.69 – 6.2 Vp: 0.00002 Pa (20 °C) Reference: (73, 179)


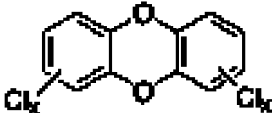



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Table I. (Continued). Chemical structure and properties of UNEP Persistent Organic Pollutants

Compound	Chemical Structure	Chemical Properties
DDT		CAS No: 50-29-3 Formula: C ₁₄ H ₉ Cl ₅ Mw: 355 g mol ⁻¹ Log K _{ow} : 6.19 Vp: 0.00003 Pa (25 °C) Reference: (73, 179)
Heptachlor		CAS No: 76-44-8 Formula: C ₁₀ H ₅ Cl ₇ Mw: 373.34 g mol ⁻¹ Log K _{ow} : 4.4 Vp: 0.0004 Pa (20 °C) Reference: (73, 179)
Hexachlorobenzene		CAS No: 118-74-1 Formula: C ₆ Cl ₆ Mw: 284.8 g mol ⁻¹ Log K _{ow} : 3.93 – 6.42 Vp: 0.0014 Pa (20 °C) Reference: (73, 179)
Lindane		CAS No: 58-89-9 Formula: C ₆ H ₆ Cl ₆ Mw: 288 g mol ⁻¹ Log K _{ow} : 3.8 Vp: 0.004 Pa (25 °C) Reference: (73, 179)
Polybrominated diphenyl ether (PBDE)		CAS No: Varied Formula: C ₁₂ H _(10-x-y) Br _{x,y} O Mw: 248.9 – 959.22 g mol ⁻¹ Log K _{ow} : 5.74 – 10 Vp: 0.1 – 1 × 10 ⁻⁶ Pa (25 °C) Reference: (180–182) Note: Ranges are given for mono to deca-BDEs
Polybrominated biphenyl (PBB)		CAS No: Varied Formula: C ₁₂ H _(10-x-y) Br _{x,y} Mw: 232.9 – 943 g mol ⁻¹ Log K _{ow} : 4.59 – 8.58 Vp: <6 × 10 ⁻⁶ – 7 × 10 ⁻⁶ Pa (25 °C) Reference: (105) Note: Ranges are given for mono to deca-PBBs

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Table I. (Continued). Chemical structure and properties of UNEP Persistent Organic Pollutants

<i>Compound</i>	<i>Chemical Structure</i>	<i>Chemical Properties</i>
Polychlorinated biphenyl (PCB)		CAS No: Varied Formula: $C_{12}H_{(10-x,y)}Cl_{x,y}$ Mw: 188 – 493.7 g mol ⁻¹ Log K _{OW} : 4.7 – 8.3 Vp: 1.1 – 1.4 × 10 ⁻⁶ Pa (25 °C) Reference: (43) Note: Ranges are given for mono to deca-PCBs
Polychlorinated dibenzo-p-dioxin (PCDD)		CAS No: Varied Formula: $C_{12}H_{(8-x,y)}Cl_{x,y}O_2$ Mw: 218.5 – 460 g mol ⁻¹ Log K _{OW} : 4.8 – 8.2 Vp: Pa (25 °C): 0.012 – 1.10 × 10 ¹⁰ Reference: (183) Note: Ranges are given for mono to octa PCDD
Polychlorinated dibenzofuran (PCDF)		CAS No: Varied Formula: $C_{12}H_{(8-x,y)}Cl_{x,y}O_2$ Mw: 202.7 – 443.8 g mol ⁻¹ Log K _{OW} : 4.8 – 8.00 Vp: 0.30* – 5 × 10 ¹⁰ Pa (25 °C) Reference: (183) Note: Ranges are given for mono to octa PCDF except vapour pressure (*) which is the di-substituted furan
Perfluorooctane sulphonate (PFOS)		CAS No: No specific number Formula: $C_8F_{17}SO_3^-$ Mw: 538.23 g mol ⁻¹ Log K _{OW} : Cannot be determined Vp: 3.31 x 10 ⁻⁴ Pa (20 °C) Reference: (184)
Perfluorooctanic acid (PFOA)		CAS No: 335-67-1 Formula: $C_7F_{15}COOH$ Mw: 184 – 460 g mol ⁻¹ Log K _{OW} : Unknown Vp: Unknown Reference: (184)

Polychlorinated Dibenzodioxins and Furans

Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are produced as by-products of chlorinated chemical production and combustion and are ubiquitous environmental contaminants (11, 17). Unlike other United Nations POPs they have never been intentionally produced for industrial purposes or deliberately released into the environment (17). Short-term human exposure to these compounds may result in skin lesions (chloracne, patchy darkening of the skin) and altered liver function (18). Long-term exposure is linked to impairment of the immune system, the developing nervous system, the endocrine system and reproductive functions (18). According to the International Agency for Research on Cancer (IARC), tetrachlorodibenzo-p-dioxin (TCDD) is classified as a human carcinogen (19).

PCDDs and PCDFs each have between one and eight chlorine atoms bound to the dibenzo-p-dioxin and dibenzofuran skeleton resulting in 75 and 135 PCDD and PCDF isomers respectively. The differences in chemical structure produce varying levels of toxicity and changes to the chemical properties (Table I). To simplify discussion, the Toxicity Equivalence (TEQ) system was developed to reduce the concentration data to a single comparable value. The TEQ system works by assigning Toxicity Equivalence Factors (TEFs) to each congener, relative the most toxic congener 2,3,7,8-TCDD, assigned a value of one. The system is based upon a number of assumptions, the most important being that the combined effects of the different congeners are dose/concentration additive and that all the congeners share a common mechanism of toxicity. The TEQ classification system was first developed in 1977 and was further refined by several scientists and regulatory agencies in the 1980s and 1990s (20), resulting in a variety of reporting schemes. The first major system was the International TEQ (I-TEQ) which was largely developed by the United States Environmental Protection Agency (US EPA) (20). The I-TEQ proposed TEFs for seven PCDDs and ten PCDFs. The isomers included in the I-TEQ system all share the base chlorine substitution of the 2,3,7,8 positions similar to 2,3,7,8-TCDD. The World Health Organization updated the system in 1998 (WHO₉₈ TEQ) to incorporate twelve 'dioxin-like' PCBs (again sharing a base chlorine substitution pattern of 2,3,7,8) and was updated again in 2005 (WHO₀₅ TEQ) (21, 22). While 'dioxin-like' PCBs are included in the calculation of the WHO₉₈ TEQ and WHO₀₅ TEQ they will not be specifically discussed in this section. Changes to the TEQ scheme by WHO are relatively few, but can make a significant difference to the overall TEQ reported, particularly in sewage sludge and soil samples that are typically dominated by octachloro dibenzodioxin (OCDD) (an example of this is provided later in the discussion). The evolution of the three main TEQ systems makes the analysis and interpretation of historical literature problematic. If raw data have been omitted, which is common, it is not possible to update historical literature to the latest TEQ system for comparison. All values quoted in this text will use the TEQ system as originally reported.

Research investigating PCDD/Fs in sewage sludge has been reported from the USA, Germany, Sweden, Canada, UK, Switzerland, Spain, China, Australia and Korea. One of the most interesting studies was also the first in the area.

Lamparski *et al.* (1984) analysed two contemporary (1981, 1982) and one archived sample (1933) from the USA and surprisingly, reported similar levels of PCDD/Fs in the two sets of samples, all containing approximately 60 000 ng kg⁻¹ dw of octachlorodibenzo-p-dioxin (OCDD) (23). It is believed waste incinerators and pentachlorophenol use (PCP) were the main sources of PCDD/F contamination in the sludges (24). However, the production of chlorinated chemicals such as PCP and PCBs were still in their infancy in the 1930s, therefore, the PCDD/Fs in the 1933 samples must have had another source. Lamparski *et al.* (1984) proposed that the PCDD/Fs may have been formed *in-situ* at the wastewater treatment plant (WWTP), but later research seems to be contradictory. One study investigating that the chlorination of potable water did not result in the formation of PCDD/Fs in the sludge (25), while another study that found evidence of the formation of PCDFs but not PCDDs as a result of water chlorination (26). The congener profiles of the potable water membrane sludges from the first study were compositionally similar to sewage sludge, suggesting a “common source of origin” (25). Other studies have found that certain PCDD/Fs can be formed through the WWTP process, with an increase of higher molecular weight PCDDs from semi-anaerobic digestion over 192 days (27). However, there is still no clear explanation of the presence of PCDD/Fs that were found in the Lamparski *et al.* (1984) archived sample (23).

The majority of studies have reported similar PCDD/Fs concentrations and TEQ values although highly contaminated sludges have also been reported (Table II). A German study published in 1986 found concentrations of 2,3,7,8-TCDD ranging from 60 to 370 ng kg⁻¹ dw and up to 200,000 ng kg⁻¹ dw for OCDD (24). Other examples of highly contaminated sludges were reported in samples from the USA where the concentration was 1240 ng I-TEQ kg⁻¹ dw (28). Changes to TEFs between the I-TEQ and WHO₉₈ TEQ systems have altered the apparent burden of ‘dioxin-like’ compounds in sludge, as the major congener typically present in sewage sludge, OCDD, had its TEF contribution reduced by a factor of ten. In the above example, 200,000 ng kg⁻¹ dw of OCDD has an I-TEQ of 200 ng kg⁻¹ dw and a WHO₉₈ TEQ of 20 ng kg⁻¹ dw (24).

Many studies have observed compositional differences when comparing PCDD/Fs in sludges from urban and rural sources (29–32). For example, Weerasinghe *et al.* (1985) found that an urban sludge contained five to ten times more PCDD/F congeners than a rural sludge. which the authors associated with PCP use in wood treatment/preservation in the urban area (29). Supporting this finding is an Australian study that reported a relationship between PCDD/F sludge levels and population in rural and urban areas (31). Similar findings were reported Swiss sludge samples (n=30) were “in general the concentrations were found to be higher in the urban areas” and that “the highest inputs could be correlated to local industrial sources” (33). Rappe *et al.* (1994) hypothesised that low level PCDD/Fs found in sewage sludge are best explained by direct PCP contamination and by the formation of PCDD/Fs from chlorinated benzenes (33, 34). While it was recognized early in this research area that PCP use in both wood preservation and textiles was a significant source of contamination, this work identified PCP as not only a primary source of contamination, but also as a secondary source (34). The low level, almost background contamination of rural samples still requires explanation.

A survey of sewage sludges from Switzerland ($n=30$) in 1994 reported the greatest burden of PCDD/Fs to this point in time, with concentrations ranging from 6 to 4100 ng I-TEQ kg^{-1} dw (33). In a follow-up survey reported in 1997, the concentration of PCDD/Fs and TEQ concentrations had decreased (35). Following on from their earlier work and the US EPA's National Survey of American Sewage Sludge, the concentrations of PCDD/Fs in USA and Swedish sludge samples had decreased by 35-50%, except at sites that had historical contamination (28). This finding was corroborated in 2001, when it was reported that TEQ levels in USA sludges had decreased from an average of 60.5 ng WHO₉₈ TEQ kg^{-1} dw in 1994 to 41.1 ng WHO₉₈ TEQ kg^{-1} dw in 2001 (36). An analysis of archived samples from the UK between the years 1940 to 1960 showed increasing concentrations of PCDD/Fs in sludge, peaking in the mid-1950s before beginning to decrease to current levels (37).

German researchers published a series of studies that aimed to identify the source of PCDD/Fs in sewage sludge (38–40). The first experiment involved measuring the contribution from street runoff and atmospheric deposition (38). In a later experiment by the same authors, PCDD/F concentrations were measured in primary sludge during periods of dry weather and directly after rain events, but no significant difference was found (40). They continued investigations and provided evidence that household waste-water contributed the majority (two to seven times) of the PCDD/Fs found in sewage sludge compared to other sources, concluding that grey water from the washing of contaminated clothing (i.e. PCP use in textiles) accounted for the majority of PCDD/Fs found in German sludges (39). Others have investigated human faeces as a source of low levels PCDD/F contamination because humans are constantly exposed to PCDD/Fs through their diet. However, it was found that the levels of PCDD/Fs found in human faeces are extremely low (i.e., ~ 3.9 ng I-TEQ kg^{-1} dw) and therefore, not likely to constitute a major input source to sludge (41, 42).

A summary of 2,3,7,8-TCDD, OCDD and TEQ concentrations found in sewage sludge is presented in Table II.

Table II. Summary of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), octachlorodibenzo-p-dioxin (OCDD) and toxicity equivalence (I-TEQ, WHO₉₈ TEQ or WHO₀₅ TEQ) in sewage sludge

Country	Year	n	2,3,7,8-TCDD ng kg ⁻¹ dw			OCDD ng kg ⁻¹ dw			TEQ ng Unit kg ⁻¹ dw				Study
			mean	me- dian	range	mean	me- dian	range	mean	me- dian	range	Unit	
USA	1984	2	13.5	13.5	11–16	55000	55000	50000–60000	-	-	-	-	(23)
USA	1985	2	NA ^a	NA	NA	33800	33800	7600–60000	-	-	-	-	(29)
Germany	1986	15	NA	NA	NA	13600	7700	2500–51000	-	-	-	-	(24)
Sweden	1989	2	0.72	0.72	<0.48–0.72	8250	8250	7400–9100	-	-	-	-	(30)
Sweden	1990	4	1.7	1.6	1.3–2.2	23200	23600	12800–32900	79	71	41–133	I-TEQ	(185)
Canada	1990	49	NA	NA	NA	NA	NA	600–30400	-	-	-	-	(186)
Sweden	1990	1	1	1	NA	7500	7500	NA	31	31	-	I-TEQ	(187)
Germany	1992	13	NA	NA	NA	NA	NA	NA	0.047	0.037	0.020–0.177	I-TEQ	(89)
UK	1995	8	2.1	2.0	1.0–3.8	16100	9250	650–63000	17	19	0.4–29	I-TEQ	(37)
USA	1998	17	1.5	1.3	0.1–5.3	33700	4300	560–480000	109	25	2.3–1270	I-TEQ	(28)
Spain	1999	18	2.3	1.1	0.4–8.7	5460	4650	1000–19000	50	33.5	6.9–150	I-TEQ	(188)
UK	2001	14	1.8	1.2	0.7–5.6	10200	4300	2320–51500	64.7 51.9	40.4 33.1	19.9–225 17.7–183	I-TEQ WHO ₉₈ TEQ	(189)

Continued on next page.

Table II. (Continued). Summary of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), octachlorodibenzo-p-dioxin (OCDD) and toxicity equivalence (I-TEQ, WHO₉₈ TEQ or WHO₀₅ TEQ) in sewage sludge

Country	Year	n	2,3,7,8-TCDD ng kg ⁻¹ dw			OCDD ng kg ⁻¹ dw			TEQ ng Unit kg ⁻¹ dw				Study
			mean	me- dian	range	mean	me- dian	range	mean	me- dian	range	Unit	
Canada	2003	26	1.1	NA	0.3–3.9	4500	NA	730–16000	40	-	5.5–250	WHO ₉₈ TEQ	(56)
Spain	2003	8	0.3	0.2	0.1–0.4	815	716	530–1600	40.3	38.6	22.7–72.5	WHO ₉₈ TEQ	(190)
Spain	2005	79	NA	NA	NA	NA	NA	NA	25.6	13.6	3.1–263.8	I-TEQ	(58)
China	2007	6	7.6	<dl	<dl–7.6	240	240	35–504	23.5	6.2	3.5–88.2	I-TEQ	(191)
Spain	2007	31	0.8	0.6	0.1–3.0	2500	240	170–27300	23.5	9.3	4.8–346	I-TEQ	(192)
Australia	2008	14	1.2	1.2	<dl–1.6	2700	940	190–20900	6.1 5.5 5.6	3.4 4.7 4.6	1.2–27.3 1.2–15.3 1.2–15.3	I-TEQ WHO ₉₈ TEQ WHO ₀₅ TEQ	(31)
Korea	2009	6	7.9	3.1	<dl–7.9	690	210	38–3200	7.6	1.8	0.3–26.6	WHO ₀₅ TEQ	(193)

^a NA – Data not available

Polychlorinated Biphenyls

Commercial production of polychlorinated biphenyls (PCBs) began in the USA in 1929. The chemical and physical stability of PCBs, their electrical resistance, low volatility and resistance to degradation at high temperatures were their commercially valued properties. The commercial product(s) were a complex mixture, used for a variety of purposes, but most commonly in dielectric fluids in capacitors and transformers. PCBs are now ubiquitous environmental pollutants, occurring in human and animal tissue as well as most environmental compartments (43). Even though PCB production has been banned in most countries since the 1970s and 1980s, it is estimated that over 1 million tonnes of PCBs have been generated and about one third of this quantity is thought to be circulating in the environment (44). PCBs have a number of configurations which give rise to varying modes of toxicity. PCBs accumulate in the fatty tissues of humans and the health effects that have been associated with exposure to PCBs include acne-like skin conditions in adults as well as neurobehavioral and immunological changes in children (45). The skin and liver are the major sites of pathology, but other sites of negative health implications are the gastrointestinal tract, the immune system, and the nervous system (46). PCDFs which are contaminants in commercial PCB mixtures, contribute significantly to their toxicity (46). PCBs are 'probably carcinogenic to humans' (IARC Group 2A) (47) and are known to cause cancer in animals (45).

Research investigating PCBs in sewage sludge has been undertaken in many nations and published research has been reported from the USA, the UK, Netherlands, Italy, Switzerland, Canada, Ireland, Greece, Spain and Australia. Much of this research was directed towards understanding the risk posed from the land application of sewage sludge as well as quantifying the environmental release of PCBs in from sludge and wastewaters. It is important to be aware of the various reporting styles for PCBs. There are a total of 209 possible individual PCBs, however, quantification of all 209 is generally not possible or necessary. Concentrations were originally reported in terms of the commercial formulation, Aroclors™, which is a mixture of various PCB isomers. Later, individual isomers were selected as representative of ΣPCB concentration.

One of the first reports of PCBs in sludge was of six USA samples in 1977 (48). The concentrations were very high compared to contemporary levels and ranged between 238,000 and 1,700,000 $\mu\text{g kg}^{-1}$ dw. At that point in history, PCBs were still being used for industrial purposes and this may account for the high concentrations. Follow-up studies of PCBs levels in USA sludges have found that the levels are far lower than the earlier study with reported concentrations ranging between 1,200 – 6,200 $\mu\text{g kg}^{-1}$ dw (49) and 150 – 3,600 $\mu\text{g kg}^{-1}$ dw (50). Research from the UK reported concentrations of up to 22,000 $\mu\text{g kg}^{-1}$ dw (51) which was not typical, as the mean concentrations were less than 1,000 $\mu\text{g kg}^{-1}$ dw in two separate UK sludge surveys (51, 52). PCB concentrations in Dutch sludges were in a similar range to the UK sludges with concentrations ranging between 390 and 1,480 $\mu\text{g kg}^{-1}$ dw). The concentrations of sludges in the 1980s were typically in the low parts-per-million range, which are far lower than the concentrations reported in the early USA study by Bergh *et al.* (1977) (48).

In the 1990s, the concentrations of PCBs in sludges were declining and often below $1,000 \mu\text{g kg}^{-1} \text{ dw}$. For example, Italian sludges had PCB concentrations between 210 and $1010 \mu\text{g kg}^{-1} \text{ dw}$ (53) and in a follow-up survey of UK sludges the total concentration ranged between 106 and $712 \mu\text{g kg}^{-1} \text{ dw}$ (54). The concentrations of the three most toxic coplanar PCB congeners (77, 126, 169) as well as seven mono and di-ortho-PCBs (PCB 8, 28, 52, 101, 118, 153, 138, 180) which are routinely measured as representatives of the PCB fraction were measured in nineteen sewage sludges from Switzerland with concentration ranges between 43 – $550 \mu\text{g kg}^{-1} \text{ dw}$ (55). Sludges that received industrial effluents clearly showed higher PCB levels than rural ones (55).

By the new millennium, the concentration of PCB in sludges around the world, had decreased to be typically less than $500 \mu\text{g kg}^{-1} \text{ dw}$. For example, a survey of Canadian sludges that was reported in 2003 found that PCBs were not detected in any of the subset of 20 samples analysed at a minimum detection limit of $50 \mu\text{g kg}^{-1} \text{ dw}$ (56). In UK studies, the concentrations of ΣPCBs in fourteen sludges ranged between 110 – $440 \mu\text{g kg}^{-1} \text{ dw}$ (57). The concentrations of PCBs in a survey of Spanish sludges ($n=139$) were lower than earlier studies (range 3 – $60 \mu\text{g kg}^{-1} \text{ dw}$) and well below the European recommended limit of $800 \mu\text{g kg}^{-1} \text{ dw}$ (2, 58). Similarly, recent French and Greek studies reported PCB concentrations below the proposed European contaminant limit; ranging from 70 to $650 \mu\text{g kg}^{-1} \text{ dw}$ (59) and 185 to $765 \mu\text{g kg}^{-1} \text{ dw}$ (60), respectively.

The concentrations of PCBs were measured in two Swedish sludge samples during a rainy and dry weather period. The concentration of PCB ($\Sigma 6$ specific congeners) increased from $54 \mu\text{g kg}^{-1} \text{ dw}$ during the dry period to $85 \mu\text{g kg}^{-1} \text{ dw}$ during the rainy period (61). Atmospheric deposition being a source of PCBs in sewage sludge was supported by French research that found it was main source of PCBs in their sludges (59). Variation of the urban environment may have implications for PCB sludge concentrations, as the concentration of PCBs in the atmosphere was found to be 40 – 500% greater in precipitation from an urban environment than background precipitation (62). Loganathan *et al.* (1997) investigated the PCB by wet and dry deposition and found that contaminated street dust was also a major PCB source, particularly in areas that had historical contamination (63).

A summary of the concentration of PCBs in international sludges is presented in Table III showing that PCBs levels have declined since measurements began. The highest reported concentrations of PCBs in sludges occurred in the USA ($1,700,000 \mu\text{g kg}^{-1} \text{ dw}$) at a point in history when PCBs were being phased out. There is little variation of PCB concentrations between nations, suggesting that PCB contamination has been similar throughout developed countries. Presently, PCB concentrations rarely exceed $1,000 \mu\text{g kg}^{-1} \text{ dw}$. That PCBs are still detected, albeit at low concentrations, provides evidence that PCBs are highly environmentally persistent. It is also worth noting that PCBs are still contained in infrastructure in many Western nations and may continue to be a source of these chemicals to the environment for some time to come.

Table III. Concentration of PCBs in international sewage sludges as reported in the scientific literature $\mu\text{g kg}^{-1}$ dw

<i>Country</i>	<i>Year</i>	<i>Method</i>	<i>n</i>	<i>Mean</i>	<i>Median</i>	<i>Range</i>	<i>Study</i>
USA	1976	GC-ECD	16	5,200	4,200	<10–23,000	(64)
USA	1977	GC-ECD	NA ^a	NA	NA	238,000–1,700,000	(48)
USA	1980	GC-ECD	4	3,400	3,200	1,200–6,200	(49)
UK	1982	GC-ECD	40	160	150	20–460	(52)
UK	1984	GC-ECD	444	340	140	10–21,500	(51)
USA	1984	GC-ECD	30	1,200	900	150–3,600	(50)
Netherlands	1984	GC-ECD	6	880	960	390–1,480	(65)
UK	1993	HRGC-ECD	12	292	NA	106–712	(54)
Italy	1993	HRGC-MS	5	628	565	210–1,010	(53)
USA	1994	HRGC-ECD	16	<250	<250	<250–4,600	(66)
Switzerland	1996	HRGC-MS	19	NA	NA	43–550	(55)
Canada	1996	HRGC-MS	11	NA	<10	<10–28	(67)
Ireland	2000	GC-ECD	9	58	7	<10–105	(68)
Canada	2003	HRGC-MS	26	NA	NA	<50	(56)
UK	2003	HRGC-MS	14	NA	NA	110–440	(57)
Greece	2004	HRGC-MS	20	550	500	180–765	(60)
Spain	2005	HRGC-MS	79	NA	30	3–60	(58)
China	2009	HRGC-HRMS	8	101	91	66–157	(69)
Australia	2010	Archived Data	829	<10	NA	<10–30	(70)

^a NA – Data not available

Organochlorine Pesticides

The term 'organochlorine pesticides' (OCPs) refers to all chlorine-containing organic compounds used for pest control. The term includes a broad range of substances and is not confined to compounds of any single specific type of chemical structure or organic functional group (71). OCPs are considered to be the second generation of pesticides that took over from the early first generation of insecticides produced from inorganic compounds (72). The most infamous of these is DDT which is remarkably active against a number of insect pests. Ironically, one of the valued properties of DDT was its persistence as there was no need for frequent applications. DDT forms two metabolites DDD (dichlorodiphenyldichloroethane) and the environmentally persistent DDE (dichlorodiphenyldichloroethylene). DDT became the prototype OCP from which other more toxic pesticides were developed viz., aldrin, dieldrin, lindane, chlordane and heptachlor. Most of these OCPs are no longer used because of their harmful effects on human health and contamination of the global environment. Despite restrictions for use in most Western nations, many OCPs and their breakdown products are still detected in environmental compartments, including human tissue throughout the world (43, 73, 74).

There is a large amount of research investigating OCPs in environmental compartments, but there are not many studies focussing on typical concentrations in sewage sludge, or the way these concentrations have changed over time. The few studies that have been reported on OCPs in sewage sludge have come from the USA, the UK, Sweden, Italy, Greece, China and more recently, Australia.

In the first of a series of studies from the UK (published in 1982), the concentrations of DDE, dieldrin and lindane in sewage sludges were measured ($n=40$) (52). DDE was detected in all samples ($10 - 490 \mu\text{g kg}^{-1} \text{ dw}$), but dieldrin and lindane were not. While dieldrin and lindane were less frequently detected, the maximum concentrations of dieldrin ($1,260 \mu\text{g kg}^{-1} \text{ dw}$) and lindane ($930 \mu\text{g kg}^{-1} \text{ dw}$) were slightly higher than the maximum DDE concentration ($49 \mu\text{g kg}^{-1} \text{ dw}$) (52). In a follow-up survey that was published in 1984, the concentration of lindane, aldrin, dieldrin and endrin in UK sewage sludges ($n=444$) (51) had increased significantly, with the major pesticides detected being lindane and dieldrin. The concentrations of these two pesticides had reported maximum concentrations of $70,000$ and $52,940 \mu\text{g kg}^{-1} \text{ dw}$, respectively. These maximum concentrations appear to be from unusual samples as the median concentrations for both chemicals were less than $150 \mu\text{g kg}^{-1} \text{ dw}$ (51).

The 1990s saw the improvement in analytical techniques and detection limits. A Swedish study reported ΣDDT (DDT+DDE+DDD), HCB and lindane at low concentrations $<100 \mu\text{g kg}^{-1} \text{ dw}$ (61). Hexachlorobenzene (HCB) and DDE were detected in Italian sludges ($n=5$) at concentrations ranging from 10 to $310 \mu\text{g kg}^{-1} \text{ dw}$ and 20 to $90 \mu\text{g kg}^{-1} \text{ dw}$, respectively (53). DDT and DDD were also detected, but interfering analytes prevented the quantification of these compounds. A survey of Canadian sludges in 1996 reported that aldrin, chlordane, heptachlor and DDT were below the survey detection limit ($<10 \mu\text{g kg}^{-1} \text{ dw}$), while DDE and HCB were detected with concentrations ranging from <10 to $13 \mu\text{g kg}^{-1} \text{ dw}$ and <10 to $33 \mu\text{g kg}^{-1} \text{ dw}$, respectively, but they were not detected in all samples (67).

By the new millennium, the detection of OCPs was becoming less common, with some countries choosing not to monitor for OCPs in national surveys (56). A survey of digested sludge from fourteen UK WWTPs measuring the concentration hexachlorohexanes (HCHs), HCB, endosulfan, DDT, DDD, DDE and chlordane reported low or non-detectable levels of all compounds (57). Only two of the compounds analysed as part of the survey were found at concentrations above the detection limit [HCB (6.4 – 260 $\mu\text{g kg}^{-1}$ dw) and DDE (6.0 – 28 $\mu\text{g kg}^{-1}$ dw)] and this observation was consistent with the declining use of OCPs in Europe (57). HCB, like other chlorobenzenes, has some industrial applications, and this may account for its presence in all the samples. It is also relatively volatile and ubiquitous in the atmosphere (57).

Sludge samples collected in 2005 from 31 WWTPs in 26 cities of China, were analysed for HCH, DDT, DDD, DDE and HCB (75). None of the four HCH isomers were regularly detected as the median concentration was <LOD for all isomers. Σ DDTs were detected frequently and suggests that DDT is still currently being used within China. The authors noted that the pesticide diclofol, containing DDT as an impurity, is still used in China and may be the source of the contamination. HCB was detected in all sludge samples (7.5 to 319 $\mu\text{g kg}^{-1}$ dw) and the source of this compound has been associated with the production of pentachloronitrobenzene, combustion and metallurgical processes involving the use of chlorine (75).

A comprehensive study of OCPs in sludge (n=829) was recently reported from Australia (76). The main OCPs detected in sludges between 2004 and 2006 were dieldrin, chlordane and DDE. This study also examined historical data over a ten year period (1995 – 2006) and the concentrations of dieldrin and chlordane were correlated over this time period, indicating a similar mechanism of movement through the environment and into WWTPs.

OCPs may enter the WWTP from industrial discharge or as a component of urban runoff or drainage into the sewer system. However, little research has investigated the environmental pathways and source of these compounds in sludges. The most illuminating work was the Swedish study discussed earlier, that measured the concentration of organic contaminants in sewage sludge following dry and rainy weather periods (61). They found that the concentrations of DDTs increased from 39 $\mu\text{g kg}^{-1}$ dw in the dry period to 68 $\mu\text{g kg}^{-1}$ dw in the wet period, demonstrating that rainfall plays a role in the environmental movement of this compound. The concentration of lindane was the same during both the dry (8.7 $\mu\text{g kg}^{-1}$ dw) and wet periods (7.8 $\mu\text{g kg}^{-1}$ dw) and it would appear that atmospheric transport does not play a major role in its movement. Oddly, the concentration of HCB decreased from 43 $\mu\text{g kg}^{-1}$ dw during the dry period to 11 $\mu\text{g kg}^{-1}$ dw during the wet period.

A number of studies have investigated the fate of OCPs within WWTPs. While most OCPs are persistent during wastewater treatment processing and concentrate in the sludge (77), one study reported that lindane was effectively degraded ($67 \pm 10\%$) (78). There is a correlation between biodegradability and water solubility, and the relative high degradation rates of lindane are likely to be a reflection of this fact. Lindane also has a higher vapour pressure and thus could have volatilized during the treatment process.

A summary of OCP concentrations in sewage sludges is presented in Table IV. Most OCPs appear to be no longer present in sludges where countries have controlled the source. However, dieldrin, chlordane and DDE are environmentally persistent and are regularly detected in sludges fifteen years after banning them in Australia.

Table IV. Summary of organochlorine pesticides concentrations ($\mu\text{g kg}^{-1}$ dw) in sewage sludge

Analyte	Year	Country	Method	n	Mean	Median	Range	Study
Aldrin	1984	UK	GC-ECD	444	30	20	<10–90,210	(51)
	1996	Canada	HRGC-MS	11	<10	NA ^a	NA	(67)
	2010	Australia	Archived Data	829	<10	NA	<10–70	(70)
Chlor-dane	1996	Canada	HRGC-MS	11	<10	NA	NA	(67)
	2003	UK	HRGC-MS	14	<dl	<dl	NA	(57)
	2010	Australia	Archived Data	829	10	NA	<10–290	(70)
Dield-rin	1976	USA	GC-ECD	16	0.19	0.27	<0.03–2.2	(64)
	1982	UK	GC-ECD	40	280	260	<10–1260	(52)
	1984	UK	GC-ECD	444	500	130	<10–52,940	(51)
	2004	Greece	HRGC-MS	20	15	<dl	<dl–86	(60)
	2010	Australia	Archived Data	829	30	NA	<10–700	(70)
Σ DDT	1993	Switzer-land	GC-MS, GC-ECD	27	70	70	nd–376	(79)
	1996	Canada	HRGC-MS	11	<10	NA	NA	(67)
	2003	UK	HRGC-MS	14	<dl	<dl	NA	(57)
	2007	China	HRGC-HRMS	31	NA	2.4	<0.10–100.7	(75)
	2010	Australia	Archived Data	829	<10	NA	<10–270	(70)
DDE	1982	UK	GC-ECD	40	20	40	<10–49	(52)
	1993	Italy	HRGC-MS	5	49	30	20–90	(53)
	1996	Canada	HRGC-MS	11	NA	<10	<10–13	(67)
	2000	Ireland	GC-ECD	9	51	5	<10–106	(68)
	2003	UK	HRGC-MS	14	13	13	6.0–28	(57)

Continued on next page.

Table IV. (Continued). Summary of organochlorine pesticides concentrations ($\mu\text{g kg}^{-1}$ dw) in sewage sludge

Analyte	Year	Country	Method	n	Mean	Me- dian	Range	Study
	2004	Greece	HRGC-MS	20	27	19	<dl-96	(60)
	2010	Australia	Archived Data	829	<10	NA	<10-270	(70)

^a NA – Data not available

Polybrominated Diphenyl Ethers

Polybrominated diphenyl ethers (PBDEs) are a class of brominated fire retardants that were used in plastics, textiles, electronic circuitry and other materials. There are 209 potential PBDE congeners and they are numbered according to the IUPAC system for PCBs (80). PBDEs were sold in three commercial formulations, each named for the prominent homologue in the mixture viz., pentaBDE (BDE 47, 99, 100, 153, 154), octaBDE (BDE 183), and decaBDE (BDE 209) (81). Despite the commercial formulation names, each contains a mixture of PBDE congeners. Due to the threat to human health and the environment, PBDEs (specifically pentaBDE and octaBDE) were listed as United Nations Persistent Organic Pollutants in 2008 (11, 15). In many countries, the use of PBDE fire retardants is being phased out – in particular, the pentaBDE and octaBDE formulations. Their use has been restricted in many parts of Europe, Japan, some states of the USA and Australia (82, 83). Recently, there has been a voluntary industry led phase-out of the decaBDE commercial formulation (83).

Assessment of health risks associated with PBDE human accumulation and exposure is complicated and has not been adequately characterized. However, the potential risks associated with exposure to the most bio-active congeners (tri- to octa-BDE) include thyroid hormone disruption, neuro-developmental defects and cancer (84). Several studies have shown that PBDEs share the general property of organo-halogenated compounds which is that *in vivo* exposure of rodents results in reduction of serum total and free thyroid hormone [thyroxine (T4)] levels (84, 85). Altered thyroid hormone function, particularly during development, are profound and have been hypothesized to lead to disrupted brain development and permanent neurological damage (86, 87).

PBDEs were first detected in sewage sludge and other environmental samples in 1979 from samples collected near a chemical manufacturing site in the USA (88). However, it was not until 1992 that the concentration of two PBDEs congeners, representative of the penta commercial formulation, were first reported in sludges from Sweden: BDE 47 concentration of $15 \mu\text{g kg}^{-1}$ dw and BDE 99 of $19 \mu\text{g kg}^{-1}$ dw (61). These levels are similar to those reported in a German study (n=13) conducted in 1992, which reported Σ penta-BDE concentrations ranging from 0.22 to $17.13 \mu\text{g kg}^{-1}$ dw (89). This study also reported the consistent presence of brominated furans (PBDF) at relatively high concentrations that ranged between 0.21 and $3.05 \mu\text{g kg}^{-1}$ dw that are similar levels to PCDFs found in sewage sludges (28). The German researchers suggested that since the

concentrations of PBDFs and PBDEs were highly correlated, PBDEs are likely to be the source of PBDFs observed in the sludges analysed. This is extremely important and requires more research, as PBDFs share the same chemical behaviour and also the same mechanism of toxicity as PCDFs (17, 90).

The sludge samples collected in 1997-1998 from Stockholm, Sweden, reflect concentrations of PBDEs in sludge more typical of levels found in contemporary sludges: BDE 47 36 – 80 $\mu\text{g kg}^{-1}$ dw, BDE 209 170 – 270 $\mu\text{g kg}^{-1}$ dw (91, 92). They found a higher burden of BDE 209 compared to the other congeners from the pentaBDE formulation, which has been consistently reported with subsequent studies (93–95).

Swedish sewage sludges (n=50) collected in 2000 and analyzed for BDE 47, 99, 100, 153, 154, 209 reported that BDE 209 was the dominant congener, with highly variable concentrations ranging from 5.6 to 1,000 $\mu\text{g kg}^{-1}$ dw (96). The congener profiles of BDE 47, 99, 100, 153 and 154 in all the Swedish sludges were similar to that of the pentaBDE technical product which was probably the original source (96). Concentrations of the lower brominated PBDEs were fairly similar in all sewage sludge samples, indicating diffuse leaching of these from products into wastewater streams (96). Similarly, a German study (n=39) in 2007, found that the total tri- to hepta-BDE concentrations (sum of BDE 28, 47, 99, 153, 154 and 183) ranged from 12.5 to 288 $\mu\text{g kg}^{-1}$ dw (mean 108 $\mu\text{g kg}^{-1}$ dw) and the BDE 209 concentrations once again varied widely, ranging from 97 to 2,217 $\mu\text{g kg}^{-1}$ dw (mean 256 $\mu\text{g kg}^{-1}$ dw) and was the most prevalent congener detected (97). The total concentration of penta-BDEs in sewage sludges from the USA (93) reported in 2001 ranged between 1,100 – 2,290 $\mu\text{g kg}^{-1}$ dw and the authors reported that the input of penta-BDEs was consistently high, regardless of the region, and irrespective of preliminary treatment. These levels are far higher than previously reported and exceed those in European sludges by a factor of 10- to 100- fold which was attributed by the researchers to the much higher use of PBDEs, both the pentaBDE and decaBDE formulations in the USA. Unlike BDE 47 & 99 (both components of the pentaBDE formulation) BDE 209 varied widely among the samples analyzed (84.8 – 4 890 $\mu\text{g kg}^{-1}$ dw). The greater variability of BDE 209 concentration compared to BDE 47 & 99 has been reported in other studies and may provide an indication of the source of PBDEs into wastewater (93, 94). Many studies have reported a lower concentration range for pentaBDE congeners compared to decaBDE congeners and this suggests that the primary source of pentaBDE congeners is the domestic environment, as it is a universal, continual source of diffuse contamination. The variable levels of decaBDE, on the other hand, suggest a variable source that is dependent upon the location of WWTP and would indicate contributions from industries such as the textiles industry. For example, a Spanish study found that BDE 209 was the major PBDE constituent of sludge (>95%), with concentrations ranging between 786 and 5,837 $\mu\text{g kg}^{-1}$ dw and were associated with the textiles industry (98). A study of sludges over a 32-month period in the USA found a consistent concentrations of PBDEs that had no seasonal variation. This again suggests that the principal source of PBDE contamination in sewage sludge is the domestic environment (99).

Chinese sludge samples collected from 31 WWTPs in 26 cities had Σ PBDE ranging from 6.2 to 57 $\mu\text{g kg}^{-1}$ dw (75). These levels are about 10–100 times

lower than those found in Europe and North America. PBDE levels in sludge were not found to depend on the location or treatment capacity of the WWTPs. Two recent studies of sludges from industrialised countries (Kuwait, Australia) reported similar PBDEs burdens to other developed nations (94, 100). The concentrations of PBDEs in sewage sludges in China may increase in future with further industrialization and improved standards of living.

A few studies have investigated the fate of PBDEs during wastewater treatment processing. As expected highly hydrophobic organic compounds such as PBDEs, are adsorbed to the suspended solids (SS) and are principally removed through sedimentation (97, 101–104). A German study investigating the transformation of PBDEs in sludges from different stages of the wastewater treatment process (primary sludge, secondary excess sludge and dewatered digested sludge) found no change in the tri- to hepta-BDE congener profile ratios (97). An Australian study showed that the concentrations of PBDEs (reported on a mass/mass basis) was consistent throughout the wastewater process (104).

A summary of the concentrations of BDE 47, BDE 99, BDE 209 and Σ PBDEs reported in sewage sludges is presented in Table V.

Table V. Summary of PBDEs concentrations ($\mu\text{g kg}^{-1}$ dw) in sewage sludge

Country	Year	n	BDE 47			BDE 99			BDE 209			Σ PBDEs			Study
			mean	me- dian	range	mean	me- dian	range	mean	me- dian	range	mean	median	range	
Sweden	1992	2	15	15	15–15	19	19	19–19	NA ^a	NA	NA	NA	NA	NA	(61)
Sweden	1999	3	65	78	36–80	85	98	56–100	220	220	170–270	NA	NA	NA	(92)
USA	2001	11	568	536	359–754	661	613	391–1157	1372	553	84.8–4890	NA	NA	NA	(93)
Nether- lands	2003	3	20	11	10–40	21	14	11–38	96	90	8.6–190	NA	NA	NA	(107)
Spain	2004	6	37	33	2–50	37	35	23–64	5968	4511	756–18632	NA	NA	NA	(98)
Germany	2004	8	47	44	25–88	70	66	37–127	326	243	100–639	NA	NA	NA	(194)
USA	2004	1	757	NA	NA	944	NA	NA	1183	NA	NA	3381	NA	NA	(101)
Sweden	2006	50	49	NA	7–100	60	NA	8.1–150	120	NA	5.6–1000	NA	NA	NA	(96)
Germany	2007	19	52	42	20–115	57	48	24–124	442	354	113–1339	555	410	142–2491	(97)
China	2007	31	5	NA	0.4–58.7	4.5	NA	<3.4–67.7	68.5	NA	<1–1109	94	43.8	5.1–1115	(75)
Australia	2008	16	126	105	0–410	141	130	0.37–400	705	320	3.4–3780	1137	NA	4.5–4230	(94)
Kuwait	2008	21	2.33	1.8	0.2–7.81	5	4	0.44–14.74	182	93	4.8–1596	191	100	5.7–1600	(100)
Antarc- tica	2008	2	776	776	132–1420	735	735	200–1270	770	770	219–1320	2664	2664	637–4690	(195)

Country	Year	n	BDE 47			BDE 99			BDE 209			ΣPBDEs			Study
			mean	me- dian	range	mean	me- dian	range	mean	me- dian	range	mean	median	range	
USA	2009	84	710	NA	73–5000	716	NA	64–4000	2180	NA	150–17000	NA	NA	NA	(196)
USA	2010	15	161	153	128–238	169	160	128–245	920	883	792–1220	1496	1420	1330–1820	(99)

^a NA – Data not available

Polybrominated Biphenyls

Polybrominated biphenyls (PBBs) are a class of brominated fire retardant and the commercial products were mixtures named after the dominant homologue present (similar to the system used for PBDEs). The use of PBBs was curbed in the 1970s largely as a result of a serious human contamination incident in Michigan, USA (105). Their production has now been phased out internationally, with the last PBBs manufactured in France in 2000 (91).

The commercial formulation commonly sold as “hexabromobiphenyl” (hexaBB) can have hexaBB concentrations between 60 and 90% (105) with the major hexaBB identified being 2,2',4,4',5,5'- or BB153 (106). The decaBB formulation is reported to have a purity of more than 98% with the remaining 2% being nonaBB (105). In the USA and Canada, hexaBB (FireMaster™) was the principal PBB product. It was used as a fire retardant in three main commercial products: ABS plastics, coatings and lacquers, and polyurethane foam (105). The decaBB formulation (Adine 0102™) was used as a flame retardant for thermoplastics and thermosets (polyesters, epoxy resins, polystyrene, ABS, polyolefines, and PVC), for elastomers (PU-elastomers and India rubber) and for cellulose (chip-board).

The concentration of PBBs in sewage sludge has received little attention, primarily because of the relatively low use of PBBs in manufacturing. In general, the few studies (n=5) that have investigated the levels of PBBs in sludge, showed PBBs to be below the detection limit or at very low concentrations (88, 94, 95, 107, 108). A study of PBBs conducted in 2000 in the Netherlands on WWTP raw water, treated effluent and other environmental samples (sediments, biota) failed to detect these compounds (95). Neither were they detected in a follow-up study conducted in 2003; detection limits ranged from < 0.1 to $< 10 \mu\text{g kg}^{-1} \text{ dw}$ (107). This result is in agreement with the negligible PBB production in Europe over the past few decades (107). On other hand, a Swedish study reported the presence of PBB-153 in sewage sludge samples (n=116) (108). Unfortunately, the results were reported on a wet weight basis making it impossible to compare the levels determined with those reported in other studies, as the water content of sewage sludge is highly variable (108). In a recent Australian study, BB153 was detected in all sludge samples (n=16) analysed, which is quite surprising, as there is no history of use of this chemical in Australia (94, 109). This finding suggests that BB153 is extremely environmentally persistent and mobile. Alternatively, given the wide geographic distribution of this compound, it is possible that it may be a contaminant in another commonly used product.

Perfluorochemicals

Perfluorochemicals (PFCs) are a family of anthropogenic chemicals that have been used since the late 1950s to make products resistant to heat, oil, stains, grease and water. Common applications include non-stick cookware, breathable membranes for clothing, stain-resistant carpets and fabrics as well as components of fire fighting foam and surfactants (110). They have been used in many industrial sectors, including the aerospace, automotive, building/construction, chemical

processing, electronics, semiconductors, and textile industries (110). PFCs are persistent and widely dispersed in the environment (111, 112). Accumulation of PFCs has been detected in ocean animals, such as birds and mammals, and in human tissues throughout the world (113, 114). The human and environmental toxicological response to such exposure is not known, but could include endocrine disruption (115).

The chemical structures of PFCs make them very resistant to degradation in the environment; the carbon-fluorine bonds are extremely strong and are stronger relative to other commonly used halogens. Consequently, perfluorocarbon chains do not readily biodegrade and any biodegradation may be limited to attached hydrocarbon moieties. The two most common groups of PFCs that are measured and detected in environmental matrices are perfluoroalkyl sulphonates (PFASs) [perfluorooctane sulphonate (PFOS), perfluorohexane sulphonate (PFHxS), perfluorooctane sulphonamide (PFOSA)] and perfluoroalkyl carboxylates (PFACs) [perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA)]. PFASs and PFACs are synthetic chemicals that do not occur naturally in the environment (110) and are employed as base chemicals in the preparation of fluoropolymers, which upon degradation can release the environmentally persistent monomers (116).

The earliest available report of PFCs in sewage sludge, reported in 2001, was undertaken by the perfluorochemical industry on sludge samples collected from six USA cities (32). PFOS and PFOA were the most common PFCs present at the low $\mu\text{g kg}^{-1}$ dw range and were detected in all samples analysed. A sludge sample was also tested from a WWTP serving a fluorochemical manufacturer and as would be expected, the concentrations were high: PFOS ($2,980 \mu\text{g kg}^{-1}$ dw) and PFOA ($173 \mu\text{g kg}^{-1}$ dw). This study demonstrated that PFCs, in particular PFOS and PFOA, are likely to be present in sludge in any countries where PFCs are manufactured and used. Domestic sources are also likely to be a major contributor of PFCs in sewage sludge. Other studies from the USA and Denmark report similar concentrations (117–120). No differences in PFC levels were apparent in sludges from urban and rural WWTPs; however, major seasonal variations in concentrations were observed (117). WWTP mass balance studies of PFCs have reported higher mass loadings of PFOA and PFOS in WWTP effluent compared to raw influent, indicating that degradation of other fluorinated organic compounds (i.e., fluoropolymers) into PFOA and PFOS may take place during wastewater treatment (117–119).

While there are restrictions on marketing and use of PFOS and also some voluntary reductions in the use of PFOA, they are still being manufactured (121). The European Union is currently assessing PFOA and, whilst there are no restrictions in place in the EU at present, a ban could be imposed in the future. However, these substances have been extensively used in the urban and domestic environments and therefore could represent a significant, long-term diffuse input into wastewater and sludge.

A summary of the concentrations of PFOS and PFOA reported in sewage sludges is presented in Table VI.

Table VI. Concentration of perfluorochemical concentrations (PFOS, PFOA) in international sewage sludges $\mu\text{g kg}^{-1}$ dw

Analyte	Country	Year	n	Mean	Median	Range	Study
PFOS	USA	2001	5	689	125	59–2980	(32)
	USA	2006	NA ^a	100	NA	81–160	(118)
	USA	2006	10	31	44	<10–65	(119)
	USA	2007	8	76	95	8.2–990	(117)
	Denmark	2008	7	NA	NA	5–74	(120)
PFOA	USA	2001	5	48	3	3–172.5	(32)
	USA	2006	NA	<3	NA	NA	(118)
	USA	2006	10	107	107	18–241	(119)
	USA	2007	8	68	20	8.3–219	(117)
	Denmark	2008	7	NA	NA	1–20	(120)

^a NA – Data not available

Risks from Sewage Sludge Land Application

Much research has been conducted internationally to assess the risks to human health and the environment when applying to biosolids land that are contaminated with POPs (7, 10, 122, 123). These assessments centre around four major exposure pathways: direct human exposure, plant accumulation, grazing animal accumulation and water contamination. Each of these pathways, including soil accumulation and persistence will be discussed below.

Studies have shown that the application of sewage sludge will increase the concentration of the POPs in biosolids-amended soil (124, 125). Furthermore, field trial studies have shown that POPs are environmentally persistent in soil with reported half lives exceeding five years for PCDD/Fs (126, 127), PBDEs (99, 128–130), OCPs (131, 132) and PCBs (133, 134). The dominant removal mechanisms for many POPs from biosolids-amended soil is thought to be volatilization (127, 133, 135); however, there are no studies that have investigated atmospheric losses of PBDEs or PFCs from biosolids-amended soil or soil generally.

Soil ingestion is thought to be the dominant exposure route for organic compounds from soil and other direct exposure routes, such as inhalation and dermal absorption, are thought to make only a very small contribution to exposure from contaminated soil (136). The direct exposure to biosolids-amended soil can be assessed for each of the chemicals by comparing typically ingested amounts of soil for a child and an adult: 100 mg and 50 mg per day respectively (137, 138). Deliberately ingested soil, referred to as pica, is not commonly incorporated into biosolids human health risk assessments or contaminated land risk assessment as it is believed that pica is a relatively uncommon childhood condition (3, 139).

The majority of experimental (laboratory and field) work demonstrates that there is little relationship between the concentration of non-ionic hydrophobic organic compounds in the soil and the concentration found in the aerial components of the plant material (140–144): PCDD/Fs (6, 145, 146), OCPs (147) and PCBs (148–150). This provides strong evidence that these chemicals are not translocated from the soil matrix through the plant and into the aerial plant components, but also, that volatilization from the soil matrix does not result in significant contamination of aerial plant components. However, there are exceptions, the translocation of PCDD/Fs and PBDEs from the soil matrix has been demonstrated for zucchini and pumpkins (151, 152).

Plant roots are known to absorb a wide variety of organic pollutants due to their lipophilicity as demonstrated with root vegetables such as carrots (153). Most of the contamination is associated with the peel of the carrots and up to seven (154) and ten-fold increases (155) in concentrations of PCDD/Fs and PCBs (141, 148, 150) have been reported when carrots were grown in contaminated soils. However, a decrease in the uptake of soil borne PCDD/Fs (155) and PCBs (148) into carrots with increasing rate of chlorination has been observed. Increasing chlorination reduces water solubility and therefore, increases adsorption of organic pollutants to the soil matrix. No uptake of PBB by radish or carrots grown in contaminated soil has been observed (156, 157).

The exposure of grazing animals to POPs from biosolids-amended soil will occur by plant uptake (158–160) as well as the direct ingestion of soil (161). The accumulation of organic pollutants in grazing animals resulting from agricultural practices is well documented and 80–95% of human exposure to POPs is thought to be derived from animal products (162). Research into this mode of exposure has included studies on PCDD/Fs (160, 163, 164), OCPs (165) and PCBs (166). Bioaccumulation of PBDEs and PFCs in grazing animals and their products has not been investigated. Management practices such as biosolids application method (i.e., surface spreading, incorporation, direct injection) and applying a withholding period for grazing animals will influence this exposure pathway. A variety of models have been proposed to estimate the accumulation of organic compounds in the tissue of grazing animals that derives from soil. Travis *et al.* (1998) used the biotransfer factor (BTF) to relate beef/milk concentrations to the chemical physicochemical properties (167). The BTF in milk and fat generally decreases with increasing halogenation (160, 164, 168) because the potential absorption of organic contaminants through the gastrointestinal tract decreases for compounds with higher octanol-water partition coefficients (K_{OW}). Thus, approximately 80 % of chemicals with a log K_{OW} between 4.5 and 7.0 is absorbed; that decreases to approximately 35% when the log K_{OW} of a chemical is >8 (169). Soil properties are not generally considered to influence the bioavailability of organic pollutants to animals (170, 171), however, it is possible (and likely) that the BTF of organic pollutants will decrease with increasing soil organic matter content as sorption with the soil matrix increases.

The contamination of groundwater may occur via two pathways by (1) horizontal movement associated with runoff into adjacent surface water bodies and (2) percolation through the soil profile leaching into the groundwater. Both pathways are related to the retention characteristics of a compound in the soil

profile determined by the soil-water partition coefficient (K_D). It is considered that compounds with a $K_D > 1000$ are immobilised by soil sorption and with the exception of the PFCs, includes all of the chemicals included in this review (89). Similarly, organic compounds with $\log K_{OW}$ greater than 3.7, again with the exception of the PFCs, includes all the chemicals discussed in this review, are thought to be immobile in soil (172). While it is clear that hydrophobic non-ionic organic pollutants are strongly retained by soil organic matter, they will dissolve in aqueous media to a small degree that can be predicted by relating the soil organic carbon content with the K_{OW} (104). Recently, field studies investigating the mobilisation organic pollutants with rainfall simulations found that organic pollutants are associated with subsurface groundwater flow (173, 174). These findings contradict the earlier established theories that POPs in biosolids-amended soil are effectively immobile and warrants further attention. It is possible that the migration of these compounds are mobilized with colloids, similar to reports of metals mobilized from biosolids-amended soil (175).

A full examination of the ecological risks associated with the land application of biosolids remains to be completed. Unlike the human health risk assessment that has one single endpoint, there are numerous potential receptors in an ecological risk assessment i.e., microbial, soil terrestrial organisms, plants and animals. Numerous studies have reported the increased concentrations of POPs and other emerging organic pollutants in earthworms (176, 177) and this provides opportunities for contamination of the food-chains.

Internationally, there appears to be no consensus over the methodology for developing contaminant limits for POPs in biosolids, or indeed if they are warranted for protection of human health and the environment. The majority of human health risk assessment studies have indicated that there is low risk of human exposure to POPs resulting from biosolids land application that contain concentrations of POPs typically reported (3, 178). Furthermore, effective source control has reduced the concentration of most POPs in sewage sludge, which will further reduce the likelihood of human exposure. However, understanding the ecological risks from sewage sludge land application has not been adequately characterized and requires more attention. A clear methodology needs to be outlined for developing contaminant limits in sewage sludge, one that is agreed upon internationally and can be applied to known and 'emerging' organic pollutants.

Conclusions

All sewage sludges in developed countries contain POPs and are a source of these chemicals to the environment when recycling this material to land. Therefore, it is important to assess the risks to human health and the environment from this practice. A large volume of research has focussed on POPs in sewage sludge over the past thirty years and many risk assessments have concluded that there is negligible risk to human health from non-ionic POPs (at concentrations typically observed) when land applying this material. However, assessments of the ecological consequences of sewage sludge land application still remains

to be completed. Of particular note are specific PFCs that are environmentally persistent, potentially toxic and have unique chemical properties that make it theoretically possible for them to enter the food chain from biosolids-amended soil. A full risk assessment (human and environmental health) of these compounds must be completed. Finally, source control of PCDD/Fs, PCBs and OCPs has been demonstrated to be an effective strategy for reducing the concentrations of POPs in sewage sludge throughout the world and this can be applied to other 'emerging' contaminants to minimize future contamination of sewage sludges and biosolids.

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Chapter 7

U.S. EPA's 2006-2007 Targeted National Sewage Sludge Survey

Rick Stevens*

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

*stevens.rick@epa.gov

1. Executive Summary

EPA's 2006-2007 Targeted National Sewage Sludge Survey (TNSSS) is a valuable step in advancing the understanding of what chemical constituents are present in treated sewage sludge. The information from the survey provides important input for EPA and others to evaluate potential uses and associated risks of biosolids generated by publicly owned treatment works. It also fulfills an important commitment under the agency's four pronged strategy for pharmaceuticals and personal care products by providing the first national estimates of which pharmaceuticals, steroids and hormones may be present in sewage sludge and at what concentrations.

This chapter summarizes the technical background, sampling and analysis activities, statistical methods, and resulting estimates of pollutant concentrations in treated sewage sludge ("biosolids") that represent Publicly Owned Treatment Works (POTWs) in the contiguous United States with flow rates of at least 1 million gallons per day (MGD). Estimates were produced using data from a national probability sample of 74 POTWs that statistically represent 3,337 POTWs that met the study criteria.

The TNSSS was designed to obtain occurrence information on select analytes of interest. The objective of the survey was to obtain national estimates of the concentrations of these pollutants in sewage sludge for use in assessing if exposures may be occurring and whether those levels may be of concern. Estimates from the survey may provide important input to EPA's efforts to evaluate biosolids generated by the nation's POTWs.

EPA conducted analyses of sewage sludge samples for 145 analytes, including four anions (nitrite/nitrate, fluoride, water-extractable phosphorus), 28 metals, four polycyclic aromatic hydrocarbons, two semi-volatiles, 11 flame retardants, 72 pharmaceuticals, and 25 steroids and hormones, and minimum and maximum

measurements that were encountered among the samples collected in this survey. For 34 of the analytes measured in this survey, this chapter summarizes an in-depth statistical analysis that yielded nationally-representative estimates. For each of the 34 analytes, nationally-representative estimates of the 50th percentile (i.e., median) of the underlying distribution of measurements across POTWs, as well as the 90th, 95th, 98th, and 99th percentiles are presented, along with the mean, standard deviation, and the minimum and maximum measurements.

The survey used both well-established multi-laboratory validated EPA procedures as well as three analytical methods that were developed or updated for the survey. The two new methods are single-lab validated methods for pharmaceuticals (EPA Method 1694, (1)), and steroids and hormones (EPA Method 1698, (2)). The updated multi-lab validated method is for flame retardants (EPA Method 1614, (3)). The percent solids in the various sewage sludge samples range from 0.14 to 94.9. To ensure comparability of results, all sample results are reported on a dry-weight basis. Efforts by the Agency to characterize potential risks associated with TNSSS results are currently ongoing.

2. Introduction and Scope

Sewage sludge is the solid, semisolid, or liquid organic material that results from the treatment of domestic wastewater by municipal wastewater treatment plants, also known as publicly owned treatment works (POTWs). The terms sewage sludge and biosolids are often used interchangeably. Biosolids are the nutrient-rich organic materials resulting from further treatment of sewage sludge to meet land application requirements. When properly treated and processed, sewage sludge becomes biosolids which can be recycled and applied as fertilizer to improve and maintain productive soils and stimulate plant growth.

Section 405(d) of the Clean Water Act (CWA) requires that the EPA establish standards for the use or disposal of sewage sludge. The Standards for the Use or Disposal of Sewage Sludge are found at Part 503 of Section 40 of the Code of Federal Regulations (40 CFR 503, hereafter simply "Part 503"). These standards establish numeric limits, management practices, and operational standards to protect public health and the environment. Sewage sludge is typically used during land application to fertilize crops or reclaim mined lands, or disposed either by landfilling or surface disposing, or by incinerating. States may adopt additional or more stringent regulations for the use or disposal of biosolids.

Additionally, Section 405(d) of the CWA requires EPA to review existing sewage sludge regulations at least every two years (i.e., biennial review). The purpose of such reviews is to identify additional toxic pollutants, and promulgate regulations, if needed, for those pollutants consistent with the requirements set forth in the CWA.

3. Targeted National Sewage Sludge Survey

The Agency periodically conducts surveys to determine what may be present in sewage sludge. EPA has conducted three previous sewage sludge surveys: 1)

a 40-city survey in 1982 to develop information on the fate and effects of priority pollutants in wastewater treatment plants and estimates of pollutant concentrations in sewage sludge; 2) a National Sewage Sludge Survey in 1988-1989 to gather information on sewage sludge use or disposal practices and to obtain updated information on the concentration of over 400 pollutants in the Nation's sewage sludge; and 3) a National Sewage Sludge Survey in 2001 to obtain updated national estimates of dioxins and dioxin-like compounds in sewage sludge managed by land application.

For the current Targeted National Sewage Sludge Survey (TNSSS), EPA included 145 pollutants to obtain updated or new concentration data are needed to assess exposure and help in evaluating whether levels of these pollutants in sewage sludge present environmental or human health concerns. Pollutants included in the TNSSS were those for which appropriate analytical methods for detecting and quantifying the pollutants either already existed or were developed for the survey.

The list of TNSSS pollutants included 28 metals; benzo(*a*)pyrene (found in coal tar, automobile exhaust fumes, tobacco and wood smoke, charbroiled food, and burnt toast); 2-methylnaphthalene (found in nonstructural caulking compounds and sealants, synthetic resins, rubber adhesives, and wall coverings); bis (2-ethylhexyl) phthalate (widely used as a plasticizer in manufacturing of items such as cosmetics, toys, tools, and laboratory equipment); fluoride (used in topical and systemic therapy for preventing tooth decay, as well as many other uses); water-extractable phosphorus (correlated with phosphorus concentration in runoff from soils amended with manure and biosolids and an effective indicator of loss that may contribute to algae buildup in surface waters); 11 PBDEs (used as flame retardants in a wide array of products, including building materials, electronics, furnishings, motor vehicles, plastics, polyurethane foams, and textiles); and 97 pharmaceuticals, steroids, and hormones.

EPA began sampling in August 2006, using the procedures described in Section 3, and completed sampling in March 2007. Analyses of survey samples were conducted as described in Section 4.

3.1. Survey Objective

The TNSSS was designed to: 1) obtain updated occurrence information on nine analytes of potential concern, and 2) obtain occurrence information on a number of contaminants of emerging interest identified by EPA and the National Research Council (4) that may be present in sewage sludge generated by POTWs.

3.2. Target Population

For this survey, EPA focused its efforts on POTWs that treat more than one million gallons of wastewater per day (MGD). This group of facilities collectively generates approximately 94 percent of the wastewater flow in the nation. To be eligible for the survey, EPA also required that a POTW be located in the contiguous United States and employ secondary treatment or better. EPA selected POTWs meeting the criteria from information in the 2004 Clean Water Needs

Survey and the 2002 version of the Permit Compliance System. From the 3,337 POTWs that met the criteria in either data source, EPA used a stratified random sampling design to statistically select 74 facilities in 35 states for the survey and collected biosolids samples from those facilities. By using statistical methods, the concentration measurements can be extrapolated to the entire population of 3,337 POTWs.

3.3. Sample Collection

EPA collected samples of the final treated sewage sludge at each of the 74 POTWs that ultimately participated in the TNSSS. EPA developed a sampling and analysis plan that was peer-reviewed and describes the sample collection procedures in detail. EPA revised the plan periodically during the survey to address the changing list of facilities and to add updated contact information for the laboratories that performed the analyses. EPA sampled between August 2006 and March 2007.

EPA began the sample collection process by identifying the number and nature of the types of sewage sludge produced at each facility. Whether the facility land applies the sewage sludge or disposes of it via incineration or surface disposal was not a consideration for selecting a facility for inclusion in the survey. Grab samples were collected from each facility using sampling equipment appropriate to the type of sewage sludge (liquid or solid) and the analytes of interest. To avoid or minimize contamination from sampling equipment, plastic equipment was used to collect samples for analyses of metals and anions, and stainless steel equipment was used to collect samples for analyses of all the organics.

Liquid samples were collected as free-flowing materials from storage tanks, transfer lines, taps, and hoses. After purging any lines used to collect samples, liquid samples were collected directly into the final sample containers shown in Table I. Where possible, plant staff turned on mixing equipment in any storage tanks prior to sampling so that the collected liquids were representative of the bulk sewage sludge.

Solid samples included dewatered sewage sludge. These samples were collected from a belt press, filter press, drying bed, centrifuge, compost pile, or other source on site. The sampler collected small grab samples from multiple areas of any large piles, or multiple grabs from any continuous processes (e.g., belt press). Small grabs were composited in a large pre-cleaned container of appropriate construction, mixed well, and the mixed sample was transferred into the final sample containers (see Table I). Several kilograms of material were collected for each type of treated sewage sludge and mixed. Any mixed material that remained after all the sample containers were filled was returned to the sewage sludge process for disposal.

Table I. Sample Containers for Solid and Liquid Sewage Sludge, by Analysis Fraction

<i>Analysis Fraction</i>	<i>Solid Sample Container</i>	<i>Liquid Sample Container</i>
Metals	500-mL wide-mouth HDPE	500-mL wide-mouth HDPE
Polycyclic Aromatic Hydrocarbons (PAHs) and Semivolatiles (as one analytical fraction)	500-mL wide-mouth glass	1000-mL wide-mouth glass
Inorganic Anions	500-mL wide-mouth HDPE	500-mL wide-mouth HDPE
Polybrominated Diphenyl Ether Congeners	500-mL wide-mouth glass	1000-mL wide-mouth glass
Antibiotics and Drugs	500-mL wide-mouth glass	1000-mL wide-mouth glass
Steroids and Hormones	500-mL wide-mouth glass	1000-mL wide-mouth glass
Archive Samples - for use in the event of breakage, lab accident, or for future EPA studies	2 500-mL wide-mouth HDPE and 4 500-mL wide-mouth glass	2 500-mL wide-mouth HDPE and 4 1000-mL wide-mouth glass
Total Containers per Sampling Point	12	12

3.4. Representative Samples

The TNSSS was designed to collect sewage sludge samples that were representative of various types of sewage sludge. For bulk sewage sludge, collecting representative samples presented a challenge at some facilities. For example, at one facility that composted its final sewage sludge, samples were collected from one of the long piles of sewage sludge mixed with woods chips. The piles were upwards of 50 feet (15.24 meters) long and over 6 feet (1.83 meters) high, with sides sloping up at roughly a 45 degree angle. Samples were collected from the oldest sections of the rows at the facility to represent the length of the typical composting period at the facility, which ranges from one to six months, depending on the season.

Samples of sewage sludge were taken by digging into the side of the compost pile at roughly six points along its length, on both sides of the pile, a foot or more off the ground to avoid materials in contact with the concrete substrate. Large chunks of wood or small branches were removed from the pile before mixing the bulk sample. Once the bulk sample was well mixed, the samples were transferred to the final sample containers. This procedure was repeated twice: 1) for samples for the organic parameters, using stainless steel equipment and glass containers, and 2) for the metals and anions, using plastic equipment and containers.

At another facility which produced liquid sewage sludge, samples were collected from a catwalk atop a 1-million gallon storage tank. Sewage sludge was introduced into the tank by water cannon with a 4-inch diameter discharge nozzle. Plant personnel turned on the water cannon and throttled back the flow to a relative trickle and the sampler held each sample container in the edge of the stream until it was full. The containers were capped once they were full and wiped down before packing. Neither of these situations means that the samples were not representative or that the Agency cannot rely on the results obtained. It simply points out the complexities and challenges with sampling sewage sludge generated by the variety of treatment processes and management options available nationally.

3.5. Field Duplicates

The sampling plan called for collection of field duplicate samples at 10% of the facilities. A field duplicate sample is a second sample collected at the facility using similar procedures and equipment as the original sample for quality control purposes. The results of the field duplicate sample can be compared to the results of the original sample as a means of assessing the overall precision of the sampling and analysis processes.

Eight facilities were originally selected for collection of field duplicates. This number was ultimately reduced to six because two of the facilities at which field duplicates were to be collected were dropped from the survey and not replaced.

3.6. Storage and Shipment of Samples to Laboratories

The sample containers were packed for shipping using procedures described in the peer-reviewed sampling and analysis plan. The sampling personnel purchased ice near each facility, or the POTW provided ice, and packaged it in one-gallon self-sealing plastic bags. Each sample container was either encased in bubblewrap bag or layers of bubblewrap sheeting to prevent its movement during shipping. Samples were packed into sturdy plastic ice chests. All of the samples from a single site could be packed, with ice and bubblewrap, in one 48-quart ice chest, or two 28-quart ice chests, depending on availability.

When samples arrived at the sample repository, the staff inspected the ice chests for external damage or leakage (none occurred) and placed them in one of two walk-in freezer dedicated to EPA samples and maintained at -11°C. Freezing at -11°C reduces microbiological activity and the rates of any chemical reactions that might lead to changes in the sample.

To streamline the shipping logistics and manage both shipping and analytical costs, EPA shipped batches of 15 to 20 samples from the repository to the contract laboratories for analyses. Additional shipments were sent to the laboratories as more facilities were sampled. In all, six shipments were sent to the laboratory performing the analyses of metals, anions, and organics, with the last shipment being the two samples collected at the last facility. For the PBDEs, pharmaceuticals, and steroids and hormones analyses, more samples had been collected and stored at the repository by the time those analyses began. Ultimately,

three shipments were made to the laboratory performing the PBDE analyses and three shipments were made to the laboratory performing the pharmaceuticals, steroids, and hormones analyses.

4. Target List of Pollutants

Pollutants identified in Table II were included in the TNSSS because EPA had a sufficient data base (e.g., human health benchmark values, and information on fate and transport in the environment); 24 additional metals could be analyzed at little extra cost at the same time as the four metals (barium, beryllium, manganese, and silver); molybdenum because of the Agency's interest in determining the need for a revised numeric standard for it in land-applied biosolids; and other analytes because of their widespread incidence and use and emerging interests (see Table II).

Table II. Primary Target Analytes for the TNSSS, by Analyte Class

<i>Analyte Class</i>	<i>Analyte</i>	
Metals	Aluminum	Manganese
	Antimony	Mercury*
	Arsenic*	Molybdenum*
	Barium	Nickel*
	Beryllium	Phosphorus**
	Boron	Selenium*
	Cadmium*	Silver
	Calcium	Sodium
	Chromium*	Thallium
	Cobalt	Tin
	Copper*	Titanium
	Iron	Vanadium
	Lead*	Yttrium
	Magnesium	Zinc*
Polycyclic aromatic hydrocarbons (PAHs)	Benzo(a)pyrene	2-Methylnaphthalene
	Fluoranthene	Pyrene
Other semivolatiles organics	bis (2-Ethylhexyl) phthalate	4-Chloroaniline

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Table II. (Continued). Primary Target Analytes for the TNSSS, by Analyte Class

<i>Analyte Class</i>	<i>Analyte</i>	
Inorganic anions	Fluoride	Water-extractable phosphorus
	Nitrate	Nitrite
Polybrominated diphenyl ethers (PBDEs), including the tetra, hexa, penta, and deca congeners	2,4,4'-TrBDE (BDE-28)	2,2',3,4,4',5'-HxBDE (BDE-138)
	2,2',4,4'-TeBDE (BDE-47)	2,2',4,4',5,5'-HxBDE (BDE-153)
	2,3',4,4'-TeBDE (BDE-66)	2,2',4,4',5',6-HxBDE (BDE-154)
	2,2',3,4,4'-PeBDE (BDE-85)	2,2',3,4,4',5',6-HpBDE (BDE-183)
	2,2',4,4',5-PeBDE (BDE-99)	2,2',3,3',4,4',5,5',6,6'-DeBDE (BDE-209)
	2,2',4,4',6-PeBDE (BDE-100)	

The 9 pollutants in **bold** are those selected in the December 2003 Biennial Review (68 FR 75531). * Metals currently regulated at 40 CFR 503. ** Phosphorus appears with metals because it was analyzed along with the metals using a “metals method” (EPA Method 200.7). Based on recent studies of the effects of adding phosphorus as a soil amendment, the survey analyzed two forms of this important nutrient. Following acid digestion procedures, phosphorus was included among the metals, while a separate water extract of the sludge was analyzed for phosphorus, along with the other inorganic anions.

Among the other new and emerging contaminants of concern identified in the NRC report were various pharmaceuticals, steroids, and hormones for which several EPA organizations were developing methods at the time that the TNSSS was being planned. EPA included certain pharmaceuticals, steroids and hormones in the TNSSS for which analytical methods were developed. Given the time required to develop and test new methods, EPA proceeded with the sample collection effort for the TNSSS as described in Section 3, and stored samples for the analyses of these analytes of interest until such time as the new methods for these classes of compounds were more fully developed. The drugs, antibiotics, steroids, and hormones added to the TNSSS are shown in Table III.

Table III. Pharmaceuticals, Steroids, and Hormones Included in the TNSSS

<i>Analyte Class</i>	<i>Analyte</i>	
Antibiotics and their degradation products, disinfectants, and other antimicrobials	Anhydrochlortetracycline	Ofloxacin
	Anhydrotetracycline	Ormetoprim
	Azithromycin	Oxacillin
	Carbadox	Oxolinic acid
	Cefotaxime	Oxytetracycline
	Chlortetracycline	Penicillin G
	Ciprofloxacin	Penicillin V
	Clarithromycin	Roxithromycin
	Clinafloxacin	Sarafloxacin
	Cloxacillin	Sulfachloropyridazine
	Demeclocycline	Sulfadiazine
	Doxycycline	Sulfadimethoxine
	Enrofloxacin	Sulfamerazine
	4-Epianhydrochlortetracycline	Sulfamethazine
	4-Epianhydrotetracycline	Sulfamethizole
	4-Epichlortetracycline	Sulfamethoxazole
	4-Epioxytetracycline	Sulfanilamide
	4-Epitetracycline	Sulfathiazole
	Erythromycin	Tetracycline
	Flumequine	Triclocarban
	Isochlortetracycline	Triclosan
	Lincomycin	Trimethoprim
Lomefloxacin	Tylosin	
Minocycline	Virginiamycin	
Norfloxacin		

Continued on next page.

Table III. (Continued). Pharmaceuticals, Steroids, and Hormones Included in the TNSSS

<i>Analyte Class</i>	<i>Analyte</i>	
Other drugs and degradation products	1,7-Dimethylxanthine	Diphenhydramine
	Acetaminophen	Fluoxetine
	Albuterol	Gemfibrozil
	Caffeine	Ibuprofen
	Carbamazepine	Metformin
	Cimetidine	Miconazole
	Codeine	Naproxen
	Cotinine	Norgestimate
	Dehydronifedipine	Ranitidine
	Digoxigenin	Thiabendazole
	Digoxin	Warfarin
	Diltiazem	
Steroids	Campesterol	Epi-coprostanol
	Cholestanol	Ergosterol
	Cholesterol	β -Sitosterol
	Coprostanol	β -Stigmastanol
	Desmosterol	Stigmasterol
Hormones	Androstenedione	Estriol
	Androsterone	Estrone
	17 α -Dihydroequilin	17 α -Ethinyl estradiol
	Equilenin	Norethindrone
	Equilin	Norgestrel
	17 α -Estradiol	Progesterone
	17 β -Estradiol	Testosterone
	β -Estradiol-3-benzoate	

4.1. Analytical Techniques

Table IV presents the analytical techniques applied to samples in the TNSSS. The target reporting limits in Table IV are based on a consensus of what might be achievable in a sewage sludge sample.

Table IV. Analytical Methods or Techniques

<i>Analyte Class</i>	<i>Method or Technique</i>	<i>Target Reporting Limit (dry weight)</i>
28 Metals, including mercury	ICP/AES, ICP/MS, and CVAA (EPA Methods 200.7, 200.8, and 245.1)	3 to 4 mg/kg
4 Polycyclic aromatic hydrocarbons (PAHs) and 2 semivolatiles (as one analytical fraction)	GC/MS, with selected ion monitoring (SIM), after solvent extraction and gel permeation chromatography (GPC) cleanup (EPA SW-846 Method 8270C)	100 to 300 µg/kg
4 Inorganic anions, including water-extractable phosphorus (WEP)	EPA Methods 340.2, 353.2, and 365.3, after leaching of the solid sample with reagent water with a study-specific protocol	2 to 8 mg/kg
11 PBDE Congeners*	High resolution GC/MS, draft EPA Method 1614	5 to 200 ng/kg
72 Pharmaceuticals	High performance liquid chromatography (HPLC) with tandem MS/MS detection, using an early draft of EPA Method 1694	Not specified
25 Steroids and hormones	High resolution GC/MS, using an early draft of EPA Method 1698†	Not specified

* The list of target PBDE analytes was limited to the following 11 PBDE congeners: 28, 47, 66, 85, 99, 100, 138, 153, 154, 183, and 209, which include those identified in the method as being of potential environmental or public health significance. mg/kg = milligrams per kilogram. µg/kg = micrograms per kilogram. ng/kg = nanograms per kilogram.

As indicated, the survey used both well-established multi-laboratory validated EPA procedures as well as three analytical methods that were developed or updated for the survey. The two new methods are single-lab validated methods for pharmaceuticals (EPA Method 1694, (1)), and steroids and hormones (EPA Method 1698, (2)). The updated multi-lab validated method is for flame retardants (EPA Method 1614, (3)). These three methods have not yet been promulgated at 40 CFR Part 136 for compliance monitoring in CWA programs, including the analysis of sewage sludge.

From an analytical standpoint, sewage sludge is a challenging matrix. The concentrations of pollutants present in samples vary depending on the nature of the inputs to the treatment plant. In addition to the pollutants of interest, sewage sludge contains a number of other components that are potential interferences in the analyses of the pollutants of interest. These components include lipids and other naturally occurring materials, as well as materials that may be added to the sewage during processing (e.g., surfactants, ferric chloride, polymeric colloids, or lime). These components can manifest themselves as interferences at all stages of the analytical process, from sample preparation through the determinative analysis.

Another analytical challenge with a national survey of sewage sludge is that the various treatment process and disposal practices used nationwide lead to differences in the moisture content of the final sewage sludge sent for use or disposal. Some of the facilities from which samples were obtained in the TNSSS produce liquid final sewage sludge, while others produce solid sewage sludge. Among the sewage sludge that were pourable liquids, the percent solids (hereafter percent solids) content ranged from less than 1% to about 4%, across treatment plants. For the solids, the percent solids content ranged from <1% to 94.9%. These differences in the form (liquid vs. solid) of the sewage sludge and the range of moisture or solid contents have direct effects on the analyses of the samples. The differences also affect how the data for the survey can be interpreted.

EPA considered these effects on sensitivity and comparability when it planned the TNSSS. EPA minimized the potential sensitivity differences by instructing the laboratories to determine the percentage of solids (percent solids) of each sample first, and then use that information to select a portion of the sample for the analysis that contains the method-specified sample weight or volume on a dry-weight basis. In addition, even when the laboratories prepared liquid samples using procedures designed for aqueous samples (e.g., liquid-liquid extraction with an organic solvent), they were instructed to report the results in weight/weight units (e.g., ng/kg, $\mu\text{g}/\text{kg}$, or mg/kg) appropriate for the class of analyte, adjusted for the moisture content of the sample (e.g., 100% dry sample).

4.2. General Review Procedures

EPA assessed the results for all of the samples analyzed during the survey using well-established quality control and data review procedures described in modest detail in the TNSSS Sampling and Analysis Technical Report (5). EPA employs standardized quality control (QC) measures described in the EPA analytical methods used for the survey. The analysis involved in the TNSSS was complex and a number of analytical challenges were faced. Biosolids is one of the most challenging environmental matrices known due to the high solids content and matrix interferences present. When conducting analyses in sewage sludge matrices it is expected that some results will have to be qualified to accurately reflect the uncertainty of the values.

To ensure that the data met the objectives of the survey, a multi-stage review process was used and designed to identify and correct data deficiencies as early as possible and maximize the amount of usable data generated:

1. EPA performed a data completeness check. Specifically, EPA evaluated elements in the laboratory submission to verify that results for specified samples were provided, that data were reported in the correct format, and that relevant information, such as preparation and analysis logs, was included in the data package. EPA initiated corrective action procedures to resolve any deficiencies identified.
2. The data review process focused on an instrument performance check. EPA verified that calibrations, calibration verifications, standards, and calibration blanks were analyzed at the appropriate frequency and

- met method or survey performance specifications. Corrective action procedures were initiated to resolve any deficiencies identified.
3. The data review process then focused on a laboratory performance check. EPA verified that the laboratory correctly performed the required analytical procedures and was able to demonstrate a high level of precision and accuracy. During this stage, EPA evaluated quality control (QC) elements such as the ongoing precision and recovery (OPR) tests, method blanks, and other QC operations. Again, corrective action procedures were initiated to resolve any deficiencies identified.
 4. EPA examined method/matrix performance data to discern whether any QC failures resulted from laboratory performance or difficulties with the method or sample matrix. As with previous steps, corrective action procedures were initiated with the laboratory to resolve any deficiencies identified.

The objective of the data review process was to document the quality of all of the data in the TNSSS and identify any limitations that might affect their end use. The EPA database contains data qualifiers applied to results from the TNSSS, individually, and by analyte class.

4.3. Overview of Statistical Methodology

Each POTW in the sampling frame had a nonzero probability of being selected for the sample. However, as a result of the stratified sampling design, some POTWs had a different probability of being selected than others. Therefore, EPA assigned a *survey weight* to each POTW contributing a biosolids sample to the survey. The survey weight corresponds to the total number of POTWs in the sampling frame that the selected POTW represents. The sum of all survey weights equals the total number of POTWs in the sampling frame. By incorporating survey weights in the statistical analysis, EPA obtained estimates that represented the entire target population.

As a first step in assigning survey weights, EPA assigned an initial “base weight” to each stratum (i.e., flow group). Because each POTW within a stratum had an equal probability of being selected for the sample, each selected POTW in a stratum received the same base weight. Because stratum and sample sizes differed among the strata, different strata had different base weights. Once all field sampling was completed, EPA calculated a final set of survey weights. This involved adjusting the base survey weights to account for deviation between EPA’s original and final sample of POTWs.

As is relatively common in sampling, EPA’s sample contained some facilities that did not fall within the survey’s initial definition of the target population. As a result, EPA replaced some facilities with others, which required an adjustment to the base survey weights. The replacement of five POTWs with other facilities had no effect on the final survey weights for the three strata. In each incidence that a replacement occurred, EPA determined that the biosolids sampled by the replacement POTW were representative of the biosolids generated by the POTW that it replaced. Thus, the replacement had no net impact on the sample size.

To each replacement POTW, EPA assigned the survey weight associated with the stratum for the POTW that it replaced.

Table V provides the final set of survey weights for each stratum. Within a given stratum, EPA assigned the final survey weight to each POTW that contributed one or more biosolids samples to the survey. The Targeted National Sewage Sludge Survey, Statistical Analysis Report (6) includes additional details on statistics used throughout the survey.

4.4. In-Depth Statistical Analysis for Select Analytes

EPA applied an in-depth statistical analysis to concentration data for 34 analytes listed in Table VI. The primary objective of the in-depth statistical analysis was to generate national estimates of the mean, standard deviation, and selected percentiles of analyte concentrations (i.e., 50th, 90th, 95th, 98th, 99th percentiles). The characterization of specific percentiles is useful for EPA's subsequent evaluation of exposure and risk.

Table V. Final Set of Survey Weights

<i>Stratum</i>	<i>Stratum Size</i>	<i>Original Sample Size</i>	<i>Base Weight</i>	<i>Final Sample Size</i>	<i>Final Weight^b</i>
> 100 MGD	51	8	51/8 = 6.375	8 ^a	51/8 = 6.375
10 to 100 MGD	543	12	543/12 = 45.25	12	543/12 = 45.25
1 to 10 MGD	2,743	60	2,743/60 = 45.7167	54	2,743/54 = 50.80

^a One of the eight POTWs performed final treatment of the partially-treated biosolids of a facility originally selected from the "> 100 MGD" stratum. Thus, this replacement facility was assigned the final weight for the ">100 MGD" stratum. ^b Assigned to each POTW within the final sample. The final weight, rather than the base weight, is utilized in all statistical analyses. For silver, the final weight for the "10 to 100 MGD" stratum was 543/11 = 49.36.

Table VI.

Table VI. Analytes Considered for In-Depth Statistical Analysis		
Metals	Barium Beryllium Manganese	Molybdenum Silver
Organics	4-Chloroaniline Fluoranthene	Pyrene
Inorganic nutrients	Nitrate/Nitrite	
PBDEs	BDE-47 (2,2',4,4'- tetrabromodiphenyl) BDE-99 (2,2',4,4',5- pentabromodiphenyl)	BDE-153 (2,2',4,4',5,5'-hexabromodiphenyl) BDE-209 (decabromodiphenyl)
Pharmaceuticals, antibiotics, disinfectants, and other antimicrobials	4-Epitetracycline (ETC) Azithromycin Carbamazepine Cimetidine Ciprofloxacin Diphenhydramine Doxycycline	Erythromycin-Total Fluoxetine Miconazole Ofloxacin Tetracycline (TC) Triclocarban Triclosan
Steroids and Hormones	Beta Stigmasterol Campesterol Cholestanol	Cholesterol Coprostanol Epicoprostanol Stigmasterol

5. Summary Results

Table VII provides a summary of the results for all 84 samples in the first phase (i.e., all analytes excluding pharmaceuticals, steroids, and hormones) of the survey, listing the number of samples in which each analyte was reported, along with the minimum and maximum concentrations. Tables VII and IX provide the results for the second phase for pharmaceuticals and steroids and hormones, respectively. The percent solids in the various sewage sludge samples range from 0.43 to 93.5 (Table VII) and 0.14 to 94.9 (Tables VIII and IX), and all sample results are reported on a dry-weight basis.

The units for pollutants vary with the class of analyte, as shown in Tables VII, VIII, and IX. The results include six field duplicate samples and four POTWs that generated more than one type of sewage sludge. The minimum concentration is the lowest value reported as present in any sample. EPA did not report a minimum or maximum value for those analytes that were not detected. That situation only occurred for some of the pharmaceuticals, steroids and hormones, and EPA used "NA" to indicate that the minimum and maximum values were "not applicable."

Table VII. Summary of Results for Metals, Anions, Organics, and PBDEs

Class	Analyte	Units	# Detects ¹	Observed Dry-weight Concentration	
				Minimum	Maximum
Solids	Percent Solids	%	84	0.43	93.5
Anions / Inorganic Nutrients	Fluoride	mg/kg	84	7.6	234
	Nitrate/Nitrite		84	1.6	6,120
	Water-extractable phosphorus		84	11.0	9,550
	Water-extractable phosphorus ratio (WEP/P)	unit-less	84	0.00065	0.33920
Metals	Aluminum	mg/kg	84	1400	57,300
	Antimony		72	0.45	26.6
	Arsenic*		84	1.18	49.2
	Barium		84	75.1	3,460
	Beryllium		83	0.04	2.3
	Boron		80	5.70	204.0
	Cadmium*		84	0.21	11.8
	Calcium		84	9,480	311,000
	Chromium*		84	6.74	1160
	Cobalt		84	0.87	290
	Copper*		84	115	2,580
	Iron		84	1,575	299,000
	Lead*		84	5.81	450
	Magnesium		84	696	18,400
	Manganese		84	34.8	14,900
	Mercury*		84	0.17	8.3
	Molybdenum*		84	2.51	132
	Nickel		84	7.44	526
	Phosphorus**		84	2,620	118,000
	Selenium*		84	1.10	24.7
Silver	84	1.94	856		
Sodium	84	154	26,600		
Thallium	80	0.02	1.7		

Continued on next page.

Table VII. (Continued). Summary of Results for Metals, Anions, Organics, and PBDEs

Class	Analyte	Units	# Detects ¹	Observed Dry-weight Concentration	
				Minimum	Maximum
	Tin		78	7.50	522
	Titanium		83	18.50	7,020
	Vanadium		84	2.04	617
	Yttrium		84	0.70	26.3
	Zinc*		84	216	8,550
Organics (PAHs and Semi-volatiles)	4-Chloroaniline	µg/kg	63	51	5,900
	2-Methylnaphthalene		39	10	4,600
	Fluoranthene		77	45	12,000
	Pyrene		72	44	14,000
	bis (2-Ethylhexyl) phthalate		84	657	310,000
	Benzo(a)pyrene		64	63	4,500
PBDEs	BDE-28	ng/kg	84	2,200	160,000
	BDE-47		84	73,000	5,000,000
	BDE-66		84	1,800	110,000
	BDE-85		84	3,200	150,000
	BDE-99		84	64,000	4,000,000
	BDE-100		84	13,000	1,100,000
	BDE-138		56	1,900	40,000
	BDE-153		84	9,100	410,000
	BDE-154		84	7,700	440,000
	BDE-183		84	2,100	120,000
BDE-209	83	150,000	17,000,000		

* Metals currently regulated at 40 CFR 503. ¹ The total number of samples analyzed was 84. ** Phosphorus appears with metals because it was analyzed along with the metals using a “metals method” (EPA Method 200.7). Based on recent studies of the effects of adding phosphorus as a soil amendment, the survey analyzed two forms of this important nutrient. Following acid digestion procedures, phosphorus was included among the metals, while a separate water extract of the sludge was analyzed for phosphorus, along with the other inorganic anions.

Table VIII. Summary of Results for Pharmaceuticals (Drugs, Antibiotics and Their Degradation Products, Disinfectants, and Other Antimicrobials)

<i>Analyte</i>	<i>Units</i>	# <i>Detects</i> ¹	<i>Observed Dry-weight Concentration</i>	
			<i>Minimum</i>	<i>Maximum</i>
Percent Solids	%	84	0.14	94.9
Acetaminophen	µg/kg	2	1,120	1,300
Albuterol		1	23.2	23.2
Anhydrochlortetracycline		1	125	125
Anhydrotetracycline		52	94.3	1,960
Azithromycin		80	10.2	6,530
Caffeine		39	65.1	1,110
Carbadox		0	NA	NA
Carbamazepine		80	8.74	6,030
Cefotaxime		0	NA	NA
Chlortetracycline		1	1,010	1,010
Cimetidine		74	7.59	9,780
Ciprofloxacin		84	74.5	47,500
Clarithromycin		45	8.68	617
Clinafloxacin		0	NA	NA
Cloxacillin		0	NA	NA
Codeine		20	9.59	328
Cotinine		39	11.4	690
Dehydronifedipine		19	3.48	24.6
Demeclocycline		3	96	200
Digoxigenin		0	NA	NA
Digoxin		0	NA	NA
1,7-Dimethylxanthine		4	1,130	9,580
Diltiazem		69	1.39	225
Diphenhydramine		84	36.7	5,730
Doxycycline		76	50.8	5,090
Enrofloxacin		14	12.1	66
4-Epianhydrochlortetracycline		0	NA	NA
4-Epianhydrotetracycline	31	126	2,160	

Continued on next page.

Table VIII. (Continued). Summary of Results for Pharmaceuticals (Drugs, Antibiotics and Their Degradation Products, Disinfectants, and Other Antimicrobials)

<i>Analyte</i>	<i>Units</i>	# <i>Detects</i> ¹	<i>Observed Dry-weight Concentration</i>	
			<i>Minimum</i>	<i>Maximum</i>
4-Epichlortetracycline		1	974	974
4-Epioxytetracycline		8	35.7	54.9
4-Epitetracycline		80	47.2	4,380
Erythromycin-total		77	3.1	180
Flumequine		0	NA	NA
Fluoxetine		79	12.4	3,130
Gemfibrozil		76	12.1	2,650
Ibuprofen		54	99.5	11,900
Isochlortetracycline		1	3,140	3,140
Lincomycin		3	13.9	33.4
Lomefloxacin		2	33.3	39.8
Metformin		6	550	1,160
Miconazole		80	14.2	9,210
Minocycline		32	351	8,650
Naproxen		44	20.9	1,020
Norfloxacin		29	99.3	1,290
Norgestimate		0	NA	NA
Ofloxacin	µg/kg	83	73.9	58,100
Ormetoprim		1	5.91	5.91
Oxacillin		0	NA	NA
Oxolinic Acid		1	39.4	39.4
Oxytetracycline		29	18.6	467
Penicillin G		0	NA	NA
Penicillin V		0	NA	NA
Ranitidine		46	3.83	2,250
Roxithromycin		3	14.3	22.8
Sarafloxacin		2	179	1,980
Sulfachloropyridazine		2	35.9	58.7

Continued on next page.

Table VIII. (Continued). Summary of Results for Pharmaceuticals (Drugs, Antibiotics and Their Degradation Products, Disinfectants, and Other Antimicrobials)

<i>Analyte</i>	<i>Units</i>	# <i>Detects</i> ¹	<i>Observed Dry-weight Concentration</i>	
			<i>Minimum</i>	<i>Maximum</i>
Sulfadiazine		3	22.9	140
Sulfadimethoxine		5	3.58	62.2
Sulfamerazine		1	5.61	5.61
Sulfamethazine		2	21.5	23.2
Sulfamethizole		0	NA	NA
Sulfamethoxazole		30	3.91	651
Sulfanilamide		8	191	15,600
Sulfathiazole		1	21	21
Tetracycline		81	38.3	5,270
Thiabendazole		58	8.42	239
Triclocarban		84	187	441,000
Triclosan		79	430	133,000
Trimethoprim		24	12.4	204
Tylosin		0	NA	NA
Virginiamycin		15	43.5	469
Warfarin		0	NA	NA

NA = Not applicable, because the analyte was not reported in any sample. ¹ The total number of samples analyzed was 84.

Table IX. Summary of Results for Steroids and Hormones

<i>Analyte</i>	<i>Units</i>	# <i>Detects</i> ¹	<i>Observed Dry-weight Concentration</i>	
			<i>Minimum</i>	<i>Maximum</i>
Percent Solids	%	84	0.14	94.9
Androstenedione	µg/kg	32	108	1,520
Androsterone		50	21.3	1,030
Campesterol		84	2,840	524,000
Cholestanol		84	3,860	4,590,000
Cholesterol		81	18,700	5,390,000

Continued on next page.

Table IX. (Continued). Summary of Results for Steroids and Hormones

<i>Analyte</i>	<i>Units</i>	# <i>Detects</i> ¹	<i>Observed Dry-weight Concentration</i>	
			<i>Minimum</i>	<i>Maximum</i>
Coprostanol		84	7,720	43,700,000
Desmosterol		58	2,730	94,400
17 α -Dihydroequilin		1	98.4	98.4
Epicoprostanol		83	868	6,030,000
Equilenin		1	60.6	60.6
Equilin		15	22.3	107
Ergosterol	$\mu\text{g}/\text{kg}$	53	4,530	91,900
17 α -Estradiol		5	16.1	48.8
17 β -Estradiol		11	22	355
β -Estradiol 3-benzoate		18	30.2	1850
17 α -Ethinyl-estradiol		0	NA	NA
Estriol		18	7.56	232
Estrone		60	26.7	965
Norethindrone		5	21	1,360
Norgestrel		4	43.8	1,300
Progesterone		19	143	1,290
β -Sitosterol		73	24,400	1,640,000
β -Stigmastanol		83	3,440	1,330,000
Stigmasterol		76	11,000	806,000
Testosterone		17	30.8	2,040

NA = Not applicable, because the analyte was not reported in any sample. ¹ The total number of samples analyzed was 84.

5.1. Summary Results for In-Depth Statistical Analyses

In addition to the results presented above in Tables VII, VIII, and IX, EPA conducted in-depth statistical analyses and obtained estimates of the mean, standard deviation, and selected percentiles (99th, 98th, 95th, 90th, and 50th percentiles) for each of the 34 analytes specified in Table VI. EPA selected the subset of 34 analytes for in-depth statistical analysis for the following reasons:

- Nine "target" analytes (i.e., barium, beryllium, manganese, silver, 4-chloroaniline, fluoranthene, pyrene, nitrate, and nitrite) from the 2003 Biennial Review were identified as needing additional evaluation

of potential risks using more up-to-date sludge concentration and occurrence data

- Molybdenum is being re-evaluated using updated information to determine the need for a revised numeric standard in land applied biosolids
- Four PBDEs (i.e., tetra, hexa, penta, and deca) have been detected in various environmental media and acceptable human health benchmarks exist that may be useful for any future risk assessment purposes
- 20 pharmaceuticals, including drugs, antibiotics, degradation products, disinfectants, and other antimicrobials, were detected at 90 percent or more of the POTWs in the target population.

The estimates are representative of the distribution of concentrations in biosolids for the entire target population (i.e., they represent “national” estimates). The characterization of specific percentiles is useful for EPA’s subsequent evaluation of exposure and risk. Table X summarizes the in-depth statistical estimates for each of the 34 analytes.

For all other analytes, Appendix B.3 of the Targeted National Sewage Sludge Survey Statistical Analysis Report (6) provides preliminary summaries and national estimates derived from the concentration data.

5.2. Comparison of Metals Results to Current Standards

EPA established the current standards for land application (40 CFR 503) as a ceiling (i.e., Ceiling Concentrations per Table I of Section 503.13) on the dry-weight concentrations for nine distinct metals. Table XI documents these nine metals and their land application ceiling standards, along with the maximum observed concentrations among samples collected in the TNSSS for these nine metals. The maximum concentrations are calculated by considering the individual sample results (presented under “un-weighted statistics”). The numbers of POTWs in the sample with data values exceeding the ceiling are reported.

As noted in Table XI, only three metals have maximum observed concentrations exceeding their respective land application ceiling concentrations: molybdenum, nickel, and zinc. The maximum observed concentration for all other metals in this table are well below their respective land application regulatory limits. If EPA were to apply the survey weights to these POTWs to obtain national estimates, it is estimated that less than three percent of POTWs in the survey’s target population might be expected to exceed the land application standards for any of these three metals. EPA notes that three percent is likely to be an overestimate, because the regulations apply only to land application, and many facilities use other methods of disposal. Of the POTWs observed exceeding these standards in the survey, one incinerated its treated biosolids on site, while the others sent their biosolids to landfills. Thus, results from this survey indicate that POTWs were generally complying with the existing land application standards for metals.

Table X. Nationally Representative Estimates of the Mean, Standard Deviation, and Selected Upper Percentiles of the Distribution of Concentrations for 34 Analytes in the TNSSS

Analyte	Observed Values		Estimates					Summary Statistics		
	Minimum	Maximum	Percentiles					Mean	Standard Deviation	Percent POTWs with Detected Conc
			99th	98th	95th	90th	50th			
Barium	77	2,117	2,230	1,848	1,396	1,088	452	572	443	100
Beryllium	0.04	2.34	1.81	1.45	1.04	0.77	0.27	0.38	0.37	98.5
Manganese	35	14,900	9,700	6,904	4,156	2,648	540	1,165	2,231	100
Molybdenum	2.51	86.4	68.7	55.6	40.5	30.6	11.4	15.3	13.8	100
Silver*	2	195	105	82	57	42	13	20	22	100
4-Chloroaniline	51	5,900	12,013	8,288	4,762	2,912	513	1,284	2,946	74.4
Fluoranthene	45	12,000	13,173	9,112	5,256	3,226	575	1,421	3,211	89.5
Pyrene	44	14,000	15,918	10,894	6,184	3,742	634	1,654	3,981	84.9
Nitrate/Nitrite**	2	6,120	6,120	2,750	960	463	14	219	828	100
BDE-47 (2,2',4,4'-tetrabromodiphenyl)	73,000	5,000,000	2,650,430	2,212,077	1,688,881	1,329,167	570,448	709,174	523,791	100
BDE-99 (2,2',4,4',5-pentabromodiphenyl)	64,000	4,000,000	2,696,928	2,248,181	1,713,370	1,346,295	574,559	716,362	533,447	100
BDE-153 (2,2',4,4',5,5'-hexabromodiphenyl)	9,100	410,000	265,395	220,098	166,454	129,902	54,117	68,334	52,685	100
BDE-209 (decabromodiphenyl)	150,000	17,000,000	15,836,435	11,645,502	7,360,103	4,898,034	1,162,523	2,181,237	3,462,942	98.5
4-Epitetracycline (ETC)	41	4,380	8,026	5,937	3,787	2,540	620	1,135	1,741	96.0
Azithromycin	8	5,205	8,717	5,811	3,172	1,853	278	831	2,342	96.0
Carbamazepine	9	6,030	1,234	856	497	306	55	135	298	96.0
Cimetidine*	4	8,330	19,128	10,975	4,789	2,294	171	1,332	10,314	89.8

Analyte	Observed Values		Estimates					Summary Statistics		
	Minimum	Maximum	Percentiles					Mean	Standard Deviation	Percent POTWs with Detected Conc
			99 th	98 th	95 th	90 th	50 th			
Ciprofloxacin	75	40,800	79,636	57,975	36,095	23,703	5,367	10,501	17,658	100
Diphenhydramine	37	5,730	5,255	4,021	2,696	1,891	541	871	1,101	100
Doxycycline	34	5,090	7,021	5,046	3,082	1,989	424	877	1,588	92.8
Erythromycin-Total	2	180	264	194	123	82	19	36	58	92.9
Fluoxetine*	10	3,130	1,555	1,178	778	539	147	245	329	96.0
Miconazole	7	9,210	16,931	10,083	4,652	2,341	207	1,239	7,311	95.8
Ofloxacin	25	58,100	85,562	57,929	32,363	19,304	3,113	8,573	21,998	98.5
Tetracycline (TC)	38	5,270	10,042	7,250	4,458	2,895	630	1,278	2,255	97.5
Triclocarban	187	441,000	276,708	205,043	131,079	88,120	21,677	39,433	59,924	100
Triclosan	334	133,000	197,288	124,176	62,217	33,693	3,862	16,097	65,135	92.4
Beta Stigmastanol	3,440	1,330,000	1,651,188	1,123,256	632,009	379,365	62,547	168,079	419,232	98.5
Campesterol	2,840	524,000	842,112	598,919	360,119	229,283	46,547	100,879	193,964	100
Cholesterol	3,860	4,590,000	7,874,368	5,071,045	2,629,149	1,467,636	187,244	680,046	2,374,369	100
Cholestanol	2,340	5,390,000	13,376,891	8,538,884	4,369,111	2,410,541	295,092	1,129,268	4,171,366	96.9
Coprostanol	7,720	43,700,000	57,794,254	35,060,035	16,626,022	8,574,467	827,108	4,366,714	22,636,715	100
Epicoesterol	868	6,030,000	25,579,800	13,441,281	5,143,938	2,193,143	108,028	1,702,708	26,783,520	98.5
Stigmasterol	455	568,500	4,606,900	2,646,615	1,157,099	555,217	41,513	321,199	2,464,383	90.1

* For these analytes, the calculations in this table excluded either one or two concentration values. For silver, one sample was considered a statistical outlier; for cimetidine, one sample did not meet the chemical quality assurance criteria; and for fluoxetine, two samples did not meet the chemical quality assurance criteria.
 ** Nitrate/nitrite estimates were generated using the non-parametric model. Estimates for all other analytes in this table were calculated using the lognormal model, which treated any non-detected outcomes as censored at the sample-specific detection limit.

Table XI. Comparison of Survey Maximums to Existing Regulatory Limits

<i>Pollutant</i>	<i>Dry-Weight Concentration in mg/kg</i>		<i># Sampled POTWs Over Ceiling</i>
	<i>Land application ceiling</i>	<i>Survey Maximum</i>	
Arsenic	75	49.2	0
Cadmium	85	11.8	0
Copper	4,300	2,580	0
Lead	840	450	0
Mercury	57	8.3	0
Molybdenum	75	86.4	1
Nickel	420	526	2
Selenium	100	24.7	0
Zinc	7,500	8,550	1
Maximum results that exceed the land application ceiling are shown in bold .			

5.3. Summary

For a variety of targeted chemicals, the primary goal of the TNSSS was to characterize mean concentration levels and selected percentiles of analytes in biosolids generated by the nation's POTWs (having flow rates of at least 1.0 MGD). EPA successfully collected 84 biosolids samples from its targeted sample size of 74 POTWs. The TNSSS database, available from EPA, contains concentration measurements for 145 different analytes. These analytes included three anions/inorganic nutrients, 28 metals, six organics (PAHs and semivolatiles), 11 PBDEs, 72 pharmaceuticals and 25 steroids and hormones. To summarize, EPA'S TNSSS found:

- The four anions were found in every sample. The analytical approach for the survey included determining the element phosphorus (P). The water-extractable phosphorus (WEP) determined using the leaching procedure of Vadas and Kleinman (7) is a useful predictor of the concentrations of phosphorus that might be available for runoff from land to which sewage sludge has been applied. The ratio of the two forms of phosphorus (WEP/P) is an indication of the proportion of the total phosphorus applied that may contribute to runoff. That ratio may be of interest to those states that regulate land application of sewage sludge.
- 27 metals were found in virtually every sample, with one metal (antimony) found in no less than 72 samples.
- Of the six semivolatile organics and polycyclic aromatic hydrocarbons, four were found in at least 72 samples, one was found in 63 samples, and one was found in 39 samples.

- Of the 72 pharmaceuticals, including drugs, antibiotics, degradation products, disinfectants, and other antimicrobials, three (i.e., ciprofloxacin, diphenhydramine, and triclocarban) were found in all 84 samples and nine were found in at least 80 of the samples. However, 15 pharmaceuticals were not found in any sample and 29 were found in fewer than three samples.
- Of the 25 steroids and hormones, three steroids (i.e., campesterol, cholestanol, and coprostanol) were found in all 84 samples and six steroids were found in at least 80 of the samples. One hormone (i.e., 17 α -ethynyl estradiol) was not found in any sample and five hormones were found in fewer than six samples.
- All of the flame retardants except one (BDE-138) were found in every sample or all but one sample.

It is not appropriate to speculate on the significance of the results until a proper evaluation (e.g., risk characterization) has been completed and reviewed for each analyte. EPA plans to evaluate the pollutants identified by the survey as being present in sewage sludge. Using the survey information, EPA has begun assessing pollutants that may warrant further consideration. The evaluations will depend on the availability of data needed to conduct exposure and hazard assessments for these pollutants. Some of the information generally needed to conduct exposure and hazard assessment includes:

- Toxicity data for human and ecological receptors (e.g., toxicity defined in terms of reference dose, reference concentrations, cancer slope factor, lethal dose, lethal concentration, or adverse effects, such as reproductive or developmental effects).
- Concentrations for which a pollutant is present in sewage sludge (e.g., data from this survey).
- Chemical and physical properties, including vapor pressure, solubility, and molecular weight, and half-life.
- Fate and transport data for pollutants that may be present in sewage sludge, including degradation rates in various media and data on the bioconcentration and biotransfer potential of the pollutant.

EPA expects to initiate evaluations of the TNSSS pollutants that may warrant further consideration. The evaluations will depend on the availability of data needed to conduct such evaluations.

The information in this chapter has been reviewed and approved for publication by the EPA's Office of Science and Technology. This chapter was prepared based on the support of Battelle and Computer Sciences Corporation, under the direction and review of the Office of Science and Technology. Additional details regarding EPA's Targeted National Sewage Sludge Survey may be found in the Targeted National Sewage Sludge Survey, Sampling and Analysis Report (5).

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Chapter 8

Pharmaceuticals and Personal Care Products in U.S. Biosolids

Kristin McClellan¹ and Rolf U. Halden^{*,1}

¹Center for Environmental Biotechnology, The Biodesign Institute at
Arizona State University, 1001 South McAllister Avenue, Tempe, AZ 85287

*Corresponding author e-mail: halden@asu.edu.

Since the 1988 U.S. ban on ocean dumping of sewage sludge, the majority of these materials are disposed of on land as biosolids. To provide fundamental data for risk assessment concerning the environment, crop plants, and humans, several studies have been conducted that aimed at quantifying the load of pharmaceuticals and personal care products (PPCPs) as well as other emerging pollutants of concern in biosolids. So far, two large studies exist that analyzed biosolids samples representing the whole U.S. Both sample sets were collected by the U.S. Environmental Protection Agency (EPA) and analyzed by the same contract laboratory that developed the EPA method 1694 for analysis of PPCPs in biosolids and other matrices. The samples were analyzed using liquid chromatography tandem mass spectrometry and quantified using isotope dilution as well as conventional use of internal and external standards. The present meta analysis scrutinizes the findings and approaches of the two studies and puts them in context to potential environmental risks that should be considered.

Both the EPA's Targeted National Sewage Sludge Survey (TNSSS) and the analysis of a comparable sample set collected in 2001 revealed concentrations of several antimicrobials and antibiotics in the mg kg⁻¹ dry weight range. Prevalent contaminants were triclocarban, triclosan, ciprofloxacin and ofloxacin, followed by a number of tetracycline antibiotics.

A comparison of the two datasets and study designs showed that combining a large number of individual samples to mega-composite samples is a suitable approach for identifying

prevalent contaminants and for obtaining representative mean concentrations. Whereas the use of mega composite samples can result in significant time and cost savings, this study design strategy tends to yield lower numbers of total analytes detected, lower detection frequencies for individual analytes and it limits the detection of spatial (geographical) patterns in analyte occurrence.

The findings of both nationwide studies provide a critical data basis for future risk assessment concerning the safety of biosolids application on agricultural and recreational land. Risks of primary concern identified in this work are the promotion of antibiotic resistance in the environment, adverse effects on soil microbial communities and plants, as well as the possibility of direct exposure of consumers to antibiotic residues contained in food crops grown on biosolids amended fields.

Introduction

Pharmaceuticals and personal care products (PPCPs) enter the wastewater stream through multiple pathways, such as ingestion and excretion of pharmaceuticals and their metabolites with urine and feces, direct disposal of unused medications, and washing off after dermal application. In many cases removal during wastewater treatment is incomplete, and trace amounts of these biologically active compounds are discharged to aquatic environments (1, 2). Even if significant removal is achieved during the treatment process, this does not always mean that the compound has been degraded during treatment (3, 4). Specifically, hydrophobic PPCPs have the potential to sorb to primary and secondary sludge and, therefore, can evade biological degradation due to their reduced bioavailability (5). The majority of sludge from wastewater treatment plants is further treated for land application and the resulting biosolids are used as soil amendment on agricultural land, in forests and at reclamation sites (6). The U.S. Environmental Protection Agency (EPA) estimates that approximately 0.1% of agricultural land is amended with biosolids annually. The remainder of sewage sludge mass produced is either disposed of in landfills or burned in municipal incinerators.

In response to the U.S. National Research Council's call for better characterization of residual contamination of biosolids, the EPA conducted the Targeted National Sewage Sludge Survey (TNSSS) in 2006/2007 to obtain national estimates of certain pollutants in biosolids. In addition to various organic and inorganic compounds, a suite of 72 PPCPs was analyzed for their occurrence in biosolids originating from across the U.S. In 2001, the U.S. EPA had conducted a similar survey focusing on dioxins and dibenzofurans in biosolids destined for land application. Later in 2008, when analytical methods for the analysis of PPCPs in biosolids became available (7), archived samples from the 2001 EPA biosolids survey were examined for PPCP contaminant levels (8). Thus,

today two recent U.S. nationwide datasets on PPCPs exist, one representing contaminant levels in biosolids originally collected in 2001 for analysis of dioxins and a second one, the TNSSS conducted specifically for PPCP determination. The sampling campaigns for both surveys were conducted by the EPA and the samples collected in 2001 and in 2006/2007 were analyzed by the same contract laboratory that developed EPA method 1694 for PPCP analysis in biosolids.

This article aims to compare the findings of the two independent studies in regards to concentrations of PPCPs found as well as the analysis approaches taken and the quality and informational value of the resulting data.

Secondary Data Analysis Strategy

Data Sources

Analytical results from the two large nationwide U.S. biosolids studies were obtained from the TNSSS website (<http://www.epa.gov/waterscience/biosolids/tnss-overview.html>) and from the peer-reviewed literature (8).

Origin of the Biosolids Analyzed in Prior Studies

In order to properly understand the commonalities and differences between the two studies compared here, it is important to consider the origin of the samples for which analytical data were obtained. Both the 2001 and the 2006/2007 sample sets were collected by the EPA according to established protocols (9).

In the spring of 2001, the EPA collected sewage sludge samples from 94 wastewater treatment plants in 32 states and the District of Columbia. Five of the 94 plants had two treatment systems, therefore, 2 samples were collected from these facilities. In addition 14 duplicate samples were collected for quality control. This resulted in 113 sewage sludge samples that were analyzed for dioxins and dibenzofurans and subsequently given to the National Biosolids Repository, currently maintained at Arizona State University, where they were stored at -20°C. In the spring of 2008, 110 of the samples were combined into five composite mega-samples, consisting of 21 to 24 randomly selected individual samples. These five composite samples were subsequently analyzed.

In 2006/2007, the EPA collected sewage sludge samples from 74 treatment plants in 35 states. In addition, 10 samples were collected for duplicate analysis or because the plant had more than one treatment system. Only treatment plants that processed more than 1 million gallons of wastewater per day (MGD) were considered for sampling. Together, this group of facilities treats approximately 94% of all wastewater in the U.S. All treatment plants sampled employed at least secondary treatment. Sewage sludge produced at these facilities was disposed of either through land application, incineration or surface disposal.

Overview of Analytical Strategies Utilized by Prior Studies

Both sample sets were analyzed by the same analytical laboratory, Axys Analytical Services (2045 Mills Road West, Sydney, British Columbia, Canada

V8L 3S8), according to the EPA method 1694 (7). A detailed description of this liquid chromatography tandem mass spectrometry method, a.k.a. EPA method 1694, can be found at www.epa.gov/ost/methods/method/files/1694.pdf.

For the purpose of compound detection, the 72 analytes were divided into four groups in EPA method 1694. All analytes were separated by liquid chromatography and detected by tandem mass spectrometry (MS/MS). For some compounds for which stable isotope-labeled analogs were available, concentrations were determined using the isotope dilution technique (7). For compounds where a labeled analog was unavailable, corresponding concentrations were determined using conventional calibration by employing internal and external standards.

Statistical Tools Employed and Literature Information Source

Data from the two large nationwide studies were extracted from the literature and entered into a Microsoft Excel data spreadsheet. Analytical results were compared using standard statistical analysis such as scatter plots and linear regressions using Microsoft Excel. Literature information for the risk assessment section was obtained from the peer-reviewed literature indexed in the Web of Science (ISI Thompson; <http://thomsonreuters.com/>).

Results and Discussion

Statistical Comparison of the 2001 Survey and EPA Targeted National Sewage Sludge Survey 2006/2007

Both surveys analyzed sewage sludge samples for a set of 72 PPCPs. The TNSSS conducted by the EPA relied on the analysis of individual samples. In contrast, the dataset on PPCP concentration present in sludge samples collected in 2001 was obtained by analyzing a small number of mega composite samples (8). In mega composites mixed from individual samples collected in 2001, 38 PPCPs were found in at least one of the mega composite samples analyzed, and a total of 26 analytes were detected in all five mega composite samples (8). In the TNSSS in which individual samples from discrete plants were analyzed, 69 PPCPs were detected in at least one sewage sludge sample. Three analytes, namely ciprofloxacin, diphenhydramine, and triclocarban, were found in each of the 84 samples examined. The observed differences in detection frequency can be linked to the very different experimental approaches of the two studies, i.e., the use of mega composite samples versus individual samples from discrete treatment plants. Despite these expected differences in detection frequency, a comparison of mean concentrations identified in both surveys shows that both studies produced similar findings (Figure 1), especially for analytes found in high concentrations, i.e., ciprofloxacin, ofloxacin, triclocarban, and triclosan.

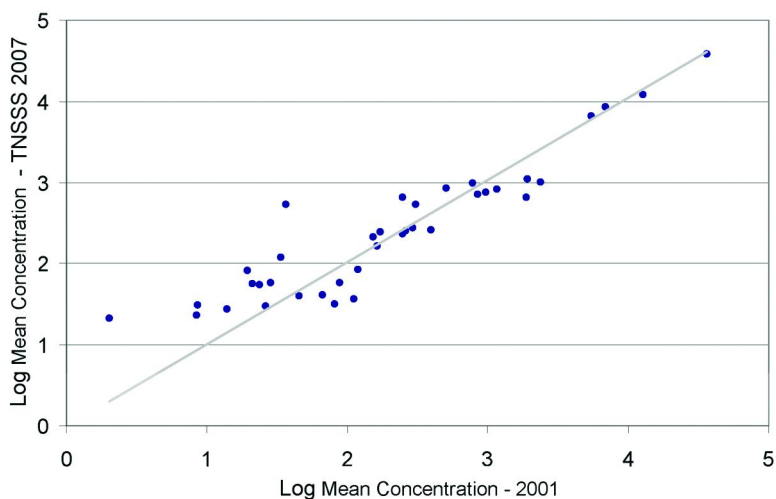


Figure 1. Comparison of mean concentrations for pharmaceuticals and personal care products in sewage sludges collected in 2001 and 2006/2007 by the U.S. Environmental Protection Agency. Excluded are mean concentrations below the detection limit; line denotes linear fit ($y = 1.012x$ with an R^2 value of 0.778). (see color insert)

A comparison of the range of concentrations detected reveals that the analysis of individual samples resulted in a much larger range of concentrations for all analytes detected (Figure 2). This comes to no surprise as the composite samples represent an average of all individual samples they are comprised of.

In addition, systematic differences in detection frequencies were revealed between the two datasets (Figure 3). These differences in detection frequency also originate from the different sampling approaches (individual vs. composite samples). Incorporation of uncontaminated materials into composite samples can lead to a dilution of concentrations of rare analytes. This may cause concentrations in composite samples to fall below the limit of detection, as evidenced by the lower number of analytes detected in any one of the composite samples when compared to the individual samples of the TNSSS. A similar but reverse effect was observed for analytes featuring a high frequency of occurrence in individual samples. For composite samples, detection frequencies of these types of analytes was 100%, whereas the analysis of individual samples from the TNSSS revealed detection frequencies of less than 100% for most analytes.

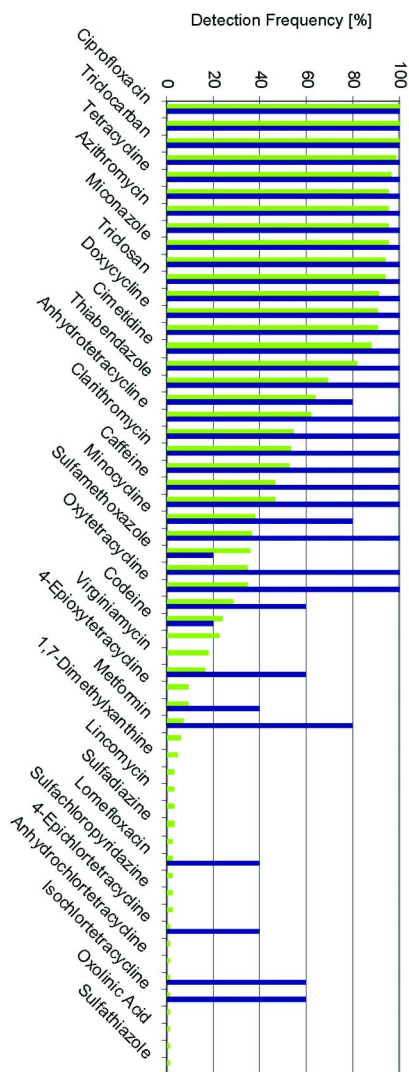


Figure 3. Comparison of detection frequencies of analytes featuring concentrations above the detection limit in mega composite samples collected in 2001 (dark blue; $n = 5$) and in individual samples analyzed as part of the EPA's TNSSS (light green; $n = 84$). (see color insert)

Comparison of the 2001 and 2006/2007 Datasets on PPCPs in Biosolids to Literature Values

Both sewage sludge surveys identified two biocides, triclocarban and triclosan, as the primary contaminants among the PPCPs analyzed. Triclocarban was found at mean concentrations of $36.1 \pm 8.0 \text{ mg kg}^{-1}$ dry weight (dw) (2001) and $39.4 \pm 60 \text{ mg kg}^{-1}$ dw (2006/2007), whereas triclosan was measured at 12.6

$\pm 3.8 \text{ mg kg}^{-1} \text{ dw}$ (2001) and $16.1 \pm 65 \text{ mg kg}^{-1} \text{ dw}$ (2006/2007). Both analytes have been found previously in sludge. Triclocarban has been found at lower concentrations ranging from 3.05 to 25.9 $\text{mg kg}^{-1} \text{ dw}$ in biosolids (10–12). In contrast, triclosan has been found in a wide range of concentrations from 0.09 $\text{mg kg}^{-1} \text{ dw}$ up to 55 $\text{mg kg}^{-1} \text{ dw}$ by different studies (3, 5, 11–15), which includes the range of values reported above. Furthermore, both surveys identified two fluoroquinolone antibiotics as prevalent contaminants. Ciprofloxacin was found at $6.8 \pm 2.3 \text{ mg kg}^{-1} \text{ dw}$ (2001) and $10.5 \pm 17.6 \text{ mg kg}^{-1} \text{ dw}$ (2006/2007), while ofloxacin was found at mean concentrations of $5.4 \pm 1.9 \text{ mg kg}^{-1} \text{ dw}$ (2001) and $8.6 \pm 22 \text{ mg kg}^{-1} \text{ dw}$ (2006/2007). These four contaminants were identified by both surveys as having the highest concentrations among all PPCPs analyzed. Other fluoroquinolone antibiotics identified in both surveys were norfloxacin, with mean concentrations between 0.1 and 1 $\text{mg kg}^{-1} \text{ dw}$, as well as enrofloxacin and lomefloxacin, both at mean concentrations of around 0.01 $\text{mg kg}^{-1} \text{ dw}$. In addition, the 2006/2007 TNSSS found sarafloxacin at a mean concentration of $0.3 \pm 0.7 \text{ mg kg}^{-1} \text{ dw}$, while it was not detected in any of the 2001 samples. The group of fluoroquinolones accounted for 12.6 $\text{mg kg}^{-1} \text{ dw}$ (2001) and 16 $\text{mg kg}^{-1} \text{ dw}$ (2006/2007) in sewage sludge samples. Ciprofloxacin has been found in concentrations from 22.6 to 46.4 $\text{mg kg}^{-1} \text{ dw}$ in biosolids from Ohio (16), which are even higher than the mean concentrations found in samples representing biosolids nationwide. Other fluoroquinolone antibiotics have so far not been reported in biosolids from the U.S.

Tetracyclines are another group of antibiotics identified by both surveys as PPCPs prevalent in biosolids. Both surveys found tetracycline and 4-epitetracycline at mean concentrations above 1 $\text{mg kg}^{-1} \text{ dw}$, and doxycycline, minocycline, anhydrotetracycline (in order of decreasing mean concentration) were found at concentrations between 0.1 and 1 $\text{mg kg}^{-1} \text{ dw}$ in both surveys. In addition, the 2006/2007 TNSSS showed mean concentrations of anhydrochlortetracycline and 4-epichlortetracycline in the same range, whereas these substances either were not detected in any of the samples from 2001 (anhydrochlortetracycline) or at concentrations below 0.1 $\text{mg kg}^{-1} \text{ dw}$ (4-epichlortetracycline). Furthermore, isochlortetracycline, oxytetracycline and chlortetracycline were detected in both surveys at mean concentrations between 0.01 and 0.1 $\text{mg kg}^{-1} \text{ dw}$. Lastly, 4-epioxytetracycline and demeclocycline were found in samples from 2006/2007 in the same concentration range, but not in any of the samples collected in 2001. Together, tetracycline antibiotics accounted for 6 $\text{mg kg}^{-1} \text{ dw}$ (2001) and 3 $\text{mg kg}^{-1} \text{ dw}$ (2006/2007). So far, only one peer-reviewed study (16) has reported tetracycline in biosolids from the U.S. Tetracycline was detected in only one biosolids sample from Ohio at a concentration of 15.7 $\text{mg kg}^{-1} \text{ dw}$, which is about one order of magnitude more than mean concentrations found for biosolids samples from the entire U.S.

Other antibiotics found by both surveys at mean concentrations above 0.1 $\text{mg kg}^{-1} \text{ dw}$ were azithromycin and sulfanilamide. Virginiamycin was only found by the 2006/2007 TNSSS at a mean concentration of $0.14 \pm 0.23 \text{ mg kg}^{-1} \text{ dw}$. Notably, only the EPA's TNSSS found 1,7-dimethylxanthine, a stimulant, at a mean concentration of $1.18 \pm 8.7 \text{ mg kg}^{-1} \text{ dw}$, while this substance was not detected in any of the samples collected in 2001. Concentrations for 1,7-dimethylxanthine

have not been reported elsewhere for U.S. biosolids, however, the compound has been found in surface water before (17). Both surveys also found a variety of over-the-counter and prescription drugs at mean concentrations between 0.1 and 1 mg kg⁻¹ dw, namely caffeine, carbamazepine, cimetidine, diphenhydramine, fluoxetine, gemfibrozil, ibuprofen, and miconazole. Most of these compounds have been found by other U.S. studies. Some PPCPs have previously been found at higher concentrations, namely caffeine which has been reported in one U.S. study at concentrations of around 5 mg kg⁻¹ dw (16), carbamazepine, which was found at concentrations of 4.7 to 12.8 mg kg⁻¹ dw by the same study and diphenhydramine, which was found at concentrations up to 22 mg kg⁻¹ dw (3, 15). Some compounds that were previously found in lower concentrations are cimetidine, which has been found in concentrations of up to 0.07 mg kg⁻¹ dw (3), fluoxetine of up to 0.06 mg kg⁻¹ dw (3) and miconazole, which has been found at concentrations of up to 0.46 mg kg⁻¹ dw (3). Gemfibrozil has been found in a wide range of concentrations from 0.06 to 3.4 mg kg⁻¹ dw (3, 16). Only ibuprofen has not been previously reported for U.S. biosolids, but has been detected in biosolids in Spain (18).

Potential Effects

Since the majority of sewage sludge is applied on land, these findings warrant further evaluation regarding the fate and effects of these contaminants in the environment. To predict the fate of biosolids contaminants in the environment, multiple physicochemical mechanisms have to be considered. Once applied on land, biosolids are typically mixed into top soil through tilling. Organic contaminants introduced into soils in biosolids can then become dissolved in soil porewater and leach out during rain events. They also may be removed in the sorbed state by soil erosion. The extent of compound migration depends on a compound's solubility in water, its sorption coefficient for soil and its tendency to form insoluble salts through complex formation. Some PPCPs have functional groups which are capable of complex formation with metallic cations, which can reduce their bioavailability.

More likely for hydrophobic chemicals is the process of sorption to soil particles. Once sorbed to soil, it cannot be easily be predicted to what extent chemicals desorb under varying environmental conditions, or to what extent they are still available to biodegradation or biologically active, in the case of pharmaceuticals.

Antibiotics and antimicrobials are designed to affect bacteria and other microorganisms. Therefore, even low concentrations in the environment might affect microbial communities. These effects can occur in the form of direct toxicity to soil bacteria, therefore influencing soil quality of agricultural land after application of biosolids. Evidence for this has been observed in a soil microbial community, where 10 µg kg⁻¹ tetracycline still had significant effects on the metabolic quotient 8 weeks after a one-time application (19). Besides direct toxicity, the potential of antibiotics to promote resistance among bacteria has to be considered. Although some details of the mechanisms leading to the spread of antibiotic resistance in the environment are still unclear, it is generally accepted that sub-therapeutic concentrations in environments with high bacterial density

(such as soil) constitute conditions for the development and spread of antibiotic resistance. However, it has been suggested that the input of already resistant bacteria into the environment may be more important for resistance occurring in the environment than the presence of antibiotics (20). Biosolids may also be a direct source of resistant bacteria. Several studies have shown that resistance against multiple groups of antibiotics have been found in bacteria contained in sewage sludge (21–23) and that these are not always being eliminated during conventional anaerobic sludge digestion (24).

Presence of pharmaceutical contamination in biosolids also constitutes a risk to crops grown on sludge-amended agricultural land. Plants may take up therapeutic agents from soil which can affect growth and development of the plants in question. Evidence for plant uptake as well as adverse effects has been found for several antibiotics. Uptake of sulfamethazine has been demonstrated for lettuce, potato and corn (25). Another study demonstrated uptake of chlortetracycline from a manure-soil mixture by corn, green onions and cabbage (26), while tylosin was not absorbed by these plants. A study focusing on veterinary medicines showed uptake of florfenicol, levamisole and trimethoprim by lettuce, while diazinon, enrofloxacin and trimethoprim were taken up by carrot roots (27). These studies show that uptake of PPCPs is dependent on the plant in question as well as the compound. Besides active uptake of dissolved PPCPs, hydrophobic compounds also have the potential to bioaccumulate in plant tissue that is in direct contact with contaminated soil. Bioaccumulation by plants has been demonstrated by several studies (28–31), which is of particular concern for root vegetables.

Toxic effects on plants also have been demonstrated. In one study 400 ppm tetracycline suppressed free-branching of poinsettia in laboratory studies (32). Also, 300 ppm sulfadimethoxine depressed growth of several plants (29, 33). It was further found that 50 ppm of enrofloxacin caused hormesis in several plant species, while 5000 ppm had toxic effects (34). However, adverse effects seem to be plant and compound specific. Another study found adverse effects on pinto beans by oxytetracycline and chlortetracycline, while the same antibiotics had no effect on corn, and even stimulated the growth of radish and wheat plants (35). In addition to the plant species and the therapeutic agent, the nature and extent to which adverse effects occur also depend on soil properties such as organic carbon, pH, mineral concentration, clay composition and temperature.

While the uptake and effects of antibiotics in crop plants are variable, these findings indicate potential risks of antibiotic contamination of food supplies. Associated health risks for consumers can include allergic or toxic reactions, chronic toxic effects due to low-level exposure, disruption of the human gut microbial flora and spread of resistant bacteria.

Conclusions

Both the EPA's TNSSS and the analysis of samples collected in 2001 revealed concentrations of several antimicrobials and antibiotics in the mg kg⁻¹ dw range.

Prevalent contaminants were triclocarban, triclosan, ciprofloxacin and ofloxacin, followed by a number of tetracycline antibiotics.

The statistical comparison of the results of the two studies showed that combining a large number of individual samples to mega composite samples is a suitable approach to identify prevalent contaminants and obtain representative mean concentrations. Shortcomings of this analysis approach potentially include unrepresentatively high frequency of non-detect values for rare contaminants, and reduced informational value concerning geographical distributions of contaminants. However, the use of mega composite samples can result in significant cost savings and it is accomplished much quicker than the analysis of many individual samples.

The two studies compared here provide a fundamental knowledge basis for further risk assessment of the practice of biosolids application on agricultural and recreational land. Areas of concern include the promotion of antibiotic resistance in the environment, adverse effects on soil microbial communities and plants, as well as direct exposure of consumers to drug residues in food crops grown on biosolids amended fields.

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Chapter 9

Removing Steroids from Contaminated Waters Using Radical Reactions

Stephen P. Mezyk,^{*,1} Edsel M. Abud,¹ Katy L. Swancutt,¹
Garrett McKay,¹ and Dionysios D. Dionysiou²

¹Department of Chemistry and Biochemistry, California State University at
Long Beach, 1250 Bellflower Blvd., Long Beach, CA 90840

²Department of Civil and Environmental Engineering,
University of Cincinnati, 765 Baldwin Hall, Cincinnati, OH
45221

*smezyk@csulb.edu

Among the most important pharmaceutical contaminants in wastewater are the estrogenic steroids. As quantitative removal of steroidal activity can be difficult, radical-based advanced oxidation/reduction processes (AO/RPs) are gaining interest for augmenting traditional water treatments. In support of the application of AO/RPs we have determined rate constants for the reactions of three representative steroids with the oxidizing hydroxyl ($\cdot\text{OH}$) and sulfate ($\text{SO}_4^{\cdot-}$) radicals. The fast $\cdot\text{OH}$ rate constants for ethinylestradiol and estradiol suggest a common mechanism of initial radical addition to the common phenol ring, whereas the slower progesterone value is more consistent with hydrogen atom abstraction. The rate constants for $\text{SO}_4^{\cdot-}$ reaction with estradiol and progesterone are identical, indicating a common initial reaction, but the faster value for ethinylestradiol suggests significant initial oxidative reaction at the triple bond.

Introduction

The adverse ecological impacts of endocrine-disrupting compounds, personal care products, antibiotics, and pesticides/herbicides in water supplies (1–3) and wastewater effluents are causing concern amongst regulatory groups and the public

around the world. Traditional water treatment relies primarily upon adsorptive and chemical-physical processes to remove or transform these unwanted organic contaminants. However, nearly a decade ago, the U.S. Geological Survey (USGS) study first documented the occurrence of hormonally active chemicals, such as reproductive steroids, at trace concentrations in multiple water sources originating from residential, industrial, and agricultural environments throughout the United States (4). The relatively high frequency in which steroids were detected in this survey implied that these chemicals persisted throughout conventional water treatment methods, although their absolute concentrations did not exceed established drinking-water guidelines.

The presence of synthetic and natural steroids such as estradiol, ethinylestradiol, and progesterone (Fig. 1) is attributed to human excretion as both natural and synthesized biological byproducts. However, human waste is not the only contributing factor in estrogen compound contamination; livestock have also been suggested as producers of similar steroid contaminants that pollute freshwater streams (5, 6). Concentrations of estrogenic compounds vary depending upon the season and location (7), but are nonetheless ever-present in the water cycle (8). Once in water, these steroid compounds interfere with normal endocrine function of various species of fish (9–13) and amphibians (14), as well as a variety of invertebrates (15).

Due to their negative environmental impact and the poorly-understood health concerns of human sensitivity to reproductive steroids, the removal of estrogens from water is necessary, and may warrant the use of special technologies. However, traditional treatment processes may not be sufficient, as quantitative removal of small (ng L^{-1}) levels of steroids may be complicated by the presence of much higher levels of other water constituents such as dissolved organic matter (DOM) and carbonate. Moreover, the presence of DOM may further complicate removal strategies as these hydrophobic compounds may be preferentially adsorbed onto them.

Therefore, the specific use of free radical species, such as the oxidizing hydroxyl radical ($\cdot\text{OH}$) or reducing electron (e_{aq}^-), to degrade trace contaminants following standard water treatment processes could be a viable approach. These processes are generally referred to as advanced oxidation/reduction processes (AO/RPs) (16–20). Radicals can be created using a variety of techniques including a combination of $\text{O}_3/\text{H}_2\text{O}_2$, $\text{O}_3/\text{UV-C}$, $\text{H}_2\text{O}_2/\text{UV-C}$, UV irradiation of titanium dioxide, sonolysis, or the irradiation of water via electron beams or γ rays.

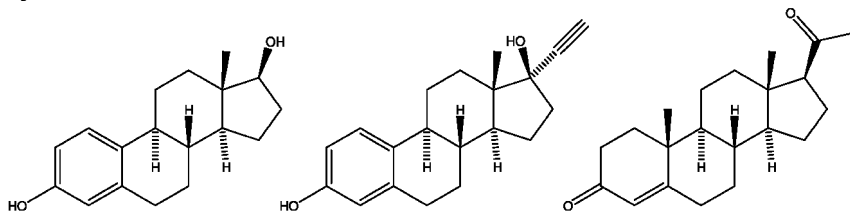


Figure 1. Structures (left to right) of estradiol, ethinylestradiol (EE2), and progesterone.

Several studies have investigated the efficiency of radical-based oxidation to destroy estrogenic steroids (17, 21–24), and the results of this effort have encouraged further investigation for the large-scale implementation of AO/RPs for this purpose. It has been demonstrated that simple oxidation of the phenolic moiety of the steroidal backbone can decrease estrogenic activity by at least 13% (22). However, optimal quantitative removal through the use of these processes requires a thorough understanding of the redox chemistry occurring between free radicals and the chemicals of concern. This can be accomplished if absolute kinetic rate constants are determined for redox reactions occurring in the AO/RP systems. Unfortunately, there has only been a single quantitative measurement (25) for the reaction of the hydroxyl radical with ethinylestradiol (EE2) utilizing ozone competition kinetics, with a reported rate constant of $9.8 \pm 1.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. This order-of-magnitude value is consistent with a subsequent attempt to predict these reaction rate constants, where Lee *et al.* estimated the overall rate oxidative constant for steroids to be in the range 10^9 – $10^{10} \text{ M}^{-1} \text{ s}^{-1}$, based on the rate constants of $\cdot\text{OH}$ reacting with phenols, single carbon-hydrogen bonds, acetylene, and carbon-hydrogen bonds of aliphatic rings of various chemicals (22).

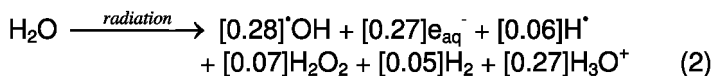


The direct measurement of absolute rate constants for estrogenic steroids (Reaction 1) reaction with oxidizing radicals is difficult due to their low aqueous solubility. In this work we describe our rate constant measurements for three representative estrogenic steroids (see Figure 1) with the oxidizing hydroxyl ($\cdot\text{OH}$) radical and sulfate ($\text{SO}_4^{\cdot-}$) radicals. These two oxidizing radicals were chosen for our study after taking into consideration that reducing AO/RP species, such as the hydrated electron ($e_{\text{aq}}^{\cdot-}$) and hydrogen atom ($\text{H}\cdot$), will predominantly react with dissolved oxygen at the real-world concentrations of these steroids.

Experimental

All chemicals used were purchased from Sigma-Aldrich Chemical Company at the highest purity available (hexafluoroacetone, >98%, steroids, >98%, KSCN, 99%, $\text{K}_2\text{S}_2\text{O}_8$, 99%). All were used as received.

There are many methods of producing radicals for AO/RP studies, but the use of an electron beam is optimal as it allows for the selective and quantitative production of $\cdot\text{OH}$, hydrated electron ($e_{\text{aq}}^{\cdot-}$), and hydrogen atom ($\text{H}\cdot$) from the direct decomposition of water (26):



The number preceding each species in Equation (2) is its absolute yield (G-value) in units of $\mu\text{mol J}^{-1}$. The secondary reaction between the produced radicals and an added solute molecule typically occurs in microseconds, far faster than for hydrogen peroxide reaction, and so the latter oxidation does not interfere with our radical kinetic measurements.

All rate constant data were collected using the Linear Accelerator facilities at the Radiation Laboratory, University of Notre Dame. This irradiation and transient absorption detection system has been described in detail previously (27). Absolute radical concentrations (dosimetry) were based on the transient absorption of $(\text{SCN})_2^-$ at 475 nm, using 10^{-2} M thiocyanate (KSCN) in N_2O -saturated solution at natural pH with $G_{\text{e}} = 5.2 \times 10^{-4} \text{ m}^2 \text{ J}^{-1}$ (28), performed daily.

Steroid solutions were made in high quality, Millipore Milli-Q, charcoal-filtered (TOC <13 ppb), deionized (>18.0 M Ω) water. To better mimic real-world AO/RP treatment conditions, these solutions were at natural water pH (measured as 6.8 – 7.2). However, under these conditions, the steroid solubility was not sufficient for its necessary concentration required for hydroxyl radical kinetic measurements (typically in the range 1-10 μM). To dissolve these steroids at this concentration, a small amount of co-solvent (hexafluoroacetone) was used. This perfluorinated species was chosen as its $\cdot\text{OH}$ radical reaction rate is very slow ($k < 10^6 \text{ M}^{-1} \text{ s}^{-1}$) and therefore it does not interfere with the steroid oxidation reaction. No solubility problem occurred for our $\text{SO}_4^{\cdot-}$ measurements, as they were conducted in 1.0 – 2.0 M *tert*-butanol, which was used as a hydroxyl radical and hydrogen atom scavenger.

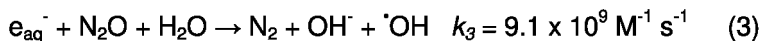
Solution flow rates were adjusted so that each pulse irradiation was performed on a fresh sample, and multiple traces (5-15) were averaged to produce a single kinetic trace. Typically, 3-5 ns pulses of 8 MeV electrons generating radical concentrations of 1-5 μM per pulse were used in these experiments. All of these experiments were conducted at ambient room temperature ($20 \pm 2^\circ\text{C}$).

Rate constant error limits reported here are the combination of experimental precision and compound purities.

Results and Discussion

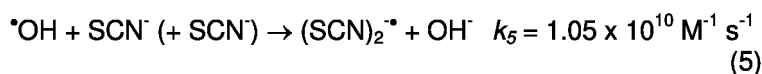
Hydroxyl Radical Measurements

To minimize interference from the reducing species also produced by the pulsed electron irradiation, in these studies the $\cdot\text{OH}$ radical was isolated by pre-sparging our aerated water with N_2O . At the relatively high ($\sim 25 \text{ mM}$) concentration of this gas, the following chemical conversions occur rapidly:



leading to quantitative conversion of the hydrated electron and some hydrogen atom to the $\cdot\text{OH}$ radical.

The relatively low steroid concentrations ($< 10 \mu\text{M}$) used meant that no direct absorbance change upon its oxidation by the hydroxyl radical reaction (Reaction 1) was possible. Instead, we determined these rate constants using SCN^- competition kinetics (26). The reaction of the hydroxyl radical with thiocyanate in N_2O -saturated solution is (26):



which occurs in competition with added steroid (such as EE2)



The transient $(\text{SCN})_2^{\cdot-}$ species has a strong absorption whose maximum is at 475 nm. Upon addition of EE2 to a standard KSCN solution, Reactions (5) and (6) occur together, which lowers the total transient $(\text{SCN})_2^{\cdot-}$ absorption intensity. The competition for the hydroxyl radicals follows the equation:

$$\frac{\text{Abs}^o(\text{SCN})_2^{\cdot-}}{\text{Abs}(\text{SCN})_2^{\cdot-}} = 1 + \frac{k_6 [\text{EE2}]}{k_5 [\text{SCN}^-]} \quad (7)$$

where $\text{Abs}^o(\text{SCN})_2^{\cdot-}$ is the peak transient absorption measured for only the SCN^- solution, and $\text{Abs}(\text{SCN})_2^{\cdot-}$ is the reduced absorbance of the $(\text{SCN})_2^{\cdot-}$ transient when EE2 is present. As this analysis depends upon the initial hydroxyl radical concentration being constant for all the measurements, low concentrations of SCN^- (typically 30–40 μM) were deliberately used in this study, which minimized the impact of intra-spur scavenging of radicals (29).

Typical data showing the reduction in $(\text{SCN})_2^{\cdot-}$ absorbance are given in Figure 2. By taking the peak intensities of this transient absorption, and transforming them according to Equation (7), the competition-kinetics plot shown in Figure 3 is obtained. This plot shows an excellent straight line, with an intercept of unity and a slope corresponding to the rate constant ratio (k_6/k_5). At this temperature $k_5 = 1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, allowing calculation of $k_6 = (1.52 \pm 0.23) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$. This value is slightly higher, but within combined experimental error, to the only previously determined rate constant of $9.8 \pm 1.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (25).

This competition kinetics approach was also used for the other two estrogenic steroids of interest in this work, and the measured rate constants are summarized in Table 1. Within experimental error, the values for ethinylestradiol and estradiol are the same, indicating a common reaction mechanism.

However, the measured value for progesterone is considerably slower, $k = (8.5 \pm 0.9) \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. This suggests that the primary $\cdot\text{OH}$ oxidation occurs at the common phenolic ring in EE2 and estradiol (see Scheme 1), consistent with the hydroxyl radical oxidation of phenol ($k = 0.7 - 1.8 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$) in aqueous solution (26, 30). For progesterone, which only has one C=C double bond, the slower rate constant instead suggests hydrogen atom abstraction from the saturated ring backbone.

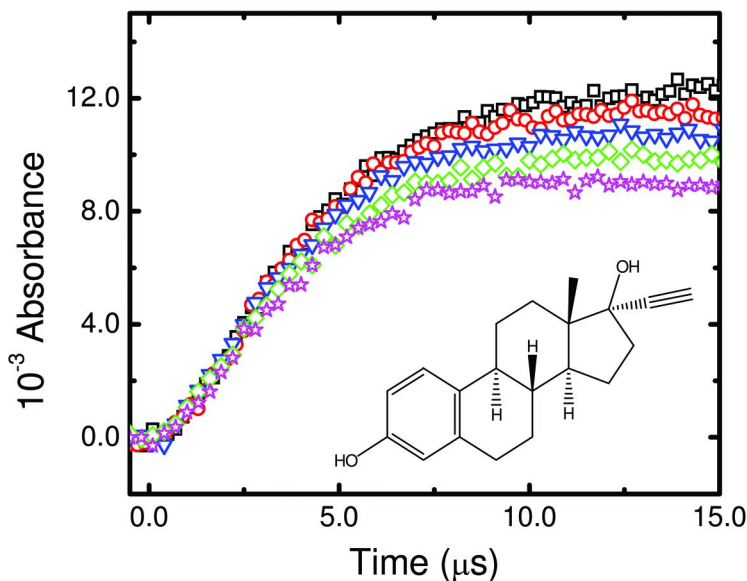


Figure 2. Reduction of transient $(SCN)_2^*$ intensity at 475 nm for $33.50 \mu M$ SCN^- in N_2O -saturated solution at natural pH and $22.7^\circ C$ with zero (\square), 1.83 (\circ), 3.80 (∇), 5.97 (\diamond) and 10.04 (\star) μM added EE2.

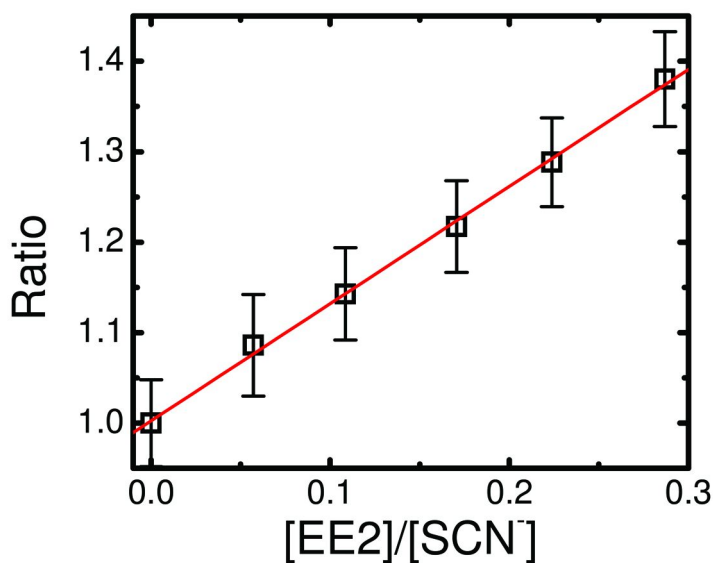
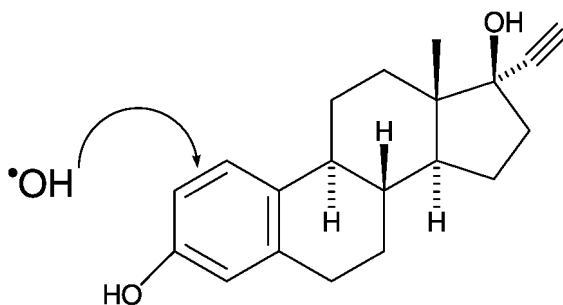


Figure 3. Transformed competition-kinetics plot for EE2. The weighted fit slope of this line corresponds to the ratio (k_6/k_5) , which gives $k_6 = (1.52 \pm 0.23) \times 10^{10} M^{-1} s^{-1}$ ($R^2 = 0.998$).

Table 1. Summary of determined rate constants for $\cdot\text{OH}$ and $\text{SO}_4\cdot^-$ oxidation of estrogenic steroids in aqueous solution

<i>Estrogen</i>	$k_{\cdot\text{OH}}$	$k_{\text{SO}_4\cdot^-}$
	$M^{-1} s^{-1}$	$M^{-1} s^{-1}$
Ethinylestradiol	$(1.52 \pm 0.23) \times 10^{10}$	$(3.01 \pm 0.28) \times 10^9$
Estradiol	$(1.15 \pm 0.28) \times 10^{10}$	$(1.21 \pm 0.16) \times 10^9$
Progesterone	$(8.5 \pm 0.9) \times 10^8$	$(1.19 \pm 0.16) \times 10^9$



Scheme 1. Suggested initial mechanism of hydroxyl radical oxidation of EE2 (and also estradiol).

Sulfate Radical Reactions

Under real-world treatment conditions, the reactions of oxidizing radicals will be favored due to the relatively easy reduction of dissolved oxygen (concentration $\sim 250 \mu\text{M}$). However, one possibility is to deliberately add chemicals that can convert these reducing radicals to oxidizing ones. While saturation by $\text{N}_2\text{O}_{(\text{g})}$ is not feasible at large scale, adding persulfate ($\text{S}_2\text{O}_8^{2-}$) would allow the conversion of produced hydrated electrons to oxidizing sulfate radicals:



This allows the oxidation of estrogenic steroids (for example EE2) to occur, through



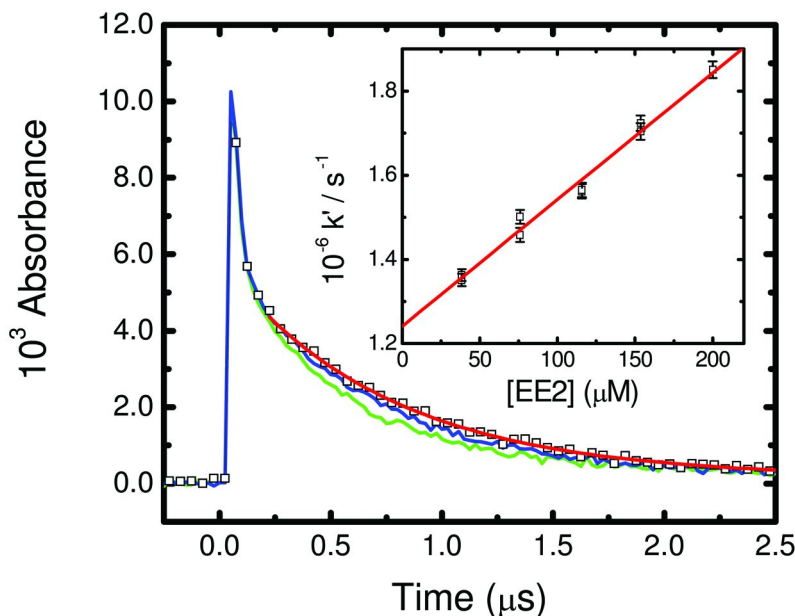
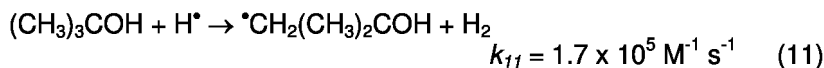
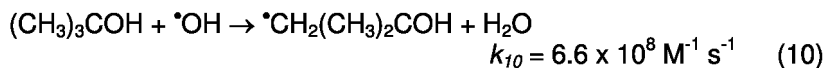
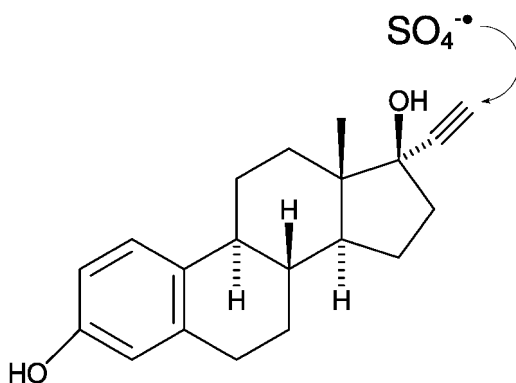


Figure 4. Decay of $SO_4^{\bullet-}$ radical at 450 nm for 38.3 (\square), 115.8 (intermediate solid line) and 200.0 μM (lowest solid line) added EE2. Solid line through data points corresponds to fitted first-order kinetics. Inset: Second order plot of first-order fitted values plotted against EE2 concentration. Solid line corresponds to second order rate constant, $k_9 = (3.01 \pm 0.28) \times 10^9 M^{-1} s^{-1}$.

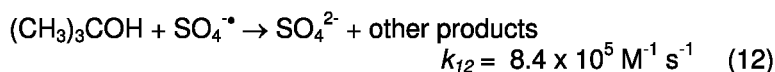
These kinetic measurements require the removal of hydroxyl radicals and hydrogen atoms in order to isolate the hydrated electron-persulfate reaction. Therefore, these experiments were conducted using a constant high concentration of *tert*-butanol, $(CH_3)_3COH$, as a co-solvent (1.0-2.0 M), which immediately scavenges the radiolytically produced hydroxyl radicals and hydrogen atoms (see Equations 10 and 11) to produce the relatively inert $\bullet CH_2(CH_3)_2COH$ alcohol radical:





Scheme 2. Suggested initial mechanism of sulfate radical oxidation of EE2.

The isolated hydrated electron will quantitatively react with added persulfate (in our experiments 5.0 mM) to give the oxidizing sulfate radical. This radical will slowly react with the added *tert*-butanol,



but a significant fraction will also react with added steroid. Under these relatively high alcohol concentrations, we were able to dissolve up to 200 μM of each of these steroids, allowing direct determination of the reaction kinetics.

The sulfate radical has a broad absorption spectrum, with a maximum near 450 nm. The kinetics of the pure sulfate radical decay are shown in Figure 4. The initial spike corresponds to second-order $\text{SO}_4^{\bullet-}$ radical decay, but this is followed by a first-order component corresponding to this radical's reaction with added steroid. By fitting the first-order tail with simple exponential kinetics, and then plotting these fitted values against the EE2 concentration (Figure 4, Inset) a second-order rate constant of $k_{\sigma} = (3.01 \pm 0.28) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ is obtained.

The sulfate radical oxidation is considerably slower than that for the hydroxyl radical, indicating different initial reaction mechanisms for these two oxidizing species. Following the same methodology, sulfate radical reaction rate constants were obtained for the other two estrogenic steroids. All rate constants are again summarized in Table 1. For both progesterone and estradiol the $\text{SO}_4^{\bullet-}$ rate constant ($\sim 1.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) was much slower than for EE2. While these kinetic data do not allow the specific mechanism of oxidation for progesterone and estradiol to be determined, the significantly faster rate constant for EE2 suggests that significant $\text{SO}_4^{\bullet-}$ oxidation occurs at the triple bond in this molecule (see Scheme 2).

The data presented above provides fundamental information necessary to apply AO/RPs to treatment of aqueous waste streams containing estrogenic steroids. It appears that both hydroxyl radical and sulfate radical oxidation could result in, or help assist, the total removal of these chemicals.

The kinetic data obtained in this study provides the fundamental information necessary to estimate the efficiency of using AO/RPs to remove estrogenic steroids from real-world waters containing high levels of dissolved organic matter

(DOM) and other hydroxyl radical scavengers through standard kinetic relative rates analyses. For example, in aerated wastewater containing 3.5 ppm DOM (290 μM DOM assuming 12 g C per mole C, $k = 2.3 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ (31)), an assumed high level of 10 ppb ethinylestradiol (3.4 nM), and a pH of 8.0 and alkalinity of 100 mg/L (as CaCO_3 , giving $\sim 1.0 \text{ mM HCO}_3^-$, $k = 8.5 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ (26)), any hydrated electrons produced will be quantitatively scavenged by dissolved oxygen, and the hydroxyl radical reaction will be partitioned to DOM (88.63%), HCO_3^- (11.30%) and the steroid (0.07%). While some enhancement from sulfate radical reaction could also be achieved through high added persulfate levels, the low total efficiency of using these radicals to destroy this steroid under these conditions suggests that further optimization would be necessary.

Conclusions

Rate constants for the reactions of oxidizing hydroxyl and sulfate radicals have been determined for three estrogenic steroids in water. The relatively fast and equivalent values for $\cdot\text{OH}$ reaction with estradiol, $(1.15 \pm 0.28) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ and ethinylestradiol, $(1.52 \pm 0.23) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, suggest a common mechanism believed to be initial addition to the phenol ring in these compounds. The much slower value for progesterone is consistent with this oxidation occurring by hydrogen atom abstraction from the steroid backbone. For the $\text{SO}_4^{\cdot-}$ oxidation kinetics, the faster rate constant for ethinylestradiol $(3.01 \pm 0.28) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, relative to the values obtained for estradiol and progesterone, $k = 1.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, suggests significant sulfate radical reaction at the triple bond for the former. These kinetic and mechanistic data provide sufficient background information to calculate AO/RP efficiencies through standard competitive relative rates analyses for these radicals. The estimated efficiencies are low in real-waters, which suggests that additional AO/RP optimization would be required.

Acknowledgments

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Chapter 10

Transport of PPCPs and Veterinary Medicines from Agricultural Fields following Application of Biosolids or Manure

Edward Topp,^{*,1} Chris D. Metcalfe,² Alistair B. Boxall,³
and David R. Lapen⁴

¹Agriculture and Agri-Food Canada, 1391 Sandford Street, London ON,
N5V 4T3 Canada

²Worsfold Water Quality Centre, Trent University, Peterborough ON,
K9J 7B8 Canada

³EcoChemistry Team, University of York, Heslington, York, United Kingdom

⁴Agriculture and Agri-Food Canada, 960 Carling Ave., Ottawa ON,
K1A 0C6 Canada

*Ed.Topp@agr.gc.ca

Biosolids from municipal treatment plants, and manure from livestock or poultry production are frequently used as fertilizer in crop production. These materials can contain human or veterinary pharmaceuticals and organic microconstituents that can pose a threat to the environment or to human health should they be transported to surface water or groundwater. Here, we consider field experiments defining the significance and mechanisms of transport of pharmaceuticals and personal care products (PPCPs) and veterinary medicines into runoff or into tile water. An emphasis is placed on experimental and analytical challenges, key rate controlling factors governing the fate of selected PPCPs, and the toxicological significance of mass loads and maximum aqueous concentrations of the exported residues. Information is provided on better management practices that reduce the risk of exposure after field applications of biosolids and manure.

Introduction

Trace concentrations of various pharmaceuticals and personal care products (PPCPs) and veterinary medicines have been widely detected in surface and groundwater. The health risks to humans and wildlife exposed to these chemicals is of some scientific and regulatory concern (1). The potential sources of environmental exposure to PPCPs include effluents from sewage treatment plants, leakage from septic systems and landfills, discharges from pharmaceutical manufacturing facilities and hospitals, and transport from land that has been irrigated with wastewater or that has been fertilized with municipal biosolids, or sewage sludge (2). Veterinary medicines may enter the environment from the application of manures and slurry, from direct excretion by pasture animals, spillage from the treatment of animals for ectoparasites, or from treatments used in aquaculture (3). Biosolids and manures are a valued source of nutrients for crop growth, and these materials are commonly used as a fertilizer in many agricultural areas worldwide. In this context, our research has focussed on characterizing the risk of contamination of adjacent surface or shallow ground water with PPCPs by evaluating the concentrations and mass transfer of PPCPs from land following the application of municipal biosolids or animal manure. This chapter gives an overview of our experimental approaches, our results that characterize the transport of selected PPCPs via key exposure pathways, and our observations within the context of assessing and managing the risk of contamination of aquatic resources from the agricultural use of these fertilizers.

Considerations and Objectives for Field Experiments

Biosolids and manures are typically applied as a slurry (total solids content usually <3%), or as a solid or dewatered material (total solids content usually >20%). The characteristics of solid and slurry material differ in ways that strongly influence the potential for transport of PPCPs following their application to soil. Slurry amendments applied to unsaturated soil will rapidly move into soil pores, ensuring contact with soil constituents such as organic matter and soilborne microorganisms that may, respectively, augment sequestration and accelerate biodegradation. In contrast, PPCP mobilization from aggregate materials (dewatered or solid waste products) to the adjacent soil environment may be limited by restricted diffusion of PPCPs out of the solid aggregates, and delayed physical breakdown of aggregates as a result of aggregate size and consistency. Slurry amendments behave as liquids when applied, and therefore can entrain PPCPs within and over the soil at the time of application. We have evaluated and contrasted the transport of PPCPs carried in both solid and slurry material.

For both slurry and solid amendments, the mode of land application and product placement in or on the soil surface will strongly control transport, biodegradation, and sequestration potential. A key element of responsible land application therefore is maintaining the applied material and associated contaminants in the rooting zone away from groundwater, artificial drainage systems, and surface water sources. These practices will permit sorptive and degradative processes that sequester and dissipate residues, attenuating off-site

movement. This can be accomplished by using better management practices (BMPs) for land application that include judicious rates of application, tillage to incorporate material into the soil and disrupt continuous macropore networks, and application of material during time periods when transport potential is low. Thus, we have systematically investigated the impact of various BMP options on the transport potential of PPCPs.

Tile drainage systems (artificial subsurface drainage) are ubiquitous in many agricultural regions that require soil drainage to foster crop production. Tile drains represent a particularly efficient means of transporting agricultural pollutants in the tile/groundwater system off site to adjacent surface water systems. Solutes can be transported to tiles at high velocity through macropores following heavy rainfall or following heavy application rates of slurry. Under these circumstances there is little contact time with the soil matrix, reducing or eliminating sorptive and degradative processes that would otherwise retard and reduce mass fluxes. Thus, the objectives of some of our field experiments has been to evaluate the off-site movement of PPCPs via tile flow.

Overland flow from fertilized land, particularly if it occurs shortly after application is another high risk transport pathway. Such a scenario will arise when saturated sloping soils receive precipitation in excess of the infiltration rate. Thus we have undertaken a series of experiments using artificially applied precipitation to promote runoff drainage shortly after the application of a commercial rate of dewatered biosolids.

PPCPs may also leach through the soil profile down to groundwaters. For example, in a recent national reconnaissance study in the US (4), concentrations of pharmaceuticals and other organic wastewater contaminants were characterized at 47 groundwater sites. In this survey, among 65 analytes targeted, 35 were detected at least once and 81% of the sites were found to show detectable levels of PPCPs. The most commonly detected pharmaceutical was the sulfonamide antibiotic, sulfamethoxazole, which is a highly water soluble compound used in both human and veterinary medicine. Over the past ten years, we have performed a range of field and semi-field experiments to understand the movement of PPCPs from the soil surface down to groundwaters. The results of these studies are also being used to validate modelling approaches for predicting movement to groundwaters in the absence of experimental monitoring data.

One of the challenges associated with these types of studies is the analysis of PPCPs and veterinary medicines in environmental matrixes (i.e. water, soil, biosolids, manure). Unlike more persistent contaminants, such as PCBs and brominated flame retardants, PPCPs and veterinary medicines are typically water soluble and in many cases are present in ionic form in the environment. These chemical characteristics make it difficult to extract the target analytes from environmental matrixes without co-extracting many organic constituents along with the compounds of interest. These co-extractives introduce “matrix effects” that complicate the analytical approach. Since PPCPs and veterinary medicines are typically not very volatile, traditional analytical approaches, such as gas chromatographic separation and detection by mass spectrometry (i.e. GC-MS) cannot be applied, and instead, liquid chromatography with tandem mass spectrometry (i.e. LC-MS/MS) is the most common analytical approach. These

LC-MS/MS systems must be capable of detecting low part per billion or part per trillion concentrations of the analytes in the small samples of water (i.e. 50-200 mL) or solid matrixes (i.e. 0.5 – 2 g dry weight) that are typically collected for field experiments or in monitoring studies.

When extracting PPCPs and veterinary medicines from aqueous matrixes (e.g. runoff, tile drainage), the samples are typically filtered or centrifuged to remove suspended particulates before extraction using solid phase extraction cartridges. Therefore, any target analytes adsorbed to suspended particulates will be lost prior to extraction, leading to underestimates of the mass transport of these chemicals from agricultural fields. Our studies have shown that the total mass of highly water soluble compounds associated with particulate material is negligible (5). However, for less water soluble compounds, such as the active ingredients in antibacterial soaps (i.e. triclosan, triclocarban), our studies have shown that transport in the particulate fraction may contribute significantly to mass transfers from agricultural fields (6). Therefore, care must be taken to extract both particulate and aqueous fractions of aquatic matrixes when studying the distribution and transport of target compounds that are poorly water soluble.

Transport of PPCPs and Veterinary Drugs to Tile Drainage following Application of Biosolids and Manure

We have evaluated tile drain contamination by PPCPs derived from liquid municipal biosolids (LMB) applied to an agricultural field (7). The LMB was applied at a rate of 93,500 L ha⁻¹ using two contrasting land application methods: one that pre-tilled the soil prior to surface application (LMB_A), and the other that surface applied the material on non-tilled soil that was subsequently tilled after land application (LMB_{SS}). Here, the majority of PPCP mass lost to tile drains occurred at the time of land application as a result of rapid macropore flow of LMB to tile drains. The macropore effect was less strongly pronounced for the pre-tillage application method because tillage disrupts the macropores and augments soil porosity (i.e. increasing liquid storage potential). In fact, for PPCPs listed in Table 1, the ratios of mass lost to tile from LMB_A relative to LMB_{SS} (LMB_A:LMB_{SS}) were 0.14, 0.06, 1.17, 0.89, and 0.83 for ACE, ATN, CBZ, TCS, and SMX respectively. Peak concentrations for the selected PPCPs in tile discharge are shown in Table 1, but these peaks were highly transient in nature (i.e. minutes).

Taking a modeling approach, Larsbo et al. (9) demonstrated that for situations where flow velocities in the macropores will be high, such as might occur during land application of LMB, net solute residence time in the macropores may be short. Larsbo et al. (9) improved considerably the MACRO model for providing estimates of Predicted Environmental Concentrations (PECs) of PPCPs in tile drainage after LMB land application by considering non-equilibrium sorption in the macropores. Non-equilibrium sorption is likely to occur when solute residence time is too short for reactions to reach ‘equilibrium’ at the solution-solid interface; the latter being an intrinsic assumption of many models regarding reactive solutes. By considering non-equilibrium sorption in the macropores, predictions of PPCP

concentrations improved by between roughly 56 to 125% for selected compounds (9). Using this modeling approach, carbamazepine and triclosan mass losses to tile drains were predicted, as a percent of mass applied to field, to be ~2 and 6%, respectively; similar to what was observed in field experiments (7).

Unlike LMB, dewatered municipal biosolids (DMB) will not move significantly to depth in the soil at land application, unless mobilized by rainfall or irrigation. However, the nature of how the material is applied to the surface will govern PPCP persistence and mobilization. We studied the transport to tile drainage of PPCPs after application of DMB (8) using either subsurface injection (DMB_{DI}) or surface spreading with subsequent shallow incorporation (DMB_{SS}). After applications of DMB at a rate of 8 Mg dw ha⁻¹, peak concentrations of most PPCPs in tile water occurred well after land application (Table 1). For instance, maximum triclosan concentrations occurred ~110 days after land application (8). DMB applied to the soil can be considered in some ways as a delayed release fertilizer, whereby environmental processes that break down the biosolids are required to release biosolid constituents into the soil environment. Peak PPCP concentrations in tile water after DMB application were never higher than those observed for the LMB study (Table 1), but in both cases, the peak concentrations were brief, over a time span of minutes to hours. Hence, from studies performed here, pulses of PPCPs in tile drain effluent resulting from both LMB and DMB applications were highly transient. Nevertheless, some target compounds demonstrated persistence in the soil-groundwater environment after LMB and DMB application, as demonstrated by the detection of residues of naproxen, atenolol, triclosan and other PPCPs in the tile water several months after application (7, 8). Triclosan showed evidence of leaching from soil in a field that was monitored after 33 years of biosolids applications (10).

We conducted similar studies of the fate of veterinary medicines after application of pig manure slurry over two years at a tile-drained field site in the UK (11). The study investigated the fate of three antibiotics: oxytetracycline, tylosin and sulfachloropyridazine. Pig slurry, spiked with the study compounds at realistic concentrations, was applied to a field in arable production in two consecutive years and the concentrations of the target compounds in the soil and tile drain water were monitored over time. Both sulfachloropyridazine and oxytetracycline were detected in soil at concentrations up to 365 and 1691 μg kg⁻¹, respectively. The oxytetracycline was seen to persist in the soil from some time, but tylosin was not detected. Oxytetracycline and sulfachloropyridazine were transported to field drains, with peak concentrations observed in drainflow in the first year of the study at 613 and 36 μg L⁻¹ for sulfachloropyridazine and oxytetracycline, respectively. These findings could be explained by the persistence and sorption characteristics of the antibiotics, as oxytetracycline has a large sorption coefficient and sulfachloropyridazine has a small sorption coefficient, while tylosin is rapidly degraded in slurry. In the second year of the study, the soil was tilled prior to application of the slurry and concentrations observed in drainflow were an order of magnitude lower than seen in the first year of monitoring. These observations are in agreement with the no tillage vs tillage results described previously for human PPCPs applied in LMB and DMB (7, 8).

Table 1. Maximum concentrations (ng L⁻¹) of selected PPCPs measured in tile drainage from fields where there were applications of liquid municipal biosolids (LMB) (7) or dewatered municipal biosolids (DMB) (8) using various techniques

	<i>ACE</i>	<i>ATN</i>	<i>CBZ</i>	<i>TCS</i>	<i>TCC</i>	<i>SMX</i>
LMB _{SS}	440*	267*	1136	3676	ND	322*
LMB _A	432*	40*	213	296	ND	22*
DMB _{DI}	104	57	32	227	2	<LOQ
DMB _{SS}	233	101	49	235	3	<LOQ

ACE, acetaminophen; ATN, atenolol; CBZ, carbamazepine; TCS, triclosan; TCC, triclocarban; SMX, sulfamethoxazole; ND, not determined; <LOQ = Below limit of quantitation. * Compounds were spiked into LMB prior to application.

Transport of PPCPs in Surface Runoff following Application of Biosolids

In two field studies, we investigated the transport of PPCPs entrained in overland flow from a sloping agricultural field that had received biosolids as LMB (12) or as DMB (6). Following biosolids application, a rainfall simulator was used to drive overland flow. PPCPs were detected in runoff from land that received surface applied LMB, but not when the same slurry was injected below the soil surface (12). The highest concentrations of PPCPs were detected one day after application, and concentrations declined in subsequent runoff events with generally first order kinetics. Triclosan, gemfibrozil and carbamazepine were still detected in runoff 9 months after application, whereas the concentrations of sulfamethoxazole, atenolol, and naproxen fell below analytical detection limits more rapidly. The time to reach concentrations below detection limits was consistent with the likely persistence of the chemicals in soil. Ibuprofen demonstrated unusual kinetics in that an initial decline in concentration over the first week post-application was followed by an increase in concentration up to day 36. We hypothesized that the unusual behaviour of ibuprofen was due to a portion of the chemical in biosolids being sequestered either chemically (e.g. conjugated) or physically (e.g. sustained-release formulations), and then subsequently released in the soil. Table 2 summarizes the maximum concentrations of selected compounds that were observed in runoff after surface application, although it must be stressed that the LMB was spiked with acetaminophen, atenolol and sulfamethoxazole at concentrations of 100 µg L⁻¹, 10 µg L⁻¹ and 10 µg L⁻¹, respectively. Overall, this study showed that injection of LMB below the soil surface was an effective BMP for reducing transport via runoff.

In a 36-day experiment with DMB, the temporal patterns for PPCP concentrations in runoff following surface DMB application were much less consistent than those following LMB application (6). The maximum aqueous runoff concentrations of triclosan, atenolol, acetaminophen, and sulfamethoxazole were detected on day 1, the maximum concentration of naproxen were detected on day 3, and the maximum concentrations of triclocarban, caffeine and

carbamazepine were detected on day 7. The maximum concentration of ibuprofen was detected on day 36, consistent with the delayed release of this chemical observed after LMB application (12). Table 2 shows that the maximum concentrations observed for selected PPCPs in runoff were significantly lower for the DMB applications than for the LMB applications, although the LMB was spiked with additional amounts of acetaminophen, atenolol and sulfamethoxazole.

Mass fluxes during the experiment were estimated on the basis of the total runoff collected, the biosolids application rate, and the PPCP concentrations in the applied biosolids. On a mass basis, very little of the total amount in the applied biosolids was transported in runoff for ibuprofen (0.27%), naproxen (0.03%), gemfibrozil (<0.5%) or sulfamethoxazole (0.51%). In contrast a larger fraction of the applied carbamazepine (19.7%) atenolol (29.1%), cotinine (14.6%), caffeine (2.5%) and acetaminophen (1.7%) was detected in runoff water. Analytes that had low transport potential all had log K_{ow} values of 3.18 or more, whereas those that were readily transported had log K_{ow} values of 2.45 or less; indicating that sorption of hydrophobic compounds to soils reduces the potential for transport in the aqueous phase (6). However, as mentioned previously, this study demonstrated that care must be taken to consider the adsorption of high K_{ow} compounds to suspended particulates and subsequent overland transport. Our observations with the antibacterial compounds, triclosan (phenolic compound) and triclocarban (weak base) also showed that sorption to particulates is highly dependent on pH for compounds that can dissociate into ionic species (6). Overall, these studies demonstrated that sorption, as well as dissipation following application are processes that will influence the transport of PPCPs via surface runoff.

Transport of Veterinary Drugs in Leachate following Application of Manure

We have employed both field and semi-field studies to explore the leaching behaviour of veterinary pharmaceuticals from soil surfaces to groundwater (13, 14). The field studies were similar to the drainflow studies described earlier and involved the use of slurry from tylosin-treated pigs which was spiked with oxytetracycline and sulfachloropyridazine, and applied to plots at a site with a sandy loam soil. Samples of soil water were then obtained over time from three depths (i.e. 40, 80 and 120 cm). Sulfachloropyridazine was found to dissipate rapidly, with DT50 and DT90 values of 3.5 and 18.9 d, respectively, but oxytetracycline was more persistent with DT50 and DT90 values of 21.7 and 98.3 d, respectively. Only sulfachloropyridazine was detected in soil water samples at a maximum concentration of 0.78 $\mu\text{g L}^{-1}$ at a depth of 40 cm, 20 days after treatment. Tylosin was not detected in any soil or water samples. These findings were consistent with the sorption and persistence characteristics of the compounds and support a number of studies that have detected these antibiotic classes in soil and water (4, 15).

Alongside the field studies, lysimeter-based studies were performed to explore the leaching behaviour of the three veterinary antibiotics under different conditions that could occur in the agricultural environment (14), including the

Table 2. Maximum concentrations (ng L⁻¹) of selected PPCPs measured in runoff from fields where there were surface applications of liquid municipal biosolids (LMB) (12) or dewatered municipal biosolids (DMB) (6)

	<i>ACE</i>	<i>ATN</i>	<i>CBZ</i>	<i>TCS</i>	<i>TCC</i>	<i>SMX</i>
LMB	146*	70*	221	258	ND	272*
DMB	20	40	60	110	3	3

ACE, acetaminophen; ATN, atenolol; CBZ, carbamazepine; TCS, triclosan; TCC, triclocarban; SMX, sulfamethoxazole; ND = not determined. * Compounds were spiked into LMB prior to application.

influence of slurry amendment and incorporation and the effects of rainfall and time to rainfall following slurry application. Sulfachloropyridazine was detected sporadically in leachate at concentrations up to 0.661 $\mu\text{g L}^{-1}$ under typical rainfall conditions and more frequently at concentrations up to 8.5 $\mu\text{g L}^{-1}$ under extreme rainfall conditions (Figure 1). Incorporation and timing of rainfall had no significant effect on leaching behaviour. Oxytetracycline and tylosin were not detected in any leachate samples. Just like the field studies, these differences in behaviour were explained by the sorption and persistence characteristics of the compounds.

The lysimeter data were also used to evaluate the performance of a leaching model, PEARL that is recommended for environmental risk assessments of veterinary pharmaceuticals ((16)1). Comparison of the Measured Environmental Concentrations (MECs) with simulations from the leaching model indicated that the model greatly underestimates the transport of antibiotics to groundwater, which raises questions over the suitability of these models for regulatory risk assessments. One potential reason for the mismatch between the model predictions and the experimental observations is that the model does not take into account the effect of the slurry matrix on the behaviour of the antibiotics. Laboratory-based studies have demonstrated that the addition of slurry can affect the sorption behavior of veterinary medicines and that they may also affect persistence (e.g., (17, 18)). These effects have been attributed to changes in pH or the nature of dissolved organic carbon in the soil/manure system. For example, our laboratory studies of the impact of slurry amendment on sorption of the sulfonamide antibiotics demonstrated that the addition of manure or slurry to soils affects the sorption of many veterinary medicines, with sorption coefficients increasing as more slurry is added (18).

Other researchers have also explored the leaching behaviour of veterinary medicines. For example, in investigations in Germany (19), soil water was collected and analyzed from four separate areas of agricultural land: two belonging to livestock farms and treated with animal slurry and two where no animal manure had been applied for approximately five years. Chlortetracycline, oxytetracycline, tetracycline and tylosin were all found at the limit of detection (0.1–0.3 $\mu\text{g L}^{-1}$) in water samples collected at 80 and 120 cm depth, independent of soil treatment. In addition, no biologically active residues could be detected with microbiological assays that had approximately five-fold higher detection limits.

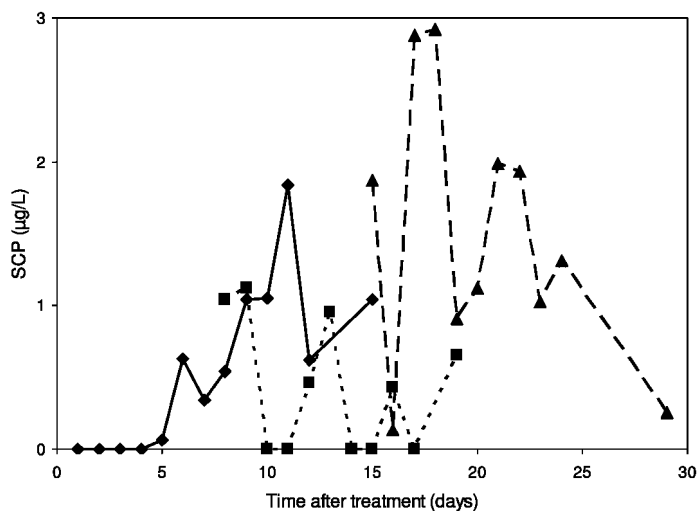


Figure 1. Concentrations of sulfachloropyridazine observed in leachate collected from lysimeters deployed in sandy loam soil following either 0, 7 or 14 d time lag between application of pig slurry and rainfall. (Adapted from (14))

Assessment of Risks for Aquatic Organisms Exposed to PPCPs and Veterinary Medicines in Runoff and Tile Drainage

Applications of pharmaceuticals in manure or biosolids can have significant impacts on organisms that make up the soil community (20), but there are also potential impacts on aquatic organisms exposed to PPCPs and veterinary drugs as a result of runoff from agricultural fields. For veterinary pharmaceuticals, most of the concern has been directed at potential biological effects in aquatic organisms exposed to either antiparasitic drugs or to antibiotics (21). The parasiticide, ivermectin is a veterinary drug that is widely used to control parasites in cattle, sheep, pigs and horses. When added to medicated feeds to control endoparasites, ivermectin is excreted primarily in the faeces. We have not studied the transport of ivermectin from agricultural fields into surface water, but Leibig et al. (22), used the FOCUS runoff model to estimate PECs following runoff events and estimated that concentrations in streams could reach up to 6.2 ng L⁻¹. Because of the high acute toxicity of ivermectin to *Daphnia*, at ng L⁻¹ concentrations (23), the PECs for ivermectin in runoff are well within the range of concentrations that could be toxic to aquatic invertebrates.

Human and veterinary antibiotics have the potential to enter surface waters after applications on agricultural fields, but the relatively low concentrations expected in surface waters are not likely to have toxic impacts on aquatic invertebrates or vertebrates (24). However, chronic exposure to antibiotics could lead to the development of microorganisms with antibiotic resistance. For instance, Sapkota et al. (25) observed higher inhibitory concentrations of four antibiotics in enterococci isolated from surface waters downgradient from leaking swine waste storage pits. Antibacterial compounds, such as triclosan have also been shown to induce cross-resistance to antibiotics (26), which raises concerns

about the continued use of large amounts of antibacterial soaps and other domestic products.

PPCPs may reach concentrations in runoff from agricultural fields that could adversely affect aquatic plants, invertebrates and vertebrates. In general, pharmaceuticals are acutely toxic to aquatic organisms at mg L⁻¹ concentrations, but some compounds (e.g. propranolol, fluoxetine, diclofenac) can induce sublethal effects in aquatic organisms exposed to concentrations in the low µg L⁻¹ range (27). Table 3 lists the Lowest Observed Effect Concentrations (LOECs) reported in selected acute and chronic toxicity studies with aquatic organisms exposed to the compounds listed previously in Tables 1 and 2. Overall, these data indicate that the thresholds for the acute and chronic toxicity of pharmaceuticals are generally several orders of magnitude above the concentrations that have been measured in runoff or leachate from fields treated with biosolids. However, there may be potential for toxic effects in aquatic organisms exposed to the antibacterial compounds, triclosan and triclocarban (Table 3). In particular, it is interesting to note that endocrine disrupting effects were observed in frogs exposed to 150 ng L⁻¹ of triclosan (28) and in mudsnails exposed to 200 ng L⁻¹ of triclocarban (29). At least for triclosan, the LOEC for toxic effects overlaps with the maximum concentrations that we observed in effluents from agricultural fields.

Table 3. Lowest observed effects concentrations (LOECs; ng L⁻¹) for acute and chronic toxicity reported in selected toxicity tests with aquatic organisms. The taxonomic classification for the organisms in the reported toxicity tests (i.e. algae, invertebrate, fish, amphibian) are shown in brackets.

Where LOECs were not available, they were estimated by dividing the L(E)C50 by a factor of 10. Toxicity data were reported by Veldoen et al. (28), Fent (27), Sabourin et al. (6), De Andres et al., (30), Fernandez et al., (31), and Guidice and Young (29).

	<i>ACE</i>	<i>ATN</i>	<i>CBZ</i>	<i>TCS</i>	<i>TCC</i>	<i>SMX</i>
Acute LOEC	2 x 10 ⁶ (invert.)	10x 10 ⁶ (fish)	1.4x 10 ⁶ (invert)	53 (algae)	1,700 (algae)	2,680 (algae)
Chronic LOEC	ND	5.6x 10 ⁶ (invert)	9 x 10 ⁶ (invert)	150 (amphib)	200 (invert)	ND

ACE, acetaminophen; ATN, atenolol; CBZ, carbamazepine; TCS, triclosan; TCC, triclocarban; SMX, sulfamethoxazole; ND = not determined.

Conclusions

Our studies have shown that PPCPs carried in biosolids and veterinary drugs carried in manure can be transported in runoff, tile drainage and leachate after application of biosolids or manure onto agricultural land. Spikes in concentrations observed in water are typically transient, and depend on the frequency and intensity of rainfall events. Water soluble compounds show the greatest potential for transport in the aqueous phase, but there is also potential for transport of less water soluble compounds sorbed onto suspended particulates. Some compounds are persistent in soils and can be transported into tile drainage and runoff up to several months after application. More work is required to validate and improve models for predicting the transport of PPCPs and veterinary medicines into water resources, particularly by taking into account the capacity of the manure/biosolids matrix to modify the sorption and persistence of target compounds, and the capacity for preferential flow through soil macropores.

Veterinary parasiticides (e.g. ivermectin) and biocidal compounds used in personal care products (e.g. triclosan) show potential for transport into surface waters adjacent to agricultural fields at concentrations that are toxic to aquatic organisms. More work is required to determine whether human use or veterinary antibiotics in runoff are contributing to the development of antibiotic resistance among microbial populations in the aquatic environment.

BMPs for the application of biosolids and manure can mitigate the transport of these compounds into water resources. These approaches include:

- a) Restricting field applications to seasons and times when runoff or leaching potential is low.
- b) Where possible, avoiding the application of liquid biosolids or manure in favour of dewatered or solid material.
- c) For surface applications, pre-tilling the soil prior to applying manure or biosolids.
- d) Utilizing subsurface injection technologies; particularly when applying manure slurry or liquid municipal biosolids.

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Chapter 11

Environmental Fate and Hazards of the Pharmaceutical Diclofenac in Aquatic Environments

Jesper Svanfelt, Jenny-Maria Kallio, Johan Eriksson,
and Leif Kronberg*

Laboratory of Organic Chemistry, Åbo Akademi University,
FI-20500 Åbo/Turku, Finland
*leif.kronberg@abo.fi

Pharmaceuticals enter the environment mainly through discharges from municipal wastewater treatment plants (WWTPs). The removal efficiency of the plants varies depending on the pharmaceutical and the treatment(s) applied. Diclofenac (DCF) has been found to be rather unaffected by the treatment process and is consequently often detected in the effluents from plants and in receiving water bodies. DCF is a dichlorinated diphenylamine containing an acetic acid group as a substituent, which makes the compound rather water soluble. The compound is known to be extremely toxic to some vulture species and to readily undergo phototransformation reactions in the environment. In the following chapter, we summarize what is known about the fate of DCF in WWTPs and in the aquatic environment with emphasis on the bioavailability of the compound to fish and on its phototransformation reactions. A few studies have shown that DCF can be taken-up by fish exposed to the compound in concentrations found in the environment ($1 \mu\text{g L}^{-1}$). Also, it has been shown that DCF even at these concentrations may impair the health status of fish and other aquatic organisms. Very recently, DCF metabolites have been identified in fish bile. Furthermore, it has been shown that the compound undergoes bioconcentration in the fish bile yielding bioconcentration factors of up to about 1000. When subjected to sunlight, DCF forms several phototransformation

products and recently the major products have been identified as carbazoles where one of the condensed rings has a fully conjugated diketone function (a quinone unit). The structures are of interest from a toxicological point of view and further work is needed before their significance is fully understood. All in all, DCF as well as other pharmaceuticals entering the aquatic environment should be considered as potentially harmful for the ecosystem and there is a need to assess the occurrence of the parent compounds and the transformation products, as well as their ecotoxicological impact.

Introduction

Thousands of tons of pharmacologically active compounds are used each year for the treatment of human and animal illnesses. The pharmaceuticals used by humans enter the environment mainly *via* influents to wastewater treatment plants (WWTPs), where they are partially removed or modified in the activated sludge process and thereafter released *via* effluents into recipient water bodies – rivers, lakes, and coastal waters. Although the compounds are detected at low concentration levels (ng to $\mu\text{g L}^{-1}$ levels) (e.g. (1–8)) they are considered potentially hazardous for the aquatic organisms and the ecosystem health (e.g. (9)). Many of the pharmaceuticals have been shown to have rather low acute toxicities (i.e. mg L^{-1} range) (e.g. (9–11)) towards aquatic organisms, but chronic and even life-long exposure caused by the continuous release of mixtures of pharmaceuticals and their metabolites to water bodies have to be considered as a risk not only for single species but for the whole aquatic ecosystem. However, data on chronic effects of pharmaceuticals on aquatic organisms are practically non-existing and the environmental risk assessment is mainly based on the acute toxicity data of the single compounds (9). A further complication to risk assessments is the occurrence of transformation products of pharmaceuticals, whose identities at present are largely unknown.

After the first reports on the detection of pharmaceuticals in surface waters, research is now focused on their fate in the aquatic environment (e.g. (1, 12–14)) and on the possible effects on aquatic organisms (e.g. (9, 15–21)). Many pharmaceuticals are not stable in the aquatic environment and undergo, in addition to biotransformation reactions, common abiotic reactions such as hydrolysis and photolysis. It has been argued, that a major route for transformation of pharmaceuticals is photolysis (4, 22–24). This process is of main importance since the energy compounds gain from the light is used for intramolecular reactions and rearrangements. The resultant compounds often are of lower energy than the parent compound. This means that the products of photolysis can be more stable and more hydrophobic than the parent compound and thus of greater environmental concern. Examples of this are the dibenzodioxin analogue acridine and the dibenzofuran analogue carbazole produced by photolysis of carbamazepine and DCF, respectively (14). Even if the drugs themselves do not absorb UV-light, there is always a possibility that they react with, or gain

energy from some other molecule that absorbs light. The most important light absorber that may induce indirect photolytic transformation in natural water is the dissolved organic material (DOM). Since the DOM concentrations in river and lake water can become very high (up to (15–25) mg L⁻¹ in the boreal zone), it is evident that DOM may play a significant role as the producer of transient photo oxidants in water (¹O₂, RO[•], HO[•], HO₂[•]), especially in the summer time.

A scenario involving pharmaceuticals, which has not been considered previously is the possibility that the drugs currently in daily use are transported through the food web. We have recently found that fish exposed to DCF may bioaccumulate and metabolize the drugs (25) and thus fish predators, for example birds, may be subjected to the drugs and drug metabolites. The extent and consequence of this transfer are not known.

DCF is an anti-inflammatory drug in general use worldwide. The compound has been reported to be poorly eliminated during wastewater treatment (e.g. (3, 6)) and is consequently a common pollutant in water receiving discharges from treatment plants. In the environment DCF seems to readily undergo photolysis reactions and transformation products are generated. Since DCF and at least some of the phototransformation products contain chlorine, the compounds may exhibit a pronounced stability in the environment and the transformation products could possess an enhanced lipophilicity, i.e. the compounds own and acquire chemical properties that render them of concern for the environment. Therefore, the environmental fate of DCF needs to be understood. In addition, a further interest into DCF is provided by the reports showing that some vulture species are extremely sensitive to the compound (e.g. (26–29)). Therefore, from an ecotoxicological viewpoint, the logical way to proceed would be to thoroughly study the uptake, metabolism and the toxicity of DCF in fish and other aquatic organisms.

DCF in Wastewater, WWTPs, and Sediments

In the influent of WWTPs, not only DCF is present, but also the DCF metabolites produced in the human body. However, the exact extent of the occurrence of the metabolites in influent water has not been determined. The main phase I human metabolites of DCF are 4'-hydroxydiclofenac (4'-OH-DCF) and 5-hydroxydiclofenac (5-OH-DCF), while the main phase II metabolites are the acyl glucuronides of the parent compound and of the phase I metabolites (e.g. (30–36)). Also sulfate conjugates of hydroxylated DCFs are formed (30, 31, 37).

Several studies have shown that DCF is poorly eliminated in WWTPs, but the degree of elimination and the fate of the metabolites are poorly understood. It has been postulated that the human phase II metabolites of DCF (6, 7, 38) and also of other pharmaceuticals and of the estrogens (e.g. (3, 39)) are at least partly deconjugated to the parent compounds or to the phase I metabolites during the treatment processes. Thus the concentration of the parent compound can be higher in the effluent than in the influent water, which may result in a negative removal rate. The DCF concentration may be further enhanced through the ester hydrolysis of aceclofenac; an anti-inflammatory drug structurally related to DCF (6). Pérez

and Barcelo (6) found that 4'-OH-DCF and DCF behave differently in wastewater treatment processes. Also, Gomes et al. (40) found a clear distinction between the elimination of sulfate and glucuronide conjugates of different estrogens in the crude sewage. These findings show that the efficiency in elimination varies between the metabolites and the parent compounds.

In WWTPs and in river sediments, the pharmaceuticals and their metabolites undergo microbiological transformations (e.g. (6, 40–44)). A few studies have been concerned with the microbial metabolism of DCF. Kosjek et al. (42) found 1-(2,6-dichlorophenyl)-1,3-dihydro-2*H*-indol-2-one (**1**), [2-[(2,6-dichlorophenyl)amino]benzyl alcohol (**2**), and [2-[(2,6-dichlorophenyl)amino]benzyl alcohol methyl ether (**3**) to be products of DCF transformations taking place in the WWTP (Fig. 1). Recently, Kosjek et al. (43) reported on the formation of several additional microbiological transformation products of DCF, but their identities remain unknown. In a laboratory experiment with a microflora of sediments, Gröning et al. (44) found that DCF is converted to 5-OH-DCF, which is further rapidly oxidized to a *p*-benzoquinone imine (**4**) derivative. Although compound **4** was found to be the major biotransformation product, it is highly reactive and undergoes further transformation/degradation reactions and is also likely to be involved in adduct formations for example with proteins. (Fig. 1).

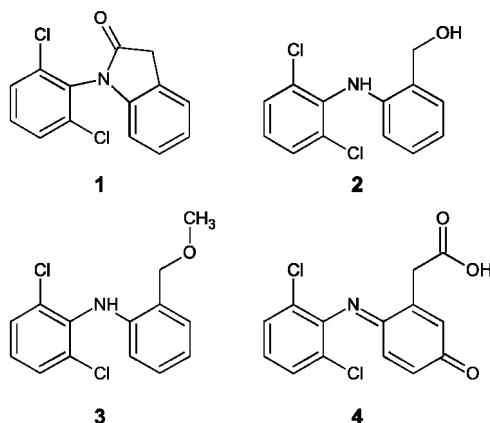


Figure 1. Microbial transformation products of diclofenac (DCF): 1-(2,6-dichlorophenyl)-1,3-dihydro-2*H*-indol-2-one (**1**), [2-[(2,6-dichlorophenyl)amino]benzyl alcohol (**2**), [2-[(2,6-dichlorophenyl)amino]benzyl alcohol methyl ether (**3**) and *p*-benzoquinone imine of 5-OH-DCF (**4**).

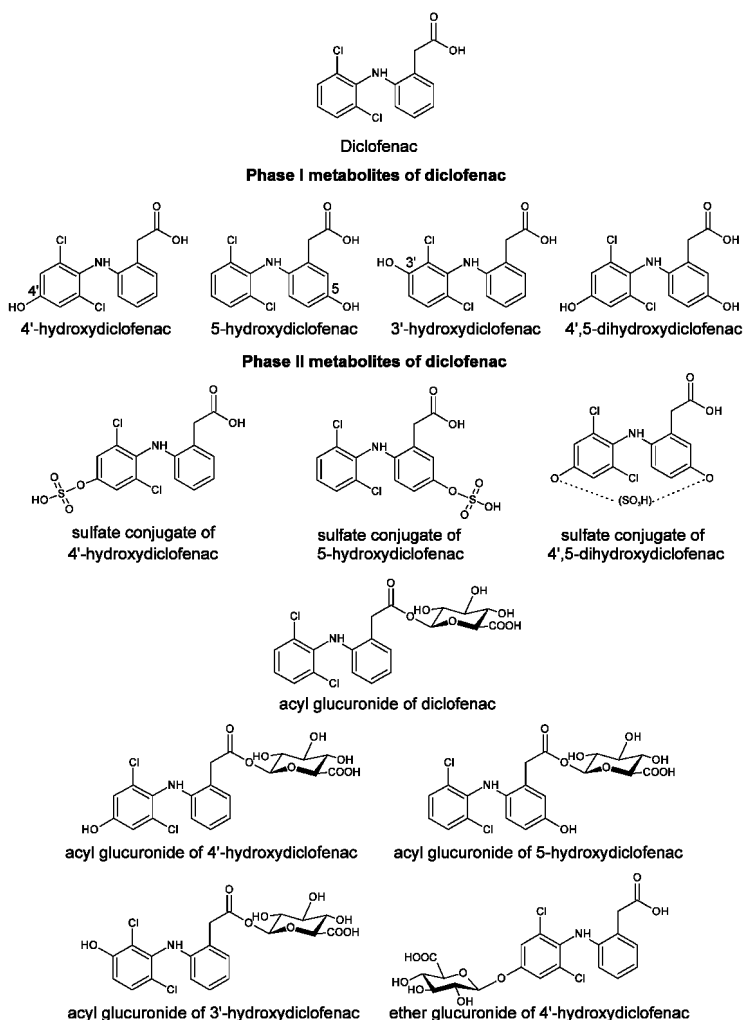


Figure 2. Diclofenac (DCF) phase I and II metabolites found in rainbow trout bile. 3'-OH-DCF and 4',5-diOH-DCF were detected only as phase II metabolites.

The ability of a compound to partition between water and organic phase and sorp to sludge is predicted by its octanol-water partitioning (K_{ow}) and solid-water distribution (K_d) coefficients. The potential of a compound to absorb to WWTP sludge is assumed to be low with $\log K_{ow} < 2.5$, moderate with $\log K_{ow} = 2.5-4.0$ and high with $\log K_{ow} > 4.1$ (45). The sorption to sludge is considered as relevant elimination pathway, if the $\log K_d$ is > 2.48 (46). The $\log K_{ow}$ of DCF is 4.51 (47), although a higher mobility of DCF than indicated by its K_{ow} value has been suggested in many studies (e.g. (48, 49)). The $\log K_d$ values depends on the characteristics of the sludge/sediment and has been determined to be 2.7 and 1.2 in primary and secondary sludge (48), $-0.27-0.67$ in sediment (49) and $-0.35-2.22$ in soil (50). To summarize, the sorption of DCF to sludge in WWTPs

and to sediments in recipient waters may take place, but it is not believed to be an important elimination mechanism (e.g. (48, 49, 51)). This is also confirmed e.g. by Buser et al. (52), who found negligible absorption rates of DCF to lake sediments.

Studies of Exposure of Aquatic Organisms to DCF

Several exposure studies of DCF to aquatic organisms have been performed. Rainbow trout was shown to accumulate DCF in tissues when exposed to a concentration of $1 \mu\text{g L}^{-1}$ (15). Brown et al. (19) noticed that rainbow trout accumulates DCF in the blood plasma when exposed to the compound through sewage treatment plant effluents. On the basis of the assumption that DCF acts on the same targets in fish and humans, plasma concentrations can be used to assess the possibility of a pharmaceutical effect of the drug. Brown et al. compared human therapeutic plasma concentration of pharmaceuticals with those found in fish plasma and concluded that DCF may present a higher risk for fish than for example ibuprofen or naproxen (19).

Kallio et al. (25) have recently studied the metabolism and bioaccumulation of DCF in rainbow trout (*Oncorhynchus mykiss*) exposed to DCF in aquaria at a concentration of $1.76 \mu\text{g L}^{-1}$. This concentration level may typically occur for example in river water downstream of WWTP discharge locations. The main metabolites found in the trout bile were acyl glucuronides of DCF and of the phase I OH-DCF metabolites (Fig. 2). The metabolites are formed through the same general metabolic pathways of DCF that are prevailing in humans, i.e. hydroxylation of the aromatic rings and the glucuronidation of the carboxylic acid group (e.g. (30–32, 34–36)). In addition, one ether glucuronide and three sulfate conjugates of OH-DCFs were detected in fish bile. Also unmetabolized DCF was found in bile. These findings show that DCF is taken up by fish and strongly indicates that this happens also in fish living downstream of WWTPs (25).

Since the pK_a of DCF is 4.15 (47), the compound occurs primarily in the ionized form in water and in biological matrices. Due to the lower lipophilicity, deprotonated DCF is not expected to readily undergo bioaccumulation. However, Hoeger et al. (53) injected intraperitoneally ^{14}C -labelled DCF into brown trout (*Salmon trutta* f. *fario*) and found DCF to enter the enterohepatic circulation, which results in a prolonged exposure to the fish. The longer residence time in the body promotes bioaccumulation and thus enhances the risks for toxic effects (53).

Schwaiger et al. (15) exposed rainbow trout (*Oncorhynchus mykiss*) for 28 days to DCF at concentrations ranging from $1\text{--}500 \mu\text{g L}^{-1}$ and found the highest bioconcentration to take place at the lowest exposure concentration ($1 \mu\text{g L}^{-1}$). At this concentration, BCFs of DCF in the liver, the kidney, the gills and the muscles were 2700, 970, 760 and 70, respectively. Brown et al. (19) reported a BCF of 5 for DCF in blood plasma of fish exposed to sewage effluent, where the DCF concentration was $2.32 \mu\text{g L}^{-1}$. For comparison, the BCF for ibuprofen was 18700 (19). Mehinto et al. (21) measured BCF for DCF in fish bile to be 657 when fish were exposed to $0.5 \mu\text{g L}^{-1}$ of DCF for 21 days. Kallio et al. (25) exposed fish for $1.7 \mu\text{g L}^{-1}$ DCF for 10 days and on the basis of estimated concentrations of

DCF metabolites and DCF in the bile found a total BCFs (including metabolites) of 320–950. These BCFs are much higher than the factor found in blood plasma and thus the analyses of biliary metabolites could be a useful way of monitoring fish exposure to DCF in natural waters. However, due to the sporadic emptying of the gall-bladder, the bile analyses have to be performed on a large number of fish. In summary, these studies show that DCF can be taken up by fish and that the compound and its metabolites undergo bioconcentration especially in the bile.

Photochemical Transformation of DCF

In the environment, interactions take place between organic compounds and sunlight and may ultimately result in chemical transformations, i.e. alteration of compound structures. Therefore, photochemistry plays an important role in understanding the behavior of organic pollutants in the environment. Most pharmaceuticals contain chromophores with the ability to absorb light in the solar UV region. DCF has been shown to undergo phototransformation when exposed to natural sunlight, which is considered to be the major transformation route of the drug in the aquatic environment (e.g. (22–24)). Recent studies have also shown that the ecotoxicological impact of DCF is enhanced due to phototransformation as some transformation products have been found to be more toxic to aquatic organisms than the parent compound (54). In this connection, concern has also been raised about the application of UV light to wastewater treatment, as it may provide an input of transformation products directly to the aquatic environment (55).

Halogenated aromatic compounds, such as DCF, are of particular interest since chemical bonds between sp^2 -hybridized carbons and halogens are known to easily break in the presence of UV-light (e.g. (56)). The free radicals formed during homolysis of photolabile carbon-halogen bonds in haloarenes may react with biological substrates and cause impaired health conditions in organisms (57). An important class of photochemical reactions of aromatic compounds is nucleophilic substitution reactions, where halides often act as leaving groups. Consequently, reactions in aqueous solutions commonly involve reactions with water. However, the electronically excited state in photochemical reactions is sufficiently energetic and sufficiently different in its electron distribution and electron donor-acceptor properties, for such reactions to rarely exhibit the characteristics of ground state processes (58). As a result, the outcome of photochemical reactions is often difficult to predict.

In a majority of the studies dealing with phototransformation of pharmaceuticals, mass spectrometry has been the sole tool for identification of unknown transformation products (59). Agüera et al. (13) reported 13 possible structures for transformation products of DCF on the basis of LC-MS and GC-MS analyses. Ideally, all compound structures should be confirmed by detailed structural analyses (e.g. NMR), but that is often both a very difficult and time consuming task, especially when dealing with complex, multi-component samples. Recently, however, synthetic routes to some of the previously reported transformation products of DCF have been described (60). With synthetic

standards available, the presence of anticipated transformation products in UV and MS chromatograms are easily confirmed. Also, through access to pure photoproducts transformation pathways and reaction kinetics can be studied more thoroughly. In this way, valuable information may be gained, which can prove helpful in the elucidation of reaction scenarios that are likely to take place in the environment and during wastewater treatment.

The absorption spectrum of DCF (Fig. 3) has a maximum at 275 nm and shows absorption up to about 340 nm, which explains its tendency to undergo transformation in natural sunlight. The aqueous photolysis of DCF has been the subject of much research over the past decade and several papers have reported the formation of a number of transformation products upon exposure of aqueous solutions of the drug to UV-light (e.g. (13, 57)). Moore et al. (57) concluded that the primary photochemical process of DCF transformation involves intramolecular cyclization to form the carbazole (8-chloro-9H-carbazol-1-yl) acetic acid (CZ1, Fig. 4) through loss of HCl. In methanolic solution, further transformation of CZ1 was found to proceed mainly *via* photoreduction, i.e. replacement of the remaining chlorine with hydrogen (57). Upon aqueous photolysis of DCF, CZ1 was almost immediately formed, but the compound underwent rapid photonucleophilic reaction with water to produce the 8-hydroxy analogue (8-hydroxy-9H-carbazol-1-yl) acetic acid (CZ2), which reacted further with water to produce mainly the *para*-quinone CZ4 (also the *ortho*-analogue, CZ5, could be traced) (61). In the study of Eriksson et al. (61), the UV and MS-chromatograms also revealed a small peak with a mass equivalent to the 1,4-dihydroxy compound CZ3. Reactions carried out under both oxygenated and deaerated conditions yielded the same carbazole products, hence the additional oxygens in compounds CZ2–CZ5 originate from water. Of the transformation products, CZ4 was formed with the highest yield following 200 min of irradiation and could be isolated by LC and structurally assigned by NMR and HRMS analyses (61).

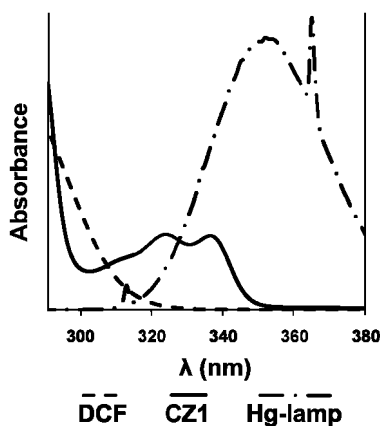


Figure 3. UV absorption spectra of diclofenac (DCF) and CZ1. The emission spectrum of the UV-lamp used in the study of Eriksson et al. (61) is also shown.

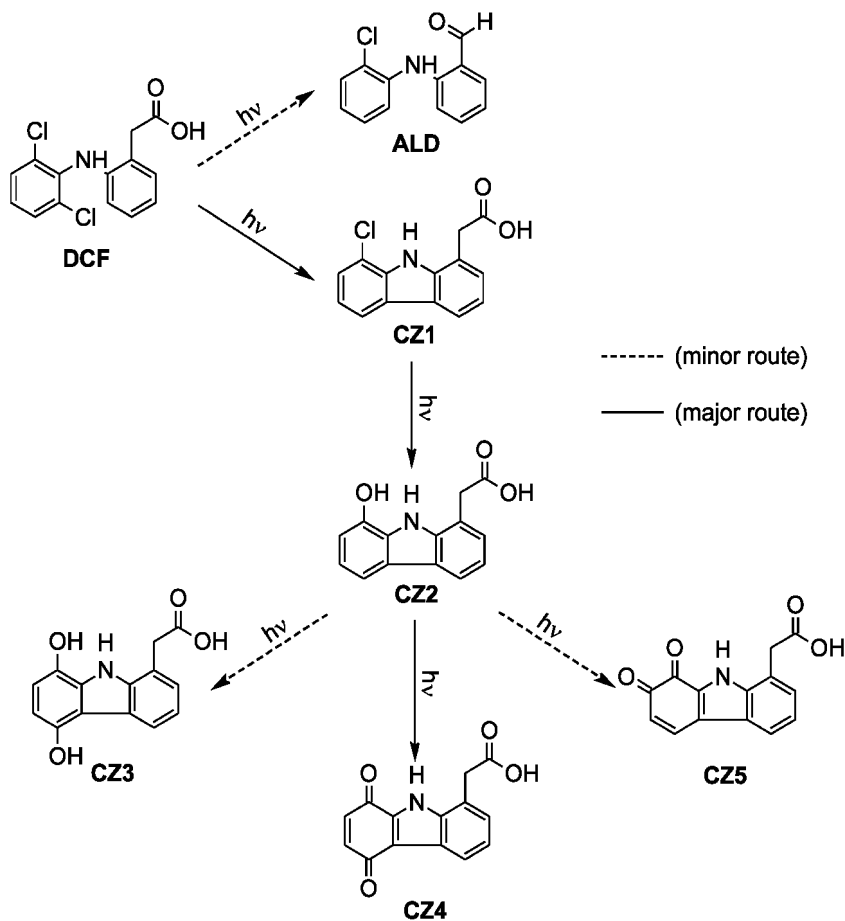


Figure 4. Aqueous phototransformation of diclofenac (DCF) under oxygenated and oxygen free conditions.

The compound CZ1 shows very high photosensitivity due to a relatively high overall quantum yield in combination with an absorption that stretches quite far into the long wave UV-region (Fig. 3) (61). As a matter of fact, the transformation rate constant of CZ1 has been shown to be > 20 times greater than that of DCF (61).

A second minor transformation route leads to the formation of the only confirmed non-carbazole product, i.e. 2-(2-chloro-phenylamino)-benzaldehyde (ALD, Fig. 4). A synthetic pathway to this compound has previously been described (60). Its photochemical formation has been proposed to involve an intramolecular mechanism, possibly including several short-lived intermediate products (61). Although the presence of dissolved oxygen has been shown to hamper its formation, this compound is the most photostable transformation product of DCF with a half-life more than 80 times longer than that of DCF (61).

Toxicological Effects in Aquatic Organisms

In long-term aquaria studies (28 days) of Schwaiger et al. (15) and Triebkorn et al. (16, 17) it was found that DCF induces cytological alteration in the liver, kidneys and gills of rainbow trout already at concentrations of 1 to 5 $\mu\text{g L}^{-1}$. Also, Hoeger et al (18) found subtle effects in the liver, kidneys and gills of brown trout at an exposure concentration of only 0.5 $\mu\text{g L}^{-1}$ for 21 days and on the basis of their findings they conclude that at the concentration levels found in surface water, chronic exposure to DCF has to be considered harmful to fish populations. In green shore crab (*Carcinus maenas*) DCF exposure at 10–100 ng L^{-1} for 7 days caused impaired osmoregulatory ability (20). In a very recent work by Mehinto et al. (21) it was documented that sub $\mu\text{g L}^{-1}$ concentrations of DCF can impair the health of rainbow trout for example through structural disruptions in the kidney and intestine. It was suggested that the damage of the intestinal tract could be caused by reactive metabolites of DCF.

Hydroxylated metabolites of DCF (5-OH-DCF and *N*,5-diOH-DCF) have been found to show hepatotoxicity in rat and human cell lines (33). It has been proposed, that the reaction behind the toxicity is the autoxidation of hydroxylated metabolites of DCF (especially 5-OH-DCF; **4** in Fig. 1) to reactive *p*-benzoquinone imines and their adduct formation with proteins (36, 62–64). Besides hydroxylated phase I metabolites of DCF, its acyl glucuronides are also known to form covalent adducts with proteins (36, 65).

At present, very little is known about the toxicity of the phototransformation products of DCF. However, the quinones CZ4 and CZ5 stand out as the most interesting with regards to potential toxic properties. Quinones form a class of toxicological intermediates capable of causing a variety of hazardous effects *in vivo*, such as acute cytotoxicity, immunotoxicity and carcinogenesis (66). As Michael acceptors, they may inflict cellular damage *via* alkylation of vital cellular proteins and/or DNA. In addition, the high redox activity possessed by quinones may lead to the formation of harmful reactive oxygen species (e.g. hydroxyl radicals) (66). The toxic properties of quinones are also utilized by the pesticide industry, where they are found as important structural units in many common commercial fungicides such as *p*-chloranil, dichlone and oxine (67).

As chemicals designed to have specific biological effects, pharmaceuticals should undoubtedly be considered potentially harmful to ecosystem processes. In addition, abiotic transformation of pharmaceuticals may lead to the formation of stable and potentially more hazardous compounds. Thus there is an increasing need to assess the occurrence and ecotoxicological impact not only of the parent compounds, but their transformation products as well. This, in turn, implies a demand for more synthetic standards for use in environmental analyses.

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Chapter 12

Fate of Caffeine in the Environment and Ecotoxicological Considerations

**Thomas Bruton,[†] Ali Alboloushi,[†] Bella de la Garza,[†] Bi-O. Kim,[†]
and Rolf U. Halden^{*}**

**School of Sustainable Engineering and the Built Environment,
Arizona State University, 1001 South McAllister Avenue,
Tempe, AZ 85287-5701**

***Corresponding author e-mail: halden@asu.edu.**

[†]These authors contributed equally.

In recent years much attention has been devoted to the occurrence of caffeine in natural waters. It has been established that caffeine is now present in a wide variety of environments, including wastewater treatment plant (WWTP) effluents, groundwater and remote mountain lakes. However, it is less clear what the practical significance of caffeine occurrence may be because recent research has only begun to evaluate the consequences of increasing caffeine concentrations for aquatic biota. The objectives of this paper are (1) to gain a qualitative understanding of the routes through which caffeine enters the environment and the mechanisms through which it is degraded, (2) to obtain quantitative data on which to base a mass balance of caffeine in wastewater treatment plant effluent, and (3) interpret environmental occurrence levels of caffeine in the context of toxicity threshold values determined for aquatic biota. To accomplish these objectives a literature review and mass balance were performed. This paper shows that caffeine concentrations are typically in the ng/L range in many freshwater environments. In certain areas levels appear to be sufficiently high to approach threshold toxicity values for aquatic biota. Primary locations of concern in urban areas are discharge points of treated wastewater. Although caffeine presents no large scale threat now, further research is needed

on the occurrence of caffeine in natural waters and its chronic toxicity to aquatic organisms.

Introduction

In recent years much attention has been devoted to the occurrence of pharmaceuticals and personal care products (PPCPs) in natural waters. The development of more sensitive analytical techniques has made it evident that a wide spectrum of drugs and personal care products have become common if not ubiquitous in the water environment, albeit at very low concentrations (1). PPCPs enter the environment through a variety of routes (2), including sewage treatment plant and septic tank discharges, as well as combined sewer overflows. As the global population increases and pressure mounts to protect the quality of the Earth's limited water resources, it will become increasingly important to understand the growing presence of PPCPs and their potential effects.

One of the most frequently detected PPCPs is caffeine (3). Caffeine, also known as 1,3,7-trimethylxanthine (Fig. 1), is a stimulant that affects the central nervous system in humans (4). It is an alkaloid that is naturally present in more than 60 plant species, including the familiar coffee, tea, and cacao (5). However, few of these plants are native to the U.S. (6). Although naturally occurring background levels of caffeine have been observed in at least one U.S. stream (7), the majority of the caffeine in the U.S. environment can be assumed to occur there as a result of anthropogenic activities. Caffeine is a constituent of many common beverages including coffee, tea, soft drinks and the increasingly popular class of beverages known as energy drinks (8). Caffeine is also present in chocolate, pastries and dairy desserts (9). It is estimated that per capita caffeine consumption in the United States is 210 mg person⁻¹ day⁻¹ (9). Additionally, caffeine is included in various pharmaceuticals as an analeptic and to enhance the effects of analgesic drugs (10). In fact, it has been suggested that caffeine is the most widely consumed drug in the world (11).

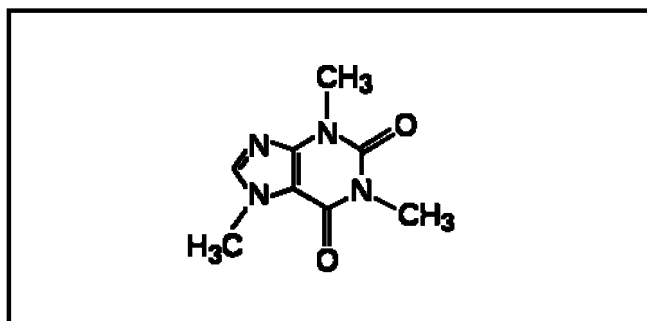


Figure 1. Chemical structure of caffeine (1,3,7-trimethylxanthine).

Numerous studies have documented the presence of caffeine in wastewater treatment plant influents and effluents (12, 13), surface water (9), groundwater (14, 15) and even in marine environments (6, 10, 16). Because caffeine has a short half-life and is present at high levels in raw sewage and at low levels in treated sewage, it can be used to indicate the presence of raw sewage in ground and surface waters (9), which in turn indicates the presence of biological contaminants that drive health risk.

Although caffeine is recognized by the United States Department of Agriculture as a Generally Recognized as Safe compound (17), at high concentrations caffeine is known to be toxic. It has been reported that one time dosages of 5 to 10 g of caffeine in humans can be lethal, and caffeine intoxication (or caffeinism) can be observed at one-time dosages of 250 mg (18). Symptoms of caffeinism include anxiety, restlessness, sleep disruption, and abnormally rapid heart rate (19). Researchers have also demonstrated its potential for use as a deterrent to a variety of agricultural pests (17, 20). With the potency of caffeine toxicity firmly established, it becomes necessary to evaluate the risk posed by its widespread environmental occurrence (10). Toxicity is of little concern for humans since human exposure to caffeine through drinking water is infrequent; however, a similar generalization for aquatic organisms cannot be made since they are continuously exposed over a lifetime.

The objectives of this chapter are (1) to gain a qualitative understanding of the routes through which caffeine enters the environment and the mechanisms through which it is degraded, (2) to obtain quantitative data on which to base a mass balance of caffeine, and (3) to understand likely modes of caffeine toxicity and the concentrations at which toxicity has been observed in aquatic biota.

Methods

An extensive review of the environmental caffeine literature was performed. Databases queried included Google Scholar, Science Direct, Elsevier, Web of Science, Springer Link and Pub Med. The following search terms were used in various combinations: *assessment, biosolids, caffeine, coffee, degradation, energy drinks, environment, groundwater, human metabolism, landfill, marine, mass balance, persistence, photolysis, PPCP, sewage, sludge, surface water, toxicity, wastewater, WWTP*.

Modeling of Caffeine Concentrations in WWTP Effluent

To estimate caffeine concentrations in wastewater treatment plant (WWTP) effluent and the aquatic environment, a mass balance was performed for a hypothetical WWTP of typical design. Average values of population normalized inputs of caffeine to WWTP influents and WWTP sludge, as well as average WWTP caffeine removal efficiencies were extracted from the literature. Using these data, the general equations to predict caffeine concentrations in WWTP influents and effluents can be written as:

$$C_{inf} = \text{population} \times 1144 \text{ pg } L^{-1} \text{ cap}^{-1}. \quad (1)$$

$$C_{eff} = C_{inf} \times (1-0.94) \quad (2)$$

Similarly, the equation to predict caffeine concentrations in sludge can be written as:

$$C_{sludge} = population \times 0.304 \text{ pg g}^{-1} \text{ cap}^{-1} \quad (3)$$

Using the equations developed above, a mass balance was established for caffeine being discharged into a river. It was assumed that wastewater with caffeine concentration C_{eff} and flow rate Q_{eff} was discharged into a river with a cross-sectional area A , upstream caffeine concentration C_o , and flow rate Q_r . The resulting concentration from the discharge is C_r , which represents the concentration of caffeine at the point where the effluent enters the river (Fig. 2). Critical assumptions of the model are that the discharged wastewater is completely mixed with surface water, that the river's flow characteristics approach those of a plug flow system, and that caffeine degradation can be approximated as a first order reaction with a half-life of 1.5 days (t). The model can be used to determine the downstream concentration profile as a function of time and distance.

$$Q_r C_o + Q_{eff} C_{eff} = (Q_r + Q_{eff}) C_r \quad (\text{Mass Balance})$$

$$C_r = \frac{Q_r C_o + Q_{eff} C_{eff}}{(Q_r + Q_{eff})} \quad (4)$$

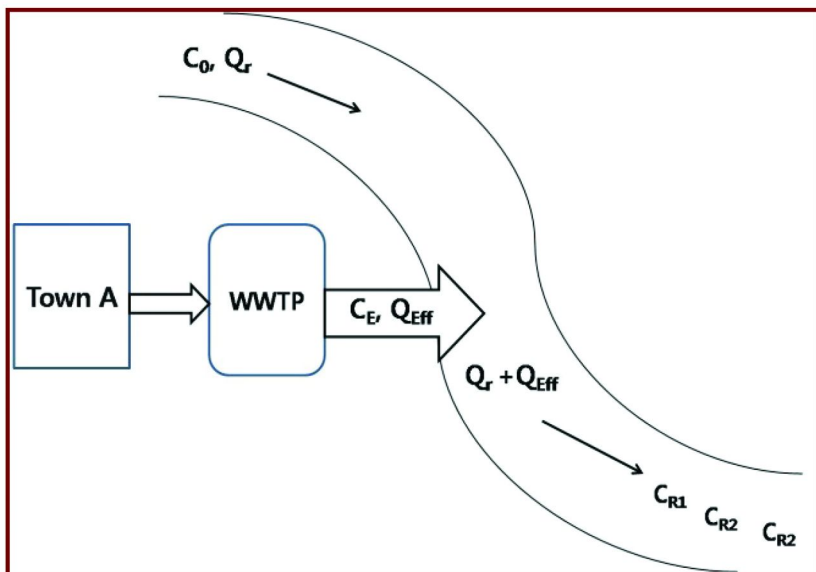


Figure 2. Conceptual model of caffeine introduction, dilution and dissipation in a river.

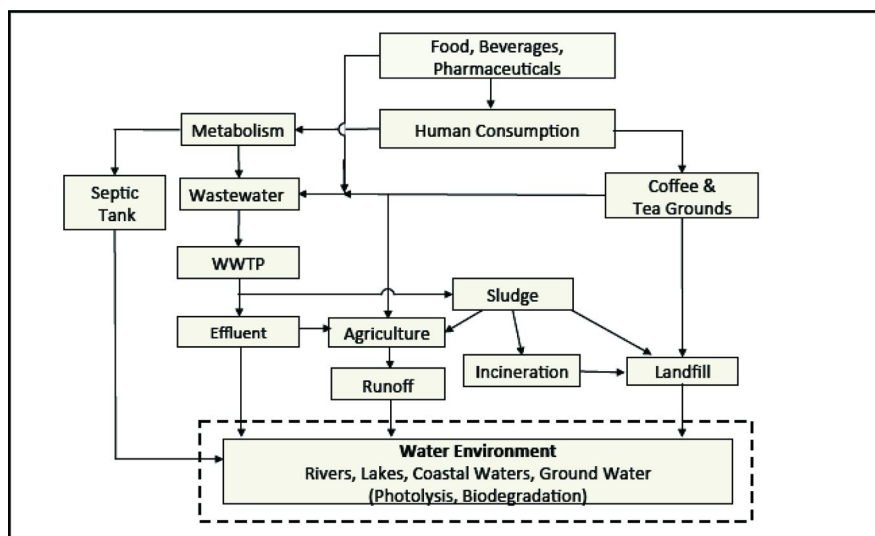


Figure 3. Caffeine routes into the environment.

Results and Discussion

The first studies regarding caffeine toxicology and metabolism were performed in the 1940s (21) and research in this area continues today. Most of the literature on the environmental occurrence of caffeine has been published within the last decade. In general, this paper uses the most recent data available.

Routes by Which Caffeine Enters the Environment

The first step in understanding the fate of PPCPs in the environment is to examine their sources. In the case of caffeine, the most important source of environmental release is human consumption of caffeine-containing foods, beverages and pharmaceuticals, and subsequent excretion of caffeine into domestic wastewater. It has been determined that human metabolism of caffeine occurs predominantly in the liver, and the pathway for caffeine metabolism in humans is well established (22). The half-life of caffeine in humans is on the order of 3.5 to 5 hours (22). In human adults caffeine is almost completely metabolized, with less than 2% of the consumed mass being detectable as non-metabolized, native caffeine in urine (23, 24). Based on these figures, it can be assumed that 2% of the total caffeine consumed will make its way into domestic sewage collection systems, where a small fraction will be degraded by microorganisms while en route to WWTPs.

In addition to human excretion, other routes exist by which caffeine may enter the environment. Runoff from agricultural areas where sewage sludge and/or coffee grounds are applied to soil contribute to the caffeine in surface water. Caffeine in food waste, drugs, municipal incinerator ash (25), etc. is disposed of in landfills. Landfill leachate may percolate into the underlying soil (15), thereby

contaminating local groundwater and nearby wetlands (26). Presumably, some portion of the caffeine produced by industrial decaffeination processes also makes its way into waste streams. Moreover, detection of caffeine in treated wastewater effluent and the increasing use of reclaimed water for irrigation may contribute to the transport of caffeine into the ground and aquatic environments (27). Figure 3 illustrates the pathways by which caffeine enters the water environment from the point of its consumption to its different probable end-points.

Fate of Caffeine during Wastewater Treatment

Table 1 summarizes the concentration per capita (in picograms L⁻¹ cap⁻¹) of caffeine in influent and effluent of different wastewater treatment plants in different regions. The analytical methods used in the studies summarized here varied significantly. For example, caffeine was measured using gas chromatography/mass spectrometry (GC/MS) (9, 31), liquid chromatography/mass spectrometry (LC/MS) (30, 32, 33), and liquid chromatography/diode array detection (LC/DAD) (28, 29). Three studies utilized internal standards (9, 30, 33) while others (28, 29, 31, 32) did not, and the reported recoveries varied from 38% to 127%. Nevertheless, caffeine is not difficult to analyze, and taken as a whole, the values in Table 1 should provide a reasonably accurate determination of the occurrence of caffeine in WWTP influents and effluents.

The deviations between influent values in Table 1 are thought to be due to different caffeine consumption rates in these regions. Several of the plants utilize conventional treatment processes (e.g., activated sludge process), whereas in other plants, advanced treatment technologies are also adopted. However, because caffeine removal is accomplished predominantly by activated sludge treatment (9, 11), it is assumed that advanced processes do not have a major impact on caffeine removal efficiency. The average concentrations per capita of caffeine in the WWTP influents and effluents are 1144 pg L⁻¹ cap⁻¹ and 67 pg L⁻¹ cap⁻¹, respectively, and the corresponding caffeine removal rate is 94% ± 14%.

The portion of caffeine that is not biodegraded and is not subject to pass-through in the effluent accumulates in the sludge which is then incinerated (15%), landfilled (30%), or used in agricultural applications (49%) based on U.S. data from 2007 (34) (Fig. 4).

Table 2 summarizes the caffeine concentration per capita in sludge in some WWTPs in different regions. The average concentrations per capita of caffeine in sewage sludge is 0.184 pg g⁻¹ cap⁻¹. Both studies summarized below determined caffeine content by LC/MS. Neither study made use of internal standards, but both reported recovery of approximately 110%.

Caffeine has an *n*-octanol water partitioning coefficient (K_{ow}) of 0.74 (10), which indicates that caffeine will partition favorably to the aqueous phase and sorption to particulate matter will not be significant. For this reason it is predicted that settling processes in the wastewater treatment plant will result in minimal caffeine removal. This prediction is confirmed by a study showing that removal of caffeine in the primary sedimentation process is on the order of just 2% (9). Because of its low K_{ow} value, another logical prediction is that partitioning

of caffeine to biosolids will be relatively low. Again, this prediction is born out by data that show the concentrations of caffeine in the sludge are three orders of magnitude lower than the dissolved concentrations (35). Although the concentration of caffeine in biosolids is relatively low, the considerable quantities of biosolids generated each year (7.2 million dry tons of biosolids in the U.S. (34)) make them a potentially relevant source of caffeine entering the environment.

Table 1. Available information on population adjusted inputs of caffeine to wastewater treatment plants in Europe

<i>Region</i>	<i>Population</i>	<i>C_{inf}</i> (μgL^{-1})	<i>C_{eff}</i> (μgL^{-1})	<i>C_{inf}</i> ($\text{pgL}^{-1}\text{cap}^{-1}$)	<i>C_{eff}</i> ($\text{pgL}^{-1}\text{cap}^{-1}$)	<i>% Removal</i>
Switz. (9)	2.03E+04	40	4.77	1975.3	235.3	88.1
Spain, North ^a . (28)	3.50E+05	4.31	1.09	12.3	3.1	74.8
Spain, South ^a . (28)	9.50E+05	3.34	1.86	3.5	2	44.5
Spain, East ^a . (28)	2.00E+05	6.1	1.51	30.5	7.6	75.2
Spain, West ^a . (28)	2.00E+05	4.74	1.58	23.7	7.9	66.7
Spain, Seville, Aug. ^b . (29)	1.52E+06	2.7	0.56	1.8	0.4	79.3
Spain, Seville, Nov. ^b . (29)	1.52E+06	6.8	2.3	4.5	1.5	66.2
Spain, NE (30)	2.50E+06	104.85	21.77	41.9	8.7	79.2
Spain, South (31)	6.20E+04	122	22.7	1967.7	366.1	81.4
Spain, NE, Catalonia1 (32)	9.40E+04	20.54	0.66	218.5	3.5	98.4
Spain, NE, Catalonia2 (32)	4.60E+04	12.31	1.01	267.6	11	95.9
Switz., Gossau ^c . (9)	1.10E+04	37	0.12	3363.6	11.2	99.7
Switz., Uster ^c . (9)	3.60E+04	42	0.07	1166.7	2.1	99.8
Switz., Pfaffikon ^c . (9)	9.20E+03	48	0.36	5217.4	38.6	99.3
Switz., Wetzikon ^c . (9)	1.90E+04	21	0.05	1105.3	2.6	99.8
Switz., Maur ^c . (9)	4.49E+03	24	0.11	5345.2	25.2	99.5
Switz., Bubikon-Durten ^c . (9)	5.65E+03	14	0.05	2477.9	8.8	99.6
Switz., Knonau ^c . (9)	5.00E+03	9	0.05	1800	10.8	99.4
Switz., Mannedorf ^c . (9)	9.04E+03	73	0.06	8071.6	6.2	99.9
Switz., Mannedorf ^c . (9)	9.04E+03	29	0.09	3206.5	10.2	99.7
Switz., Kusnacht ^c . (9)	1.65E+04	42	0.04	2549.3	2.5	99.9

Continued on next page.

Table 1. (Continued). Available information on population adjusted inputs of caffeine to wastewater treatment plants in Europe

<i>Region</i>	<i>Population</i>	<i>C_{inf}</i> (μgL^{-1})	<i>C_{eff}</i> (μgL^{-1})	<i>C_{inf}</i> ($\text{pgL}^{-1}\text{cap}^{-1}$)	<i>C_{eff}</i> ($\text{pgL}^{-1}\text{cap}^{-1}$)	<i>% Removal</i>
Switz., Kusnacht ^c (9)	1.65E+04	7	0.03	424.9	1.7	99.6
Switz., Horgen ^c (9)	2.06E+04	19	0.5	924.1	24.4	97.4
Switz., Meilen ^c (9)	2.20E+04	50	9.48	2272.7	430.9	81
Switz., Meilen ^c (9)	2.20E+04	30	1.24	1363.6	56.4	95.9
Switz., Thalwil ^c (9)	1.95E+04	33	0.12	1688.2	6	99.6
Switz., Wädenswil ^c (9)	1.90E+04	26	2.36	1368.4	124.2	90.9
Germany ^d . (33)	3.12E+05	147	1.05	471.2	0.3	99.9
Average		34.95	2.70	1144.2	66.9	94.2
Max.		147.00	22.70	8071.60	430.90	99.90
Min.		2.70	0.03	1.80	0.30	44.50
STD.		36.43	5.85	1941.57	109.94	14.18
Median		25	0.61	1265.15	8.3	97.9

^a Treatment processes: primary, secondary biological, sometimes tertiary. ^b Treatment processes: conventional treatments with primary settling (settling and flotation) and biological processing (activated sludge). ^c Treatment processes: mechanical, biological (activated sludge, mostly with nitrification and partially with denitrification), and chemical treatment (with phosphate precipitation by iron salts, without chlorination) and, in most cases, subsequent sand filtration. ^d Treatment processes: preliminary clarification followed by an aeration tank with addition of Fe(II)chloride for phosphate elimination and a final end point clarification.

Table 2. Available information on population adjusted inputs of caffeine to sewage sludge in different regions of the world

<i>Region</i>	<i>Population</i>	<i>Caffeine Input</i> ($\text{pg g}^{-1}\text{cap}^{-1}$)
Japan (35)	1.14E+06	0.091
Spain, Zaragoza (36)	1.40E+05	0.407
Spain, Zaragoza (36)	1.40E+05	0.414
Average		0.304
STD.		0.184

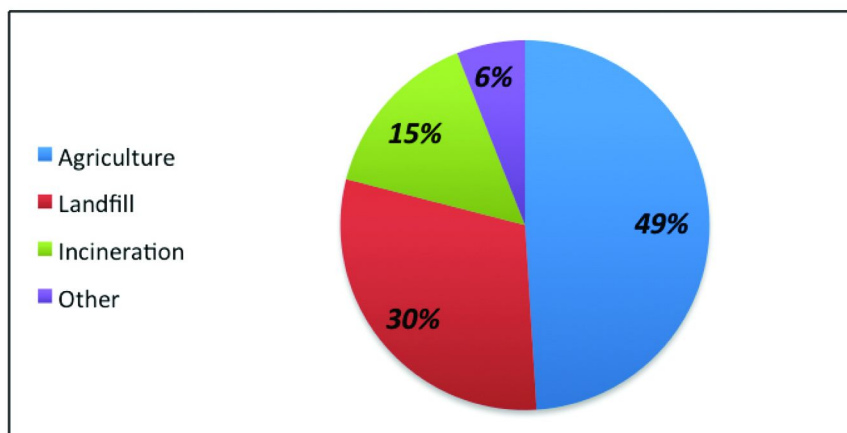


Figure 4. Disposal routes of municipal sludge produced in U.S. wastewater treatment plants, 2007 (34).

Caffeine in the Aquatic Environment

Once caffeine enters the aquatic environment through any of the routes discussed above, there are a number of processes that can affect its fate. Two major examples are biodegradation and photolysis. It has been shown in lab experiments that photolysis can be a significant mechanism of caffeine degradation (1, 9). Laboratory persistence experiments conducted by Lam et al. demonstrated similar caffeine degradation rates for sterile and non-sterile pondwater exposed to sunlight, indicating that biodegradation was negligible. Conversely, Buerge et al. concluded that biodegradation is likely to play a more important role due to the limited penetration depth of light in natural waters. Results of field microcosm experiments yielded half-life values of caffeine in the environment ranging from 0.8 to 2.3 days, with a mean of 1.5 days (1). Caffeine is as rapidly released as it is degraded, and in areas of continuous release such as downstream of sewage treatment plants, environmental concentrations can be expected to reach a narrow steady-state range, thereby creating conditions for an apparent “pseudo-persistence” (1). As a result of the balance between rapid release and rapid dissipation, a variety of studies have shown that measurable quantities of caffeine are present in different surface waters. Table 3 lists the results of several of these studies. The values in the table show that caffeine concentrations range from 3 to 100 ng/L and in some cases values of up to 800 ng/L have been reported. Of the studies summarized in Table 3, five used GC/MS (3, 7, 9, 10, 16) and two used LC/MS (3, 37). Five studies (3, 7, 9, 16, 37) utilized internal standards, and reported recoveries ranged from 34% to 127%.

Table 3. Occurrences of caffeine in surface water

<i>Sample location</i>	<i>Mean Concentration (ng/L)</i>
Swiss lakes (9)	44
Swiss rivers (9)	103
Coastal Spain (9)	3
U.S. streams ^a , (3)	14 80
Baltimore streams (37)	869
North Sea (10)	7.9
Coastal North Sea (16)	34
Georgia (US) rivers (7)	25
Mississippi River (38)	30

^a The two values reported reflect two distinct analytical methods.

Modeling Results

The following example demonstrates the calculation of C_r for a town of 50,000 people that produce 70 gal d⁻¹ cap⁻¹ of sewage that is discharged into a river with a depth (D) of 2 m and width (W) of 10 m, $Q_r=350$ mgd, with a negligible background caffeine concentration ($C_0=0$).

Equations (1) and (2) developed above were used to calculate the concentration of caffeine in the effluent:

$$C_{inf} = Pop. \times 1144 \text{ pg } L^{-1} \text{ cap}^{-1} = 50,000 \text{ cap} \times 1144 \text{ pg } L^{-1} \text{ cap}^{-1} = 57.2 \text{ } \mu\text{g } L^{-1}$$

$$C_{eff} = C_{inf}(1-0.94) = 57.2 \text{ } \mu\text{g } L^{-1}(1-0.94) = 3.4 \text{ } \mu\text{g } L^{-1}$$

Equation (4) uses a mass balance approach to calculate the concentration of caffeine in the river at the effluent discharge point after complete mixing with the receiving surface water:

$$C_r = \frac{Q_r C_o + Q_{eff} C_{eff}}{(Q_r + Q_{eff})} = \frac{0 + (1.32 \times 10^7 \text{ L } d^{-1} \times 3.4 \text{ } \mu\text{g } L^{-1})}{1.32 \times 10^9 \text{ L } d^{-1} + 1.32 \times 10^7 \text{ L } d^{-1}} = 0.034 \text{ } \mu\text{g } L^{-1}$$

By assuming that caffeine degradation in the river has first-order kinetics, the caffeine concentration can be modeled as a function of time. This is demonstrated below in Equation 5 using the mean half-life for caffeine from (1).

$$1^{st}\text{-order reaction: } C_r(t) = -kt + \ln C_o$$

$$k = \frac{\ln 2}{t_{\frac{1}{2}}} = \frac{\ln 2}{1.5d} = 0.46 \text{ } d^{-1} \quad (5)$$

The rate of caffeine degradation will vary spatially and temporally due to differences in environmental factors such as incident sunlight and temperature, as well as microbial community composition. Using the minimum and maximum half-life values reported by Lam et al., the expected range of the decay constant (k) for caffeine in the river is $0.30 \text{ d}^{-1} < k < 0.87 \text{ d}^{-1}$.

Similarly, by relating the flow rate of the river to the distance travelled by the water, the caffeine concentration can be modeled as a function of distance, as shown below in Equation 6.

$$x(t) = \frac{(Q_r + Q_{\text{eff}})t}{DW} \quad (6)$$

Equations 5 and 6 can be used to calculate the time and distance required for caffeine levels in river water to fall below the analytical detection limit. For the purposes of this example, a detection limit of 5 ng L^{-1} is assumed, as was found in (7). In this case the level of caffeine in the river will reach the detection limit 4.2 days after discharge, in which time a given parcel of river water will have flowed 277 km from the point of discharge.

A key limitation of this model is the assumption that the WWTP effluent is completely mixed with receiving waters. This approach would not be suitable for discharges into coastal environments and other surface waters where mixing may be incomplete for extended periods of time.

Toxicology of Caffeine in Aquatic Environments

As the general understanding of caffeine's presence in the water environment has improved, questions about the compound's potential effects on aquatic ecosystems and organisms are slowly gaining the attention of researchers. Relatively little is known to date, although several valuable studies have been published showing that the toxicity thresholds of caffeine vary greatly among species (Table 4). For example, changes in the growth of the African clawed frog, a species that has been introduced in the United States and is currently present in over 11 states, were observed after four days of exposure to caffeine concentrations of $0.11 \text{ } \mu\text{g L}^{-1}$ and death at $0.27 \text{ } \mu\text{g L}^{-1}$. The African clawed frog is viewed as an invasive species in the United States, however, its susceptibility to caffeine indicates that native amphibian populations may also suffer as a result of caffeine exposure.

Conversely, higher caffeine toxicity thresholds have been reported for marine organisms. For instance, early research tested the effects of caffeine and three of its metabolites in seawater on sea urchin gamete development (21) and egg fertilization. The results obtained from those tests demonstrate that caffeine at concentrations greater than 0.2 g L^{-1} in sea water have an inhibitory effect in the uptake of oxygen in the urchin eggs (39, 40) resulting in retardation of metabolism and decrease in rate of division of the cell (41). Moreover, it was observed that zygotes that were exposed to caffeine and then transferred to seawater free of caffeine were not able to survive after the early stages of cell division. Other

studies on the effects of exposure of aquatic species to caffeine concentrations show adverse metabolic consequences; these results are summarized in Table 4.

Tests performed on unicellular flagellates such as green algae showed that caffeine disrupts the structure of dicytosomes, which are responsible for the processing of proteins and lipids. Inhibition of dicytosome vesiculation activity was observed after treatment with caffeine concentrations of 200 - 300 mg L⁻¹. Although cytosome disruption resulting from short-term caffeine exposure has been shown to be reversible, irreversible cellular alterations occur with longer exposure (45).

Table 4. Effects of caffeine in aquatic species

<i>Name</i>	<i>Effect</i>	<i>Caffeine ($\mu\text{g L}^{-1}$)</i>	<i>Exposure Duration</i>	<i>Media type</i>
African clawed frog (42)	Growth change	1.1E-1	4 d	Fresh water
African clawed frog (42)	Mortality	2.7E-1	4 d	Fresh water
Water flea (42)	Intoxication	1.6E+5	1 d	Fresh water
Brine shrimp (42)	Mortality	3.5E+6	1 d	Fresh water
Fathead minnow (42)	Growth change	7.0E+4	5 d	Fresh water
Fathead minnow (42)	Mortality	7.2E+5	5 d	Fresh water
Rotifer (42)	Mortality	4.7E+6	1 d	Fresh water
Hydra (43)	Cellular damage	2.0E+2	48 h	Fresh water
Coral reefs (44)	Bleaching	3.0E+4	10-40 d	Sea water
Sea Urchin eggs (39, 40)	Oxygen uptake inhibition	2.1-9.7E+5	2-3 h	Sea water
Green algae (45)	Inhibition of dicytosome vesiculation	2.0-3.0E+5	1-12 h	Sea water
Sea anemone (46)	Alteration of protein phosphorylation	4.9E+6	2.5 h	Sea water

A more recent study showed that caffeine from marine wastewater discharges acts as a stressor factor in coral bleaching (44). Coral bleaching is a process in which the algal symbionts of corals are reduced, resulting in a loss of carbon and energy supplies to the coral host. It was also reported that the inhibitory effects of caffeine in coral algae may be dependent on the type of algae, since it was observed that more resistant organisms were able to continue growing.

Previous studies on sea anemones show caffeine contributes to the problem of coral bleaching by causing the detachment of host cells from corals. This detachment occurs due to alterations that caffeine causes in the level of protein phosphorylation in anemones. Such effects are reportedly induced at ambient temperature and caffeine concentrations of 4.9 g/L (46). This concentration is orders of magnitude higher than environmentally relevant concentrations (see Table 3).

It is important to note that much of the data in Table 4 is incomplete in the sense that the duration of the toxicity assays was short in comparison to the lifetime exposure that organisms receive in the environment. This lack of longer-term toxicity data on aquatic biota is a critical data gap that needs to be filled.

Conclusions

Caffeine in the environment originates mostly from the consumption of foods, beverages, and caffeine enhanced pharmaceuticals. The major route in which caffeine enters the environment is from wastewater treatment plant discharges. Other contributing routes include landfill leachate, runoff from biosolids-amended soils, and coffee grounds added directly to the soils as fertilizers.

The removal efficiency of caffeine in wastewater treatment plants averages $94\% \pm 14\%$ and is due mainly to biodegradation. Once caffeine enters the environment, it is subject to breakdown by biodegradation and photolysis, with a half-life of 1.5 days (1). However, because caffeine is as rapidly released as it is degraded, environmental concentrations can be expected to reach a steady state level.

Several European studies show that average caffeine concentrations are 1144 pg per liter per capita for WWTP influent. Based on these data, equations were developed to model the fate and transport of caffeine produced from effluent discharged by a town of 50,000 people. The results from this modeling compare favorably to reported data.

Numerous studies have been performed on the toxic effects of caffeine in organisms. These studies show that current caffeine levels are approaching toxicity thresholds for a few aquatic organisms, while many species appear to be quite tolerant to environmental levels of caffeine. Concerns in urban areas center on discharge points of treated wastewater. Although caffeine presents no large scale threat today, further research is needed on the occurrence of caffeine in natural waters and the chronic toxicity it may exert on vulnerable aquatic organisms.

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Chapter 13

Perfluorinated Chemicals in Drinking and Environmental Waters

**Paul C. Rumsby,* Wendy F. Young, Tom Hall,
and Clare L. McLaughlin**

**National Centre for Environmental Toxicology, WRc plc, Frankland Road,
Swindon SN5 8YF, UK**

***paul.rumsby@wrplc.co.uk**

Perfluorinated chemicals (PFCs) have been used for many years as surfactants in a variety of industrial and consumer products with the main ones being perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA). However, owing to their persistent, bioaccumulative and toxic (PBT) characteristics, PFOS has been phased out by its principal producer and the use of PFOA has been severely reduced. In their place, a number of newer PFCs are being introduced and, while they appear to have a shorter persistence in humans and the environment, their toxicity is at present uncertain and further studies are required. Recent monitoring studies suggest that these other newer PFCs and previously unmonitored breakdown products, are detected in environmental waters, in some cases at levels similar to those of PFOS and PFOA. Some new data are described which indicate that PFOS, PFOA and other PFCs can be removed by granular activated carbon absorption, such as that seen in advanced drinking water treatment, under appropriate controlled conditions, although these conditions vary with different PFCs.

The data suggest that the toxicology of PFCs is complex with PFOS and PFOA having different effects at varying concentrations in different species. Cancer, developmental delays, endocrine disruption, immunotoxicity and neonatal mortality are all potential toxic endpoints. Contamination and occupational exposure have led to a number of ongoing epidemiological studies in populations exposed to high levels

of PFOS or PFOA, as well as on those with background exposure. Many of these studies are examining reproductive and developmental endpoints. The work is ongoing but at present, the results are inconsistent with only small effects, if any, being observed in populations exposed to high levels of PFOA. Monitoring suggests that, although PFOS and PFOA are persistent, controls on their manufacture and use since 2000 have led to a decrease in their presence in the human population and the environment.

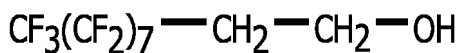
Manufacture and Usage

Perfluorooctane sulphonate (PFOS; Figure 1) perfluorooctanoic acid (PFOA; Figure 1) and related compounds are members of a large family of perfluorinated chemicals (PFCs) that have been produced for at least 50 years. These PFCs have been increasingly used as surfactants in a number of industrial and consumer products, mainly to repel dirt, water and oils (1). Their use has included performance chemicals such as photographic film, surfactant in fire-fighting foams, surfactant for alkaline cleaners, emulsifiers in floor polish, mist suppressant for metal plating baths, surfactant for etching acids for circuit boards, pesticides, and dirt repellent treatments for textiles (e.g. carpets, home furnishings and leather) and paper (e.g. food containers and masking tape).

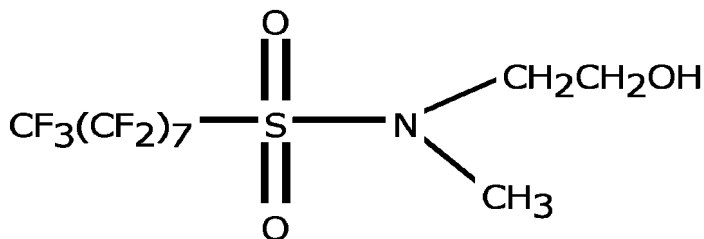
PFOS-related chemicals are manufactured from a precursor material known as perfluorooctanesulphonyl fluoride (POSF). It has been estimated that the total global production/use (from 1970-2002) has been 96 000 tonnes of POSF with total global emissions being 650-2600 tonnes of POSF and 6.5-130 tonnes of PFOS. Most of the environmental release is to water (98%) and the remainder to air (2). They are immobile in soil and are non-biodegradable in, for example, activated sewage sludge (1). PFOS is actually a degradation product of perfluorooctane sulphonamide derivatives (such as perfluoroalkyl sulphonamidoethanols; Figure 1), components of the original stain protection product Scotchguard, made by 3M (3).

In the year 2000, between 3665 and 4500 tonnes of POSF were produced globally, and 3M was the dominant producer. In that year, 3M announced that they would phase out the use of POSF after data revealed that PFCs are extremely persistent in the environment, are bioaccumulative, and pose a risk to the environment and human health. Global production and use by 3M ceased in 2001. 3M also phased out production of PFOS, PFOA and related chemicals by 2002. However, other manufacturers have filled the deficit of 300 tonnes/year production of the ammonium salt of PFOA.

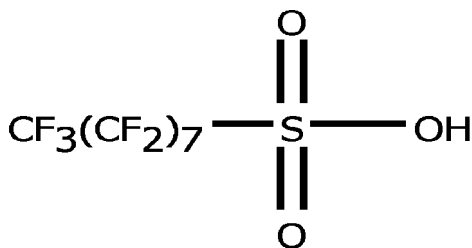
In 2006, the European Union announced severe restriction on the use of PFOS, with member states adopting national measures by June 2008. PFOS cannot be placed on the market or used as a substance or constituent of preparations in a concentration equal to or higher than 0.005% by mass, or 0.1% in semi-finished articles or preparations, or 1 $\mu\text{g}/\text{m}^2$ in textiles and coated material (4). Exemptions to this restriction include its use in anti-reflective coatings for photolithography,



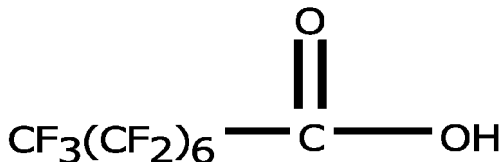
8:2 Fluorotelomer alcohol



Perfluoroalkyl sulphonamidoethanol



PFOS



PFOA

Figure 1. Structure of some PFCs

mist suppressants for non-decorative hard chromium (VI) plating and hydraulic fuels for aviation. However, any PFOS-containing fire-fighting foams in existence are still permitted for use until mid-2011.

PFOA has been a component in surfactants used in the production of DuPont's Teflon and is also a degradation product of long chain fluorotelomer alcohols (such as 8:2 fluorotelomer alcohol; Figure 1; (3)).

Persistence, Bioaccumulation and Toxicity

It has become clear in the last few years that PFCs are very persistent in the environment. The chemical nature of fluorine means that the carbon-fluoride bond is the strongest found in nature, making fluorinated compounds resistant to chemical and biochemical reactions. Fluorinated compounds are stable against many degradation processes found in the environment such as hydrolysis, photolysis, acid and basic attacks, oxidising and reducing agents, and biodegradation (5).

PFCs have been shown to bioaccumulate in animals, including humans. Traces of PFCs are found in blood (where they bind to serum proteins) and organs such as liver and kidneys, as well as in muscle tissue. This is in contrast to other persistent organic pollutants (e.g. polychlorinated biphenols), which are lipophilic and tend to accumulate in the fatty tissues. PFCs have been detected globally in the environment, having been found in polar bears in Greenland, giant pandas in China and albatrosses in the middle of the Pacific Ocean (6). This suggests atmospheric transport of these compounds, although their potential to volatilise is low. However, PFOS and PFOA seem more prevalent in the more industrial areas such as the Baltic Sea, The Mediterranean, the Great Lakes and along Asian coasts (5).

There is some evidence that in humans, blood concentrations of PFOS and, to a lesser extent, PFOA, have declined from 2000. Olsen *et al.* (7) using plasma samples from Minnesota American Red Cross blood donors have shown, in a small sample, that PFOS levels have decreased from 2000 (33.1 ng/l geometric mean) to 2005 (15.1 ng/ml). The geometric mean for PFOA was 4.5 ng/ml in 2000 and 2.2 ng/ml in 2005. The decline in PFOS and PFOA levels may be due to the phase-out of POSF by the principal global manufacturer, 3M, in 2000-2002 and the reduction in the use of PFOA.

Although the acute toxicity of PFCs is moderate, its persistence in the body (average half-life for PFOA in humans of 3.5 years and up to 8.7 years has been determined in retired production workers) has led to increasing concerns over long-term effects. The toxicity of PFOS and PFOA is not clearly understood at present (reviewed in (6, 8)). Different animal species appear to have different sensitivities to these compounds which make interpretation of experiments difficult, (e.g. Rhesus monkeys are more sensitive to PFOS than rats, while mice are the least sensitive). The species variability may be due to the different handling of these compounds in the body. At present, it is unclear whether PFOS and PFOA act by the same mechanisms, and high and low doses may differ in their toxic endpoints and effects. High dose studies on animals have indicated that cancer, developmental delays, endocrine disruption, immunotoxicity and neonatal mortality are potential toxic endpoints. Recent research has also suggested that receptor binding may be an important general mechanism. PFOS and PFOA both

bind to peroxisomal proliferator-activated receptor (PPAR). Activation of such receptors may alter fatty acid metabolism and play a role in cancer, foetal growth, and hormone and immune functions (reviewed in (6, 8, 9)).

There have recently been a number of human studies published looking at toxicological endpoints in populations exposed to both background and increased levels of PFCs, particularly of PFOA. The increased exposure is mainly in workers involved in the manufacture of PFCs and neighbouring populations to plants where contamination incidents have occurred (see below).

Contamination Incidents

The presence of PFCs and in particular PFOS and PFOA in environmental waters has been the subject of much interest in recent years. Important information has emerged following contamination incidents: in the USA, Germany and the UK.

USA

Little Hocking, West Virginia

PFOA has been detected in the drinking water supply of Little Hocking near Washington, West Virginia, USA (10, 11). This village is across the Ohio River from and downwind of the Dupont fluoropolymer manufacturing facility. The extent of exposure to residents of the village was assessed by questionnaire and measuring PFOA in blood samples. Levels of PFOA in drinking water averaged 3.55 µg/l during 2002-2005. The blood PFOA levels were 60-75 times higher than in the general population. Serum PFOA was particularly high in individuals who consumed more home-grown fruit and vegetables. It is unclear whether PFOA was present in the produce itself or in water used for cooking.

A further study on the exposure of residents living near this facility (11) used historical emission records for 52 years to estimate the potential intake of 50 000 residents. PFOA detected in groundwater was deemed to have originated by particulate deposition from air emissions to the soil and then transfer to the water. Maximum concentrations were estimated to occur at up to one mile from the site, with maximum air, surface soil and drinking water levels estimated to be 200 ng/m³, 11 µg/kg and 4 µg/l, respectively.

An independent team of scientists, C8 Assessment of Toxicity Team (CATT), has been assembled to conduct human health and ecological risk assessments and communicate health risk information to the public. Their preliminary risk assessment concluded that average daily intakes of PFOA within 5 miles of the plant over a 50 year time span was 10 000 less than an intake that was not considered a risk to human health (11).

The contaminated population in this area has been further assessed in a number of ongoing epidemiological studies, the results of some of which have recently been published.

Nolan *et al.* (12) compared birth weights and gestational ages of neonates born to mothers residing in zip codes with water supplies provided completely, partially or not at all by the Little Hocking Water Association (LHWA). The incidence of low birth weight, preterm birth, mean birth weight and mean gestational age of neonates did not significantly differ among water service categories. The authors concluded that markedly elevated PFOA exposure was not associated with any increased risk of lowered birth weight or gestational age.

Stein *et al.* (13) examined the association of serum PFOA and PFOS with self-reported pregnancy outcome in a similar population highly exposed to PFOA. Data on birth outcomes were compared to serum PFOA and PFOS levels for 1845 and 5262 pregnancies, respectively. Neither serum PFOA nor PFOS showed any association with miscarriage or preterm birth. There was a modest association of serum PFOA with preeclampsia and birth defects, and of serum PFOS with preeclampsia and low birth weight. However, associations were small, limited in precision, and based solely on self-reported health outcomes and so, while there was an association, no firm conclusion could be drawn.

Nolan *et al.* (14) examined the associations between PFOA exposure, congenital anomalies, labour and delivery complications, and maternal risk factors in neonates and their mothers exposed to PFOA-contaminated residential drinking water from the Little Hocking Water Association (LHWA). Increased PFOA exposure was not associated with an overall increase in the likelihood of congenital anomalies nor any specific diagnosis and delivery complications and maternal risk factors.

Therefore it appears that any effects on reproduction and development from increased exposure to PFOA are very small. Olsen *et al.* (15) recently qualitatively reviewed the published epidemiologic literature as to the potential association of exposure to PFOS and PFOA with human foetal development. The published research has focused on birth weight and other measurements that reflect human foetal development. A total of eight epidemiologic studies were reviewed that focused on six general (non-occupational) and two occupational populations. Of the six general population studies, five examined associations between birth weight and other anthropometric measurements in relation to maternal blood and/or umbilical cord concentrations of PFOS and PFOA. In the sixth study, three geographical areas in Washington County, Ohio (Little Hocking), were categorized by their public drinking water sources that contained PFOA and which had resulted in higher serum concentrations than observed in other general population studies. The occupational studies focused on another perfluorochemical manufacturing site (Decatur, Alabama, see below), with exposure categorized from work history and biomonitoring data. There were inconsistent associations reported for several different birth outcomes, including birth weight, birth length, head circumference, and ponderal index, among the five general population studies that measured PFOS and PFOA in the study subjects (16, 17). No association with birth weight or gestational age was reported in the community drinking water study (see above; (12)). Only one general population study examined infant Apgar scores and developmental milestones at 6 and 18 months of age, with no associations reported. No association with self-reported

birth weight and occupational exposure to PFOS materials was observed among female perfluorochemical production workers.

There have also been two Danish epidemiological studies (18, 19) investigating the association between plasma levels of PFOS and PFOA in pregnant women and motor and mental developmental milestones of their children and time to pregnancy (fecundity). The authors found no convincing associations between developmental milestones in early childhood and levels of PFOA or PFOS as measured in maternal plasma early in pregnancy, but did find some reduction in fecundity at levels of plasma PFOS and PFOA seen in the general population in developed countries. There has also been a further small Danish study which found that high serum PFOS and PFOA levels were associated with fewer normal sperm, although the authors indicated that their findings would need to be corroborated in larger studies (20).

There is a further ongoing study in residents of Little Hocking (21). It is investigating the relationship between serum PFOA and PFOS concentrations and total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), the ratio of total cholesterol to HDL, and triglycerides. In multivariate models adjusting for other factors, all lipid outcomes except HDL were higher when serum PFOA and PFOS were higher. The odds ratio for high cholesterol (defined by ≥ 240 mg/dl), by increasing quartile of serum PFOA concentration, were 1, 1.21, 1.33 and 1.4, respectively. The corresponding adjusted odds ratio by quartile of PFOS, were 1, 1.13, 1.28 and 1.51, respectively. These odd ratios indicated that those with serum levels in the top 25% of the two chemicals had a 40-50% increased risk of having high cholesterol compared to those in the lowest 25%. The C8 Science Panel will only assess if a probable link to the disease exists when all the relevant data have been collected and analysed, which is expected to be completed in 2011.

A recent paper, using data from the US Health and Nutrition Examination Survey (NHANES), found an association between the highest levels of PFOS and PFOA in the general population and current thyroid disease (22). Effects on the thyroid have been observed in animal studies on PFOS and PFOA. This endpoint is also being examined in the Little Hocking population exposed to much higher concentrations of PFOA and the results of this study will clarify this finding.

Cottage Grove, Minnesota

3M produced PFCs at a site in Cottage Grove, Minnesota, from the late 1940s until 2002 (23). During this time, there were air emissions of PFCs, waste from production was deposited in an on-site pit, and wastewater treatment plant effluent containing PFCs was discharged into the nearby Mississippi River. There was also a fire-training area on site where PFC-containing foams were used. Monitoring indicates that groundwater beneath the site was contaminated with PFOS and PFOA at significant levels (PFOS and PFOA concentrations up to 120 $\mu\text{g/l}$ and 105 $\mu\text{g/l}$, respectively). Much of this contaminated groundwater was then processed through the wastewater plant on site which was unable to remove PFCs. However, more recently (approximately 2004 onwards), the addition of a

large granular activated carbon (GAC) system has eliminated PFC discharge into the Mississippi River.

Decatur, Alabama

A further 3M PFC production plant is situated in Decatur, Alabama. Monitoring of the nearby Tennessee River upstream and downstream of this plant showed PFOS present throughout the 80-mile stretch of the river studied, with levels of 32+/-11 ng/l upstream of the facility and 114+/-19 ng/l downstream. Concentrations of PFOA were below the level of detection upstream, but 394+/-128 ng/l downstream (24)(Hansen *et al.*, 2004). Workers at this plant have also been the subject of epidemiological studies on reproductive and developmental outcomes (reviewed by Olsen *et al.* (15); see above).

Germany

In the summer of 2006, 12 perfluorinated surfactants were sampled in various surface water and drinking waters in Germany (25). Surface water sampled included the rivers Rhine, Ruhr and Moehne as well as some of their tributaries, whilst the drinking water samples were from public buildings in the Rhine-Ruhr area. The sum of the seven compounds most frequently found in the River Rhine and its tributaries was <100 ng/l. The highest concentrations of the 12 PFCs detected were in the Ruhr River at 94 ng/l, with PFOA being the major component. However, samples from the rivers Ruhr and Moehne (a tributary of the Ruhr) showed high (446 and 4385 ng/l, respectively) concentrations in their upper reaches. By tracking these high concentrations, the main source of contamination was localised to an area of agricultural land near Brilon-Scharfenberg in the Sauerland region. The main source of the substantial environmental PFC contamination was concluded to be industrial waste with high concentrations of PFCs that was applied as a 'soil improver' on agricultural land. It is estimated that the contamination lasted for several years.

The maximum drinking water concentration of the total PFCs detected in Arnberg Heheim in this area was 598 ng/l, with PFOA being the most prevalent compound. Drinking water concentrations were comparable to those found in surface water, suggesting that these compounds were not being significantly removed by water treatment, although approximately 50% of the water works on the Ruhr River were equipped with GAC. A further study was conducted on the blood plasma levels of PFCs in adult and children residents of Arnberg Heheim who were exposed to PFC-contaminated drinking water. PFOA was the major PFC in blood with concentrations being 4- to 8-fold higher than non-exposed controls (26).

A previous study conducted in Germany in 2004 indicates that the normal background levels of PFCs in environmental waters are in the low ng/l range (5).

Hölzer *et al.* (27) have reported on a subsequent follow-up study in order to determine the decline of the PFOA concentrations in blood plasma. The (geometric) mean PFOA concentrations in blood plasma of Arnberg's residents

decreased from 22.1 to 17.4 $\mu\text{g/l}$ in children, from 23.4 to 18.8 $\mu\text{g/l}$ in mothers and from 25.3 to 23.4 $\mu\text{g/l}$ in men within one year. The average (geometric mean) changes in each individual's PFOA-concentrations were reported as approximately 10 (men), 17 (mothers) and 20 (children) percent/year. The observed decline in PFOA concentrations indicated a slow elimination in humans. This finding in groups of the general population is in agreement with data on long elimination half-lives observed in occupationally exposed workers.

United Kingdom and the Channel Islands

In December 2005, there was an explosion at the Buncefield Oil Depot, north of London. This was the largest post-war explosion in Europe. The use of PFOS-containing fire-fighting foams led to the widespread contamination of the local aquifer and surface waters. In the months following this event, there was continuous monitoring of local waters for PFOS. Boreholes (except for one deep borehole) around the Buncefield site remained contaminated after sampling in June 2006. In general, the surface water sources (the local rivers) remained uncontaminated (levels below 1 $\mu\text{g/l}$) with just a few samples above this. However, there were a number of groundwater sites around Buncefield and in the surrounding area which showed raised levels of PFOS (up to 5.91 $\mu\text{g/l}$), starting in April 2006 and continuing until June 2006. PFOS was not detected in drinking water samples. However, at this time, the methodology used had a limit of detection (LOD) of 1 $\mu\text{g/l}$ dropping to 0.1 $\mu\text{g/l}$ after about April 2006 (reported in Atkinson *et al.* (28)). This relatively high limit of detection reflects the fact that there was no standard method of detection already developed and available in the UK when the Buncefield incident occurred. This and the large number of samples generated meant that a number of laboratories had to rapidly develop at least some screening technique for detecting PFOS in water. This contrasts with the low LODs (ng/l) which have been developed for the more recent planned research monitoring exercises described in Rumsby *et al.* (29).

During June 2006, there was an accidental release of stored contaminated surface water (held following the clean-up of the Buncefield site) from a sewage treatment work into a local river. Monitoring at the nearest downstream water abstraction point some days later, detected PFOS for two days (0.9 and 0.8 $\mu\text{g/l}$), but the concentrations then returned to below the limit of detection (<0.1 $\mu\text{g/l}$).

In the 1990s, foam used by practising fire-fighters at the airport on the island of Jersey, one of the Channel Islands, off the coast of France, polluted the surrounding area. It seriously contaminated local land and boreholes with PFOS that can still be detected at 10+ $\mu\text{g/l}$ levels in certain boreholes, ditches and ponds in the area, confirming the persistence of PFOS in the environment. Of 9 seriously contaminated sites, 4 had shown some evidence of decreased PFOS concentrations (reported in Atkinson *et al.* (28)). Drinking water obtained from boreholes was also seriously contaminated (the highest PFOS level measured in one property was 98 $\mu\text{g/l}$, with other samples mainly over 20 $\mu\text{g/l}$; most other properties had levels below 1 $\mu\text{g/l}$) and affected residents now have mains water or are supplied with bottled water.

Drinking Water Guidelines

In the light of the pollution incidents, a number of authoritative bodies have issued guidance, based on human health effects, for PFOS and PFOA if found in drinking water.

United Kingdom

In 2007, the Drinking Water Inspectorate of England and Wales issued guidance for PFOS and PFOA (30). After consideration of further toxicological data, the values were revised in 2009 based on a 4-tiered system, and the values for PFOA were lowered (the current values for PFOS and PFOA are shown Table 1; (31)). Action was initiated at concentration of 0.3 µg/l and above.

Following the use of PFOS-containing foams on the fire at the Buncefield Oil Depot, the Environment Agency for England and Wales and the Department for Communities and Local Government issued a circular to the fire-fighting authorities entitled 'Guidance on the phasing out of PFOS based foams for Class B fires'. This document sought a voluntary ban on the future use of PFOS based foams by the UK fire and rescue service, and highlighted ways in which emissions to the environment can be prevented, or if this was not possible minimised, during any further use of such foams (32).

Germany

In June 2006, following the high PFOA contamination of drinking water in Arnsberg Heheim, the Drinking Water Commission of the German Ministry of Health at the Federal Environment Agency issued guidance on maximum values for combined PFOA and PFOS concentrations in drinking water (33). This guidance is outlined in Table 2. In some ways, these values are similar to the UK values for a single PFOS contamination event. However, for action on PFOA contamination, the German combined value is more precautionary.

USA

For US States where contamination around production plants has been identified, a site specific action level of 0.5 µg/l (500 ng/l) has been set for West Virginia, where the Dupont Little Hocking plant is situated. The same value is set for a safe level for PFOA in drinking water in Minnesota, where the 3M Cottage Grove production plant is situated (34).

After detection of PFOA in two New Jersey (NJ) public water systems at concentrations of up to 190 ng/l, a monitoring study of PFOA in 23 other NJ public water systems was conducted in 2006 (35). PFOA was detected in 65% of the systems at concentrations ranging from 5-39 ng/l. The contribution of drinking water to human exposure to PFOA was evaluated, and a health-based drinking water concentration protective for lifetime exposure of 40 ng/l derived. The exposure assessment was based on a 100:1 ratio between the concentration of PFOA detected in serum and that in drinking water in a community with highly

Table 1. Drinking Water Inspectorate (DWI) Guidance Levels for PFOS and PFOA (30, 31)

PFOS			
Tier 1	Regulation 27 (Risk assessment)	Potential hazard	Ensure considered as part of statutory risk assessment
Tier 2	Regulation 10 (Sampling: further provisions)	>0.3 µg/l	Consult with local health professionals; monitor levels in drinking water
Tier 3	Regulation 4(2) (Wholesomeness)	>1.0 µg/l	As tier 2 plus: put in place measures to reduce concentrations to below 1.0 µg/l as soon as is practicable
Tier 4*	Water Industry (Suppliers' Information Direction) 2009 (notification of events)	>9.0 µg/l	As tier 3 plus: ensure consultation with local health professionals takes place as soon as possible ; take action to reduce exposure from drinking water within 7 days

* Note – notification to the Inspectorate under the Information Direction may also be triggered at lower levels due to Tier 1,2 or 3 activities

PFOA			
Tier 1	Regulation 27 (Risk assessment)	Potential hazard	Ensure considered as part of statutory risk assessment
Tier 2	Regulation 10 (Sampling: further provisions)	>0.3 µg/l	Consult with local health professionals; monitor levels in drinking water
Tier 3	Regulation 4(2) (Wholesomeness)	>5.0 µg/l	As tier 2 plus: put in place measures to reduce concentrations to below 5.0 µg/l as soon as is practicable
Tier 4*	Water Industry (Suppliers' Information Direction) 2009 (notification of events)	>45.0 µg/l	As tier 3 plus: ensure consultation with local health professionals takes place as soon as possible ; take action to reduce exposure from drinking water within 7 days

* Note – notification to the Inspectorate under the Information Direction may also be triggered at lower levels due to Tier 1,2 or 3 activities

Table 2. German Maximum Value Guidance for Combined PFOA and PFOS concentrations in drinking water (33)

<i>Type of Maximum value</i>	<i>Abbreviation</i>	<i>Value (µg/l)</i>
Health-based precautionary value (long-term minimum quality goal) for non-genotoxic substances	HPV ₁	0.1
Strictly health-based guidance value for safe lifelong exposure for all population groups	GV	0.3
Precautionary action value for infants	PAV _i	0.5
Precautionary action value for adults	PAV _o	5.0

contaminated drinking water (Little Hocking). The applicability of this ratio to lower drinking water concentrations was confirmed using data on serum levels and water concentrations from other communities. The health-based concentration of 40 ng/l was derived using the US Environmental Protection Agency (EPA) PFOA draft risk assessment and newer information. In additional sampling of 18 NJ

public water systems in the period 2007-2008, PFOA ranged between <10 to 140 ng/l.

In 2009, US EPA issued provisional advisory health limits for levels of the perfluorinated pollutants PFOS and PFOA in drinking water of 0.2 and 0.4 $\mu\text{g/l}$, respectively, which are designed for protection against short-term exposure for a 10 kg child drinking 1 litre of water/day (36).

Tardiff *et al.* (9) recently derived a drinking water equivalent level (DWEL) for PFOA based on extensive relevant information from human and laboratory animal studies. It was reported that PFOA has been identified at 3.5 $\mu\text{g/l}$ (mean) in tap water in proximity to a manufacturing facility (Little Hocking), although in most water supplies, the levels were below 7.5 ng/l (usual limit of detection). PFOA has an average half-life in humans of 3.5 years, and from animal studies, PFOA is considered a possible liver toxin and developmental toxicant for humans. Based on two chronic animal studies, PFOA was judged to be a possible human carcinogen, whose mode-of-action was likely to be related to receptor activation not genotoxicity. The Benchmark Dose-Uncertainty Factor approach was selected for dose-response for non-cancer and cancer endpoints. Based on an internal dose of PFOA, the DWEL protective against cancer was 7.7 $\mu\text{g/l}$, and the non-cancer DWELs ranged from 0.88 to 2.4 $\mu\text{g/l}$ (based on different species and endpoints). The authors suggest that these DWELs can be considered a reliable, albeit conservative, basis to set a Maximum Concentration Level Goal under the US Safe Drinking Water Act.

Concentrations of PFOS and PFOA in Environmental Waters

Following the contamination incidents and the heightened interest in PFCs and their PBT, there has been recent global interest in monitoring of environmental water for the presence of PFOS and PFOA, in particular. We have previously published a review of PFOS and PFOA which included substantial details of the concentrations of PFOS and PFOA detected in rivers, lakes and in drinking water (29). In this review, focus is given to the recent monitoring studies on PFCs other than PFOS and PFOA.

Earlier data with higher Limits of Detection (LOD; around 100 ng/l) appeared to be less reliable and often gave levels in 10s $\mu\text{g/l}$ particularly in the United Kingdom where the methodology was quickly developed following the Buncefield Oil Depot fire in 2005. Later UK monitoring gave similar results to other parts of the world (37). Earlier analysis was based on Liquid Chromatography and Mass Spectrometry while most modern methods employ Liquid Chromatography and Tandem Mass Spectrometry operated in negative ion electrospray mode.

The recent studies have LODs several orders of magnitude lower, with some reporting levels <1 ng/l. Data from many countries including USA, Japan, Italy, South Korea, Germany, UK and China, indicate that levels in environmental waters are generally below the low 100s ng/l for both PFOS and PFOA, with levels in drinking water about ten times lower (for details see (29)). These levels are below the health-based values derived by countries such as USA, Germany and the UK.

Higher levels are usually seen with identified contamination incidents such as those outlined earlier in the review.

More recent studies have included the measurement of a wide range of other PFCs besides PFOS and PFOA. These have included:

Perfluorooctane sulphonamide

1H,1H,2H,2H-Perfluorooctane sulphonic acid

N-ethyl perfluorooctane sulfonamidoacetate

Different chain lengths of perfluorosulphonates: ethane, butane, propane and hexane

Different chain lengths of perfluoroacids: propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, nonanoic acid, decanoic acid, undecanoic acid, dodecanoic acid, tridecanoic acid, tetradecanoic acid.

Mak *et al.* (38) recently reported on a monitoring study which involved analysis of 20 PFCs in tap water collected in nineteen cities in China, Japan, India, the USA, and Canada between 2006 and 2008. The water samples from Shanghai (China) contained the greatest concentrations of total PFCs (mean value 130 ng/l), whereas those from Toyama (Japan) contained only 0.62 ng/l. In addition to PFOS and PFOA, short-chain PFCs such as perfluorohexane sulphonate, perfluorobutane sulphonate, perfluorohexanoic acid and perfluorobutanoic acid were found to be prevalent in drinking water.

Murakami *et al.* (39) recently reported on a monitoring study on PFCs in 20 river samples and 5 wastewater secondary effluent samples in Japan. PFCs were detected in all rivers and, in particular, 11 out of 20 river samples exceeded 40 ng/l for PFOA. PFOS, perfluoroheptanoic acid (PFHpA), PFOA, and perfluorononanoic acid (PFNA) were major PFCs in Japanese rivers, with concentrations generally being correlated with population density, suggesting that the chemicals were derived from urban activities. The authors using crotamiton, a marker of sewage effluent, concluded that PFOS, PFHpA, and PFNA in rivers were derived from sewage effluent. On the other hand, PFOA was found at high levels (54-192 ng/l) in seven river samples containing low levels of crotamiton, suggesting that it was derived from non-sewage point sources, as well as sewage effluent.

Ericson *et al.* (40) studied the concentrations of 13 PFCs in municipal drinking water samples collected at 40 different locations from five different zones of Catalonia, Spain. The most frequent compounds were PFOS (mean concentration 3.7 ng/l; maximum 58.1 ng/l), perfluorohexane sulphonate (0.6 ng/l; 5.3 ng/l), perfluorobutane sulphonate (4.5 ng/l; 69.4 ng/l), perfluorohexanoic acid (1.5 ng/l; 8.6 ng/l), and PFOA (4.6 ng/l; 57.4 ng/l). From this it can be seen that a number of these other PFCs may be present at levels similar to PFOS and PFOA.

The environmental prevalence of a new class of PFCs, the perfluorinated phosphonic acids (PFPAs), was recently determined in Canadian surface waters and wastewater treatment plant (WWTP) effluent (41). Three PFPa congeners were monitored (6, 8 or 10 PFPa). PFPAs were observed in 24 of the 30

surface water sites investigated and in six of the seven WWTP effluent samples. Concentrations of PFPAs in Canadian surface waters were in the pg/l to low ng/l range with the C8 PFPAs dominating the concentration profile at all sites. The authors report that whilst this is the first observation of PFPAs in the environment, the finding that they were present in the majority of samples analysed suggests they are prevalent environmental contaminants at very low concentrations. Given their structural similarities with PFOS and PFOA they might be expected to be persistent.

In conclusion, PFOS and PFOA have been widely found in environmental waters globally at concentrations below those considered to be a concern for human health if found in drinking water, although there is evidence of their persistence. Other PFCs have now been detected in environmental waters in a number of countries, particularly perfluorohexyl compounds and perfluorobutane sulphonate. The presence of the latter may reflect the presence of perfluorobutane sulphonamide in the new formula of Scotchguard since 2003 (3). Perfluorobutane sulphonate is the ultimate degradation product of this compound.

Removal of PFCs by Drinking Water Treatment Processes

With increasing detection of PFCs in environmental waters and concerns over their possible health effects, the potential for these compounds to be found in drinking water became important and its likelihood needed to be addressed. There is some conflicting evidence from the monitoring studies on the ability of drinking water treatment processes to remove PFCs.

A study of Lake Maggiore in Italy gave similar very low levels of PFOS (3 ng/l) and PFOA (9 ng/l) in both lake and drinking water abstracted from the lake and the authors concluded that the basic treatment used (slow sand filtration and chlorination) was ineffective in removing these chemicals (42).

The use of reverse osmosis membranes to remove PFOS from wastewater has been assessed by Tang *et al.* (43). PFOS at feed concentrations ranging from 0.5-1500 mg/l was removed by 90% or more. In a further study, Tang *et al.* (44) investigated the use of reverse osmosis (RO) and nanofiltration (NF) to remove PFOS from wastewater. A feed concentration of 10 mg/l over 4 days was employed with 5 RO membranes and 3 NF membranes. The rejection of PFOS was concurrent with that of sodium chloride. Greater than 99% and 90-99% removal was achieved with RO and NF membranes, respectively. However, this treatment is quite advanced and would not be routinely used except where a particular contamination problem had been identified.

The use of granular activated carbon (GAC) to treat water used for drinking is more widespread, although still only common where specific contaminants have been detected. The effectiveness of GAC in removing PFCs has been studied where contamination incidents have occurred. The structure of PFCs would suggest that they would not be well-adsorbed by GAC. However, batch GAC tests gave much better adsorption than might be expected (WRc, unpublished observations).

The installation of GAC to the wastewater treatment system of the PFC production facility in Minnesota, USA, successfully removed PFCs from the effluent discharged into the Mississippi River (23).

Following the contamination in the Sauerland region of Germany (see Contamination Incidents section), drinking water concentrations detected were comparable to those in surface water, which suggested that PFOA was not being significantly removed by treatment, although approximately 50% of the Ruhr river water works were equipped with GAC (25). However, a further publication described a study on the Mohnenberg waterworks in this region, drinking water from which gave the highest level of PFOA (519 ng/l). This works installed GAC filters in June 2006 (33). This treatment proved to be effective at removing PFOA with levels decreasing from 0.9 µg/l to below the level of detection (0.01 µg/l). However, a steady increase occurred after about 3 months with levels exceeding 0.1 µg/l after about 5 months. Exchange of filters and reactivation of the carbon resulted in effective removal of PFOA again. PFOS levels were below the level of detection (<0.01 µg/l) except for two occasions (0.034 and 0.025 µg/l).

In December 2006, Anglian Water, the local water company in the East Anglia region of the UK, was made aware of the use of fire-fighting foam containing PFOS and other PFCs at an airbase in the region. Upon sampling at water treatment works adjacent to this airbase, it was discovered that raw water contained 3.7 µg PFOS/l with lower concentrations of other PFCs. The water treatment works had five F400 GAC absorbers. The water is blended with other groundwater sources before being delivered to approximately 57 000 people. At the time of the incident, the site was designed to give an Empty Bed Contact Time (EBCT) of the water with the GAC of 30 minutes, with a regeneration frequency of 24 months. Besides PFOS and PFOA, other PFCs were also detected:

Perfluorobutane sulphonate (PFBS)
Perfluorohexane sulphonate (PFHS)
Perfluorohexanoic acid (PFCA6)
6:2 Fluorotelomer sulphonate (6:2 FTS)

With dilution and blending with water from other sources, the final concentration of PFOS in drinking water was never above the lowest guideline value (0.3 µg/l) set by the UK Drinking Water Inspectorate (Table 1).

The level of PFOS in the raw water has remained at approximately the same level since the incident in December 2006 (ranging from 1.7-3.8 µg/l), showing no evidence of decreasing. PFOA has been detected at levels between 0.2 and 0.5 µg/l. Perfluorohexane sulphonate has also been detected at approximately 1.5 µg/l (maximum concentration, 1.9 µg/l). These results confirm the persistence of these PFCs in water.

Pre and post filtration sampling indicated that GAC was effective in the removal of PFOS at the levels detected in the raw water. The effectiveness was dependent on the bed life of the carbon, i.e. the period since the last regeneration. The GAC was originally installed to give a minimum EBCT of 30 minutes with two of the five units out of service for maintenance or washing. Upon detection of PFOS in the raw water, the plant flow was reduced to increase substantially

the EBCT to between 65 to 110 minutes. The GAC regeneration frequency (staggered between the five absorbers) was increased from 24 to 12-monthly. The GAC is regenerated by heating at high temperatures which are in excess of the 600 °C where PFOS and PFOA are expected to be completely transformed into carbon dioxide and hydrogen fluoride (45). There were approximately 5500 bed volumes between annual regenerations. PFOS and PFOA showed breakthrough after approximately 8000-9000 bed volumes. This was measured prior to the increase in regeneration frequency. Figure 2 shows the levels of PFOS detected in one GAC absorber with time and number of bed volumes. The effect on PFOS levels of replacing the GAC in October 2007 is clearly seen.

Other PFCs showed different breakthrough rates with perfluorobutane sulphonate showing breakthrough after 8000-9000 bed volumes (Figure 3), perfluorohexane sulphonate after 6000 bed volumes, and 6:2 fluorotelomer sulphonate after 11 000 bed volumes. Perfluorohexanoic acid was least absorbed onto GAC F400 and showed breakthrough after approximately 2000 bed volumes (Figure 4).

Other options were also tested in this study, including UV and hydrogen peroxide treatment. Results showed no measurable photolysis with UV alone or hydroxyl radical oxidation (from the addition of hydrogen peroxide). Other laboratory studies with extreme conditions using photocatalysis (reaction time up to three days) and advanced oxidation (with high temperature and pressure) have proved effective in completely degrading PFOS and PFOA to carbon dioxide and hydrogen fluoride (45). In general, without the presence of C-C double bonds, PFCs would not be expected to react with chlorine, ozone, UV and would be resistant to degradation by oxygen free radicals.

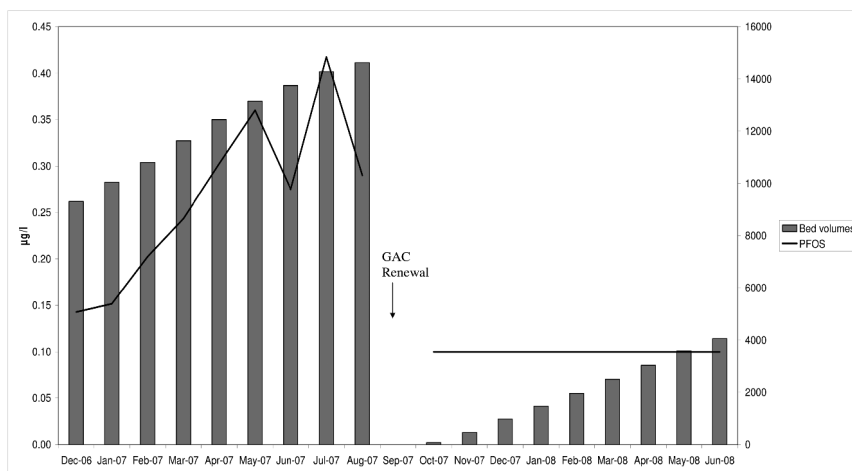


Figure 2. Effect of water volume processed, expressed in bed volumes treated, on the efficacy of PFOS removal by GAC filtration (29, 46)

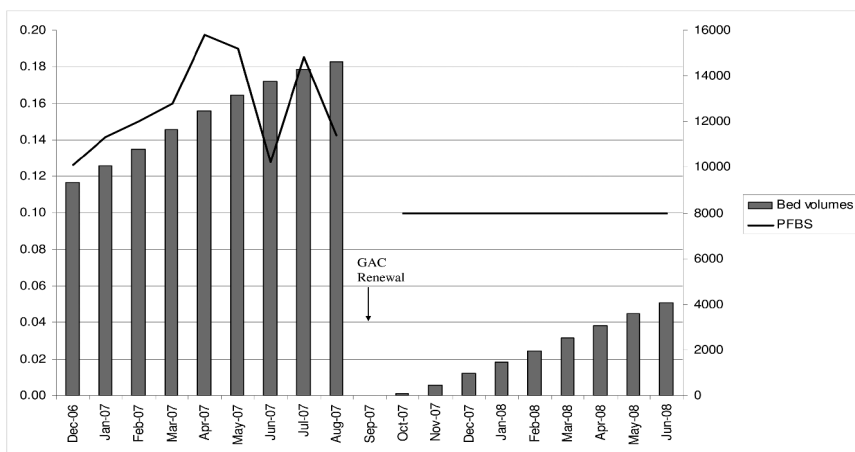


Figure 3. Effect of water volume processed, expressed in bed volumes treated, on the efficacy of PFBS removal by GAC filtration (46)

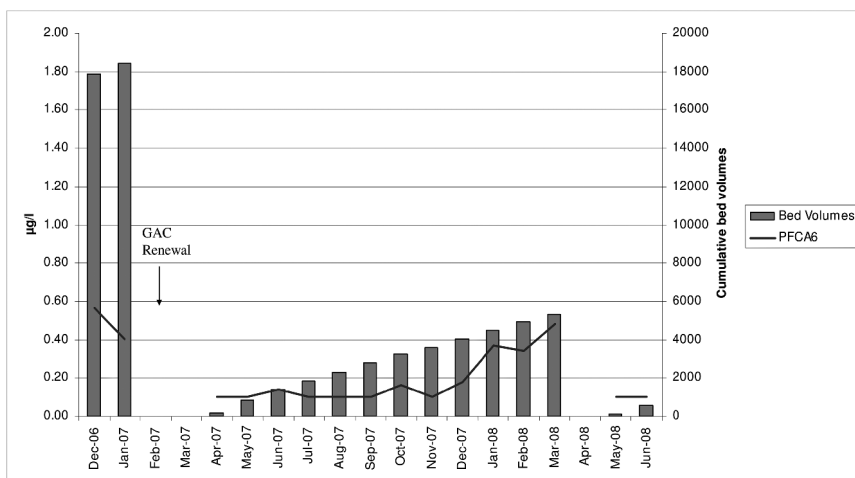


Figure 4. Effect of water volume processed, expressed in bed volumes treated, on the efficacy of PCA6 removal by GAC filtration (46)

Discussion

PFOS and PFOA have been found in environmental waters all around the world. However, in general, detected levels have been low, below the values that have been set for drinking water by various international authoritative bodies for the protection of human health of around 300-500 ng/l. Continuous raised levels were found in specific industrial areas or where known contamination had taken place. In fact, in the Sauerland area of Germany, the specific contamination was identified from the discovery of PFOA in nearby rivers. Therefore, in general,

PFOS and PFOA are present in environmental waters due to specific point source pollution.

There are only limited data on the levels of PFOS and PFOA in drinking water. Detection has been mainly confined to pollution incidents, where there is evidence that local environmental water have been contaminated (USA, Germany, UK and Jersey), although data are limited for background levels. Even when groundwater contamination is severe, such as the case following the use of PFOS-containing fire-fighting foams following the Buncefield Oil Depot fire, the amount reaching surface waters and drinking water appears to be limited.

Early studies suggested that levels of PFOS and PFOA in source waters and drinking water were similar and that PFCs were not removed by water treatment, and this appears to be true for sand filtration and chlorination, and also for UV and hydrogen peroxide treatment. However, it now appears from recent careful studies in the UK and Germany that the use of GAC, with long EBCT and appropriate regeneration regimes to prevent breakthrough, is successful in removing both PFOS and PFOA and other PFCs. Reverse osmosis and nanofiltration are also successful in removing PFCs from water.

There is some evidence that PFOS levels in the human population may have decreased since the phase-out by 3M in 2000-2002 and that PFOA may also be following, as its use is now being voluntarily reduced.

New PFCs are being introduced as replacements, such as perfluorobutane sulphonamide and its alcohol in the new Scotchguard launched in 2003. Its breakdown product, perfluorobutane sulphonate (PFBS), appears to be less bioaccumulative, having a much shorter half-life (one month in humans) and it also appears to be less toxic. The potassium salt of PFBS is more soluble than PFOS and so remains in the water column, but is less toxic to aquatic organisms, is persistent but is not bioaccumulative (47). In general, it seems that the PFCs only bioaccumulate if there are at least 7 fluorocarbons in the chain, with this property increasing considerably with each additional group (3).

So while the alternatives to PFOS and PFOA may be less bioaccumulative and toxic, they may still be persistent in the environment, so studies on their degradation products and their fate and behaviour will be required, together with their monitoring in environmental water. However, the use of these short chain chemicals may only be considered a short-term replacement for the traditional PFOS and PFOA-like compounds while non-fluorinated alternatives are sought.

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Chapter 14

Earthworms: Diagnostic Indicators of Wastewater Derived Anthropogenic Organic Contaminants in Terrestrial Environments

**Chad A. Kinney,*¹ Edward T. Furlong,² Dana W. Kolpin,³
Steven D. Zaugg,² Mark R. Burkhardt,⁴ Joseph P. Bossio,⁵
and Stephen L. Werner²**

¹Colorado State University-Pueblo, Chemistry Department,
2200 Bonforte Blvd, Pueblo, CO 81001

²U.S. Geological Survey, National Water Quality Laboratory,
Methods Research and Development Program, P.O. Box 255857,
Building 95, Denver Federal Center, Denver, CO 80225-0585

³U.S. Geological Survey, 400 S. Clinton St., Suite 269,
Iowa City, IA 52240-4105

⁴U.S. Environmental Protection Agency, Region 8 Laboratory,
16194 W. 45th Drive, Golden, CO 80403-1790

⁵Eastern Washington University, Department of Chemistry and
Biochemistry, 226 Science Building, Cheney, WA 99004
*chad.kinney@colostate-pueblo.edu

Analysis of earthworms for anthropogenic organic contaminants (AOCs) offers a potential diagnostic tool for assessing the presence and transfer of AOCs from wastewater sources into terrestrial environments and biota. Earthworms and soil samples were collected from one minimally affected agricultural field (soybean, Site 1) with no known history of biosolids or manure amendment and three agricultural locations amended with municipal biosolids. The biosolid-amended sites consisted of a soybean field amended for the first time with municipal biosolids (Site 2), a hay field with an extended history of amendment with municipal biosolids (Site 3), and a grassland pasture used for cattle grazing (Site 4) with an extended history of biosolids amendment with municipal biosolids from the same source as Site 3. Forty-two of the 72 AOCs monitored in

this study were detected in quantifiable concentrations in one or more of the biosolids, soil, or earthworm samples. In most of these samples, the biogenic sterols had the highest concentration among measured AOCs, but the biosolids and biosolid-amended samples contained a variety of AOCs indicative of human use such as the disinfectant triclosan, detergent metabolites, and the synthetic fragrances galaxolide and tonalide. A number of AOCs were detected in the earthworm tissue samples, some of which bioaccumulated to concentrations greater than found in the soils from which were collected. Unexpectedly, some AOCs were detected in the soil and earthworms from the minimally impacted Site 1. However, the relative abundance of uniquely anthropogenic contaminants, such as personal care products, is much less in the earthworms from Site 1 compared to those from the biosolids amended sites. When possible, bioaccumulation factors (BAFs) on a dry mass basis were calculated for the AOCs detected in the earthworms. Triclosan and monoethoxy-nonylphenol detergent metabolites were measured to have BAFs as high as 41.0 and 21.7, respectively. Many of the AOCs measured in the earthworm tissue samples were below detectable concentrations in the corresponding soil samples. This study documents that some AOCs present in land-applied biosolids can be transferred to earthworms and that earthworms may serve as a diagnostic tool for assessing the presence of AOCs in terrestrial environments.

Background

Municipal wastewater treatment produces liquid and solid products, treated effluent and sewage sludge, respectively. Treated effluent is generally discharged into surface water, although increasingly, treated wastewater is used as a source of nonpotable water or for indirect drinking water reuse. Treated sewage sludge that meets regulatory standards for pathogen and metal content, can be classified as biosolid. Once classified as biosolid it may be land applied as an organic carbon- and nutrient-rich soil amendment or in land-reclamation projects (1).

Numerous organic contaminants, including pharmaceuticals, detergents, fragrances, antimicrobials, pesticides, and industrial products have been detected in wastewater end products (2–4) and are collectively referred to herein as anthropogenic organic contaminants (AOCs). Many AOCs are unaltered or incompletely removed in wastewater treatment plants (WWTPs), and subsequently have been identified in the environment, especially in surface waters receiving wastewater effluent (2, 5–7). Many AOCs entering WWTPs have moderate to large log K_{OW} values and can be predicted to undergo hydrophobic partitioning into the organic-rich solids phase during treatment (8). This prediction is consistent with recent observations of concentrations of various AOCs, such as

detergent metabolites, synthetic fragrances, disinfectants, and pharmaceuticals, in biosolids destined for land application (4, 9–13).

Various studies have raised concerns about the potential impacts of the environmental presence of AOCs on humans and wildlife including reproductive impairment, immune deficiencies, and antibiotic resistance among pathogenic bacteria (14–22). Research has documented uptake of various AOCs by plants and animals (23–26), including humans (27–29). Most research investigating the effects or bioaccumulation of AOCs has focused on aquatic environments and organisms (30–33). There is a paucity of data on the movement of these compounds into terrestrial organisms.

The U.S. Environmental Protection Agency estimates that more than 8×10^6 dry tons of biosolids are produced in the United States annually (34); with more than 50% of the biosolids produced each year being land applied (1). In Europe an estimated 37% (2.39×10^6 dry tons) of the biosolids produced are land applied each year (35). Biosolids are predominantly applied on agricultural soil, but are also used for large-scale landscaping, home landscaping and gardens, remediation of abandoned mining sites, and revegetation projects (36–38).

In addition to human biosolids, thousands of tons of animal manure are generated annually from about 92 million swine, 109 million cattle, 292 million turkeys, and 7.5 billion chickens in the United States (39). Like biosolids, manure is generally applied as a source of nutrients to agricultural soil. Most concerns about the practice of land application of manure have focused on the quantity of nutrients (40). Recently, however, other constituents of manure such as veterinary pharmaceuticals (41–43) and natural and synthetic hormones (44–46) have raised many of the same concerns as those that exist regarding land application of biosolids.

The presence and distribution of AOCs in biosolids as well as in environments amended with biosolids have been well established (4, 47–50). However, the range of sources and loadings to terrestrial and aquatic environments, exposure of humans and other organisms, and the effects of exposure are only beginning to be identified and understood.

Earthworms are common primary consumers of organic matter in soil and as such may be exposed to organic and inorganic contaminants contained in the organic fractions within soil. Earthworms can comprise as much as 60–80% of total soil biomass in some locations, and at least one species of earthworm can be found in most soils. Earthworms tend to migrate only for short distances and thus may be ideal sentinel terrestrial organisms for identifying AOCs in the food web (51–53). Earthworms can accumulate organic contaminants both by the passive equilibrium partitioning of contaminants present in the dissolved phase of soil and actively through ingestion (54, 55). The relative importance of equilibrium partitioning and accumulation of organic compounds through the skin and uptake through the gut is compound dependent. Jager et al. (55) observed the relative importance of uptake through the gut increased with increasing hydrophobicity of the organic contaminant.

Earthworms are known to biomagnify inorganic and organic soil contaminants, including mercury, polycyclic aromatic hydrocarbons (PAHs), brominated fire retardants, and pesticides through soil contact or consumption

(54, 56–62). As primary consumers of contaminated soils, earthworms have been determined to introduce organic contaminants into the terrestrial food web. Harris et al. (59) determined that earthworms in orchard soils contaminated by historic application of DDT can bioaccumulate DDT and its metabolites. The earthworms from these orchard soils served as a primary source of nourishment for American Robin (*Turdus migratorius*) populations in proximity to the orchards and thus acted as the route of exposure to total DDT for the robins. In general, total DDT was determined to bioaccumulate in robins, as measured by total DDT concentrations in robin eggs. Earthworms may serve as a route of exposure to other terrestrial organisms besides birds, such as reptiles and small mammals (63).

Earthworms collected from WWTP percolating filter beds have been found to contain the anthropogenic endocrine disrupting compounds (EDCs) phthalate plasticizers, bisphenol-A, and 17 β -estradiol (64). Moreover, these synthetic EDCs were found to elicit physical changes in the brain and brain physiology of European Starlings (*Sturnus vulgaris*) (22). These examples serve to illustrate the potential importance of earthworms to bioaccumulate and introduce a variety of organic contaminants into the terrestrial food web.

The research described herein assesses the potential transfer of AOCs from land applied biosolids into biota (earthworms) and the use of earthworms as diagnostic indicators of wastewater-derived AOCs in terrestrial environments. The sites selected for these field studies include agricultural soil receiving agronomic application rates of biosolids. Agronomic application of biosolids typically results in surface soil that contains 1 – 4% biosolids by mass. Furthermore, biosolids application method (broadcast application, post application tillage, subsurface injection, etc) and weathering may affect the availability of AOCs as a result of leaching, degradation, or changes in bioavailability (49, 65–67).

Methods

Field Sites

Four field sites were used for this project (Table I). Three sites were agricultural fields (Sites 2-4) that were amended with biosolids within 31 days prior to sample collection. The final site (Site 1) was a minimally affected site with no known history of biosolids or manure amendment. All four sites were in use for commercial agronomic production at the time of this study. Site 1, which was located in the Midwestern United States, was a nonirrigated soybean field and was previously described in Kinney et al. (3). Soil and earthworm samples were collected from Site 1 on June 6, 2005. A second set of soil and earthworm samples were collected on September 29, 2005, and whereas the data are not presented here they are available elsewhere (3).

Table I. Field Site Soil Characterization

Field Sites	Organic Carbon (%) ^a	Sand ≥ 2 mm (%) ^b	Sand < 2 mm (%) ^c	Silt (%) ^c	Clay (%) ^c	Earthworm Density (worms/hole) ^d
Site 1: Minimally Affected (soybean crop)	4.5	6.3	42.0	30.8	20.9	6.1 ± 3.4
Site 2: Midwest Biosolid-Amended (soybean crop)	1.9	2.0	60.1	23.1	14.8	12.3 ± 6.7
Site 3: Northwest Biosolid-Amended (hay crop)	2.7	3.1	38.2	26.0	32.7	14.2 ± 7.3
Site 4: Northwest Biosolid-Amended (pasture/grazing)	2.1	3.6	31.9	29.3	35.2	5.6 ± 4.3

^aEstimated by loss on ignition

^bDetermined by sieve

^cDetermined by hydrometry

^dNumber of earthworms per hole ($3.14 \times 10^4 \text{ cm}^3$) ± 1 standard deviation

Site 2, one of the biosolids amended sites, was also located in the Midwestern United States and was a no-till, nonirrigated soybean field receiving biosolids as a fertilizer for the first time in spring 2005. The biosolids were from a local WWTP that processed, on average, 3.98×10^7 liters per day of wastewater influent from residential, university, hospital and medical facility, industrial, and landfill leachate sources. The biosolids produced by this WWTP results from sludge that is processed through three anaerobic digestion steps at 130, 95 and 95°C, respectively. Prior to land application, the biosolids is pressed to decrease water content and stored on an outdoor pad for 3 to 6 months. The biosolids applied to Site 2 on April 18, 2005 had been stored for about 6 months then applied at a rate of 1.8 Mg/1000 m². Soil and earthworm samples were collected from Site 2 on May 19 (31 days post-application) and again September 21, 2005 (data not shown, (3)).

Sites 3 and 4 are located in the Northwest United States. Unlike Sites 1 and 2 that had no prior biosolids or manure application, Sites 3 and 4 had an extended history of biosolids application, and were specifically selected for this reason. Sites 3 and 4 received biosolids amendment on a regular schedule of 2 consecutive years of amendment followed by one year of no biosolids amendment. Site 3 was a no-till, nonirrigated hay field. The WWTP that produces the biosolids applied at Sites 3 and 4 employs secondary treatment, trickling filter and activated sludge, to prepare the biosolids. The sludge spends about 28 days in a digester at 36 °C. The biosolids is then transferred and stored in a facultative lagoon for 8 to 9 months prior to land application as a slurry (about 6% total solids). Application of biosolids to Site 3 was completed on July 18, 2006 at a rate of 1.0 Mg/1000 m² and soil and earthworm samples were collected on August 15, 2006 (28 days post-application). Site 3 was not amended in 2005.

Site 4 was amended with biosolids from the same WWTP as Site 3. Site 4 was a no-till, nonirrigated grassland pasture used for cattle grazing. Site 4 was amended with biosolids in 2005 and again in 2006. Biosolids application was completed on July 18, 2006 at a rate of 1.1 Mg/1000 m². Soil and earthworm samples were collected on August 16, 2006 (29 days post-application).

Field Sampling

At each field site, earthworms were removed from 40-cm diameter circular holes to a depth of about 25 cm in a manner similar to that described by Salogovic et al. (68). The soil was removed using a pre-cleaned metal-blade spade and placed on a clean tarp (only used at a single field site). The spades used in this study were cleaned using soap and water followed by deionized (DI) water and isopropanol or methanol rinses. The soil was carefully sorted by hand while wearing nitrile gloves to remove all earthworms observed. To the extent possible, plant material was removed from the soil in the field, and any remaining plant material was removed in the laboratory prior to analysis. Undamaged worms were placed in a shipping container with air holes and loosely packed native soil. The samples were returned to the laboratory in an ice-filled cooler within 24 hours of collection. In the laboratory, the earthworms were cleaned using cool DI water and allowed to dehydrate on wet filter paper for 24 hours (69, 70) to assure that AOCs detected in the earthworms originated from tissue and not gut contents. This was necessary to avoid overestimating AOC content and bioaccumulation factors (BAFs). After depuration, the worms were gently cleaned with cool DI water and dried then frozen for later extraction and analysis.

Soil subsamples for AOC analysis were collected once the earthworms were separated from the soil from each sampling hole. The soil placed on the tarp was homogenized by hand in the field. Any biosolids in the soil sample were therefore distributed throughout the homogenized soil prior to subsampling. Subsamples of the soil homogenate from each hole were placed into a glass bowl, thoroughly mixed, apportioned into trace-clean glass jars, and returned to the laboratory for soil texture, soil organic carbon, and triplicate AOC.

Samples of the biosolids applied to Sites 2 – 4 were collected at the time of field application or field sample collection and frozen for later AOC analysis. Biosolids applied to Site 2 was collected from the drying pad at the WWTP, and the biosolids applied to Sites 3 and 4 was sampled directly from the well-mixed WWTP retention pond while a tanker truck used to transport and surface apply the biosolids was being filled.

Solvent Extraction of Soil, Biosolids, and Earthworm Samples

Earthworm, soil, and biosolids source samples were prepared in triplicate for AOC quantification. Two different extraction, cleanup, and quantification methods were required to encompass the range of compounds determined in this study. Both methods are based on previously published pressurized liquid extraction (PLE; Dionex-100 & 200, Dionex Corp., Sunnyvale, Calif., USA) methods developed for AOC determination in soil and sediment samples (71, 72).

The nonpolar AOCs were extracted from 1–2 g wet weight samples of homogenized soil, biosolids, or earthworm by PLE using mixtures of isopropanol and water (72). The sample was loaded into a 10-mL PLE cell and the void volume was filled with ashed Ottawa sand (400°C for 4 h). Prior to extraction method-performance surrogates were added to the top of the material to be extracted. Each sample was extracted twice; first at 120°C using 50:50

isopropanol:water and second into a separate receiving vial at 200°C using 80:20 isopropanol:water. Both extractions were at 10300 kPa and consisted of three 5-min static cycles. The two resulting extracts for each sample were combined during solid-phase extraction (SPE) preconcentration and clean-up step using a modified polystyrene-divinylbenzene (PSDVB) phase SPE cartridge (1-g, 20-mL Oasis HLB Waters Corp., Milford, MA). Once loaded, the PSDVP cartridge was eluted with three 10-mL aliquots of 80:20 dichloromethane:diethyl ether through a Florisil SPE cartridge (1-g, 6-mL International Sorbent Technologies, Mid Glamorgan, U.K.) that had about 4 g of sodium sulfate added to the top of the cartridge. The resulting eluent was brought to a final volume of about 1 mL by evaporation under a gentle stream of nitrogen. Prior to transferring the final extract to a 2-mL autosampler vial and quantitation, an internal standard mixture was added.

Pharmaceuticals in the samples were extracted by PLE using a 70:30 acetonitrile:water solvent mixture (48, 71). About 10 g wet weight of soil or biosolids or 3–5 g wet weight of earthworms was loaded into a 10-mL PLE cell. Any void volume in the cell was filled with ashed Ottawa sand to maintain consistent extraction volumes. Prior to extraction a method performance surrogate was added to the sample. Each sample was extracted for five static cycles (10-min each) at 130°C and 10,300 kPa. One mL of the extract was filtered through a 0.20- μ m syringe filter into a 2-mL autosampler vial. The acetonitrile was then evaporated off under a gentle stream of nitrogen. The sample was reconstituted to 1 mL using 100 μ L internal standard solution and a balance of 10 mM aqueous ammonium formate buffer.

Chemical Analysis

Extracts from the two PLE methods were analyzed using separate instrumental methods. The largest subset of analytes, the nonpolar AOCs, were quantified by gas chromatography/mass spectrometry (GC/MS, Agilent Technologies Model 5973, Hewlett-Packard/Agilent, Palo Alto, CA) following a protocol described by Burkhardt et al. (72). The GC/MS was operated in the full-scan mode [from 45 to 550 mass/charge ratio (m/z)], using electron-impact ionization (70 electron volts) and external calibration (72). A detailed description of chromatographic conditions and ions monitored are described elsewhere (72).

The pharmaceuticals were analyzed by high-performance liquid chromatography coupled with electrospray ionization/quadrupole mass spectrometry (HPLC/ESI/MS, Hewlett-Packard/Agilent Model Series 1100 LC/MSD) operated in the positive ion mode using selected-ion monitoring to improve sensitivity and minimize chemical interferences. The chromatographic conditions, ions monitored, and other method parameters are described in detail by Cahill et al. (73).

Table II. Concentrations of AOCs (ng/g) Detected in Biosolid, Soil, or Earthworm Samples^a

Anthropogenic Organic Contaminants	Common Use/Source	Biosolid 1	Biosolid 2	Site 1	Site 1	Site 2	Site 2	Site 3	Site 3	Site 4	Site 4
		Applied to Site 2	Applied to Sites 3 & 4	Minimally Affected Soil	Minimally Affected Earthworm	Biosolid Amended Soil	Biosolid Amended Earthworm	Biosolid Amended Soil	Biosolid Amended Earthworm	Biosolid Amended Soil	Biosolid Amended Earthworm
acetophenone	fragrance (PCP)	3450 (51)	ND	627 (12)	ND	110 (42)	ND	291 (35)	ND	135 (41)	ND
α-limonene	fragrance (PCP)	1600 (14)	1877 (16)	393 (40)	ND	ND	ND	ND	ND	32 (26)	ND
galaxolide (HHC8)	fragrance (PCP)	427000 (19)	25900 (20)	633 (23)	81 (43)	1050 (18)	3340 (24)	39 (29)	ND	55 (64)	ND
indole	fragrance (PCP)	8 800 (22)	11 900 (12)	ND	2320 (32)	285 (23)	1950 (29)	214 (25)	545 (29)	55 (37)	743 (28)
isobornol	fragrance (PCP)	ND	ND	287 (25)	ND	ND	ND	ND	ND	ND	ND
isquinoline	fragrance and flavor (PCP)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
tonalide (AHTN)	fragrance (PCP)	177000 (13)	6503 (34)	113 (38)	19 (73)	287 (26)	279 (22)	27 (18)	ND	34 (23)	ND
camphor	flavor and odorant (PCP)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
menthol	cigarttas, cough drops, and mouthwash (PCP)	ND	ND	177 (36)	< 42	ND	ND	ND	ND	ND	ND
4-cumylphenol	detergent metabolite (PCP)	ND	ND	37 (87)	ND	ND	140 (73)	ND	ND	ND	ND
4-n-octylphenol	detergent metabolite (PCP)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4-tert-octylphenol	detergent metabolite (PCP)	ND	7823 (25)	ND	ND	570 (57)	< 22.9	ND	< 22.9	ND	ND
para-nonylphenol-total	detergent metabolite (PCP)	483000 (20)	316100 (18)	ND	ND	5200 (37)	861 (26)	4938 (45)	944 (27)	3176 (14)	ND
nonylphenol	detergent metabolite (PCP)	25300 (28)	11 650 (24)	ND	ND	ND	158 (37)	1647 (21)	75 (05)	1626 (16)	ND
monooctylxytolal	detergent metabolite (PCP)	760 (48)	12820 (27)	ND	ND	ND	230 (32)	6432 (21)	ND	3542 (27)	ND
nonylphenol diethoxy-total	detergent metabolite (PCP)	5030 (21)	ND	ND	ND	ND	ND	ND	348 (52)	ND	ND
octylphenol, monoethoxy	detergent metabolite (PCP)	ND	ND	ND	ND	74 (22)	ND	ND	ND	ND	ND
octylphenol, diethoxy	detergent metabolite (PCP)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
N,N-diethyltoluamide (DEET)	mosquito repellent (PCP)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
phenol	deterfact (PCP), manuf. numerous products	6270 (16)	18810 (28)	5 970 (15)	ND	ND	ND	104 (35)	288 (48)	ND	148 (52)
triclosan	deterfact (PCP)	10500 (17)	8048 (11)	833 (87)	ND	160 (38)	1740 (31)	86 (28)	3854 (31)	41 (39)	1679 (32)
benzophenone	fixative perfumes and soaps (PCP)	ND	1 977 (28)	ND	< 31.8	ND	ND	ND	226 (46)	ND	123 (25)
3-beta-coprostanol	biogenic sterol	467000 (33)	1262000 (21)	ND	ND	1 910 (77)	ND	1834 (28)	1439 (18)	1702 (17)	758 (27)
cholesterol	biogenic sterol	86700 (24)	2898000 (18)	18 900 (69)	253000 (48)	7700 (51)	168000 (49)	4532 (41)	238788 (48)	4242 (39)	123990 (25)
beta-sitosterol	biogenic sterol	177000 (31)	302800 (15)	24 000 (17)	11800 (51)	4570 (37)	7030 (56)	613 (52)	15281 (48)	1731 (26)	8108 (43)
stigmasterol	biogenic sterol	77700 (28)	21 100 (25)	4 900 (18)	ND	1500 (33)	ND	572 (22)	2882 (36)	552 (17)	851 (27)
anthracene	PAH	329 (24)	ND	ND	ND	ND	ND	ND	ND	ND	ND
benzo(a)pyrene	PAH	ND	386 (31)	ND	ND	ND	ND	< 24.6	ND	< 24.6	ND
naphthalene	PAH, moth repellent	610 (21)	ND	ND	ND	ND	ND	ND	ND	ND	ND
fluoranthene	PAH	950 (27)	1057 (32)	ND	ND	ND	ND	33 (28)	40 (70)	26 (38)	< 23.5
phenanthrene	PAH	1730 (27)	773 (19)	ND	ND	ND	ND	< 20.7	ND	ND	ND
pyrene	PAH	740 (24)	1082 (28)	ND	ND	ND	ND	24 (5.6)	33 (23)	< 20.8	< 20.8
1-methylpyrene	Alkyl-PAH	ND	ND	ND	< 27.8	ND	ND	ND	ND	ND	ND
2-methylpyrene	Alkyl-PAH	ND	ND	ND	< 27.8	ND	ND	ND	ND	ND	ND
2,6-dimethyl-naphthalene	Alkyl-PAH	915 (44)	1579 (31)	ND	ND	ND	ND	ND	ND	ND	ND
bisphenol A	Fixative, polycarbonates	4600 (8)	1279 (13)	147 (17)	ND	ND	ND	< 31.6	512 (20)	ND	318 (22)
diethylhexyl phthalate	plasticizer	3330 (22)	444030 (16)	ND	ND	ND	2261 (34)	288 (28)	529 (23)	154 (28)	ND
diethyl phthalate	plasticizer	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
tetrabromo diphenylether	flame retardant	ND	1012 (9)	ND	ND	ND	ND	ND	ND	ND	ND
tri(2-chloroethyl) phosphate	plasticizer, flame retardant	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
tributylphosphate	antiflaming agent and flame retardant	ND	ND	2 130 (17)	200 (27)	923 (21)	250 (23)	ND	ND	ND	ND
tri(dichloroethyl) phosphate	flame retardant	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
triphenyl phosphate	plasticizer	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
para-cresol	wood preservative	4970 (34)	29370 (12)	2 200 (8)	< 161	ND	270 (17)	< 161	396 (16)	ND	1185 (22)
1,4-dichlorobenzene	pesticide, moth repellent	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
atrazine	pesticide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
biomethol	pesticide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
carbaxole	pesticide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
chlorpyrifos	pesticide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
diazinon	pesticide	ND	ND	ND	99 (26)	ND	ND	ND	ND	ND	ND
metolachlor	pesticide	ND	ND	320 (22)	ND	ND	720 (16)	ND	ND	ND	ND
prometon	pesticide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
3-methyl-1H-indole (skatol)	Focal indicator	5170 (23)	1878 (33)	ND	260 (20)	143 (41)	230 (27)	< 30.1	863 (36)	ND	482 (12)
anthraquinone	Manuf. Of dyes/textiles, bird repellent	ND	ND	ND	ND	ND	ND	< 24.3	ND	ND	ND
isophenone	Industrial solvent	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
isopropylbenzene	Manuf. of phenolic resins	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
acetylacetophen	Antifungal (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
caffeine	Stimulant (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
1,7-dimethylxanthine	caffeine metabolite (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
carbamazepine	Antiepileptic (PH)	390 (16)	16 (11)	ND	ND	ND	ND	ND	ND	ND	ND
codeine	Analgesic (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
cotinine	Nicotine Metabolite (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
dehydrochloridene	Antianginal (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
diltiazem	Antihypertensive (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
diphenhydramine	Antihistamine (PH)	7000 (12)	227 (16)	ND	ND	< 1.4	ND	2 (27)	ND	3 (35)	ND
fluoxetine	Antidepressant (PH)	ND	30 (19)	ND	ND	ND	ND	ND	ND	ND	ND
nicotinic acid	Antifungal (PH)	ND	110 (12)	ND	ND	ND	ND	3 (19)	ND	3 (22)	ND
sulfasalazine	Antisclerotic (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
sulfamerazoxole	Antibiotic (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
thiabendazole	Anthelmintic/Pesticide (PH)	5000 (17)	ND	ND	ND	ND	ND	ND	ND	ND	ND
trimethoprim	Antibiotic (PH)	ND	ND	< 1.6	ND	ND	127 (5)	ND	90 (11)	ND	18 (8)
veratrin	Anticoagulant (PH)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

^a Average from three replicate composite samples, individual compound concentrations in ng/g dry wt. ND = not detected. PCP = personal care product. PAH = polycyclic aromatic hydrocarbon. PH = pharmaceutical. Values in parentheses is percent relative standard deviation.

Quality Assurance and Quality Control

Several measures of quality assurance and quality control were used during the field and laboratory work. In the field, pre-ashed Ottawa sand was used as a field blank and was handled identically to the other samples to ensure that there were no artifacts resulting from the sampling and analytical procedures.

At least one pre-ashed sand reagent spike and laboratory blank sample were analyzed with each set of extractions and quantifications. Method-performance surrogate compounds (GC/MS: decafluorobiphenyl, fluoanthene-*d*₁₀, bisphenol A-*d*₃; LC/MS: carbamazepine-*d*₁₀, ethyl nicotinate-*d*₄) were added to all samples, spikes, and blanks. Multiple ion monitoring for each compound and chromatographic retention time were compared to authenticate standards for compound verification. Internal standards (GC/MS:1,4-dichlorobenzene-*d*₄, naphthalene-*d*₈, acenaphthene-*d*₁₀, phenanthrene-*d*₁₀, chrysene-*d*₁₂, perylene-*d*₁₂; LC/MS: nicotinamide-*d*₄) were added to each sample to correct for any differences in sample volume and as a time marker. *Eisenia foetida*, a commercially available earthworm, was selected as a reasonable surrogate matrix to validate method performance for the earthworms collected in the field. Earthworm matrix spike recoveries (n = 3) were determined for clean *Eisenia foetida* fortified with AOCs as reported elsewhere (3). The performance of the methods for sediment, soils, and biosolids has been previously assessed, including method spike and matrix spike recoveries as well as statistically determined method detection limits (MDLs, (48, 71, 74)). Trace quantities of cholesterol, galaxolide, phenanthrene, and tonalide were detected in one or more of the blank samples, but these compounds were at concentrations 1 to 4 orders of magnitude lower than that detected in any of the corresponding environmental samples, and therefore not excluded from the data. AOCs detected in samples at concentrations lower than the MDL are preceded by a “<” followed by the existing MDL (Table II). All of the compounds detected below the MDLs qualify as positively identified compounds by meeting all reporting qualifications, namely chromatographic retention time and detection of quantitation and confirmation ions within acceptable ion ratio limits.

Results and Discussion

Biosolids

The biosolids that were land applied at the field sites used in this study had a similar number of AOCs detected; 28 and 27 AOCs were detected in Biosolids 1 and 2, respectively (Table II). Twenty-three of the AOCs were detected in common in the two biosolids. The fragrances galaxolide and tonalide were detected in high concentrations, between 6.5 and 427 µg/g, in both biosolids. Nonylphenol detergent metabolites were detected in the biosolids at over 315 µg/g. The four target biogenic sterols were detected at individual concentrations as high as 2.86 mg/g. A number of PAHs and pharmaceuticals were also present in the land applied biosolids.

For ease of comparison and discussion, the AOCs included in this study have been grouped into four general categories; personal care products (PCPs), biogenic sterols, pharmaceuticals (PHs), and others (e.g. PAHs and alkyl-PAHs, wood preservative, skatol, etc.). Table II can be used to identify which compounds compose each of these groups. Figure I shows the relative contribution of each group of AOCs to the overall AOC composition in the source biosolids. On a relative mass basis the AOCs in the biosolids as well as the soil and earthworms samples were dominated by the presence of biogenic sterols (Figure I), with the

exception of the biosolids applied to Site 2. However, it is important to note that many other compounds and groups of compounds were detected in substantial concentrations as mentioned above (Table II).

Amended Soils

All of the field sites included in this study contained a variety of AOCs. Unexpectedly, Site 1, the minimally affected soil that has no known history of biosolids or manure amendment, contained detectable quantities of 17 of the target AOCs (Table II). Some of the AOCs detected at Site 1 are strictly anthropogenic compounds, such as galaxolide, tonalide, and triclosan, whereas others that have human uses or sources like d-limonene and the biogenic sterols also may originate from natural environmental sources. This may in part explain the presence of the AOCs in the soil at Site 1. The source of the strictly anthropogenic compounds at Site 1 is unknown, but may reflect runoff from fields upgradient from Site 1 or atmospheric transport and deposition (75–78), which also could affect the biosolid-amended fields. The array of AOC detections at Site 1 documents the difficulty in identifying a true control site given the ubiquitous nature of many AOCs.

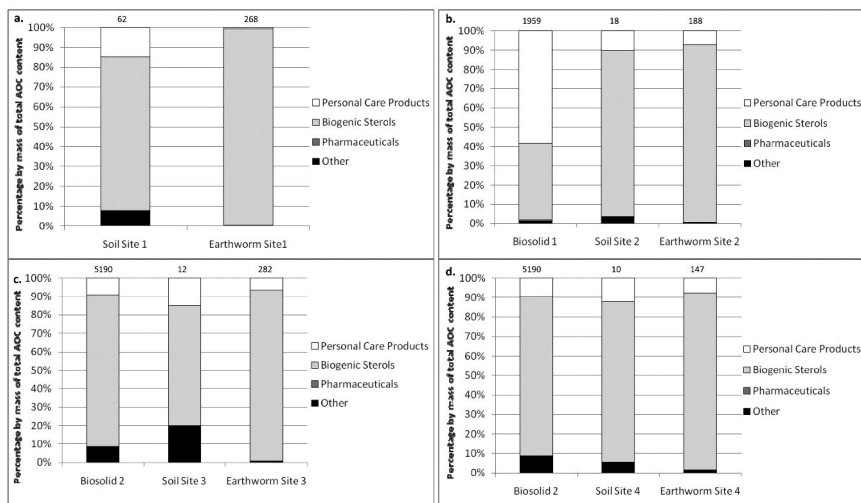


Figure 1. Relative contribution of personal care products, biogenic sterols, pharmaceuticals, and other AOCs to the overall AOC composition of biosolid, soil, and earthworms from (a) Site 1 – nonirrigated soybean, (b) Site 2 – biosolid amended (1.8 Mg/1000 m²), no-till, nonirrigated soybean, (c) Site 3 – biosolid amended (1.0 Mg/1000 m²), no-till, nonirrigated hay, and (d) Site 4 – biosolid amended (1.1 Mg/1000 m²), no-till, nonirrigated grassland pasture. The total quantity of AOCs (µg/g) detected in each sample appears above each bar.

The soil at Site 2 contained detectable quantities of 12 of the target AOCs, 10 of which were detected in the biosolids applied to the site. The presence of two AOCs in the soil at Site 2 not detected in Biosolids 1 indicates possible chemical heterogeneity in the product. On a mass basis, the AOCs detected in Site 2 are dominated by the biogenic sterols, but there are important contributions of synthetic fragrances and the disinfectant triclosan to the total AOCs at Site 2 (Figure I). The detergent metabolites detected at relatively high concentrations in the biosolids were not detected in the corresponding biosolid-amended soil samples (Table II). The soil samples were collected 31 days after biosolids amendment and the biosolids was not incorporated into the soil. Therefore, the detergent metabolites and perhaps some of the other AOCs were subject to photodegradation following application (79, 80).

Soil samples from Site 3 contained detectable quantities of 24 of the target AOCs, all but one of which was present in Biosolid 2. Unlike Site 2, many of the detergent metabolites present in the biosolids were also detected in soil at Sites 3 and 4 (Table II). However, the biogenic sterols were still the dominant group of AOCs detected in the soil (Figure I). As noted previously, Sites 3 and 4 were amended with the same biosolids. All 17 of the AOCs detected at Site 4 were present in Biosolid 2. It is unknown why there is such a sizeable discrepancy in the number of AOCs detected at Sites 3 and 4. Five of the AOCs positively identified at Site 3 were below the MDL for the compound, and thus may have been present at concentrations below the MDL at Site 4. Two compounds, nonylphenol diethoxy-total and phenol, were detected in the soil from Site 3 at concentrations substantially above the MDL and not detected at Site 4. Although the biosolids applied to Sites 3 and 4 originates from the same source, the select differences in the AOCs detected at these sites and in the earthworms from each site may originate from chemical heterogeneity in the biosolids. The biosolids on the soil surface at both Sites 3 and 4 was observed to be heterogeneously distributed suggesting that the relatively small areas from which the samples were collected may be subject to heterogeneous quantities of biosolids. This may contribute to the observed differences. Despite the differences in the total number of AOCs detected at Sites 3 and 4, the relative composition of AOCs are similar between the two sites (Figure I).

Earthworms

Earthworms collected from all four field sites, including those from Site 1 with no known history of biosolids or manure amendment, had AOCs present in their tissue. The highest concentrations of AOCs in the earthworms at Site 1 were the biogenic sterols (Figure I), particular cholesterol, which may have originated from natural sources and is naturally present in earthworms. There were also some uniquely anthropogenic AOCs detected at low concentration in the earthworms from Site 1 such as galaxolide (61 ng/g), tonalide (19 ng/g), and tributylphosphate (200 ng/g). In total, 14 of the target AOCs were detected in earthworms from Site 1.

Fifteen of the AOCs included in this study were detected in earthworms from Site 2; many of which were not detected in the soil from Site 2 and in

some instances AOCs that were below detectable concentrations in Biosolid 1. This may indicate the ability of earthworms to accumulate AOCs that were below detectable concentrations in the biosolids or that there is perhaps another anthropogenic source of these compounds to this site. Whereas the biogenic sterols cholesterol (166 $\mu\text{g/g}$) and beta-sitosterol (7030 ng/g) were the most dominant AOCs in the Site 2 earthworms, substantial quantities of nonylphenol detergent metabolites (5200 ng/g), galaxolide (3340 ng/g), and triclosan (1740 ng/g) were also detected (Table III).

Twenty AOCs were detected in the earthworms from Sites 3 and 4; 18 of which were detected in earthworms from both sites. This likely reflects the fact that both soils were amended with biosolids from the same source within a close timeframe. Both sites have an extended history of biosolids amendment, but only Site 4 was amended with biosolids the year prior to sample collection. This difference, however, does not appear to be reflected in the concentration of AOCs in the earthworms or the soil from each site (Table II). These observations likely indicate continuous weathering, degradation, volatilization, or leaching is occurring following biosolids application (81–83).

For many AOCs, the AOC composition profile of the earthworms is largely similar to the soil from which they were collected (Figure I). Moreover, the AOC profile in the soil and earthworms from Sites 2–4 generally reflect the composition of the biosolids used to amend the soil, especially at Sites 3 and 4. Moreover, the relative abundance of uniquely anthropogenic AOCs are substantially less in the earthworms from Site 1 indicating a lesser influence from anthropogenic AOC input. Therefore, some differences in AOC content of the source biosolids are reflected in the affected environment.

When possible bioaccumulation factors (BAFs, the ratio of mean AOC concentration in the earthworms to the AOC concentration in the corresponding soil) was calculated as a measure of AOC transfer into soil biota (Table III). Bioaccumulation factors were calculated instead of biota-to-soil accumulation factors because the individual species of earthworms collected from each site were not determined. Many of the AOCs had BAFs > 1 , which indicates the potential for magnification in earthworms and perhaps transfer up the terrestrial food web (22, 59, 64). A few of the AOCs that are uniquely anthropogenic in source had consistently high BAFs including triclosan (10.9–41.0) and detergent metabolites (3.4–28.0).

Calculating BAFs in this study is complicated by the fact that many of the AOCs detected in the earthworms were below detection in the corresponding soil samples (Table II), which makes it impossible to calculate BAFs. Compounds for which this is the case are designated with a “ > 1 ” in Table III. In all such instances where BAFs could not be calculated (> 1 , Table III) due to an AOC not being detected in the soil sample, the BAF by definition must be > 1 . In many such instances, such as total para-nonylphenols in Site 2, monoethoxy-octylphenol in Site 3, and para-cresol in Site 4, sizable quantities of the AOC were measured in the earthworm.

Table III. Bioaccumulation Factors (BAFs)^a of detected AOCs

Anthropogenic Organic Contaminants	Site 1 BAF	Site 2 BAF	Site 3 BAF	Site 4 BAF
acetophenone	0.2	> 1	> 1	> 1
d-limonene	0	--	--	> 1
galaxolide (HHCB)	0.1	3.2	0	0
indole	> 1	6.8	2.5	13.3
isoborneol	0	--	--	--
tonalide (AHTN)	0.2	1.0	0	0
menthol	NA	--	--	--
4-cumylphenol	0	> 1	--	--
4-tert-octylphenol	--	> 1	NA	NA
para-nonylphenol-total	--	> 1	5.7	3.4
nonylphenol monoethoxy-total	--	--	10.4	21.7
nonylphenol diethoxy-total	--	--	28.0	> 1
octylphenol, monoethoxy	--	--	> 1	--
octylphenol, diethoxy	--	0	--	--
phenol	0	--	2.8	> 1
triclosan	0	10.9	38.9	41.0
benzophenone	NA	--	> 1	> 1
3-beta-coprostanol	--	0	0.8	0.5
cholesterol	13.4	21.6	52.7	29.2
beta-sitosterol	0.5	1.5	29.8	4.7
stigmastanol	0	0	5.0	1.5
anthracene	--	--	--	--
benzo[a]pyrene	--	--	NA	NA
naphthalene	--	--	--	--
fluoranthene	--	--	1.2	NA
phenanthrene	--	--	NA	--
pyrene	--	--	1.4	NA
2,6-dimethyl-naphthalene	--	--	--	--
bisphenol A	0	--	NA	> 1
diethylhexyl phthalate	--	--	0.1	0.3
tetrabromo diphenylether	--	--	--	--
tributylphosphate	0.1	0.5	--	--
para-cresol	NA	> 1	NA	> 1
diazinon	> 1	--	--	--
metolachlor	0	> 1	--	--
prometon	--	--	--	--
3-methyl-1 <i>H</i> -indole (skatol)	> 1	1.6	NA	> 1
anthraquinone	--	--	NA	--
carbamazepine	--	--	--	--
diphenhydramine	--	NA	0	0
Miconazole	--	--	0	0
thiabendazole	--	--	--	--
trimethoprim	NA	> 1	> 1	> 1

^a BAF is calculated as the ratio of the average concentration of AOC in the earthworm to the average concentration in the soil.

-- : BAF not calculated because compound was not detected in the soil or the earthworm.

NA: BAF was not available because the compound was detected below the MDL in the soil or earthworm.

> 1: Although the compound was detected in the earthworm, a BAF was not calculated because the compound was not detected in the corresponding soil sample. By definition the BAF must be > 1.

In addition, BAFs for many AOCs could not be calculated because the compound, although positively identified, was detected at concentrations below the MDL in the soil or corresponding earthworm sample, which is designated with an “NA” in Table III. Much like some of the compounds designated as “>1”, there were some compounds, such as bisphenol-A at Site 3, designated as “NA” for which the concentration of the AOC is sizable in the earthworm but positively detected below the MDL in the corresponding soil.

The highest concentration of AOCs in the samples were the biogenic sterols, which do not have known direct ecological or human health threats. However, biogenic sterols can be transformed into sex hormones in the environment, such as the microbial transformation of cholesterol to testosterone (84). Results of controlled laboratory exposure experiments, data not included, in which earthworms (*Eisenia foetida*) were exposed to differing quantities of biosolids indicate that the concentration of the biogenic sterols measured in the earthworms is directly related to the quantity of biosolids exposure.

Although this study was not designed to directly consider potential human or ecological health, some of the AOCs detected in earthworm tissue are known or suspected EDCs, including nonylphenol detergent metabolites and benzophenone (85). It has been demonstrated that some AOCs with estrogenic activity in earthworms at relevant environmental concentrations can adversely affect bird populations (22). In addition, the synthetic fragrances galaxolide and tonalide, which have been observed to accumulate in human tissue, are suspected to result in liver disorders (86). Triclosan, which was calculated to bioaccumulate in earthworms at all three biosolid-amended sites, is known to elicit an estrogenic response in fish eggs (87) and cause changes in expression of thyroid hormone receptor genes in tadpoles (21). It is unknown if the presence of triclosan in material consumed by earthworms will adversely affect earthworms or microorganisms in their gut.

The fact that many AOCs were detected at measurable concentrations in earthworm tissue despite not being detected in the corresponding soils illustrates the potential for earthworms to serve as a sentinel organism and diagnostic tool to detect the presence of AOCs in terrestrial environments. This is further supported by the ubiquitous nature of earthworms in soils globally and the fact that they do not tend to migrate over substantial distances (52, 53).

Earthworms occupy a low trophic position in the terrestrial food web and therefore their ability to accumulate organic contaminants present in soils can facilitate the movement of these contaminants into higher trophic levels (59, 70, 88). Earthworms are known to be consumed by many bird species, representing up to 90% by weight of the diet of some species (89). In addition, species of mammals, reptiles, amphibians, fish, and other invertebrates feed upon earthworms (88). In fact, Markman et al. (22) demonstrated that male European starlings (*Sturnus vulgaris*) consuming a diet of mealworms containing a mixture of a select group of EDCs at concentrations consistent with those observed in earthworms (*Eisenia fetida*) collected from WWTP filterbeds (64) resulted in significant enlargement of the portion of the brain (HVC) controlling song production, increased song production and complexity, and a decrease in immune function. The presence of AOCs in earthworms in the biosolid-amended soils and the measured bioaccumulation of some AOCs suggests the presence of biosolids and the AOCs they contain are not immediately toxic to earthworms for exposure concentrations at the sample sites. However, it might be prudent to consider potential chronic effects of these substances on earthworm behavior, growth, and reproduction that might also indirectly affect soil fertility or terrestrial food webs (70, 90, 91).

Conclusions

The results of this work demonstrate that some organic contaminants, many of which are distinctly anthropogenic, can be transferred from source materials, such as biosolids, to soil-dwelling earthworms. Whereas many researchers have reported the bioaccumulation of specific organic contaminants in a variety of earthworm species, particularly in laboratory controlled experiments (54, 57, 61, 66, 70, 92, 93), the results of this work demonstrate that earthworms in common agricultural soil environments amended with biosolids can accumulate AOCs. Moreover, by virtue of bioaccumulation earthworms may represent a more robust sample for the detection of soil contaminants than the soil itself. This phenomenon was observed in soil amended with biosolids for the first time only 31 days prior to earthworm collection, as well as in soil with multiple biosolids applications. Based on these findings some AOCs present in biosolids are bioavailable for uptake, and therefore future consideration of the effects of AOC bioaccumulation and exposure on earthworms is warranted. This finding suggests that through predation of earthworms, these compounds could be further dispersed beyond the point of application in terrestrial ecosystems.

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Chapter 15

Potential Implications of Amending Agricultural Soils with Biosolids

Evelyn Walters and Rolf U. Halden*

Center for Environmental Biotechnology, The Biodesign Institute at Arizona State University, 1001 S. McAllister Avenue, Tempe, AZ 85287-5701

*Corresponding author e-mail: halden@asu.edu. Tel.: 480.727.0893.

Fax: 480.727.0889.

Municipal biosolids recycled on agricultural land are known to contain contaminants such as pharmaceutical and personal care products (PPCPs) at parts-per-million levels. A literature review was conducted to extract from peer-reviewed sources half-life data for 16 PPCPs often detected in biosolids. These data were then used in modeling simulations to determine the accumulation potential of various compounds ($t_{1/2} = 40, 130, 360$ d) when applied to land in the form of biosolids. Finally, threshold half-life values were calculated at which compounds are expected to accumulate in soil when applied tri-annually as well as once, twice or three times annually. The literature review revealed notable data gaps with respect to the availability of half-life information relevant to field situations. In addition, half-lives determined in field studies were found to be longer than those obtained in laboratory studies and from quantitative structure activity relationships (QSAR) that predict the fate of a single chemical spiked into agricultural soil. If these more rapid, but inappropriate, half-life values are used in chemical safety decisions or risk assessments, the result will be an underestimation of the actual concentrations and risks posed by biosolids-borne chemicals. The modeling exercise indicated that even a compound with a short half-life (e.g., 40 d) can accumulate when applied three times annually. Contaminants with a moderate half-life (e.g., 130 d) are predicted to show a multi-year net accumulation when applied only once annually. For compounds with a long half-life (e.g., 360 d), accumulation

was forecasted to occur even at an application frequency of once every three years. Predicted threshold half-lives for accumulation of biosolids-borne compounds such as PPCPs varied between 12 d for 3 applications per year (assuming no losses due to runoff and wind erosion) and 331 d for a single application every three years, when assuming additional annual losses of 10% in mass from chemical leaching and wind erosion. It is concluded that for risk and safety assessments of chemicals contained in recycled biosolids, it is essential to consider the effect of chemical accumulation over time due to slow degradation under field conditions and repeated application of biosolids.

Introduction

In the second half of the 20th century the pharmaceutical industry rapidly developed into the billion-dollar industry of today. It goes without question that modern medicine has significantly improved our quality of life and extended life expectancy. However, within the past two decades it has become apparent that medical drugs also can become unwanted contaminants detectable in terrestrial and aquatic environmental compartments (1–9). Unlike pesticides, pharmaceuticals have been thoroughly characterized concerning their human health risks, but not concerning their potential adverse environmental effects (1). In recognition of existing knowledge gaps, research has increased into the occurrence and fate of pharmaceuticals and their metabolites in the environment. Included within this group of compounds are also the active ingredients found in personal care products. Pharmaceutical and personal care products jointly are captured under the acronym PPCPs.

When used in residential settings, PPCPs may be disposed of into wastewater or they may be ingested, partially metabolized, and excreted in urine as a mixture of parental compounds and metabolites. Sewerage systems convey PPCP-laden wastewater to municipal sewage treatment facilities. Literature information shows many compounds are not effectively biodegraded during wastewater treatment (1) and as a result, may enter aquatic environments via wastewater reclamation (1, 4, 10, 11) or terrestrial environments via land application of biosolids (12–14). In the event of heavy rain events, combined sewer overflows may result in the direct introduction of wastewater into the aquatic environment. Moreover, aging sewage infrastructure may unintentionally allow raw wastewater to leak into the aquatic environment. Passage of PPCPs through wastewater treatment plants, sequestration of these compounds in biosolids, and sewage by-passing wastewater reclamation infrastructure all account for the identification of these micropollutants in surface water (6), groundwater (3, 7), drinking water (8), and agricultural soils (5).

Treatment of wastewater results in significant production of sewage sludge which necessitates disposal. The U. S. Environmental Protection Agency (USEPA) defines biosolids as nutrient-rich organic residuals that, when subjected

to proper treatment and processing, may be recycled and applied as fertilizer (15). Biosolids are widely recognized as being a valuable source of nutrients for crop growth and thus, have been used extensively in farming practice in many countries (16–20). In 2009, the USEPA published the Targeted National Sewage Sludge Survey (TNSSS) which provides comprehensive data pertaining to the occurrence of PPCPs and a large number of other manufactured chemicals in U.S. biosolids collected between 2006 and 2007 (21). The survey indicated that for a handful of compounds, concentrations detected in biosolids were in the parts-per-million (ppm) range (e.g., several antibiotics and the antimicrobial agents triclocarban and triclosan). These findings were supported in a number of smaller U.S. studies on sewage sludge (5, 22–24).

In a 2004 U.S. survey on biosolids regulation, quality, end use and disposal, it was reported that roughly 6.5 million dry metric tons of sewage sludge was produced annually in the United States, of which approximately 49% was applied to soils (25). Of the mass of biosolids added to soils, 75% was used on farmlands for agricultural purposes (25). According to the USEPA, biosolids application is dependent on the type of agricultural cropland (e.g., corn, small grains, soybeans, hay, etc.) and ranges between one and three applications annually at rates of 0.4 to 4.5 kg dry weight m⁻² application⁻¹ (26). Corn and soybean fields receive the largest mass of biosolids in one application because amendment occurs typically only once per year. Hay and small grain fields however may be amended three times annually, but at reduced rates (26). If PPCP degradation rates are slow and applications of biosolids frequent, one expects the compounds to accumulate and slowly increase in concentration over time. Recognition of this possibility has resulted in regulation for non-degradable heavy metal constituents contained in biosolids, but no similar regulations exist in the U.S. for organic compounds. Despite the knowledge of PPCP occurrence in agricultural soils, there are still significant data gaps regarding the half-lives of these compounds as well as their toxicities to indigenous microorganisms. Of course, for a meaningful risk assessment, knowledge of the concentration of PPCPs in soils over time is absolutely essential.

The aim of this study was to identify the accumulation potential of compounds having half-lives of 40, 130, and 360 days in biosolids-amended agricultural soils with the help of simulations based on typical real-world scenarios. In order to accomplish this, half-life data were compiled for PPCP microcontaminants detected in soils amended with biosolids to serve as input for the simulations. Since there is very little data available on the fate of contaminants in agricultural soils, half-lives were also collected for media other than biosolids-amended soils. Finally, threshold half-life values were calculated based on biosolids application frequencies typical of practices in the U.S. Information reported here should help to conduct risk assessments of the safety of PPCPs contained in biosolids destined for application on land.

Methods

Literature Review

The following databases were queried for information pertaining to the fate of pharmaceutical and personal care products in biosolids-amended soils:

- ISI Web of Science (<http://isiwebofknowledge.com>)
- Science Direct (<http://www.sciencedirect.com>)

Keywords used in the search included pharmaceuticals, personal care products, PPCPs, half (for half life, half-life, half lives and half-lives), DT₅₀, biosolids, soil, land amendment, triclocarban, triclosan, ciprofloxacin, ofloxacin, norfloxacin, azithromycin, tetracycline, doxycycline, 4-epitetracycline, miconazole, diphenhydramine, gemfibrozil, fluoxetine, carbamazepine, thiabendazole, and oxytetracycline. These 16 compounds were selected based on a yet unpublished study by Walters et al. who identified the compounds as having the highest concentrations in biosolids-amended agricultural soils (27).

Modeling PPCP Accumulation in Agricultural Soils

The accumulation of pharmaceutical and personal care products in agricultural soils was modeled for a time period of three years based on half-life and frequency of biosolids application. Half-lives of 40 d, 130 d, and 360 d were selected corresponding to a short, moderate, and long half-life, respectively. These values were chosen based on a range of values for PPCPs in soil, as found in the literature review. With the half-lives ($t_{1/2}$) and the assumption of first-order degradation kinetics, the elimination rate constants (k_{elim}) were determined using the following equation:

$$k_{elim} = \frac{\ln(2)}{t_{1/2}}$$

Four different application frequencies were considered corresponding to biosolids applications occurring one-time only, as well as once, twice and three times per year. This was the range given by the EPA for corn, small grains, soybeans, and hay fields. The assumption was made that if more than 10% of the applied chemical mass was projected to still be present in the soil prior to biosolids reapplication, the compound's concentration would have the potential to accumulate.

Determination of PPCP Threshold Half-Life Values in Agricultural Soils

In addition to modeling different accumulation scenarios, threshold half-life values were predicted for the different application frequencies. These values, corresponding to the half-lives at which compounds will begin to accumulate, were calculated for four different scenarios: physical, chemical and biological removal

only (i.e., no [0%] leaching and wind erosion) and the above loss mechanisms plus additional annual losses of 1%, 5%, and 10% due to chemical leaching, runoff and loss of chemicals with dust as a result of wind erosion.

Results and Discussion

Half-Lives of PPCPs in Agricultural Soils and Data Gaps

A literature review was performed with the aim of collecting half-life data for 16 of the 72 pharmaceutical and personal care products identified in biosolids as per the US EPA's Targeted National Sewage Sludge Survey, for PPCPs applied to agricultural soils in the form of biosolids. Table I presents the results of nine of the 16 PPCPs for which half-life data were found. Data gaps became evident, as only a handful of half-life values were available from studies conducted with biosolids applied on land. The majority of data came from laboratory experiments in which soil samples were spiked with environmentally-relevant concentrations of PPCPs or from laboratory studies in which biosolids were mixed with soil and monitored for chemical concentrations over time.

Table I. Predicted and experimentally determined half-lives in different soils, concentrations in biosolids, as well as sorption potential (expressed as log K_{ow}) of nine PPCPs

<i>Compound</i>	<i>CAS RN</i>	<i>Half-life [d]</i>	<i>Source</i>	<i>Mean Concentration in Biosolids [$\mu\text{g kg}^{-1} \text{ dw}$]</i>	<i>Source</i>	<i>Log $K_{OC}^{dimensionless}$</i>	<i>Source</i>	
carbamazepine	298-46-4	462-533 ^b	(28)	160 ± 60	(32)	3.12	(31)	
		a,b	(29)	180 ± 670	(21)			
		328 ^{w/s}	(30)	15 – 1 200	(33)			
		75 ^p	(31)					
ciprofloxacin	85721-33-1	1155-	(28)	7 000 ± 2 000	(32)	1.00	(31)	
		3466 ^b	(31)	8 200 ± 8 700	(21)			
		120 ^p n,b	(34)					
doxycycline	564-25-0	533-578 ^b	(28)	1 000 ± 400	(32)	1.69	(31)	
		120 ^p	(31)	680 ± 780	(21)			(35)
fluoxetine	54910-89-3	^{o,b}	(28)	170 ± 50	(32)	4.97	(31)	
		a,b	(29)	230 ± 380	(21)			
		120 ^p	(31)					
norfloxacin	70458-96-7	990-1386 ^b	(28)	300 ± 70	(32)	1.27	(31)	
		120 ^p	(31)	250 ± 630	(21)			(36)
		n,b	(34)					
ofloxacin	82419-36-1	866-1733 ^b	(28)	5 400 ± 2 000	(32)	1.09	(31)	
		360 ^p	(31)	6 200 ± 8 200	(21)			

Continued on next page.

Table I. (Continued). Predicted and experimentally determined half-lives in different soils, concentrations in biosolids, as well as sorption potential (expressed as log K_{ow}) of nine PPCPs

<i>Compound</i>	<i>CAS RN</i>	<i>Half-life [d]</i>	<i>Source</i>	<i>Mean Concentration in Biosolids [$\mu\text{g kg}^{-1} \text{dw}$]</i>	<i>Source</i>	<i>Log $K_{OC}^{\text{dimensionless}}$</i>	<i>Source</i>
oxytetracycline	79-57-2	29 – 56 ^{ss}	(37)	90 ± 20	(32)	1.89	(31)
		33 ^m ; 56 ^{ss}	(38)	60 ± 50	(21)		(35)
		2.3 –	(39)				
		270 ^{ss,d}	(40)				
		15.4-90.5 ^{ss,l}					
triclocarban	101-20-2	^{o,b}	(28)	36 000 ± 8 000	(32)	3.61	(31)
		120 ^p	(31)	35 500 ± 54	(21)		
		87 –	(41)	200			
		231 ^{ss&sb}					
triclosan	3380-34-5	182-193 ^b	(28)	13 000 ± 4 000	(32)	4.37	(31)
		120 ^p	(31)	12 300 ± 16	(21)		
		20 –	(41)	800	(33)		
		58 ^{ss&sb}	(42)	1 170 – 32 900			
		18 ^{ss}	(43)				
		14 ^{ss}					

^a no observed reduction in 60 days. ^b biosolids-amended soil. ^d performed in the dark. ^e experimentally determined in soil. ^l exposed to light. ^m manure-amended soil. ⁿ no observed reduction in 21 months. ^o no observed reduction in 994 days. ^p predicted for soil. ^{ss} spiked soil. ^{ss&sb} spiked soil & spiked biosolids-amended soil. ^{w/s} water/sediment. K_{ow} , *n*-octanol/water partitioning coefficient.

Half-lives for nine of the 16 PPCPs for which data were available ranged from 14 to >1000 days (i.e., no appreciable degradation over 994 days). Large deviations in values were found for studies using spiked soil versus those in which chemicals were applied to soil entrained in biosolids (Table I). Additionally, half-lives predicted using quantitative structure activity relationship (QSAR) models tended to be lower than those found empirically for soils amended with biosolids.

The poor performance of the QSAR model suggests that correction factors need to be applied to account for degradation-limiting parameters such as diminished bioavailability and the co-occurrence of multiple contaminants. For the six compounds from Table I that had field and predicted half-life values, compound-specific correction factors were approximated by taking the average compound concentration in the field and dividing it by the predicted value. By averaging the compound-specific correction factors, an overall correction factor of 7.6 ± 6.4 was obtained. In other words, the environmental half-life of PPCPs applied in biosolids on land, on average, is 7.6 times longer than what the EPI Suite model output data suggest. This does not imply that the EPI Suite model is

invalid, however. It simply indicates that the half-life of a neat chemical spiked into pristine soil (as assumed by the EPI Suite model) is substantially shorter than when the same chemical is applied along with other trace contaminants contained in biosolids that have been weathered extensively by prior treatment processes.

Also included in Table I are typical concentrations of the various PPCPs detectable in biosolids. These values can be used to obtain reasonable estimates of the potential mass of PPCPs being applied on land during biosolids recycling. Concentrations of the nine PPCPs in biosolids were found to range between 15.0 and 36,000 $\mu\text{g kg}^{-1}$ dry weight. Further provided in Table I are values of the logarithmically transformed organic carbon/water partitioning coefficient ($\log K_{oc}$) of the nine PPCPs for which empirical half-life information was available. $\log K_{oc}$ values can serve to indicate a compound's sorption tendency. $\log K_{oc}$ values ranged from 1.00 to 4.97.

An additional data gap identified was the toxicity of PPCPs to terrestrial biota. At present there are very few studies available that deal with aquatic toxicity of chemicals. The authors found no data for ecotoxicity of the identified compounds on terrestrial organisms. Table II outlines the lowest effect concentrations for a variety of aquatic biota. Toxicity threshold concentrations ranged from 0.1 to $1.1 \times 10^6 \mu\text{g L}^{-1}$. Chronic ecotoxicity levels of PPCPs for soil dwelling biota are lacking, as are comparable data for terrestrial organisms. With the majority of studies focusing on aquatic toxicity, it is difficult to infer the potential impacts of land applied biosolids on soil-dwelling micro- and macroorganisms including plants. Moreover, studies predominantly focus on acute toxicity. However, with half-lives of certain compounds being notably longer in the field than in the laboratory, and when considering multiple biosolids applications occurring annually, it is imperative to also examine chronic toxicity and to incorporate such information into risk and safety analyses.

Bioaccumulation and biomagnification potentials of the contaminants throughout the food chain represent an additional area lacking sufficient data. Bioaccumulation of triclocarban and triclosan in aquatic and terrestrial environments was recently demonstrated for worms (5, 54). Data are absent on whether these and other lipophilic compounds also biomagnify up the food chain in birds and mammals or in fish via uptake of chemicals from biosolids-amended soils or from aquatic environments receiving PPCP inputs from soil runoff. Finally, documentation exists regarding the uptake of antibiotics, specifically tetracyclines and fluoroquinolones, by crop plants (55, 56). However, more extensive research is needed to determine the potential for micropollutant uptake by plants and crops and associated ecological and human health risks.

Table II. Lowest effect concentrations for various aquatic organisms with respect to PPCP

<i>Compound</i>	<i>CAS RN</i>	<i>Lowest effect concentration for aquatic biota</i>	<i>Value [$\mu\text{g L}^{-1}$]</i>	<i>Organism</i>	<i>Source</i>
carbamazepine	298-46-4	LOEC	100	green algae	(44)
ciprofloxacin	85721-33-1	EC ₅₀	17	cyanobacteria	(45)
doxycycline	564-25-0				
fluoxetine	54910-89-3	LC ₅₀	510	crustacean	(46)
norfloxacin	70458-96-7	LD ₅₀	1 000 000	fish	(47)
ofloxacin	82419-36-1	EC ₅₀	21	cyanobacteria	(45)
oxytetracycline	79-57-2	EC ₅₀	4.18	green algae	(40)
triclocarban	101-20-2	LOEC	0.101	crustacean	(48)
triclosan	3380-34-5	LOEL	0.12	green algae	(49)

EC₅₀, half maximal effective concentration. LOEC, lowest observable effect concentration. LC₅₀, lethal dose for 50% of a sample population.

PPCP Accumulation in Agricultural Soils

The accumulation of chemicals in agricultural soils amended with biosolids was modeled based on three different half-lives and four different application frequency scenarios (Figure 1). The results in Figure 1 are generic and applicable to any PPCP or chemical compound featuring the respective half-lives identified in the plot. Accumulation was defined to be possible if more than 10% of the applied mass was projected to still be present in the soil at the time of the next scheduled reapplication of biosolids. The Y-axis of the plot shows the mass of chemical *i* present in soil normalized to the mass of compound *i* contained in a single biosolids application. Values of less than one indicate a net loss of chemical from soil over time. A value of unity implies chemical persistence without any net removal, and a value of greater than 1 indicates accumulation of compound *i* in soil over time.

From Figure 1, it is evident that compounds having a short half-life of 40 d exhibit no accumulation potential when applied either once every three years, once annually or twice annually. At an application frequency of three times per year, however, chemical accumulation becomes possible because approximately 14% of the initially applied chemical mass is still present at the end of the third year. This analysis shows that organic compounds can accumulate in agricultural soils over time when biosolids are applied repeatedly in accordance with EPA guidelines.

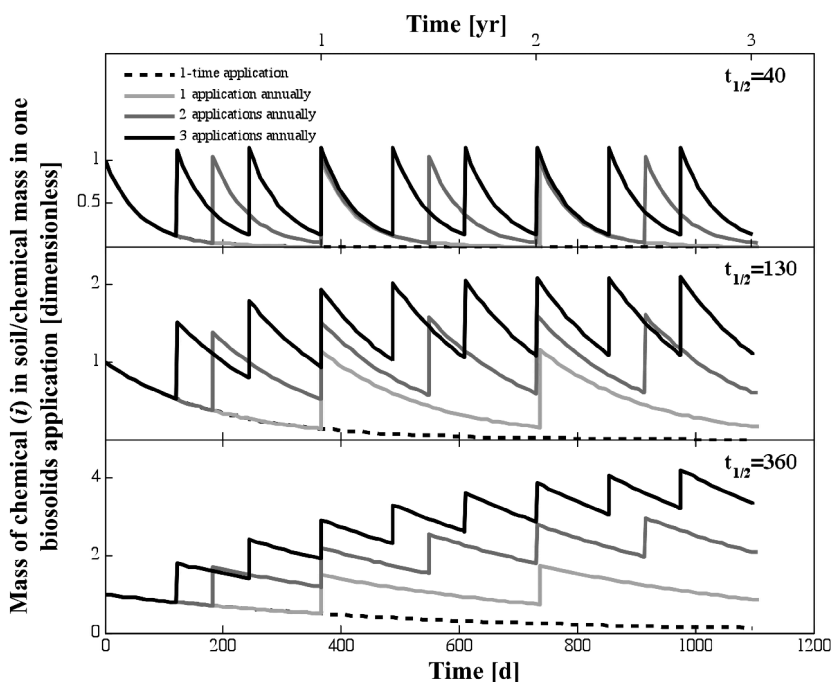


Figure 1. Predicted accumulation of organic contaminants having different environmental half-lives in agricultural soils amended with biosolids. (see color insert)

Compounds with a moderate half-life of 130 days do not have any accumulation potential when applied only once every three years. However, when the application frequency is increased to once per year, accumulation after the first, second, and third year is predicted to occur, as indicated by residual chemical concentrations of 14%, 16%, and 17%, respectively, compared to those extant after the initial application. For the subsequent scenario ($t_{1/2}=130$ days) when biosolids are land applied twice per year, 52% of the initially applied chemical mass remain after the first year and 60% and 61%, respectively, at the end of the second and third year. The model predicts that if agricultural soils are amended three times annually, residual levels of the compound in soil will be 94% of the initially applied mass at the end of the first year and 108% and 110%, respectively, of the initial levels at the end of the second and third year.

The lower panel of Figure 1 depicts the persistence of a compound in agricultural soils having a long half-life of 360 d. In contrast to the slow (40 days) and moderate (130 days) half-live scenarios discussed earlier, accumulation is possible even at a low application frequency of once every three years; at this application rate, 12% of the initial chemical mass is predicted to remain at the end of the third year. Semi-annual amendment with biosolids leads to residual chemical levels in soil equivalent to 50%, 75%, and 86% of initial levels, respectively, at the end of the first, second and third year. At a tri-annual application frequency, the residual levels at the end of the first, second and third

year increase to 192%, 287% and 334%, respectively, of those extant after a single initial application to pristine soils. This indicates the notable potential of compounds with longer half-lives to persist and increase in concentration over time in soils subjected to repeated biosolids application.

Inherent to a discussion about the fate of a chemical in soil is the phenomenon of chemical aging. The aging mechanism is believed to entail the migration of molecules into very small sites within the soil matrix (e.g., nanopores) (50). Once chemicals have become lodged in these small pores, microorganisms are unable to access the chemicals due to size exclusion. The main sorbent for hydrophobic molecules is the organic matter that comprises soils. Thus, hydrophobic contaminants become tightly bound within small pores rich in organic matter after an extended residence time in soil (50). Although aging reduces exposure of microorganisms to the pollutants (and therefore the toxicity and risk posed by chemicals to microorganisms and other biota), the chemicals may become accessible again should the structure of the soil matrix be altered (e.g., by soil erosion or tilling events).

Bioavailability is understood to represent how accessible a chemical is for assimilation by micro- and macroorganisms. Parameters which can influence a compound's bioavailability include sorption (including chemical aging), mass transfer, intrinsic degradation potential, and the presence of other compounds (29, 51, 52). It is known that organic contaminants aged in the field are considerably less bioavailable than the identical contaminants when freshly added to samples of that same soil (50). As a result of the reduced bioavailability, the capacity for biodegradation under environmental conditions likewise diminishes. This trend has been shown in several studies (28, 29, 53) and becomes evident when half-lives of field and laboratory studies are compared (Table I). Moreover, if inhibitory effects do result from co-contaminants such as antimicrobial agents or antibiotics, chemical recalcitrance in the environment may be exacerbated.

Overall, it is evident that for an accurate determination of contaminant half-life data, laboratory degradation studies performed on either spiked soils or biosolids-soil mixtures should be complemented by *in situ* studies, *i.e.*, actual field experiments.

Implications of Land Application of Biosolids

From Table I and Figure 1 it can be concluded that the majority of compounds investigated here (carbamazepine, ciprofloxacin, doxycycline, fluoxetine, norfloxacin, ofloxacin) tend to have long environmental half-lives. This signifies that these contaminants will potentially increase up to three-fold over three years due to different biosolids application scenarios. Depending on which dataset is considered, triclosan half-lives fall either into the short or moderate value range, and triclocarban in the moderate or long value range. Oxytetracycline dissipates rapidly and is thus categorized as having a short half-life with minimal potential for accumulation.

A comparison of the predicted environmental concentration (PEC) to the predicted no effect concentration (PNEC) provides a risk quotient which can be applied to determine possible risks to terrestrial plants and organisms. If

this risk quotient exceeds the value of unity, posed risks are deemed excessive and intolerable (57). According to the European Commission, the PEC may be calculated with the following equation (57):

$$PEC_{soil} = \frac{C_{biosolids} \times Rate_{application}}{Depth_{soil} \times \rho_{soil,bulk}}$$

where $C_{biosolids}$ is the chemical concentration in biosolids, $Rate_{application}$ is the application rate of biosolids to agricultural lands, $Depth_{soil}$ is the incorporation depth to which tilling occurs, and $\rho_{soil,bulk}$ is the soil bulk density.

The predicted no effect concentration represents the chemical's environmental concentration below which exposure is not expected to cause adverse effects.

As an example, a PEC_{soil} value of $57 \mu\text{g}_{\text{TCS}} \text{kg}_{\text{soil}}^{-1}$ was calculated for triclosan, based on a $C_{biosolids}$ value of $13,000 \mu\text{g} \text{kg}^{-1}$ (Table I), $Rate_{application}$ of 1.5 kg m^{-2} (26), $Depth_{soil}$ of 20 cm (58), and $\rho_{soil,bulk}$ of 1700 kg m^{-3} (58). This value is within the range of 7.4 to 88, as calculated by Reiss *et al.* (2009) for initial application. As data regarding terrestrial toxicity are scarce, Liu and Ying (2009) estimated a PNEC value of 0.01 mg kg^{-1} based on an assessment factor of 100 and a no effect concentration (NOEC) published for rice as a crop (59).

Using these values, the corresponding PEC/PNEC ratio for triclosan was calculated to be 5.7. This indicates that biosolids containing triclosan at a concentration of $13,000 \mu\text{g} \text{kg}^{-1}$ would exceed the acceptable risk. Based on the other values used in the calculation of PEC_{soil} remaining the same, the triclosan concentration in biosolids would need to be less than $2,300 \mu\text{g} \text{kg}^{-1}$ in order to achieve a PEC/PNEC ratio of less than 1. However, if triclosan-laced biosolids are applied semi-annually and assuming a moderately long half-life of 130 d, the risk quotient would be roughly 1.6 by the end of year three.

The result of the present analysis indicates that PPCPs such as triclosan may pose unacceptable risks to terrestrial organisms, but that these scenarios arise primarily when considering repeated application of PPCP-laden biosolids on land. Therefore, future risk assessments for PPCPs and other trace contaminants should take into account the accumulation of chemicals over time in soils subjected to repeated biosolids applications, as well as the typically longer half-lives that result when chemicals are added to soils contained in biosolids. The validity of future risk assessments for PPCPs could be strengthened further by generating and applying robust ecotoxicity data for terrestrial organisms.

Threshold Half-Lives of PPCPs in Agricultural Soils

The threshold half-life is understood to be the longest possible half-life at which no accumulation of the contaminant would occur. Again, accumulation is defined here as a value of greater than 100% relative to the level of PPCPs found in soil after an initial biosolids application. For the calculation of threshold half-life values it is necessary to consider the natural transport of biosolids and their associated PPCPs away from the site where application occurred. Phenomena such as leaching with water or erosion of chemicals by wind and blowing of dust from the fields may be accounted for when determining threshold half-life values.

It may be desirable to make allowances for such loss mechanisms when using half-life values determined in laboratory studies in which erosion by wind and overwatering are absent by design.

Table III identifies the predicted threshold half-life values for different biosolids application frequencies, taking into account a range of percent losses due to natural transport mechanisms. It is evident that for any given mass-loss scenario, as the application frequency is increased from once every three years to three times annually, the threshold half-life values decrease. Moreover, as the annual percentage of loss due to natural transport of chemical *i* increases from 0% to 10%, the corresponding threshold half-life values increase by up to a factor of 3. The percent values chosen here are examples only, and the actual loss rate caused by leaching and wind erosion will be determined by a number of factors, including sorption capacity of the compound, composition of the soil, annual rainfall patterns, and wind conditions throughout the year. If half-life values are available from field studies, phenomena such as leaching and wind erosion will be incorporated in the empirical measurement and thus no correction factor should be applied. When using half-life values determined in batch microcosm studies in contrast, additional mass removal mechanisms exist and may have to be accounted for. These include wind erosion and chemical leaching, and their combined effect could be negligible or much greater than the arbitrary values (2%, 5%, and 10%) shown in Table III.

Regulation of Organic Compounds

At present, organic compounds such as PPCPs are not regulated by the USEPA when applied as biosolids to agricultural soils. However, future regulation for PPCPs may be considered for wastewater treatment effluent discharged into surface waters, for effluent discharge underground for groundwater recharge, and for biosolids applied on land. Modeling results (Figure 1) show that even compounds with moderate half-lives have the potential to accumulate significantly over time. Antimicrobials and antibiotics represent potential model compounds for investigating the need for regulations based on their possible adverse effects such as the development of drug resistance in human pathogens, negative impacts on ecological biodiversity and biogeochemical cycling of nutrients, as well as bioaccumulation and biomagnifications potential.

When examining the need for potential regulations, one needs to conduct an in depth analysis of exposure levels and corresponding health effects, analytical detection capabilities, as well as the effectiveness of treatment technologies. However, as previously indicated, there are significant data gaps with respect to exposure levels and the ecotoxicity of PPCPs. Moreover, at present there are considerable analytical challenges associated with detecting trace compounds in biosolids as well as biosolids-soil matrices (28, 60). Only after these data gaps have been filled and more reliable detection methods have been made available, will it be possible to examine the need for and feasibility of regulating PPCP inputs into the environment.

Table III. Threshold half-life ($t_{1/2 \text{ crit}}$) values for PPCPs in agricultural soils when taking into account different loss percentages due to leaching and wind erosion. Half-lives greater than $t_{1/2 \text{ crit}}$ will lead to accumulation of chemicals over time following repeated application of biosolids.

<i>Application frequency</i>	<i>Threshold half-lives [d]</i>			
	<i>0% loss</i>	<i>1% loss</i>	<i>5% loss</i>	<i>10% loss</i>
<i>Effect from leaching and erosion</i>				
1 time every 3 years	99	164	253	331
1 time per year	36	54	83	105
2 times per year	17	27	41	53
3 times per year	12	18	27	35

Conclusions

This study sought to compile half-life data for 16 microcontaminants detected in soils amended with biosolids. On the basis of available environmental persistence data, simulations were performed to investigate accumulation potentials of various compounds that are land-applied in the form of biosolids. Since the simulations are anchored to biosolids application frequency and chemical half-life rather than the chemical's identity, the results furnished by the model are applicable not only to PPCPs, but also to any other chemical compound exhibiting first-order degradation kinetics at the rates used in the model. Threshold half-life values for organic compounds were predicted based on a single application of biosolids every three years, and at higher frequencies of one, two, and three annual applications. Calculated and tabulated values account for different percent losses due to leaching and wind erosion. The threshold values represent the half-lives at which accumulation of a given contaminant will occur. The following conclusions can be drawn:

- Significant data gaps were identified both for PPCP toxicity with respect to terrestrial microorganisms and for PPCP half-life data in soils amended with biosolids. Toxicity information is available for many compounds, but mainly for aquatic organisms. Moreover, available data deal predominantly with acute toxicity. For persistent compounds that do not necessarily degrade before biosolids reapplication, the acquisition of chronic toxicity data is imperative. Additionally, half-life data of PPCPs in biosolids-amended agricultural soils are limited. This literature review shows that there are significant discrepancies between PPCP degradation rates determined in laboratory and field studies.
- Modeling results show that accumulation of compounds can occur even when these have relatively short half-lives (40 d). This scenario arises when biosolids are applied three times annually, a frequency that is in accordance with EPA recommendations. This half-life threshold value represents the lower end of the half-life spectrum for the 16 compounds

included in this study. In contrast, more persistent compounds featuring a moderate half-life of 130 days may accumulate even if biosolids applications occur only once annually. For compounds exhibiting a long half-life of 360 days, at a rate of three biosolids applications annually, accumulation at the end of year three is expected to be 3 times greater than the initial concentration. These findings call into question the conclusions drawn from risk assessments in which only a one-time biosolids application is assumed (58, 61).

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Chapter 16

Manifestation of Ecotoxicity in Parts per Trillion Contaminant Levels in Natural and Simulated Environmental Settings

Hans Sanderson* and Martin Rudbeck Jepsen

University of Aarhus, National Environmental Research Institute,
Department Policy Analysis, 399 Frederiksborgvej, 4000 Roskilde, Denmark

*E-mail: hasa@dmu.dk; Tel. +45 4630 1822.

Over the past decades a blend of different active pharmaceutical ingredients (APIs) have been detected in environmental settings at trace amounts across the globe. The continual release of APIs, primarily into the aquatic environment, has earned the APIs a status as ubiquitous and pseudo-persistent contaminants of emerging concern. The question is however, if parts per trillion levels of APIs may manifest measureable toxicities and risks to the environment? The acute risks are typically negligible low from these low exposures, but the chronic risks may potentially be overlooked. This paper demonstrates three case-studies where unexpected and long-term effects were observed for three different APIs. This illustrates that chronic toxicity may occur in some cases even at very low concentrations. To detect the manifestation of toxicity at parts per trillion levels more effectively will require more specific and tailored tests relevant to the individual drug and organism of concern. There are currently being developed novel methods and frameworks that will allow a prioritization of APIs of specific concern for further tailored testing, which are briefly presented herein.

1. Introduction

Contaminants of emerging concern challenge environmental toxicology as we know it (*1*). We are able to detect manmade compounds in the environment at ever lower concentrations and at increasing speed; therefore, the list of emerging

environmental contaminants in water and other compartments is growing (2). Detection of trace amounts of individual compounds and mixtures raises the question of whether they may cause toxicity or even present an appreciable risk to humans and the environment? Both the awareness of the presence of the compounds in the environment are emerging, as well as concern surrounding the impacts of these compounds. The latter is uncertain and questions arise with regard to what the hazards are, whether risk is present at low concentrations, and whether this risk can be detected with current techniques and methods? This chapter will review three case studies from natural and semi-natural environmental settings and demonstrate how pharmaceuticals among contaminants of emerging concern bring about impacts, and even potential risks to the environment at very low concentrations. The first case study examines how trace amounts of the non-steroidal anti-inflammatory drug, diclofenac resulted in significant reductions in the Pakistani population of oriental white-backed vulture (*Gyps bengalensis*), as reported by Oaks et al. in 2004 (3). The second case study presents experiments undertaken by Kidd et al. (4) with the synthetic estrogen 17 α -ethynylestradiol (EE2), used in birth-control pills. Kidd et al. (4) treated a Canadian lake with traces of EE2 comparable to the levels found downstream of municipal wastewater treatment plants, and found a collapse of the fathead minnow (*Pimephales promelas*) population in the lake. The third and last case study is a mesocosm test under semi-field conditions with the antiparasitic drug, ivermectin undertaken by Sanderson et al. (5). During this study, the fate and effects of the drug was monitored for one year in 12,000 litre aquatic mesocosms, with focus on the partitioning of the drug between water and sediment as well as on the ecosystem effects. Finally, we will reflect upon research needs for improved testing of APIs.

2. Case Studies

The purpose of the three case studies is to illustrate how trace amounts of active pharmaceutical ingredients manifested ecotoxicity and risks towards non-target organisms. There are several studies that demonstrate ecotoxicity at low levels (3–6). However, the three studies presented here also illustrate risks at these levels and cover widely used drugs, two of which are used both for veterinary and human purposes (diclofenac and ivermectin), and the remaining drug is a hormone (EE2) widely used in the human population. The case studies are large scale and long term, with focus ranging from very large ecosystems in Pakistan to experimental lakes facility in Ontario, Canada and an experimental mesocosm study facility. In the latter the ecosystem was replicated, allowing more robust statistical evaluation of the observations, as does the experimental lake facility in Ontario.

2.1. Diclofenac and Pakistani Vultures

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) used to reduce pain and inflammation in humans and animals. It was introduced as a treatment

for livestock on the Indian subcontinent in the early 1990s, coinciding with the beginning of a rapid decline in Gyps vulture populations. It took approximately 10 years to demonstrate that this was more than a coincidence. In 2004, Oaks et al. published a study in *Nature* (3) in which very clear evidence that diclofenac is lethal to Gyps vultures was presented. In their study undertaken in Pakistan in 2000-2003, the authors examined 219 dead oriental white-backed vultures (OWBVs), of which 85% showed characteristics of visceral gout in their internal organs (as evidenced by subsequent deposition of uric acid on and in their organs). Of the 219 OWBVs, 42 were found within 24 hours after death and detailed necropsies were performed. 14 of these showed no signs of visceral gout (other causes of death could be established in eight cases), while the remaining 28 with visceral gout displayed severe, acute renal tubular necrosis. Among the 28 cases, only one had an identifiable infection and no toxic concentrations of heavy metals, pesticides or pathogens were detected. For 25 of the 28, liver samples were tested for diclofenac residues. Residuals were found in all samples at concentrations ranging from 0.051 to 0.643 $\mu\text{g g}^{-1}$ (unadjusted wet weight (w.w.)). Of the 14 OWBVs without visceral gout, samples were taken from 13, and none of them showed diclofenac residues.

The next step by Oaks et al. (3) was to administer veterinary diclofenac orally to 4 captive vultures. The recommended mammalian dose of 2.5 mg kg^{-1} was given to two vultures while the remaining other two received a smaller dose of 0.25 mg kg^{-1} . All four birds developed hyperuricaemia within 24 hours, and three of the four birds died within 36-58 hours after administration. Finally, 10 juvenile OWBVs were fed meat from cattle or goats that had received the recommended therapeutic dose of diclofenac intramuscularly once daily for three days and were slaughtered 4 hours after the last injection. The resulting diclofenac residues in meat and organs of these livestock ranged from 0.19 to 5.7 $\mu\text{g g}^{-1}$ (w.w.). Additionally, 10 OWBVs were fed meat containing 6.4 $\mu\text{g g}^{-1}$ of diclofenac. By accounting for the meat consumed by the birds, eight OWBVs received doses between 0.005 and 0.3 mg g^{-1} , resulting in death by renal failure in two of these. Two OWBVs received doses of 0.5 to 0.6 mg g^{-1} , one of them died of renal failure. Ten birds consumed meat corresponding to doses of 0.8 to 1.0 mg g^{-1} , all of which died from renal failure. The resulting mortality rate of 13 deaths among 20 vultures (65%) demonstrates a significant relationship between exposure to diclofenac and renal failure.

It is now accepted that scavenging on livestock carcasses treated with diclofenac shortly before death poses a serious threat to Gyps vultures, and contamination with diclofenac of just one out of 760 livestock carcasses is sufficient to explain the collapse in Gyps vulture populations on the Indian subcontinent (7). The 80% to 95% collapse of the OWBV population lead to a rise in the number of scavenging stray dogs, which in turn resulted in an increase in rabies incidents in humans due to contact with these dogs. As a result, on 11 May 2006, the Drug Controller General of India ordered the withdrawal of all licenses granted for the manufacture and veterinary use of diclofenac in India (8).

2.2. EE2 and the Fathead Minnows in a Canadian Lake

It is well known that the presence of estrogenic substances is associated with reproductive endocrine disruption in male fish populations (9), leading to vitellogenin (VTG) production and early-stage egg development in their testes. However, other synthetic estrogen mimicking compounds and pathways have been suggested as other causative agents (10). Estrogens have been found in surface waters and wastewater effluents at concentrations of 17 and up to 147 ng l⁻¹, measured as equivalents of the natural estrogen 17 β -estradiol (E2) (11).

In a recent whole-lake study conducted from 1999 to 2005 in Ontario's Experimental Lakes Area, Kidd et al. (4) describes the effect of constantly exposing a population of fathead minnow (*Pimephales promelas*) to the synthetic estrogen 17 α -ethynylestradiol (EE2) for three consecutive years (2001-2003). The fathead minnow has a lifespan of up to 4 years and reaches sexual maturity during the second year of life. EE2 was added at concentrations similar to, or below those found in rivers receiving water from wastewater treatment plants, with seasonal mean values in the experimental lake kept from 4.8 to 6.1 ng l⁻¹. The fathead minnow population was regularly sampled, and gonadal development as well as VTG mRNA and protein concentrations in the exposed fish were compared to populations of fathead minnow in two non-exposed experimental lakes. Seven weeks after the experiment started, whole-body concentrations of VTG in males were three orders of magnitude greater than reference samples, and normalized liver VTG mRNA values in males were approximately a factor 100 greater than values found in males in reference lakes and more than an order of magnitude greater than the levels found in female fish in the reference lakes. In the spring of 2002, less than a year after the EE2 additions began, testicular tissues from sampled males showed delayed spermatogenesis, widespread fibrosis, and malformations of the tubules. The following year, 2003, almost half (~45%) of the males captured had ova-testes displaying primary-stage oocytes. The impact of EE2 additions was striking. In the second year of exposure, no young-of-the-year were caught, and the adult population had severely declined compared to abundance pre-exposure and in the reference lake. After the three years of EE2 additions the fathead minnow population had completely collapsed (4).

2.3. Ivermectin and the Pelagic Ecosystem Structure and Function

Antiparasitic drugs are among the most important groups of veterinary pharmaceuticals in the European Union (EU), with a market volume of more than 200 million euro. Ivermectin (CAS# 70288-86-7) is one of the most widely used macrocyclic lactones and has been known as a potent, effective and safe antiparasitic drug since 1981. The compound inhibits the signal transmission at GABA-gated and glutamate-gated chloride channels in the target parasite causing death. GABA receptors respond to the neurotransmitter gamma-aminobutyric acid (GABA), which is the the major inhibitory neurotransmitter in the vertebrate central nervous system (CNS). Ivermectin shuts down the CNS in the target organism thus causing death. The compound is commonly used for treatment and

prevention of internal and external parasites of cattle, horses, and other animals on pasture (e.g. gastrointestinal and respiratory tract nematodes, flies, grubs, ticks, lice, and mites) (5). Environmental assessment of ivermectin found that *Daphnia magna* was the most sensitive species with a 48 hr leathal concentration for 50% of the test organisms (LC_{50}) = 25 ng L⁻¹, and a no observed effect level (NOEL) at ~ 10 ng L⁻¹. Due to a high organic carbon adjusted sorption coefficient (K_{oc} > 12,600), and rapid dissipation in water, with a half-life (DT_{50}) of between 12 and 39 hrs, the calculated and measured worst-case environmental concentrations in surface waters ranged from 2 to 25 ng L⁻¹.

The investigation described in this case study commenced in August 2004 at the University of Guelph Mesocosm Facility in Ontario, Canada (latitude 43.5 ° N), and ended in May 2005. The facility consists of 30 mesocosms that are sunk into the ground and designed to replicate natural pond systems. The mesocosms are approximately 1.2 m deep with a water depth of 1 m, a diameter of 3.9 m and a surface area of 11.95 m², with a volume of approximately 12,000 l of water. The treatment regime was in triplicate ($n = 3$): Controls; 30; 100; 300; and 1,000 ng l⁻¹ (parts per trillion (ppt)) nominal concentrations). Water and sediment concentrations were monitored as well as the biotic and abiotic parameters of interest. All the measured ivermectin concentrations at the beginning of the study, after treatment, were between 10 and 25% below the nominal concentrations. The aquatic DT_{50} was determined to be 4 days. Ivermectin built up over the first 3-4 weeks following treatment and then stabilized at between 20 and 30 µg kg⁻¹ for the highest treatment levels. Cladoceran and Copepod species richness, Ephemeroptera abundance, DO and pH were significantly impacted during the chronic phase (day 10 to 100), where the measured ivermectin water concentration was below the detection limit (1 ng l⁻¹), and approximately 25 ng kg⁻¹ in the sediment, suggesting potentially severe chronic risk (aquatic risk quotient = 25) to the zooplankton communities as well as more indirect ecosystem functional parameters such as pH and dissolved oxygen. The long-term effects (day 100 to 265) remained evident only for Ephemeroptera abundance and the cladoceran *Chydorous* spp after the winter and into the spring, and at this time ivermectin was no longer detectable in the water phase, only in the sediment. Ephemeroptera and *Chydorous* spp. are relatively more active in the sediment compartment than the majority of the other species monitored (12) and a relative, increasing, long-term sensitivity is thus assumed due to relatively elevated exposure via the sediment. Ephemeroptera and *Chydorous* spp. had not recovered by the end of the study, almost one year after treatment (5).

3. Discussion and Conclusions

On the basis of these three case studies, we can conclude that ecotoxicity can manifest itself at parts per trillion levels. Some effects are unexpected, such as the collapse of the vulture population in Pakistan, some are potentially severe at realistically low levels as demonstrated by EE2 and the fathead minnow collapse, and some may show long-term structural and functional effects in aquatic ecosystems (ivermectin). Impacts have received the attention of regulators, as

we heard from India with regards to diclofenac, but also targeted removal of endocrine disrupting compounds (hereunder EE2) from municipal wastewater has come under consideration (13). Continued veterinary use of ivermectin has also been scrutinized, both with regards to its impact on dung beetles and thus pasture productivity, but also with regard to its use in aquaculture to treat sea lice in salmon (see e.g. the FP6 project ERAPharma where ivermectin was the subject of one of three case studies <http://www.erapharm.org/index.html>).

How can ecotoxicology in future address similar surprises and unintended impacts on the environment as well as help avoid them? Can we adapt our testing regimes to elucidate these subtle effects? As previously mentioned, pharmaceuticals in trace amounts present a challenge for ecotoxicology, but there are actually a number of tools available that can guide future chronic impact assessments. One of these tools is the Comparative Toxicogenomics Database (CTD) (<http://ctd.mdibl.org/>). Here it is possible to identify the gene interactions of individual compounds as well as subsequent proteins and signalling pathways. For diclofenac interaction is with CYP2CP, and we can see that diclofenac shares 19 common gene interactions with another NSAID naproxen, suggesting that naproxen might not be a suitable substitute. Meloxicam would probably be a better substitute from a Gyps vulture protection perspective as it does not share gene interactions with diclofenac (14). Ivermectin interacts with the ABCB1 (ABC being the ATP Binding Cassettes), a widespread protein family in many species and levels of biological organization, and ivermectin may cause liver cirrhosis in humans. EE2 is well known to interact along the hypothalamic-pituitary-gonadal (HPG) axis.

Furthermore, the ToxCast program (<http://www.epa.gov/nct/toxcast/>), hereunder the Aggregated Computational Toxicology Resource (ACToR) (<http://actor.epa.gov/actor/faces/ACToRHome.jsp>) is a user friendly resource that rapidly can provide an overview of a compound's toxicity. Armed with screening analyses using these and other similar databases and tools (15), it is possible to address and investigate concerns about individual active pharmaceutical ingredients as well as other chemicals more specifically, and to suggest more specific toxicity assessments for each compound, thereby avoiding a one-size-fits-all testing system. Huggett et al. (2003) (16) suggested a screening approach based on a comparison between the human therapeutic plasma concentration of a drug to the measured or calculated steady-state plasma concentration in fish, and thereby derive a relative risk ranking. Ankley et al. (2007) (17), suggest screening and prioritization of APIs for further investigation based on assessment of the exposure potential based on production volume and predicted environmental concentrations. The next tier would be assessment of the presence target pathways that may lead to toxicologically relevant responses, i.e. an assessment of the Modes of Action. This approach has recently been further developed, in a systems biological framework designed to elucidate the adverse outcome pathway (AOP), which portrays the linkage between molecular events and an adverse outcome at a biological level relevant to risk assessment (18). With these analysis and prioritization it would be possible to determine relevant assessment endpoints tailored for the germane specific questions concerning the manifestation of APIs chronic risks towards ecosystems at parts per trillion levels.

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Chapter 17

Presence of Pharmaceuticals and Personal Care Products in the Environment - A Concern for Human Health?

Sara Bachman Ducey¹ and Amir Sapkota^{*,2}

¹Montgomery College, Rockville, MD 20850

²University of Maryland College Park, School of Public Health,
College Park, MD 20742

*amirsap@umd.edu

Pharmaceutical and personal care products (PPCPs) have changed the way we live. However, their widespread use has led to unintended challenges to our society; some of which are well documented, while others are poorly understood. Systematic evaluation of the current literature indicate that unintended human exposures to PPCPs are inadequately characterized, and the resulting health outcomes among general population are scantily investigated. Data on occupational exposures at PPCPs manufacturing facilities are not readily available. In the absence of this, studies have relied on sewage workers, who are potentially exposed to higher level of PPCPs via dermal and inhalation routes. Occupational studies of this population have shown increased risk of cancer among exposed population, however, there is poor concordance of cancer sites between studies. Furthermore, lack of adequate adjustment for co-exposures to other chemicals and microbial agents makes it difficult to attribute the increased risk to PPCPs alone. Risk assessment studies performed thus far have concluded no appreciable risk to humans, however these studies overlook non-cancer endpoints, do not take into consideration the most recent data, and are often commissioned by the manufacturers of PPCPs. This chapter summarizes the human health aspects of PPCPs that are present in the environment. Current data gaps and future research priorities are identified, along with a

special focus on human exposure and potential adverse health outcomes.

Introduction

Pharmaceuticals and personal care products (PPCPs) have become an integral part of modern day society. This broad array of chemicals includes prescription and over the counter (OTC) drugs; veterinary drugs; fragrances; cosmetics; sun-screen (ultra violet block) products; diagnostic agents; and nutraceuticals (e.g. vitamins) (1). They have changed the way we live and altered our capacity to fight diseases in ways that would have been hard to imagine, even a generation ago. However, their widespread and continuous use combined with improper disposal presents a new set of challenges – to the environment, to public health and to regulators who seek to assess and reduce human health risks.

Large quantities of PPCPs and their metabolites are released into the environment on a daily basis. This continual infusion into the environment enables PPCPs to behave as though they are persistent chemicals – *pseudopersistent* (2). Over the past two decades, an increasing body of literature has documented a widespread distribution of these pollutants throughout the global environment, as documented in a comprehensive database maintained by US-EPA (1). Once in the environment, most PPCPs are re-distributed through various media, ultimately resulting in unintended human exposures, potentially resulting in health risks that have yet to be fully understood and quantified. Although concentrations of each of these chemicals are typically low, concerns about human exposures continue to mount. This is because scientific knowledge about the wide variety of PPCPs present in the environment is improving and expanding, and data gaps remain regarding cumulative exposure across different exposure pathways and how different PPCPs may interact with each other. The risk assessment paradigm that is being utilized today does not adequately address subtle health outcomes, and lacks the scientific knowledge to deal with the chronic exposure to mixture of large variety of PPCPs and the potential interactions between them. The standard protocol for deriving toxicity values uses single chemical exposure under controlled settings, a situation that is not realistic in everyday life. In addition, the risk assessment applied in this particular field is vulnerable to bias, as industry risk assessment that is often relied on the decision-making process is based on data that are not public, and as such cannot be independently verified.

This chapter will discuss PPCPs from the human health perspective. It will describe sources of PPCPs and pathways of exposure, review available human exposure data and risk assessments, summarize occupational studies of sewage workers, identify gaps in existing research and suggest areas for future research. This chapter focuses on contaminants that arise from human activity: anthropogenic environmental contaminants. Natural chemicals from plants and animals will not be addressed here. Ecological health, which has been studied far more extensively, will not be discussed here. To learn more about ecological health concerns, readers are encouraged to refer to (3, 4).

Sources of Environmental PPCPs

Pharmaceuticals are released into the environment in a variety of forms. Some are released as the active pharmaceutical by a consumer who discards the drug down a sink or toilet. Others may have undergone a range of metabolic transformations by the human user, and are excreted as a mixture of parent drug and its metabolites. Because the uses of PPCPs are unremitting, their excretion and release into the environment is continuous. As a result, while some pharmaceuticals break down readily in the environment, they are constantly replenished, allowing for persistent environmental presences. A steady stream of research over the past two decades has documented the sources of environmental PPCPs, as summarized by Daughton, et al. (5). These sources include: individual use, hospital use, veterinary and agricultural use, and pharmaceutical manufacturing and respective wastes from these processes. While some of the human use PPCPs end up in wastewater treatment plants (WWTP), others are directly released into the environment. At WWTP, some of the PPCPs are removed, others remain untreated, and re-released into the environment as wastewater effluent (3). The major sources of environmental PPCPs are described below.

Individual Use and Waste

Global sales of pharmaceuticals were estimated to be \$820 Billion in 2009 (6), consisting of more than 3000 different substances used as medicines including painkillers, antibiotics, contraceptives, beta-blockers, lipid regulators, tranquilizers, and impotence drugs (7). It is estimated that the industry will continue to grow 1-2% per year in mature markets, such as the US, while the projected growth in developing countries may be as high as 5% (6). Statin drugs, described as one of the “most widely prescribed and most lucrative sectors in the pharmaceutical industry” provided total revenues exceeding \$26 billion in 2008 (8). In the US alone, nearly 12 million women used oral contraceptives in 2002. Such usage contributes to the buildup of PPCPs in the environments in a number of forms.

Pharmaceuticals that are directly consumed are metabolized to one or more daughter products and are excreted along with the non-metabolized parent compound, ultimately ending up in the WWTP and the surface water that receive the WWTP effluents (9). In addition, topical PPCPs that are externally applied end up in the water systems during bathing or recreational swimming (10, 11). Dermal applied PPCPs may also enter the environment via bathing and washing of clothing (10). Additionally, many consumers discard their unused pharmaceutical products by flushing them down the toilet, which are ultimately in the environment with WWTP effluent (8).

Hospital Use and Disposal

A broad range of powerful cleaners (disinfectants and solvents); pharmaceuticals (antibiotics, and chemotherapy agents) and diagnostic chemicals

(radionuclides) are used in hospitals (13). Hospital patients excrete active pharmaceutical ingredients and their metabolites in the urine. End of life care often involves heavy sedation and/or other therapeutic pharmaceuticals; once the patient dies, the drug and chemical residues in and on their body may enter the environment (12, 13). In addition, many hospitals, hospices and caretakers may flush drugs left behind by patients who die (10, 11). Many of these chemicals find their way down the hospital drains, untreated, thus hospital wastewater is a major sources of these PPCPs (14), as evidenced by the presence of antineoplastic (chemotherapy) drugs in the Thames River and in hospital wastewater in Haiti (14)

In addition to hospitals, funeral parlors that practice embalment commonly discharge withdrawn body fluids directly into municipal sewage systems (12, 13). These fluids contain drugs administered during heroic lifesaving or palliative care. Following burial, the embalming fluid and other PPCPs remaining in the body can be released into groundwater, especially in facilities that were poorly designed with respect to local hydrology (12, 13).

Veterinary and Terrestrial Food Animal Waste

Range of pharmaceuticals are used in terrestrial food animals (16). Such use of medication can result in the release of active pharmacological agents or their metabolites into the environment. These pharmaceuticals or their metabolites may be released in the environment during direct run-off of the unused residue and/or their metabolite in the manure. Alternately they move into the environment when the manure or slurry are land applied (18, 19). Pastured animal agriculture relies on far fewer medications, if any, and results in less environmental contamination (19). In addition to terrestrial food animals, domestic companion animals may serve as additional source of pharmaceuticals in the environment, as they are often treated with medications. These are administered orally, or may be applied topically. The excreta of these animals is often introduced into the environment, directly, without treatment (20).

Land Application of Municipal Biosolids

Biosolids are human sewage sludge that has been treated to meet the land-application standards as outlined in the Code of Federal Regulations, Title 40 Part 503. The standard was established by US EPA to oversee the national biosolids program, which is often land applied to serve as an inexpensive source of nutrients (21). Part 503 sets limit on concentration of chemical pollutants including metals and requires reductions of pathogens. In 2002, it was estimated that 5.6 million tons of dry sewage sludge was used or disposed of annually in the US. Of that, an estimated 3.36 million tons were land applied (21). Recent studies suggest that the particle-bound PPCPs can survive the extensive treatment process at the WWTP without undergoing any net transformation, entering the environment when the treated municipal sludge (biosolids) are land applied on agricultural fields (22). Once the particle bound PPCPs are land applied, precipitation will influence the rate and amount of movement of PPCPs from the municipal biosolids into the

drainage (23). Thus, biosolids can act to transport PPCPs from the wastewater stream, back to the land, and to the surrounding watershed.

Pharmaceutical Production and Waste

Direct discharge and/or irresponsible disposal from manufacturing sources are point sources of PPCP contamination of the environment. This will be a greater problem wherever regulations do not exist, or are not enforced. A study conducted near Hyderabad, India (a region with significant pharmaceuticals manufacturing) found that local lakes were contaminated with milligrams per liter of drugs (a concentration 100,000 to 1,000,000 times higher than reported levels of fluoroquinolones in surface water in the US and China (24). In two lakes, concentration of ciprofloxacin, cetirizine, norfloxacin and enoxacin were 6.5, 1.2, 0.52 and 0.16 mg/L respectively, exceeding the human therapeutic blood plasma concentrations (2.5 mg/L and 20 µg/L, for ciprofloxacin and cetirizine). Furthermore, analysis of well water in that area indicated that the analyzed drugs can contaminate groundwater over large areas – posing a direct route for human exposure, and a pathway for contamination of local food fish (24).

WWTP Effluent

Wastewater treatment plants (WWTP) do not completely remove PPCPs (22). A three year-long study of seven WWTPs in Spain found that conventional wastewater treatment processes were unable to completely remove most of the 73 pharmaceuticals studied. As a result, tributaries receiving the effluent from WWTPs have elevated levels of PPCPs (25, 26). The PPCPs released in wastewater effluents include the gamut of human pharmaceuticals, personal care products (PCPs), pesticides, as well as phenolic estrogens (27). The types of chemicals in the wastewater stream will vary by community, based on demographic characteristics. Some existing water treatment technologies can effectively remove ‘certain’ PPCPs (28, 29) but concentrations of PPCPs in wastewater effluent is dependent upon the degree/effectiveness of the treatment employed (29–31). Research by Sui, et al. studying removals of PPCPs by wastewater treatment found that most compounds were hardly removed by primary treatment, removal by secondary treatment was highly variable (ranging from 12–100%) and tertiary treatment showed “discrepant performance” (32). Gros et al. suggest that higher hydraulic retention times should be required, as more time in the system facilitates more complete degradation (26). Future studies need to focus on optimum conditions required to removal of different classes of PPCPs.

Pathways of Human Exposure

Human exposures to PPCPs are many and varied, with water playing a central role in the transport of PPCPs. They include: the consumption of water, plant and animal based (land and aquatic) food products, as well as dermal and inhalation

exposures. Currently there are very few published studies that have attempted to quantify human exposures (33).

Drinking Water

Wastewater treatment plants have variable effectiveness in removing pharmaceuticals and personal care products (26, 29, 30, 32). As a result, surface waters receiving effluent from WWTP often contain measurable amounts of PPCPs (25). These surface waters are the dominant sources of drinking water in most areas. However, the surface waters undergo additional treatments before they are distributed as point of use (POU) drinking water. These wastewater treatment processes may change the form as well as concentration of PPCPs in the drinking water, compared to the surface water (33). Most of the current literature examining PPCPs contamination of water has provided concentrations in surface waters. For the most part, neither the concentration at the tap/POU, nor the relationship between surface water and POU water concentrations are known, which hamper risk assessment.

Food

Food products containing PPCP residues may represent another significant pathway of human exposure. Unfortunately, the level of contamination in our foods from these unintentional residues is poorly characterized (33). This presents a very significant data gap as we attempt to assess risk to human health from environmental PPCPs. Food related human exposure to PPCP may occur via consumption of plant crops grown with biosolids or reclaimed wastewater, fish from contaminated water bodies, aquaculture food products raised using antibiotics or human and animal excreta, and terrestrial food animals raised using antibiotics or exposed to PPCPs via their diet.

Plant Crops Grown with Biosolids

The uptake of veterinary medicine, PAHs, and pesticides from soil, into plants, is well documented (27, 34, 35). Boxall et al. (34) have shown that PPCPs present in the manure can persist in soil for up to five months after land application. The authors chose carrot and lettuce to study the uptake, and observed that the two plant types accumulated different drugs. Florfenicol, levamisole, and trimethoprim were taken up by lettuces, while Carrot root took up diazinon, enrofloxacin, and florfenicol. This study employed typical food consumption figures from the World Health Organization's Global Environment Monitoring System/Food database. Typical dietary intakes for crops grown above ground (using lettuce data) were 0.512 kg/ day and 0.333 kg/day of plant crops grown below ground (using carrot data) In most cases they found the accumulated concentrations to be lower than the acceptable daily intakes (ADI). In a few cases, they found concentrations accounting for ~10% of the acceptable daily intake values (34). The authors concluded that there is little evidence of risk, from such

food items. However, it is unlikely that these food items would be the only source of exposures. In, reality, exposure to PPCPs will take place across a number of additional pathways, including ingestion of water, animal based food products, as well as dermal and inhalation exposures, which were not accounted for in the study.

It is unclear, what fraction of the PPCPs will be degraded in either the soil or in the plant, leading to transformation products. Some of these substances are less toxicologically active, but others may have toxicity that is similar to, or even greater than the parent compounds (34). However, it remains unknown what exact transformation may take place throughout this process. Regardless, the behavior of PPCPs in the environment may be determined by a range of factors, including: sorption kinetics of the respective compound, soil organic matter, and soil pH (18), as well as H-bonding potential, cation exchange, cation bridging at clay surfaces, and complexation (34). Research into which chemicals are most likely taken up, and how to manage them will be highly useful to prevent potential exposure. For example, Boxall et al. showed that the majority of the veterinary medicines were taken up by the outer layer of the carrot, thus deep peeling could potentially reduce exposures (34). It may also be useful to study how cooking and other food preparation techniques may help to manage/reduce PPCPs in the food, as eaten.

Plants Grown with Reclaimed Wastewater

Agriculture places tremendous demands on local water supplies. The United Nation (UN) estimates that agriculture accounts for 70 percent of all water use (36). In some developing countries this percentage climbs as high as 95 percent (36). The demand to produce more crops to feed ever increasing population will place even greater pressure on scarce water supplies. Given that water shortages are becoming increasingly frequent and intense, we can predict increasing interest in, and use of, recycled wastewater in irrigation of agricultural land (27). Reclaimed wastewater for irrigation is a practice already in use in many parts of the world (27). Exposures to PPCPs can be expected to be higher in areas where reclaimed wastewater is used for food crops, as reclaimed wastewater can contain significant amounts of PPCPs. For example, Calderón-Preciado et al. (27) reported ibuprofen concentration of 28.5 ng/g in lettuce samples purchased at a local market, where the practice of using reclaimed wastewater is common.

Fish from Contaminated Water Bodies

Fish and other seafood products from the surface water contaminated with PPCPs can have elevated levels of PPCPs. Routine consumption of such contaminated food product may increase individual's exposure. Chu and Metcalfe (37) studied the accumulation of selective serotonin reuptake inhibitors in fish tissue gathered from Hamilton Harbour in Ontario, Canada. The authors reported concentrations of up to 1 $\mu\text{g}/\text{kg}$ wet weight for three analytes (paroxetine, fluoxetine and norfluoxetine) in fish tissue (37).

A national pilot study conducted by Ramirez, et al. (31) assessed the accumulation of PPCPs in fish sampled from five effluent-dominated rivers that receive direct discharge from wastewater treatment facilities (Chicago, Dallas, Orlando, Phoenix and West Chester). They compared these with fish collected from the Gila River in New Mexico as a reference group, expected to be “minimally impacted by anthropogenic influence.” They found norfluoxetine, sertraline, diphenhydramine, diltiazem and carbamazepine at ng/g concentrations in fillet composites from the effluent-dominated locations (31). The drug residues were present in both muscle (fillet) and liver of fish, but the concentrations were much higher in the liver than in fillet. In response to this pilot study, the US-EPA has added sampling for PPCPs to its national survey of rivers and streams. This study will analyze fish and surface water samples from 150 randomly-selected urban river sites, across the United States. Results from this study are expected in 2011 (38)

Aquaculture Food Products Raised Using Antibiotics or Human and Animal Excreta

Potential exposure to pharmaceuticals resulting from the consumption of aquaculture products has been previously reviewed in detail (39). As a result of the non-hygienic and stressful conditions (40) present in aquaculture facilities—including high fish densities, high farm densities in coastal waters and lack of appropriate barriers between farms (41)—the risk of bacterial infections among aquacultured fish is high. Therefore, heavy amounts of antibiotics are administered in fish feed for prophylactic (disease prevention) and therapeutic (disease treatment) purposes in aquaculture facilities worldwide (42–45). A study conducted in Norway, determined levels of oxolinic acid residues in wild fauna that were affected by medicated fish ponds (46). Results from this study indicated that wild coalfish, mackerel, haddock and crabs from areas impacted by a medicated fish pond had elevated concentrations of oxolinic acid in plasma, liver and muscle tissue (46).

In addition, there is a long history of administering wastewater, animal waste and human excreta to fish ponds (47). The waste is consumed directly by the fish and provides nutrients for the growth of photosynthetic organisms (48, 49). Currently, this intentional application of fecal wastes to fish ponds is in decline in many Asian countries as a result of shifts in land use and shifts towards the production of “high-value” species such as shrimp, which cannot be effectively grown in wastewater (47). However, unintentional use of wastewater and excreta in aquaculture systems may be increasing as surface water pollution continues to intensify in areas where aquaculture facilities are situated (47). In addition, in some Asian countries such as Vietnam, the use of wastewater and excreta in aquaculture is still widespread, and, in some northern Vietnam cities, the use of sewage in aquaculture ponds remains the only available method of wastewater treatment and disposal (47). Thus, consumption of aquaculture products grown in such facilities are likely to increase exposures to PPCPs.

Terrestrial Food Animals

The consumption of terrestrial food animals also provides an exposure pathway for PPCPs. The animal feed used to grow these animals contain pharmaceuticals (16), and the food animals will accumulate veterinary medicines either through consumption of these feed, or through the food chain (34). Furthermore, these animals may get additional exposure to PPCPs when they consume plant crops from soils treated with reclaimed wastewater and/or animal waste/human biosolids. The National Residue Program operated by the United States Department of Agriculture's Food Safety and Inspection Service (FSIS) provides monitoring of animal foods destined for human consumption. This program was responsible, for example, for a 2010 recall of 87,000 pounds of beef products, including canned corn beef, from Brazil. They were found to be contaminated with the antiparasitic drug, Ivermectin in excess of the 10 parts per billion tolerance level. (50).

Dermal Exposure

Most pharmaceuticals have poor dermal transport, so they generally pose a smaller risk than do personal care products (33). The pharmaceuticals that will pose the greatest risks will be those that were specifically designed to be administered via dermal routes, such as hormones (patches, rings, and topical gels) and other lipophilic preparations. Because the delivery device may still contain a large percentage of the drug at the completion of treatment, the discarded devices pose a route of exposure for anyone handling it post use (11). Fentanyl patches, for example, retain 28-84% of their original content after 3 days of use (10, 11). Such pharmacologically active devices pose a risk further down the waste stream if they are flushed, or discarded to solid waste. Some drugs are excreted in sweat; some are excreted via the pores that line the fingertips (10, 11).

Personal care products can be absorbed through the dermal route while individuals shower or bathe with water containing PPCPs. Dermal exposure to PPCPs may also occur while swimming or boating in contaminated streams or lakes (1). Kayakers are potentially exposed to the water as they flip and surf, and thus likely encounter higher frequency of exposure. Because swimming pools are fairly small, the concentrations of PPCPs may be appreciably higher than in larger, natural bodies of water (10, 11). For both bathing and recreation, the actual exposure would vary according to factors such as concentration of PPCPs, temperature of water, pH of the water, contact duration, and condition of dermis.

Inhalation Exposure

Agricultural workers may be exposed through the inhalation of dust containing PPCP contaminants in intensively-reared livestock facilities (34). Workers working in agricultural fields where biosolids have been applied may be exposed to particle bound PPCPs, when biosolids get re-suspended in the air. Residential use of biosolids to nourish lawns and non-food gardens can also lead to inhalation exposures. Health care workers may get exposed to vapors

of therapeutic drugs (e.g. antineoplastics) that may off-gas from patients during treatment (10, 11).

Issues Related to Human Health

An extensive body of literature describes adverse ecological effects associated with the presence of PPCPs in the environment, though these are beyond the scope of this chapter. Data regarding human health effects are scarce. It must be acknowledged that the current studies available in the literature are far from optimal, and significant proportion of them focuses on cancer endpoints. We have evaluated the current available literature on human health effects associated with PPCP exposure, with emphasis on occupational studies of sewage workers, as most studies have focused on this group. It should be noted that future studies exploring the health effect associated with PPCP exposure should not rely exclusively on sewage workers as they are exposed to host of other chemical and biological agents in their workplace beside PPCPs. This section will also evaluate the risk assessment literature and emergence of antibiotic resistant bacteria.

Cancer Risks among Wastewater Workers

The challenges with conducting epidemiological studies focusing on PPCPs stems from the fact that the endpoints and the exposure-disease latency period is poorly understood. Furthermore, the environmental exposure levels are relatively low, so the likelihood of observing effects with small scale study is very small. The best epidemiological evidence may come from workers involved in the PPCP manufacturing process. However, such data are not made available by industry. In the absence of this, epidemiological studies of workers in sewage treatment facilities, as well as workers involved in land application of biosolids and communities living around areas where biosolids are applied may provide meaningful insights. These populations may be exposed to elevated levels of PPCPs via inhalation and dermal routes. However, such epidemiological studies need to carefully account for confounders such as additional contaminants (other than PPCPs) as well as co-exposures to microorganisms.

Occupational studies so far have reported an increased, but statistically non-significant, risk of overall cancers risk among workers employed in sewage related occupation (21). Importantly, when the analyses were stratified by specific sites, increased risks were observed by numerous investigators, some of which were significant (51–54). Using a retrospective cohort study of 477 sewage workers who had worked at least 1 year at BASF's wastewater treatment plant in Germany, Nasterlack et al. (51) reported a standardized incidence ratios (SIR) of 6.82 (95% confidence interval (CI): 1.86-17.46) for bladder cancer among subgroup of workers involved in sewage sludge treatment and incineration process. However, the co-exposure resulting from incineration process was not accounted for. Similar risks were not observed among the maintenance workers or the wastewater processing workers. In a separate study of 487 sewage workers in Buffalo NY, Lafleur and Vena (52) reported an increased standardized mortality

ratio (SMR) of 7.93 for laryngeal cancer (95% CI 1.59-23.96) and liver cancer (SMR 5.4, CI: 1.10-16.05), although the SMR for all cancers were not elevated. Friis et al. (53) reported an increased risk of stomach cancer (SMR 2.73, CI: 1.00-5.94) and kidney cancer (SMR 2.60, CI: 1.00-6.80) among Swedish sewage treatment plant workers. Hansen et al. (54) examined the cancer risk among 591 Danish wastewater workers, using either water supply workers or general Danish population as controls. Using water supply workers as control population, the authors reported an increased risk of respiratory cancer (RR 1.65, CI: 1.00-2.74) and primary liver cancer (RR 8.9, CI: 1.5-51.5). When the authors compared the wastewater workers to general Danish population, they observed increased risk of respiratory cancer (SIR 2.09, CI: 1.27-3.22), lung cancer (SIR 2.03, CI: 1.18-3.25) and primary liver cancer (SIR 5.26, CI: 1.43-13.48).

Risk Assessment Studies

Risk assessment is often used to derive a safe level of exposure that is “without appreciable health risk” in the most susceptible sub-population. These safe levels of exposures are agency specific, and include measures such as acceptable or tolerable daily intake (ADI, TDI: World Health Organization, reference dose (RfD) or reference concentration (RfC) as defined by US-EPA. These are derived dividing the no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) obtained from chronic/subchronic animal studies with appropriate uncertainty factors. To characterize risk associated with exposure to non-carcinogenic agent, additional estimates such as margin of exposure (MOE) is used, which is defined as NOAEL divided by the observed exposure, expressed in terms of average daily dose (ADD). A MOE > 100 is considered to be acceptable to protect human health. Similarly, risk associated with carcinogenic agents is characterized using excess cancer risk obtained by multiplying individual’s lifetime average daily dose (LADD) with Cancer Slope Factor (CSF). CSF and Cancer Potency Factor (CPF) are used interchangeably. The US-EPA adopted the form CSF to identify risk associated with a unit dose of a carcinogen exposure, expressed as risk per mg/kg-day. The acceptable risk designation is regulation specific, and may vary from 1 in 10,000 to 1 in million.

A compound specific risk assessment was performed for synthetic estrogen (17 α -ethinylestradiol: EE2), antibiotic (phenoxymethylpenicillin: Pen V) and antineoplastic drug (cyclophosphamide: CP) in Denmark using worst-case scenario (55). The authors concluded that there was no appreciable human health risk associated with environmental exposure to EE2, PenV and CP. To arrive to the stated conclusion, the authors compared the calculated daily exposure of EE2 with the endogenous estradiol (E2) production among prepubescent boys; daily Pen V exposure to the maximum tolerable residue levels of penicillin in food (5.9 μ g/day) established by The Committee for Veterinary Medicinal Products (CVMP). However, the daily Pen V exposure of 4.2 μ g/day reported by the authors is very close to the ADI of 5.9 μ g/day, and the worse case exposure of 86 μ g/day is 15 times higher than the CVMP recommended ADI. Thus, the data are not supportive of the author’s conclusion of no risk.

In a separate study, Schwab et al. (56) performed risk assessment on 26 different active pharmaceutical ingredients (APIs) manufactured by the major pharmaceutical companies, where the authors were employed. The authors first estimated acceptable daily intakes (ADIs) for each pharmaceuticals by dividing point of departure (POD, often the lowest single therapeutic dose for the pharmaceuticals) with five uncertainty factors (LOAEL instead of NOAEL, duration of exposure, interspecies extrapolation, intra individual susceptibility, and data quality). Using the ADI, the authors calculated the predicted no effect concentration (PNEC), which was compared to the concentration data from US waterways as well as those found in other parts of the world. Since all the ratios of estimated exposure to PNEC were less than one, the authors concluded that there was no appreciable human health risk. The major strength of this study is that it uses actual POD data on each pharmaceutical available to the authors (although through the manufacturer), as well as the use of concentration data from the published literature. Although authors mentioned that they used five UFs, with possible value of 100,000, in reality, the assigned UFs were 1% of the maximum value only in one instance (Dehydronifedipine, UF=1000). For all other pharmaceuticals, the assigned UFs were less than 0.2% of the total possible UF value. It is arguable that any data used in risk assessment is so perfect that it requires only 0.2% of the total allowable UFs. The authors' calculations are extensive, but the results are influenced solely by the extremely small UFs selected by the authors. Thus, appropriate use of UFs will alter the findings drastically. In addition, more recent data from developing countries, with lack of oversight of the direct industrial release, have shown that the surface water concentration can be up to 10^6 times higher than those used in the Schwab study (24). Use of these new data alone will likely alter the conclusions reached in the Schwab et al. study.

Review of the risk assessment literature also suggests that the majority of the studies are conducted or funded by the pharmaceutical industry, and thus far have concluded that there is no human health risk. Some of the studies conducted thus far have used the risk estimates as black and white number. For instance, if the ratio of daily exposure to ADI/TDI is less than one, the authors have interpreted it as absolutely protective, no matter how close to 1 these estimates are. In truth, these numbers are not meant to be used as such, as there is significant uncertainties involved in their derivation, and as such, estimates slightly below 1 does not indicate absence of risk just as estimates slightly above one does not indicate presence of risk in absolute certainty. Therefore, independent risk assessment studies using the most recent available data are warranted.

Antibiotic Resistance

Bacterial antibiotic resistance arises and is maintained through mutations in bacterial DNA or through horizontal gene transfer mechanisms including conjugation with other bacteria, transduction with bacteriophage, and the uptake of free DNA via transformation (57, 58). There is increasing concern that the ubiquitous low level presence of pharmaceuticals in the environment may lead

to the development of resistant pathogens (30). Antibiotic-resistant bacteria are found in significantly higher number in wastewater compared to surface waters (59–61). These studies have further shown that among rivers that receive WWTP effluent, the prevalence of resistant bacteria are higher downstream of effluent entry as compared to upstream. Thus, if the pumping stations for drinking water are located downstream of WWTP effluent release, the likelihood of human exposure to antibiotic resistant bacteria may increase via drinking water. The actual amount of antibiotic resistant bacteria at the POU water may depend upon the disinfection process employed at the treatment facility. The presence of antibiotic-resistant bacteria at the drinking water poses concern. Additionally, the exists the possibility of gene transfer to the more dangerous human pathogenic bacteria and cross resistance to human antibiotics (62).

Recommendations for Reducing Human Exposures

There are a number of steps that can be taken to reduce the load of PPCPs in the environment, which will result in reduction of human exposures. It will require concerted effort from all stakeholders, including the pharmaceutical industry, medical community, individual consumers, crop as well as animal agriculture, and local and state government.

Encourage Prudent Use of PPCPs

Pharmaceuticals can offer tremendous value to the practitioner who seeks to manage patient illness. But, often in the risk-benefit analysis, the long term impact on the environment and in human health is not considered. It is time to introduce the “prudent use of pharmaceuticals” concept in medical school and continuing medical education (5). Currently, many drugs are dosed at levels that are higher than needed for the individual patient (5). Doctors may need additional training to guide them in tailoring doses to individual patients, an effort that helps the patient (decreasing likelihood of side effects) and reduces the drug burden on the environment (12, 13). A companion strategy would be to re-design drug delivery methods such that they apply the drug directly/closest to the target organ, employing the least amount of active ingredient to accomplish the therapeutic goal. In this manner, we can increase effectiveness and reduce amount of drug needed.

An examination of how drug marketing practices and internet drug sales impact drug use and over-use should be considered (5). Consumers should be encouraged to avoid excessive use of PPCPs. “Environmental-Burden” ratings for PPCPs could be developed and used in packaging materials, to change consumer behavior, and drive industry to develop more environmentally safe products. Industry and pharmacies can collaborate with communities to develop recycling, reuse and donation programs which minimize the tonnage of un-used drugs that enter the waste system drugs (12, 13).

Implement Best Practices at WWTP

To reduce release of PPCPs to the environment, it is necessary to minimize direct discharge of untreated sewage into the environment (5). Waters that are directed into wastewater treatment facilities should undergo treatments that maximize the degradation of the PPCPs into less harmful byproducts (5, 30). The development of new water treatment technologies should be directed towards the highest priority chemicals, not just the ones we can easily measure. In addition, the older and less efficient WWTPs should be upgraded to tertiary WWTPs.

Crop and Animal Agricultural Practices

The use of antibiotics as growth promotors should be banned. In addition, sick (treated) animals should be kept away from healthy (untreated animals) to reduce contagion and to enable the waste from the treated animals to be managed more carefully (34). The use of biosolids in agricultural fields should be closely monitored and prudently done following the recommended practices outlined by the National Research Council in their 2002 report, *Biosolids Applied to Land: Advancing Standards and Practices* (21). Future efforts will also include enforcement of the NRC practices. As the current understanding of priority chemicals improve, it will be necessary re-consider the wisdom of currently accepted practices.

Improve Disposal Management

The improper disposal of medications is a contributor to the release of PPCPs into the environment (9). Nations can respond to this by formulating clear and standardized approaches for proper disposal of medications and personal care products (9). It is vital to these efforts to discourage disposal of unwanted/expired pharmaceuticals to the domestic waste (sewer and septic) system (5). Pharmaceuticals and personal care products should contain a clear and understandable label on all packaging materials (12, 13, 34).

Reduce Direct Release from Manufacturing Facilities

The direct release from manufacturing facilities is less of an issue in developed countries where regulations are stringent. However, in developing countries where such regulations are non-existent, the results are alarming. Recent data from India suggest that the concentration of pharmaceuticals in two lakes are up to 1 million times higher than those reported in the US and China (24).

Research Needs To Better Assess Human Health Risks from PPCPs

The research needs required to assess human effects associated with PPCP exposure are numerous, including: identification of “priority chemicals” posing the greatest risks; direct assessment of population level exposures to such

chemicals including biomarker based assessments; unbiased risk assessment studies that incorporate the most recent data, and toxicological as well as epidemiological studies to investigate the acute and chronic outcomes associated with multiple PPCP exposures.

Identification of Priority Chemicals

The most resource effective strategy to tackle the issue may be to identify the agents that pose the greatest risk to human health, and concentrate our management and educational efforts on those chemicals. This prioritization scheme should evaluate PPCPs based on their potency, chemical structures and functions, not the category of use. This is because the chemical composition will determine disposition, bioaccumulation, and ultimately risk. Such an approach guides us to *risk-based* as compared with an *exposure-based* assessment, and thus to risk-based management (3). Once the priority chemicals are identified, monitoring could be initiated using an indicator PPCP.

Comprehensive Assessment of Human Exposure

Reliable exposure estimates are necessary to derive any meaningful assessment of human health risk. It is particularly problematic that so little data exist regarding PPCPs types and concentration in foods, particularly plant foods. More data are also needed on point of use drinking water (33). A coordinated framework to measure PPCPs, including real-time GIS database for usage data, standardized analytical facilities, technical capabilities and resources as well as a group of scientists at the local level with shared goals and aims (63). Such datasets need to be incorporated within the existing framework such as the National Environmental Public Health Tracking Program, so that location specific data are available to boarder scientific communities engaged in addressing public health impact of PPCP exposures. In addition, expanding the available dose response data to include emerging PPCPs will be of significant help for risk assessment.

Future studies investigating human exposure measurements need to include all routes and pathways of exposure. Therefore, a biomarker based exposure estimates would be ideal as they could potentially incorporate all inhalation, ingestion and dermal exposures. A national assessment of human exposure may be possible by incorporating the targeted “indicator” PPCPs identified under the priority scheme, in existing surveys. For example, the National Health and Nutrition and Examination Survey (NHANES) conducted by Centers for Disease Control (CDC) continuously collects biological samples from a representative sample of non institutionalized US population (5,000 respondents/year). In the past, this survey has been used successfully used for national level exposure assessment to various contaminants such as volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), phthalates, pesticides, PCBs, to name few (<http://www.cdc.gov/nchs/nhanes.htm>). With enough support, such biological samples may be used to ascertain human exposures to PPCPs. Since the NHANES survey also collects detailed health outcome data on this population,

the exposure measurements can be directly linked to multiple health outcomes, which would be difficult to measure otherwise.

Epidemiological Studies of PPCPs

One of the challenges is to determine the best and most sensitive endpoints for epidemiological study. This may be accomplished with carefully designed studies of sewage workers, biosolids applicators or communities around agricultural lands where biosolids are applied. Various studies have investigated cancer risk among this population with limited success. Additional health outcomes including non-cancer endpoints need to be investigated using cross-sectional surveys. Outcomes identified in these surveys need to be further investigated using a more robust study design, such as cohort studies. As described previously, incorporating PPCP exposure measurements in ongoing national studies such as the NHANES or the National Children's Study (NCS) will enable one to investigate range of health outcomes associated with PPCP exposure within specific population strata that are considered most vulnerable. Such efficient use of ongoing studies (NHANES) may enable us to answer many important questions, such as the risks to young boys exposed to birth control hormones from consuming contaminated drinking water.

Toxicology Studies of PPCPs

Toxicological studies are needed to determine best or most appropriate animal models for various PPCPs classes. They also need to be designed to assess acute, chronic and multigenerational outcomes. Toxicological data on mixture effects will be highly useful in risk assessment as well as in informing policy (64). Such studies may shed light on synergistic as well as antagonistic effects of PPCP mixtures. In addition, such studies will be highly useful in identifying the most common endpoint for acute as well as chronic exposures.

Finally, it is critical to develop a systematic and coordinated effort to evaluate PPCPs before approving use (5). This will require collaboration across US and international agencies. There is benefit in having one single agency with responsibility for coordination and communication on PPCPs research. This is also the time to re-examine the safety of pharmaceuticals and personal care products that are in current use, looking for eco-toxicity and human health risks from environmental exposures. If unacceptable risks are found, we may need to recommend the removal of some PPCPs from the marketplace, if they prove to be particularly harmful. As we develop regulatory responses to PPCPs in the environment, we should assure "a place at the table" for all stakeholders including PPCPs manufacturers, university researchers, and regulators (5). Such regulations may bring about a large social and economic impacts, thus it is vital that regulations are informed by accurate data, and thoughtful risk analysis to minimize environmental damage and protect human health.

Abbreviations

Abbreviations: Acceptable Daily Intake (ADI), Pharmaceuticals and Personal Care Products (PPCPs), Pharmaceuticals and Personal Care Products in the

Environment (PiE), Point of Use (POU), Total Daily Intake (TDI), Wastewater Treatment Plant (WWTP)

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Chapter 18

Pharmaceutical and Personal Care Products in the Environment and Potential Risks of Emerging Antibiotic Resistance

Rebecca M. Coulborn, Erin Rees Clayton, and Allison E. Aiello*

University of Michigan School of Public Health, Department of
Epidemiology, Center for Social Epidemiology and Population Health,
1415 Washington Heights, SPH Tower, Ann Arbor, MI 48109

*Corresponding author: Phone: 734-615-9213, Fax: 734-763-5706,
E-mail: aielloa@umich.edu

Personal hygiene and cleanliness continue to represent important strategies for the maintenance of good health. Yet the introduction, within the community, of personal care products containing antimicrobial chemicals merits further review. While use of antimicrobial chemicals is both valid and appropriate in the healthcare setting where the potential for infection is great, its benefit in the community is unclear. In this chapter, we discuss personal care products containing the antimicrobial chemicals triclosan, triclocarban, and benzalkonium chloride, assessing their effectiveness for reducing disease in the community setting and growing concerns regarding the potential hazards to individuals and communities, especially the threat of emerging antibiotic resistance.

Introduction: Personal Hygiene and Cleanliness

In 1847, Ignác Semmelweis, a Hungarian physician working at the Vienna General Hospital in Austria, attributed an epidemic of “childbed fever” (puerperal fever) to the transmission of infection by the hands. Semmelweis postulated that the introduction of aseptic techniques, involving the immersion of hands in chlorinated lime solutions prior to physical contact with the patients, significantly reduced the incidence of childbed fever among women giving birth at the obstetric clinic (*J*). The work of Semmelweis, along with that of Oliver Holmes, Sr., Louis

Pasteur, Joseph Lister, Robert Koch, and many other great researchers helped establish the “germ theory of disease” which hypothesized microorganisms as the cause of illness.

Widespread acceptance of this theory contributed to sanitary and health revolutions in many parts of the world. Beginning in the late 18th and early 19th centuries, Western Europe and the United States entered a “health revolution” that continues to this day (2). Marked by numerous medical, environmental, technical, and political innovations, the health revolution gradually eliminated many of the sources and transmission routes of infectious diseases that inevitably led to infant mortality and early death for young adults in these two geographic regions. Countless disease prevention strategies were developed for the advancement of health, including the invention of antibiotics—organic chemical substances capable of destroying or inhibiting bacterial growth (3)—and significant sociocultural transformations in hygiene and cleanliness practices (1, 4). In particular, during the twenty-five years from 1890 to 1915, major changes in bathing, laundering, and domestic hygiene practices occurred in the U.S. and England (2, 5, 6), with increasing acceptance of the ‘hygiene barrier’ prevention model, which seeks to reduce risk from infections by limiting exposures to harmful microorganisms in one’s surroundings or on one’s person (1, 4). In the decades following, classic supply-and-demand mechanisms resulted in dramatic increases in the availability and development of household and personal hygiene products, such as synthetic soaps (detergents), for cleaning (7, 8).

While cleaning refers to the *mechanical removal* of a contaminant from an object, disinfection (of inanimate objects) and antisepsis (of animate objects) involves the *chemical inactivation or killing* of microorganisms with the use of products containing antimicrobial substances (i.e. biocides) (3, 4, 9). In many community settings, including normal household conditions, cleaning with detergents (soap) and water is considered an adequate first line of defense against infectious diseases (10). However, in instances involving ill persons or the handling of contaminated food or objects, disinfection/antisepsis may be necessary in order to control infectious microbes (4, 11). Though originally designed for use in the healthcare setting as surgical hand scrubs, preoperative skin preparations, healthcare professional hand washes, and medical instrument sterilizers, disinfectants/antiseptics—referred to as antimicrobial chemicals, biocides, or microbicides—have since been significantly marketed to consumers for nearly half a century (12). Today, the health revolution continues, with prolific use in the community setting of products designed for cleaning, as well as, more recently, those developed for disinfection or antisepsis in the hospital setting (3).

The explosive proliferation and marketing to consumers of these disinfecting chemicals and antiseptic personal care products has resulted in much debate in recent years over potential risks associated with their widespread use by the general population. While antiseptic use is both valid and appropriate in the healthcare setting where the potential for infection is great, its benefit in the community is unclear. In this chapter, we discuss personal care products containing antimicrobial chemicals, assessing their effectiveness for reducing disease in the community setting and growing concerns regarding the potential hazards to individuals and communities, especially the threat of emerging

antibiotic resistance. We focus on those consumer products containing the chemicals triclosan, triclocarban, or benzalkonium chloride, given the prevalence of these specific antimicrobial chemicals in personal care products and the dearth of evidence available for other microbicides in the community setting.

Proliferation of Personal Care Products Containing Microbicides

The number of consumer products that now contain an antimicrobial substance is enormous. According to research conducted by Perencevich et al. from December 1999 to April 2000, over 75% of liquid consumer soaps and 30% of bar consumer soaps contained an antimicrobial. For those soaps containing an antimicrobial, the predominant chemical was either triclosan (100% of liquid consumer soaps) or triclocarban (84% of bar consumer soaps) (13).

2,4,4'-Trichloro-2'-hydroxydiphenyl ether, more commonly known as triclosan (Fig. 1a), is a synthetic, nonionic broad-spectrum antimicrobial, first introduced in the 1960s for use in personal care products. It is an off-white crystalline powder that is odorless and tasteless, with virtually no solubility in water but high solubility in many nonpolar organic solvents (15). Initial safety data indicated that triclosan did not appear to have chronic toxicity and is not a mutagen, teratogen, or carcinogen (15, 16). Due to the numerous studies demonstrating its tolerance by humans and many other species, as well as its efficacy at low concentrations compared to many other antimicrobials, triclosan has become the most common antimicrobial chemical used in commercially available soaps, as mentioned above (17). For over 30 years, triclosan has been added as an antiseptic chemical and preservative to countless consumer products including, but not limited to: hand soaps, laundry detergents, fabric softeners, mouthwashes, toothpastes, medications for acne, wound disinfection solutions, deodorants, facial tissues, plastic kitchen utensils, medical devices, and toys (18, 19). When incorporated into acrylic fibers and clothing/plastics, triclosan is advertised under the trade name Biofresh® and Microban®, respectively (16). Ubiquitous use of triclosan in consumer products is not limited to the U.S. In 2006 and 2007, the Canadian Broadcasting Corporation reported the registration, by the Canadian federal government, of 1,200 brands of triclosan-containing cosmetics (20).

3,4,4'-Triclorocarbanilide, or triclocarban (Fig. 1b), is an antimicrobial carbanilide that has been used primarily in soaps and deodorants since the middle of the twentieth century. Triclocarban is most often used in solid formulations, rather than liquid formulations as with triclosan (13). Triclocarban is a fine white powder that, like triclosan, is insoluble in water. Though initial toxicity studies in rats demonstrated no evidence for chronic toxicity and *in vitro* mutagenicity studies showed no effect from exposure to triclocarban, recent *in vitro* bioassays suggest triclocarban may be an endocrine-disrupting compound (21, 22).

Another antimicrobial chemical commonly found in today's consumer products is benzalkonium chloride (BAC) (Fig. 2), a nitrogen-based quaternary ammonium compound (QAC) (23) used in various antimicrobial personal care

products such as wet wipes and eyedrops (24). Additionally, BAC is added to many waterless hand sanitizers; these alcohol-free formulations of hand sanitizer have been advertised extensively to schools, promoted in part on the basis of reduced flammability and likelihood of ingestion (25, 26). The final safety report on BAC issued in 1989 stated that this chemical was safe for use as an antimicrobial chemical at concentrations up to 0.1% wt/vol, but at concentrations above this, BAC was found to be an ocular and skin irritant (27).

Accumulation of Microbicides in the Body and Environment

The proliferation of triclosan in products across the world coincides with mounting evidence of its bioaccumulation and persistence in human fat tissue (20). Researchers have also detected triclosan in umbilical cord blood, human breastmilk, and urine (20). A Swedish study of 36 mothers, for whom personal care products represented the dominant systemic exposure to triclosan, documented high levels of triclosan in human plasma and breastmilk (29). The US Centers for Disease Control and Prevention (CDC), in a trial conducted from 2003-2004 among a representative sample of the U.S. general population age 6 years and older, found triclosan at unadjusted concentrations of 2.4-3,790 $\mu\text{g/L}$ in the urine of 75% of Americans (30). The significance of this finding remains unknown. However, despite the initial safety data, more recent studies have found that triclosan may have deleterious effects on the central nervous system and may be linked to allergies and asthma (16, 31). To our knowledge, no published research has examined the bioaccumulation of triclocarban in humans.

There is little available evidence on the bioaccumulation of BAC and other QACs in humans. Due to the high solubility of these compounds in water and their high molecular weight (> 200), it is thought that they are not easily transported across cellular membranes and thus bioaccumulation may be minimal (32). Nevertheless, QACs have been shown to be acutely toxic to aquatic plant and animal species even though the bioaccumulation of these chemicals was found to be substantially lower than that of other neutral organic chemicals, such as polychlorinated biphenyls (PCBs) (33).

The increase of triclosan- and triclocarban-containing products and other microbicide-containing products on store shelves and in homes, workplaces, and schools inevitably means an increasing amount of these chemicals are also found in the environment (34). In 2001, the U.S. Environmental Protection Agency (EPA) conducted its National Sewage Sludge Survey, collecting and archiving biosolids from 94 representative wastewater treatment plants in 32 states and the District of Columbia (35). Recently, independent researchers have analyzed these archived biosolid samples from the 2001 EPA survey using EPA-certified methods, such as liquid chromatography tandem mass spectrometry, in order to determine the mean concentrations of pharmaceuticals and personal care products (PPCP) in environmental samples. Fifty-four percent of the PPCP tested were found in at least one sample, and 65% of the total PPCP mass detected was comprised of triclosan and triclocarban (35). Triclocarban was found in every sample that was analyzed; of the two chemicals, triclocarban is more persistent

in the environment than is triclosan (36). While treatment plants are able to remove much of the triclosan and triclocarban prior to discharging wastewater, concerns mount over contamination of water resources and agricultural soils from wastewater and other sources of chemical pollutants (34, 35). A survey conducted by the U.S. Geological Society which assessed 95 different organic wastewater contaminants found triclosan as one of the most frequently detected compounds in American streams (37).

The prevalence of these antimicrobial chemicals in environmental samples calls for a critical investigation into their potential impact on the environment (38). A careful assessment of whether the benefits of their use outweigh the risks, both in terms of environmental and health effects, is urgently needed. We turn now to briefly discussing the mechanisms of action and existing research on the potential impact of antimicrobial use on human health.

Mechanisms of Action of Biocides

The mechanisms by which biocides function at high concentrations are well characterized, and include nonspecific actions on the intracellular components and membranes of bacteria (12). These nonspecific actions include inhibition of oxidative phosphorylation, interactions with macromolecules, alterations of biosynthetic processes, interference with electron transport, hindrance of enzyme activity, denaturing of proteins, and damage to cell walls and cytoplasmic membranes through binding to phospholipids (12, 39). Ultimately these processes result in leakage of intracellular components and induction of cell lysis (12, 39).

Conversely, complete characterization of the mechanisms of action of biocides at low concentrations remains unknown (12). In the laboratory setting, researchers have demonstrated that biocides, at low concentrations, attack specific target sites within the microbial cell and thus share the same mechanisms of action as antibiotics (12). Recent research suggests that if this specific mode of action persists in the community setting, then biocides and antibiotics may select for and confer resistance to one another through mutation or overexpression of target sites, and active transport of toxic substances from the interior of cells by plasmid-mediated efflux pumps (12). A more detailed discussion of these specific mechanisms follows.

Antimicrobial Efficacy and Shared Mechanisms of Action with Antibiotics

In order for a product to claim antimicrobial activity, certain *in vitro* laboratory tests must first be performed. These tests are designed to provide a basic characterization of the potency and spectrum of activity for a given antimicrobial chemical (3). In addition, diagnostic laboratories can use these tests to confirm antibiotic resistance (40), the ability of microorganisms, including pathogenic bacteria, to tolerate the effects of antibiotics and survive and replicate in their presence (41). The test often considered as the “gold standard” for measuring the susceptibility of microorganisms to antibiotics and antimicrobials

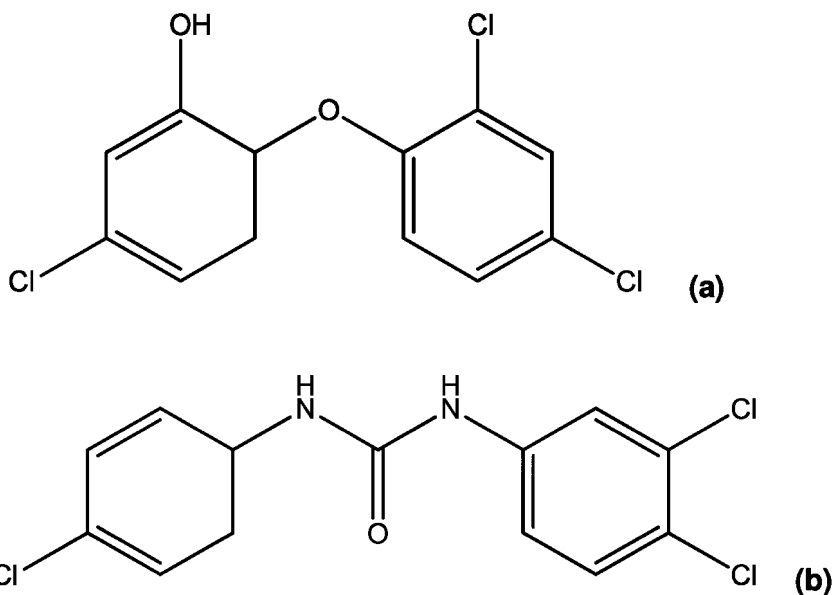


Figure 1. Molecular structures of (a) triclosan and (b) triclocarban (14)

is the determination of the minimum inhibitory concentration, or MIC (40). The MIC is defined as the lowest concentration of a substance that will inhibit visible growth of an organism in its planktonic, free-floating form (3). It is important to note that a given antimicrobial product may be more efficacious against one species or type of organism than others. Often, therefore, a panel of organisms will be selected as challenge organisms for MIC testing of a particular product, and the MIC will be defined as the minimum concentration of the product that produces no growth of all challenge organisms *chosen for testing* (3).

Triclosan acts primarily against Gram-positive bacteria, though it has also shown antimicrobial effects against Gram-negative bacteria, viruses, and other microorganisms such as fungi (42). The antimicrobial activity (whether specific or nonspecific) of triclosan is dependent upon the concentration and formulation used (15). At concentrations above the MIC, triclosan acts on multiple nonspecific cellular targets to disrupt bacterial cell wall functions (42). Thus, initially triclosan was considered to work like bleaches, peroxides, and alcohols, all of which have multiple nonspecific mechanisms of action not associated with conferrance of resistance. However, many of the consumer products containing triclosan are formulated with low concentrations of this chemical (43) or leave residues and below-lethal concentrations of triclosan on surfaces and in the environment (44). At these low concentrations, triclosan has been shown to affect specific cellular targets of bacteria (42). One such target is a bacterial protein involved in fatty acid biosynthesis known as the enoyl-acyl carrier protein (ACP) reductase. Enoyl-ACP reductase is conserved across many species of bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, in which it is called the FabI enzyme, and its homologue InhA is found in *Mycobacterium* species (42, 45). The conservation of FabI across several bacterial species and

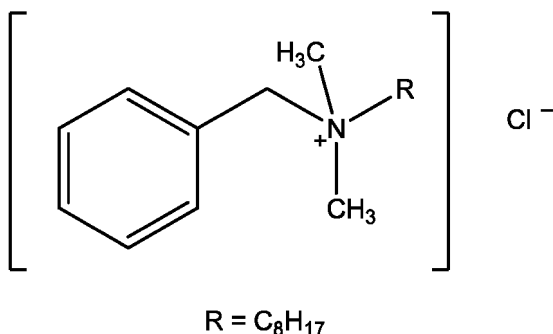


Figure 2. Molecular structure of benzalkonium chloride (BAC) (28)

the importance of fatty acid biosynthesis to the survival of the organism makes this protein an attractive target for many antibiotics. Thus, triclosan's mechanism of action is similar to that of systemic antibiotics that are commonly used in the medical community (46–49). This similarity represents, in part, the basis for concerns over cross-resistance (see below).

Many studies designed to investigate the efficacy of triclosan have involved triclosan-containing hand soaps. However, at least one study has suggested that triclosan may have a higher efficacy in other types of products. This study found that a triclosan-containing lotion, when placed on the skin, would reduce the bacterial load on the target area after a 12-hour period (50). The study also demonstrated that using a hand lotion containing triclosan for an extended period of time was more successful in reducing bacteria than hand soap containing triclosan (50). Importantly, the concentration of triclosan in the lotion (0.3%) was higher than the concentration of triclosan in most consumer soaps (43). Although the use of antimicrobial lotions should not replace hand washing, this study suggests that these topical leave-on chemicals may complement hand washing and other personal hygiene behaviors.

Like triclosan, triclocarban acts primarily against gram-positive bacteria, with less activity against gram-negative bacteria and fungi (51). To our knowledge, the exact mechanism of action of triclocarban on bacterial cells has not been determined. Given that triclocarban is an analide, however, it is possible that it promotes bacterial cell death via permeability changes that result from adsorption of triclocarban to the cell membrane (51).

Similar to triclosan and triclocarban, the efficacy of BAC is dependent on its formulation and concentration. In general, QACs are bacteriostatic, algistatic, tuberculostatic, sportostatic, and fungistatic at low concentrations (i.e., they slow organism growth), and at higher concentrations (~10–50 ppm) are bactericidal, algicidal, fungicidal, and virucidal against lipophilic viruses (3) (i.e., they kill the organisms). BAC, in particular, is considered bacteriostatic at concentrations of 4–16 ppm and bacteriocidal at higher concentrations (3). Laboratory tests to examine the efficacy of BAC as a virucidal chemical have shown that it has the ability to inactivate influenza virus, measles virus, Herpes Simplex viruses, Newcastle disease virus, and avian infectious bronchitis virus (52, 53). BAC, like triclosan and triclocarban, leaves long-lasting residues of low concentration on surfaces

(54). Just as failure to complete a full course of antibiotics allows bacteria to persist and evolve, as residues, BAC may damage benign bacteria important to normal skin flora and allow for expression of genes which encode for multidrug-resistant efflux pumps (54).

Antimicrobial Effectiveness

Despite the *efficacy* of antimicrobials in inhibiting bacterial growth in laboratory tests, there is question as to their *effectiveness* in “real life” situations in the community setting. Several research studies examining the ability of triclosan- or triclocarban-containing soaps to prevent infectious illnesses have demonstrated less than desirable results. Available data show that many common personal care products with the current concentration of triclosan do not reduce the infectious disease symptoms or bacterial counts of the individuals that use them (43). Community-based randomized intervention trials have been conducted in both the U.S. and Pakistan to examine whether there is a benefit to using triclosan- or triclocarban-containing soap compared with plain soap (43, 55–59). Interestingly, even when used for extended periods of time (over 1 year), these studies found no supportive evidence that antimicrobial soap containing triclosan or triclocarban is a better alternative to plain soap in preventing infectious illnesses. Even a difference in the reduction in the number of bacteria on the hands has generally only been observed after longer hand washes (>20 seconds) with relatively high concentrations of triclosan ($\geq 1.0\%$ wt/vol) (43). These concentrations are much higher than the concentration of triclosan in consumer products, which is usually between 0.1% and 0.45% weight/volume (wt/vol) (43). Increasing the concentration of triclosan or triclocarban found in soap, however, may have little positive public health impact on the effectiveness of these products. This is due in part to concerns over skin irritation and residues of the chemicals that may be left behind on surfaces, as mentioned above. In addition, inefficient or improper hand washing and hygiene behaviors will reduce the effect of these antimicrobials (43). While population-based estimates are unavailable, in an observational study of 7,836 individuals in 5 major U.S. cities, sponsored by the American Society for Microbiology, only 67% of participants, after using a public restroom, washed their hands (60). These data suggest that hand hygiene in the United States is suboptimal, and antimicrobial soaps may provide little benefit over plain soap in community settings, regardless of whether the community is upper Manhattan or a squatter settlement in Karachi, Pakistan.

As with triclosan and triclocarban, there are limited data with which to evaluate the effectiveness of BAC in the community; a recent systematic literature review conducted by Aiello et al. (61) retrieved only two published studies (62, 63) which implemented a hand hygiene intervention with BAC. Both studies (62, 63) were conducted among students aged 5-12 years in U.S. schools. Aiello et al. (61) generated pooled rate ratios from these two studies using meta-analyses to quantify the effectiveness of BAC-hand sanitizers against infectious diseases. Compared to a control group which received nothing, students provided with BAC-hand sanitizer had statistically significant reductions of 40% in respiratory

illnesses alone and 41% in combined gastrointestinal and respiratory illnesses (61). Similar reductions were found for gastrointestinal illnesses alone, but these results were not statistically significant (61). As both BAC studies (62, 63) suffered from methodological flaws, these findings warrant careful interpretation; importantly, the unit of intervention did not equal to the unit of analysis (in fact Dyer et al. (62) did not randomize entirely) and there was no control for clustering (61).

Possible Explanations for Differences between Data on Efficacy and Effectiveness

There are several potential reasons for the differences observed in the efficacy and effectiveness of antimicrobial-containing personal care products. First, unlike the effectiveness studies presented above, many research studies that have been conducted to examine the efficacy of triclosan investigate triclosan concentrations that are higher than the normal concentration in consumer products (43). Second, several tests of the antimicrobial activity of triclosan, triclocarban, and BAC have been performed on the planktonic-phase organism, which refers to the free-living form of bacteria. However, the natural living condition of many of these bacteria is in biofilms, which are complex aggregates of microorganisms that adhere to one another or a surface, forming a microcolony that exhibits structural and physiological characteristics distinct from planktonic-phase cells of the same organisms (3). In biofilm form, bacteria can have significantly greater (ten to thousand fold) resistance to anti-microbial chemicals (42, 64). To evaluate this, Wong et al. tested the efficacy of several antimicrobial chemicals against planktonic *Salmonella enterica* serovar Typhimurium compared to 3-day old *Salmonella* biofilms (65). The authors found that the biofilms were less susceptible to the disinfectants used, including BAC and other QACs, than were planktonic *Salmonella* cells. Higher concentrations of the disinfectants and longer exposure times were required in order to diminish biofilm growth; BAC and most of the other disinfectants tested were unable to completely eliminate the biofilms (65), leaving viable cells that, if this were not a laboratory test, could continue to grow and lead to contamination and infection. Similarly, Romanova et al. investigated the sanitizing efficacy of BAC against *Listeria monocytogenes*, a food-borne pathogen associated with an annual incidence of death eight times greater than that caused by infection with *Escherichia coli* O157:H7 (64). Compared to their planktonic form, biofilms of *L. monocytogenes* strain C719 were at least 1000 times more resistant to BAC (64).

A third possible reason that triclosan has shown greater benefit in lab tests compared to randomized trials in the community is that many infectious diseases are viral and triclosan is primarily an antibacterial chemical (15). Moreover, some disease-causing bacteria have shown resistance to triclosan. Some of the triclosan-adapted bacterial species, such as *E. coli* and *P. aeruginosa*, have demonstrated the ability to grow in culture in the presence of concentrations of triclosan of up to 1024 $\mu\text{g/mL}$, which is close to the concentrations added to many consumer soaps (i.e., 1000 $\mu\text{g/mL}$ = 0.1% triclosan [wt/vol]) (43). This resistance may be through

the ability of the organism to prevent triclosan's action on the FabI enzyme (42). However, triclosan is also a substrate for multidrug efflux pumps, the mechanism by which cells extrude toxic substances/antibiotics, and these efflux mechanisms are often shared and transmitted among several different species of bacteria. This efflux pump can confer resistance to a multitude of antibiotics, including triclosan (45). The following section discusses in more detail the potential role of triclosan in emerging antibiotic resistance.

Emergence of Resistance

“Antimicrobials are uniquely societal drugs because each individual patient use can propagate resistant organisms affecting entire health facilities, the environment and the community.”

-Alliance for the Prudent Use of Antibiotics (66)

Emerging antibiotic resistance within the community setting is becoming an increasing threat to public health (41). Infection with antibiotic-resistant bacteria is associated with increased morbidity and mortality in the community setting, due in part to prolonged duration of illness and failure in treatment (67–70). In addition, antibiotic resistance can lead to alterations in natural microbial ecology, which in turn lead to more severe infections from transmission of multi-antibiotic-resistant bacterial pathogens (71).

At least two main factors are known to contribute to the emergence of antibiotic resistance in the community (41). The first of these is person-to-person transmission, especially from childcare centers, crowded settings, and exposures among household members or other contacts (41). The second factor is selective pressures on the bacteria that result from individuals' antibiotic use and ingestion of antibiotic-treated food stuffs (41).

However, infection with antibiotic-resistant organisms has been reported among people in the community who lack these traditional risk factors (41, 72–79). In addition, prevalence studies have identified healthy individuals (i.e. “carriers”) carrying antibiotic-resistant bacteria in the absence of known risk factors (41, 72–79). Research suggests that there are factors contributing to antibiotic resistance within the community that have yet to be identified.

One potential risk factor for carriage and spread of antibiotic resistance within the community setting is the use of antimicrobial cleaning and personal care products containing triclosan, triclocarban, (41, 48) or benzalkonium chloride (23). In triclosan-containing products, the in-use concentration of the antimicrobial chemical has been considered higher than the MIC for many bacteria. Many of these products, however, leave residues on bathroom and kitchen surfaces that are diluted to non-lethal concentrations, leading to the development of resistant strains (42). One study demonstrated that the short term exposure of bacterial cells to 0.5 MIC of triclosan resulted in the death of weaker bacterial strains, but a fivefold increase in the number of resistant strains (80). Long term exposure of bacterial cells to triclosan resulted in a progressive

decrease in susceptibility. After four treatments at a concentration of 0.5 MIC, the bacterial cells showed an increase in resistance that was 128 times its initial value. Treatments at 0.25 MIC also showed an increase in resistance, as did 0.125 MIC, though to a lesser extent (80). These results suggest that products containing triclosan at effective concentrations below the MIC should not be used. However, one study found that using triclosan with a combination of non-triclosan containing soaps lowers the risk of promoting the development of resistant mutants (81).

It is possible that widespread and unregulated use of triclosan in personal care products may promote selection of multi-drug resistant bacteria which can lead to the emergence of more resistant strains (45). Emerging resistance is rarely due to the organism inactivating the biocide, and is more commonly achieved by changes in the cell envelope permeability or enhanced efflux of the biocide (82). Changes in the cell envelope permeability that prevent one antibiotic compound from diffusing into the bacterium often also prohibit entry of other drugs and chemicals. Similarly, enhanced efflux mechanisms are often non-specific and are better able to remove antibiotics of multiple classes from the interior of the cell. Thus, insusceptibility to triclosan may confer resistance to many of the other antibiotics that are commonly used today. For example, a mutation in the *InhA* gene leads to reduced susceptibility to triclosan in *Mycobacterium tuberculosis* and also causes cross-resistance to antibiotics such as isoniazid, a first-line antituberculosis drug which has the same bacterial target as triclosan (46, 47, 49, 82).

Corresponding studies for triclocarban, which examine whether exposure to this chemical promotes cross-resistance to clinically relevant antibiotics, have not yet been carried out, to our knowledge. Likewise, the issue of emergence of cross-resistance in the environmental setting from exposure to triclocarban has yet to be studied. Only a few laboratory studies have attempted to assess whether exposure to triclocarban among bacteria leads to changes in antibiotic susceptibility (83, 84). No clear relationship between exposure to triclocarban and antibiotic susceptibility was identified in either of these studies.

Similarly to triclosan, ubiquitous use of BAC in disinfecting products raises the concern of emerging antibiotic resistance. Recent evidence suggests that this is a legitimate concern. A laboratory strain of *Pseudomonas aeruginosa* was cultured continuously in the presence of BAC to select for spontaneous mutants that would grow in the presence of the biocide (23). Mutants that developed were twelve times as resistant to BAC as the original strain, due largely to the development of more efficient efflux mechanisms. Even more alarming was the 256-fold increase in resistance to ciprofloxacin, a commonly prescribed antibiotic that the *P. aeruginosa* "superbugs" had previously never encountered (23). The studies discussed here suggest that while BAC has the potential to kill a wider variety of pathogens than triclosan and triclocarban, potentially reducing viral illnesses as well as bacterial infections, there are significant risks with its widespread use in the community setting.

Regulatory Concerns

Products containing triclosan, triclocarban, and BAC are approved and regulated in the U.S., depending on their intended use, by two U.S. governmental agencies, the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA). When used as antimicrobial pesticides or disinfectants for the destruction or suppression of bacterial, fungal, or viral growth on surfaces or inanimate objects, triclosan-/triclocarban-/BAC-containing products are registered by the EPA. However, when destined for use on animals or humans (i.e. as personal care products/topical antiseptics), despite often identical active ingredients and toxicological sequelae, triclosan-/triclocarban-/BAC-containing products are approved and regulated by the FDA (19). The first patent for triclosan was awarded in 1964, and in 1974 an FDA advisory panel published recommendations on over-the-counter antimicrobial drug products that stated there was insufficient evidence to support the safety and effectiveness of triclosan (85). Now, over three decades later, the evidence continues to be such that the FDA has been unable to issue a final monograph with regulatory standards for triclosan products. In contrast, with the same available evidence, Finnish, German, and Danish governments have discouraged regular use of antimicrobial products by their citizens, and four major grocery chains in the UK have banned triclosan from their merchandise (31). Overall, European nations have taken an aggressive approach toward limiting the use of triclosan-containing products in both community and hospital settings, as well as educating consumers about the potential risk of spreading antibiotic resistance (16).

Implications and Future Research Needs

Ultimately, the health revolution has empowered individuals to protect themselves from many common infectious diseases. Personal hygiene and cleanliness continue to represent important strategies for the maintenance of good health. Yet the introduction, within the community, of personal care products containing antimicrobial chemicals merits further review. Growing evidence suggests the widespread use of triclosan, triclocarban, and BAC in the community setting is at best controversial and at worst detrimental to the environment and human health. The use of these chemicals could be leading to acquired antimicrobial resistance and the generation of “superbugs,” which in turn affects the usefulness of systemic antibiotics to treat infectious illnesses. More research is needed in order to identify the development and spread of triclosan/triclocarban/BAC-related resistance. In addition, little is known about the effect of triclosan, triclocarban, and BAC on non-pathogenic/commensal bacteria and pathogenic bacterial biofilms. Future studies must also elucidate the effects of long-term exposure to triclosan, triclocarban, and BAC, on the environment and human health, as well as the risks of the interaction of multiple antimicrobial chemicals. These data are urgently needed for the completion of a finalized FDA monograph. If the evidence indicates that the risks from use of these chemicals outweigh the benefits, then the FDA must mandate safe and effective personal care products for today’s consumers.

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Chapter 19

Empirical Models for Predicting the Occurrence and Concentration of Organic Chemicals in Biosolids

Randhir P. Deo and Rolf U. Halden*

Center for Environmental Biotechnology,

The Biodesign Institute at Arizona State University, Tempe, AZ 85287

*Corresponding author: School of Sustainable Engineering and the Built Environment, Arizona State University, 1001 South McAllister Avenue, P.O. Box 875701, Tempe, AZ 85287-5701. E-mail: halden@asu.edu.

Tens of thousands of organic chemicals are used by consumers on a routine basis for multiple applications. Despite an increasing number of publications in this area, not enough is known about their fates during wastewater treatment and upon release into the environment. For example, hydrophobic organic compounds have been shown to persist in digested municipal sludge (biosolids), an abundant by-product of wastewater treatment. The sequestration of persistent contaminants in biosolids is of concern due to the widespread practice of land application of these materials for inexpensive disposal or for use as a soil conditioner and fertilizer. Land application of biosolids represents a potential pathway for the contamination of agricultural soils, uptake into food crops, bioaccumulation in terrestrial ecosystems and human exposure. Several thousand high production volume (HPV) chemicals are produced or imported in the U.S. each year at rates exceeding 450 000 kg (1 million pounds) per chemical. Since monitoring at wastewater treatment plants of such a large number of compounds is impractical and cost-prohibitive, modeling approaches have been proposed to estimate the likely behavior of compounds during wastewater treatment. In this literature review, we present an overview of recent empirical modeling approaches for estimating both the occurrence and concentration of

hydrophobic organic compounds (HOCs) in biosolids destined for application on land.

Introduction

A large number of organic chemicals are used by consumers on a routine basis for multiple applications and, following disposal into domestic sewage, are conveyed to wastewater treatment plants (WWTPs) and may enter the environment contained in treated effluent. An alternative pathway for persistent wastewater contaminants to reach the environment is their accumulation in digested sewage sludge (biosolids), and subsequent application on land of this abundant by-product of conventional wastewater treatment. Hydrophobic organic chemicals (HOCs) are particularly prone to become sequestered and persist in biosolids during municipal wastewater treatment (1–3). Sequestration of persistent contaminants in digested sewage sludge is problematic because approximately 49% of the 6.9 million metric tons of sewage sludge produced annually in the U.S. is applied on land for inexpensive disposal and as a fertilizer and soil conditioner (4).

A growing body of literature indicates the occurrence of toxic or potentially harmful organic compounds in biosolids, and their subsequent transfer to agricultural soils, tile drainage and terrestrial biota (5–12). Thus, application of biosolids on land represents a potential pathway for the contamination of agricultural soils, uptake into food crops, bioaccumulation in terrestrial ecosystems, contamination of aquatic systems through surface runoff, and harmful exposure of animals and humans to biosolids-borne compounds (5, 13). From a risk management perspective, of particular concern are high production volume (HPV) chemicals that are produced in quantities exceeding 450,000 kg (1 million pounds) per year (14). Their massive use and release can lead to adverse effects even if these compounds have only moderate or low toxicity.

Monitoring at wastewater treatment plants of the thousands of HPV chemicals currently in use may be highly desirable but ultimately is impractical (15). Instead, modeling has been suggested as an alternative approach that can be informative and economically attractive for predicting the fate of problematic organic compounds during wastewater treatment and their accumulation in biosolids.

This literature review presents an overview of contemporary empirical approaches for predicting the occurrence and concentration of organic chemicals in biosolids destined for land application.

Models for Predicting the Occurrence of Organic Chemicals in Biosolids

Modeling has been suggested as an economical and technically sound approach for assessing the fate of hydrophobic organic compounds (HOCs) in biosolids (2). Available models rely on one or more of the following physicochemical properties of HOCs: volatility, organic carbon partition

coefficient (K_{OC}), hydrolysis, etc. (1). Other models consider parameters specific to individual WWTP (2, 15–18), including specific rate of biodegradation (19).

In situations where such detailed information on a specific WWTP is not available, universally applicable empirical models can provide value. This was demonstrated in a meta-analysis of mass balances conducted at WWTPs around the world (15). The study produced a simple empirical model relying on readily available information as input, for predicting the fraction of the total mass loading of a given HOC expected to persist in biosolids after treatment (15):

$$f_{\text{biosolid}} = p_{\text{fit}} \times \frac{K_{\text{OW}}}{1 + p_{\text{fit}} \times K_{\text{OW}}} \quad (1)$$

where f_{biosolid} is the mass fraction of HOC expected to persist in biosolids, K_{OW} is the HOC's *n*-octanol-water partition coefficient, and p_{fit} is a dimensionless fitting parameter. The p_{fit} value was obtained by fitting equation 1 to the actual mass fractions of organic compounds accumulated in biosolids against the respective compound's log K_{OW} , which gives a value of 6.51×10^{-6} . Where pH is important, the individual compounds' log D_{OW} value is used. For example, at pH 7.5 and using log D_{OW} values, the new p_{fit} value is 1.76×10^{-6} (14).

Since the p_{fit} value is an empirical parameter determined from observations at full-scale sewage treatment plants, it reflects the combined effects of all relevant removal processes including biodegradation.

Use of such models is most useful for predicting the fate during wastewater treatment of compounds for which no measured information is available. For example, the empirical relationship shown in Eq. 1 was recently applied to predict the mass fractions of 207 selected HPV chemicals destined to accumulate in biosolids (20). Modeling results suggested that two thirds of the HPV chemicals examined in the study were destined to persist in biosolids with mass fractions of at least 50% relative to their initial mass loading to the WWTPs. The corresponding log K_{OW} threshold value was ~ 5.2 . For chemicals exceeding this critical threshold value, more than half of their mass loading to the plant was predicted to persist in biosolids (20).

The model in Eq. 1 can be easily adapted to account for the effect of pH on hydrophobicity. This is done by replacing the parameter for hydrophobicity, namely K_{OW} , with the pH-dependent *n*-octanol water partition coefficient, i.e., D_{OW} . The resultant p_{fit} value for use of Eq. 1 with D_{OW} substituted for K_{OW} was estimated to equal 1.76×10^{-6} at pH 7.5 (14), which is the typical pH of domestic raw wastewater in the USA (21).

The model was further developed to enable the prediction of concentration of organic chemicals destined to accumulate in biosolids (14). The model's sole input requirements included the fitting parameter (p_{fit}), and a given organic chemical's concentration in raw wastewater as well as its D_{OW} value. The model then takes the form as shown below:

$$C_{\text{biosolids}} = C_{\text{T(influent)}} \times f_{\text{biosolids}} \times \frac{1}{Y} \quad (2)$$

where $C_{\text{biosolids}}$ represents an organic chemical's concentration in biosolids, $C_{T(\text{influent})}$ its total concentration in influent wastewater, and Y the characteristic average yield of biosolids per volume of raw wastewater treated, i.e., $1.296 \times 10^{-4} \text{ kg L}^{-1}$ (15).

In instances where only the dissolved concentration (but not the particle-associated, sorbed mass of an organic chemical in raw wastewater is available, the desired total concentration can be estimated using the following relationship (14):

$$C_{T(\text{influent})} = C_{\text{aq}} (1 + f_{\text{oc}} \times D_{\text{oc}} \times C_{\text{ss}}) \quad (3)$$

where $C_{T(\text{influent})}$ and C_{aq} are the organic chemical's total and dissolved concentrations, respectively, in influent wastewater, C_{ss} is the concentration of suspended solids in influent wastewater, f_{oc} is the fraction of organic carbon in suspended solids, and D_{oc} is the organic carbon normalized sorption coefficient.

Table 1. Compound names, Chemical Abstract Service (CAS) registry number, log D_{ow} values, total influent concentrations and concentrations predicted for biosolids on a dry weight basis^a

<i>Compound</i>	<i>CAS #</i>	<i>Log Dow (at pH=7.5)</i>	<i>Ref.</i>	<i>Total Influent (ng L⁻¹)</i>	<i>Predicted Conc. in Biosolids (μg kg⁻¹)</i>
Benzophenone	119-61-9	3.18	25	72	2
				2,686	55
Isobutylparaben	4247-02-3	3.15	24	93	2
				438	8
Menthol	89-78-1	3.20	24	3,628	78
				17,010	365
Oxybenzone	131-57-7	3.30	24	367	10
				2,812	76
Propylparaben	94-13-3	2.81	24	810	7
				2,131	19
Simazine	122-34-9	2.28	24	5	<1
				10	<1
Traseolide	68857-95-4	6.14	24	1,417	7,700

^a Table adapted from data contained in reference (14).

This expanded model was found to produce acceptable results at the 99% confidence level in a paired *t* test that compared predicted concentrations in biosolids to empirical measurements reported in the literature (14). This was true on both occasions, when the influent and biosolids concentrations for specific organic compounds were measured at the same plant, as well as when measured at different plants. This expanded model was applied towards the prediction of concentrations in biosolids of organic compounds for which occurrence data had not been reported yet. The predicted concentrations are shown in Table 1. As expected, assuming equivalent influent loading, concentrations in biosolids are predicted to be higher for organic compounds that are more hydrophobic (14).

This simple empirical model has utility for screening but its use cannot replace actual measurement of organic compounds in biosolids. It is recommended to be applied for narrowing down the list of organic chemicals that are likely to persist in biosolids (14, 20). Further streamlining of the candidate list of HOCs could result from focusing on only those organic chemicals that represent significant (e.g., > 50%) accumulation relative to total mass loading, are highly persistent (longer half-life values), and are toxic (20). Following this approach, it is possible to reduce a large number of HPV compounds down to a small set of candidate analytes that merit actual monitoring at full-scale sewage treatment facilities (20).

Underlying Assumptions of Universally Applicable Empirical Models

Despite reported successes in applying empirical models, predictions of the fate (i.e., mass fractions or concentrations) of organic chemicals in an environment as complex as WWTP cannot be accurate in every case when using simple, universally applicable models. Differences are expected to exist in the rates and extents of biological, physical and chemical reactions occurring in different WWTPs across the nation, and its diverse climatic and geographical settings. Thus, universal models are bound to have certain underlying assumptions and limitations (14).

For example, in the expanded empirical model (Eq. 2), the authors assumed a sludge-to-sewage-treated ratio (*Y*) of 1.296×10^{-4} kg L⁻¹, which represents the calculated average for conventional activated sludge treatment facilities in the U.S. Since the *Y* value can differ by plant, actual measured *Y* value specific to WWTP should be used whenever available (14).

Furthermore, use of an organic chemical's total concentration in influent wastewater is critical for accurately predicting its concentration in biosolids. In most cases, however, only dissolved concentrations (22–26) are reported, due to the customary filtration of influent wastewater prior to chemical analysis. While the filtration step is important to remove particulates that may clog the small capillaries of analytical instruments (15), a fraction of the organic chemical can be sorbed to the organic carbon fraction of the particulate matter, and thus may be excluded from measurements (27, 28). Total concentration, however, can be estimated using Eq. 3 by multiplying *f*_{OC} (fraction of organic carbon) with *C*_{SS} (suspended solids concentration) (29–33).

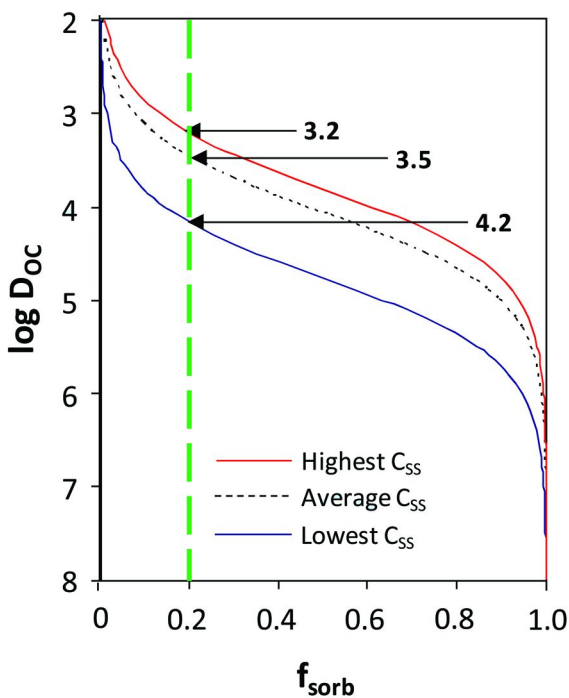


Figure 1. Effect of hydrophobicity (expressed as $\log D_{OC}$ at pH 7.5) of a given organic chemical and concentration of suspended solids (C_{SS}) in raw wastewater on the mass fraction of the organic chemical expected to sorb (f_{sorb}) onto filterable particulates. $\log K_{OC}$ is interchangeable with pH-dependent K_{OC} (i.e., $\log D_{OC}$). Also shown are threshold values of $\log D_{OC}$ at different C_{SS} , assuming f_{sorb} exceeding 0.2 (or 20%) as unacceptable. (Adapted from data in reference (34)) (see color insert)

A recent study characterized the sorption of organic chemicals to particulate matter contained in raw wastewater (27). Figure 1 shows the effect of a compound's hydrophobicity ($\log K_{OC}$ or $\log D_{OC}$) and concentration of suspended solid (C_{SS}) on the mass fraction sorbed, which can be calculated using the following equation:

$$f_{sorb} = 1 - \frac{1}{1 + f_{OC} \times K_{OC} \times C_{SS}} \quad (4)$$

where $f_{OC} = 0.43$, which is the fraction of organic carbon present in particulate matter, K_{OC} is the organic chemical's organic carbon-water partition coefficient, and C_{SS} is the concentration of suspended solids in raw wastewater. Since the K_{OC} can change as a function of pH, use of the pH-dependent K_{OC} , i.e., D_{OC} , is recommended (21).

The results in Figure 1 illustrate that both the C_{SS} and D_{OC} can have a significant effect on the mass fraction of sorption. For example, for an organic

chemical with a log D_{OC} value of 4.2, sorption can be limited to only 20% of its total mass (at the lowest C_{SS} level of 40 mg/L) but also can be as high as 70% (considering the highest C_{SS} level of 363 mg/L). Similarly, the more hydrophobic the organic chemical under consideration is, the greater will be its extent of sorption.

This implies that for accurate determination of organic chemicals in influent wastewater, both the dissolved and sorbed mass should be determined, especially since data inaccuracy in excess of 20% (or when $f_{sorb} > 0.2$) is deemed unacceptable (34). In Figure 1, this threshold of 20% sorption corresponds to a C_{SS} value of at least 363 mg/L. Thus samples containing suspended solids in excess of this value should be subjected to chemical analysis of both the dissolved and sorbed mass for all target organic chemicals featuring log D_{OC} values of ≥ 3.2 .

The significance of accurate quantification of organic chemicals in influent wastewater becomes apparent when considering the various uses of monitoring data. Besides estimating the fate of organic chemicals during wastewater treatment (more specifically, the mass fractions and concentrations in biosolids), influent wastewater concentrations also are used for tracking rates of consumption and discharge loads from point sources, for estimating mass loadings to WWTPs, for calculating organic chemicals' removal efficiencies in WWTP, and for estimating ecological and/or human health risks of the amount of organic chemicals expected to persist after treatment (27).

For example, a 2010 study (27) estimated the impact of using dissolved concentrations instead of total concentrations of organic compounds when calculating removal efficiencies in WWTPs. The authors used the following relationship for estimating the absolute removal efficiency:

$$R_{\text{absolute}} (\%) = \frac{[M_{\text{infl diss}} + M_{\text{infl part}}] - [M_{\text{effl diss}} + M_{\text{effl part}}]}{[M_{\text{infl diss}} + M_{\text{infl part}}]} \times 100 \quad (5)$$

where M_{diss} and M_{part} are the masses of organic chemical's (diss – dissolved phase, part – particulate (sorbed) phase) in influent (infl) and effluent (effl). However, when considering only masses present in the dissolved phase, the removal efficiency equation is simplified to:

$$R_{\text{aqueous}} (\%) = \frac{M_{\text{infl diss}} - M_{\text{effl diss}}}{M_{\text{infl diss}}} \times 100 \quad (6)$$

After adjusting measured dissolved concentrations to total concentrations, and using these two equations, the calculated removal efficiencies for select organic chemicals differed by as much as 20% for the very same chemical and plant examined (27). The authors concluded that the removal efficiency of a WWTP is not a fixed number but can take different values as a function of the method of mass accounting. From their secondary data analyses, the authors pointed to potential implications in data interpretation and formulated the following recommendations to ensure proper use of environmental monitoring data (27, 28):

- (a) To provide an accurate estimate of the total mass of a given HOC arriving at a WWTPs in raw sewage, mass loadings of sorptive substances should be determined in unfiltered samples or by processing the sample via liquid/liquid extraction, to capture the sum of both the dissolved and sorbed mass present.
- (b) To enhance transparency in data presentation, measured concentrations or masses of compounds determined in WWTP influent should be reported as either dissolved or total levels, consistent with the nature of sample preparation. For example, legends of Figures should state “concentrations and mass flows of target analytes determined via analysis of filtered [or unfiltered] samples.”
- (c) To eliminate uncertainty and inadvertent misinterpretation of monitoring data, removal efficiencies at WWTPs should be reported either as absolute or as aqueous removal efficiencies, consistent with the nature of sample preparation and analysis strategy (for example, filtration of samples or liquid–liquid extraction of unfiltered samples).

Unambiguous reporting of environmental monitoring data is key because once the data are entered into databases, the critical link between sample processing information and reported value typically is broken (27). Failure to follow these guidelines for reporting potentially can result in unscientific use in the regulatory context of scientifically sound data that were obtained with considerable investment of time and financial resources.

Conclusions

A review of empirical models predicting the fate of HOCs during wastewater treatment shows the utility and limitations of these approaches. Empirical models can serve to narrow down the list of potentially problematic organic chemicals earmarked for monitoring due to their presumed tendency to accumulate and persist in biosolids. Although being valuable for *in silico* screening, model prediction should be followed up with actual measurements performed on biosolids from WWTPs of interest. While model predictions can be an important tool in determining the fate of organic chemicals during wastewater treatment, the processes in WWTPs are complex, and thus plant-specific parameters should be used whenever available.

Further identified was the need for improvements in the reporting of environmental monitoring data to eliminate uncertainty and preempt misinterpretation of collected data. This applies particularly to the reporting of concentrations of organic chemicals present in the dissolved phase when indeed a significant fraction of the compound is expected to be sorbed to particulate matter. For compounds exhibiting significant sorption potential (*i.e.*, $\log D_{OC} \geq 3.2$), chemical analyses should be performed on the dissolved and sorbed phases of influent wastewater, such that total concentrations can be reported that reflect the entire mass of the analyte of interest. Secondary data analyses have suggested that the removal efficiencies of organic compounds in WWTPs may vary by as

much as 20% when considering dissolved concentrations only instead of the total chemical mass contained in a sample volume in both the dissolved and sorbed phase.

The implications of this review is that modeling can be a powerful tool in prescreening problematic organic chemicals before their actual measurement in biosolids. However, the quality of modeling results is a function of several factors, including the quality of the data used as model input. Formulae provided here also can be applied in secondary data analyses to estimate the total concentration of sorptive compounds that were measured and reported for the dissolved phase only.

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Chapter 20

Comparing the Phenomenological and Hydrodynamic Modeling Approaches for Describing the Rejection of Emerging Nonionic Organic Contaminants by a Nanofiltration Membrane

Christopher L. Bellona and Jörg E. Drewes*

Advanced Water Technology Center (AQWATEC),
Environmental Science and Engineering Division,
Colorado School of Mines, Golden, CO 80401-1887, USA
*jdrewes@mines.edu

As a barrier for emerging organic contaminants, integrated membrane systems using reverse osmosis or nanofiltration have been favored for drinking water augmentation projects using reclaimed water or other impaired water sources. Although these membranes are an efficient barrier to the majority of emerging trace organic chemicals, various organic solutes, especially non-ionic compounds, have been detected in membrane permeate samples. This study investigated the use of three models, the phenomenological, the hydrodynamic, and a newly developed geometric-hydrodynamic model, to describe the mass transport of non-ionic organic solutes during nanofiltration. For compounds not expected to display strong membrane interactions, the modeling approaches examined during this study can provide a good estimation of rejection as a function of permeate flux when the rejection performance of a given membrane is characterized.

Introduction

High-pressure membrane processes, such as reverse osmosis (RO) and nanofiltration (NF) are becoming increasingly widespread in water treatment,

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industrial processes and wastewater reclamation/reuse applications where a high product water recovery is desired (1). For drinking water augmentation projects in the United States, Singapore, and Australia that use reclaimed water, treatment using an integrated membrane system (IMS), such as microfiltration (MF) pretreatment followed by RO, is considered the industry standard. Research on occurrence and fate of organic contaminants in wastewater effluents over the past 15 years has shown that many organic chemicals present in municipal sewage are incompletely removed by wastewater processes posing a potential threat to human health in drinking water augmentation projects (2, 3).

Past laboratory research projects have demonstrated that one limitation of RO and NF membranes is the incomplete removal of various organic solutes, such as certain disinfection by-products, pharmaceutically active compounds, chlorinated flame retardants, steroid hormones, and pesticides (4–7). Bellona et al. (4) investigated the organic compound removal efficiencies of eleven commercially available membranes and while the rejection of uncharged organic solutes was variable and depended on the molecule of interest and the membrane, the rejection of ionic trace organic compounds was greater than 90 percent for all compounds and membranes tested. A review of past work investigating the removal of a variety of organic contaminants at full-scale water reuse applications employing RO revealed that nonionic organic contaminants are most frequently detected in permeate samples and included: 1,4-dioxane, bisphenol-A, caffeine, chloroform, N,N-diethyl-m-toluamide (DEET), galaxolide, iopromide, meprobamate, N-nitrosodimethylamine (NDMA), oxybenzone, pentoxifylline, and tris(2-chloroethyl)phosphate (TCEP) (4, 6, 8). Therefore, there is significant interest in the efficacy of RO and NF membranes to reject nonionic organic contaminants during water reuse applications.

Considerable work has been performed over the past fifty years modeling the rejection of solutes (mainly inorganic ions) by NF and RO membranes. A good review on various modeling approaches undertaken and transport equation derivation is given by Mason and Lonsdale (9). Early work performed by Teorell (10) and Dresner (11), and Spiegler and Kedem (12) laid much of the groundwork for modeling approaches based on the extended Nernst Planck equation and irreversible thermodynamics, respectively. Corrections for hindered solute transport through cylindrical membrane pores have allowed for the consideration of hydrodynamic conditions in membrane pores (13). More recently, significant work has been undertaken using the Donnan steric pore model (DSPM, (14–17)), the solution diffusion model (18, 19), and the phenomenological model based on irreversible thermodynamics (17, 20).

In comparison to inorganic ions, however, less work has been performed evaluating and developing models for organic solutes. The pore size of a membrane for the DSPM is often characterized by non-ionic organic solutes, such as glycerin or glucose, (often called the hydrodynamic model when the electrochemical portion of the DSPM is neglected, (21)) and Verliefe et al. (22) recently empirically modified the hydrodynamic model to predict the rejection of organic contaminants. Van der Bruggen and Vandecasteele (23) and Agenson et al. (24) used the phenomenological model to evaluate the properties of membranes and describe the rejection of various organic compounds. Other researchers have

modified the surface force pore flow model (25) and the solution diffusion model (26) to account for organic solute-membrane interactions. Additional methods for predicting rejection have been investigated, such as empirical relationships between rejection and solute and membrane properties (27), and quantitative structure property relationships (28, 29).

Similar to the purpose given by Mason and Lonsdale (9) for conducting their review on membrane transport models, the purpose of this study is to investigate the applicability of two modeling approaches for describing the rejection of nonionic organic solutes by NF membranes. In this work, two commonly employed membrane models, the phenomenological model utilizing an effective pore size distribution method for calculating the reflection coefficient as proposed by Van der Bruggen and Vandecasteele (23) and the hydrodynamic (steric-hindrance) model, were evaluated as a method to predict the rejection of nonionic organic solutes by a NF membrane. The nonionic organic solutes employed to develop and assess the two modeling strategies were comprised of two groups, one termed ‘characterization compounds’ comprised of sugars, alcohols, urea, and methyl tert-butyl ether (MtBE) and environmentally relevant solutes, termed trace organic compounds (TOrc) comprised of pharmaceutically active compounds (PhACs), personal care products (PCPs), a pesticide, a preservative, and a plasticizer.

Theory

Phenomenological Model

The phenomenological model as proposed by Spiegler and Kedem (12) can be used to describe solute transport across high-pressure membranes. The mass flux equation is expressed as:

$$J_s = -\bar{P}\Delta x \frac{dc}{dx} + (1 - \sigma)J_v c \quad (1)$$

where J_s is the solute flux, \bar{P} is the local solute permeability coefficient, σ is the reflection coefficient, and J_v is the solvent or volumetric flux. Integrating Eq. (1), introducing the solute permeability coefficient P :

$$P = \frac{\bar{P}}{\Delta x} \quad (2)$$

and rearranging yields the following equation to calculate rejection:

$$R = \frac{\sigma(1 - F)}{1 - \sigma F} \quad (3)$$

where:

$$F = \exp\left(-\frac{1-\sigma}{P} J_v\right) \quad (4)$$

The model requires three solute and membrane dependent parameters, J_v , P and σ . The reflection coefficient, σ , is defined as maximum solute rejection at infinite volumetric flux where convective transport dominates, whereas, the solute permeability, P , corresponds to purely diffusive transport as solvent flux approaches zero. For a given solute, both parameters can be calculated from experiments evaluating solute rejection as a function of the solvent (i.e., permeate) flux. The solvent flux is calculated by:

$$J_v = L_p (\Delta P - \sigma \Delta \Pi) \quad (5)$$

where L_p is the membrane solvent permeability constant, and $(\Delta P - \sigma \Delta \Pi)$ is the net-driving pressure. The phenomenological model is often considered a 'black-box' model because the actual mechanisms by which a solute is retained by a membrane are hidden within the model parameters P and σ , as opposed to the hydrodynamic model, which considers frictional hindrance of solutes within membrane pores. The hydrodynamic model, however, treats NF membranes as a bundle of cylindrical pores whereas the phenomenological model describes membrane transport as a combination of convection and diffusion with no assumption made about the nature of the pore structure.

Van der Bruggen and Vandecasteele (23) proposed that the permeability coefficient (P) for a given solute could be calculated by:

$$P_s = \frac{\rho}{d_s} \quad (6)$$

where d_s is a solute's molecular diameter and ρ is a membrane specific diffusion parameter that can be determined from solute rejection experiments. Additionally, Van der Bruggen and Vandecasteele (23) applied a log-normal probability distribution approach for the determination of the reflection coefficient as a function of the average pore size of the membrane, the standard deviation of pore size, and solute size. This method assumes that each pore will reject or pass the solute depending on the size of the solute in relation to the pore size (e.g., each pore has a reflection coefficient of 1 or 0). When all the pores in the membrane are combined, the reflection coefficient is the percent of pores small enough to reject the solute (30). The reflection coefficient can be expressed in terms of the log-normal cumulative density function:

$$D(x) = \sigma = \frac{1}{2} \left[1 + \operatorname{erf} \left(\frac{\ln(r) - \ln(\bar{r})}{S_p \sqrt{2}} \right) \right] \quad (7)$$

To use the probability or cumulative density function to calculate the reflection coefficient for a given membrane, a membrane's average pore size (\bar{r}), and standard deviation of pore radius (S_p) must first be known. These parameters can be determined by performing rejection experiments with several solutes of different sizes.

Hydrodynamic Model

The hydrodynamic model has been commonly employed to describe the rejection of uncharged solutes by porous NF membranes and detailed descriptions of the derivation and application can be found in various publications (5, 14, 31–33). This modeling approach assumes that NF membranes are composed of a bundle of cylindrical pores with the same radius, and that the transport or flux of a nonionic solute within a pore is due to hindered convection and diffusion:

$$J_s = -K_d D_\infty \frac{dc}{dx} + K_c c J_v \quad (8)$$

where $K_{i,c}$ and $K_{i,d}$ are hindrance coefficients. The average solute flux is obtained by integrating Eq. (8) over the length of the pore and relating the pore concentration to the external feed and permeate solute concentration through solute partitioning expressions ($c_m = \Phi C_m$, $c_p = \Phi C_p$). The solute steric hindrance (λ) is defined as the ratio between the solute radius and the characterized average pore radius:

$$\lambda = \frac{r_s}{r_p} \quad (9)$$

By assuming a parabolic profile of the Hagen-Poiseuille type (31, 34), the solute hindrance factors for convection and diffusion are given as:

$$K_c = A + B\lambda + C\lambda^2 + D\lambda^3 \quad (10)$$

$$K_d = E + F\lambda + G\lambda^2 + H\lambda^3 \quad (11)$$

For the case of $0 < \lambda \leq 0.8$, the coefficients in Eqs. (10 and 11) are defined as: $A = 1.0$, $B = 0.054$, $C = -0.988$, $D = 0.441$, $E = 1.0$, $F = -2.30$, $G = 1.154$, and $H = 0.224$. For the case of $0.8 < \lambda \leq 1.0$ the coefficient are defined as: $A = -8.830$, $B = 19.348$, $C = -12.518$, $D = 0$, $E = -0.105$, $F = 0.318$, $G = -0.213$, and $H = 0$. For relatively narrow and long pores with fully developed velocity profiles, Deen (13) reported that Eq. (10) should be multiplied by $(2-\Phi)$ where:

$$\Phi = (1 - \lambda)^2 \quad (12)$$

As it is speculative to assume the nature of the pore structure of NF membranes, Eq. (12) was used in combination with Eq. (10) to determine the

convective hindrance factor (K_c). By rearranging and integrating Eq. (8) and introducing the Peclet number, the ratio between the bulk permeate and feed solute concentrations, rejection can be defined as:

$$R = 1 - \frac{\Phi K_c}{1 - [1 - \Phi K_c] \exp(-Pe)} \quad (13)$$

As has been reported by Bowen et al. (35) and Otero et al. (31) the Peclet number can be described as:

$$Pe = \frac{K_c r_p^* J_v}{8 D_p \eta_0 L_p} = \frac{K_c r_p^* \Delta P}{8 D_p \eta_0} \quad (14)$$

This representation of the Peclet number eliminates the need to characterize the thickness to porosity ratio of a membrane. The rejection of an uncharged solute (Eq. 13), therefore, is a function of pore radius, solute size and diffusivity, and permeate flux.

Geometric-Hydrodynamic Model

Santos et al. (32) developed a method to transform a molecule into a prolate ellipsoid and determine the radius of the prolate ellipsoid based on the orientation angle of the ellipsoid to a membrane surface. After calculating a solute's length (length between the two most distant atoms), depth, and width, the radius of the ellipsoid can be calculated using:

$$r = \frac{LW}{2\sqrt{W^2 \cos^2(\omega) + L^2 \sin^2(\omega)}} \quad (15)$$

where L is the molecular length, and W is the average width ($W = \sqrt{\text{depth} \times \text{width}}$). The internal angle (ω) of a prolate ellipsoid can be calculated according to Santos et al. (32) by:

$$\omega = a + b \exp\left(-\frac{\theta}{c}\right) \quad (16)$$

where a, b, and c are empirical equations based on the ellipsoid ratio. The geometric radius, defined as the radius formed by a solute approaching the membrane at a given angle, can then be calculated by:

$$r_{geom} = r \cos(\theta - \omega) \quad (17)$$

where θ is defined as the orientation of a molecule as it approaches the membrane surface. The geometric radius can then be substituted for the Stokes radius in the

hydrodynamic model, allowing for an extra degree of freedom in the determination of hindrance coefficients based on size.

Concentration Polarization

Real versus observed rejection was determined by applying a mass transfer correlation determined by Geraldés and de Pinho (36) for Reynolds numbers (Re) in the range of 70–270 (based on the calculated hydraulic diameter and superficial feed velocity, our conditions yielded $Re \approx 240$) for a Dow/Filmtec NF membrane (NF200) that utilized the same feed space as used in our experiments. The mass transfer correlation is given as:

$$Sh = 0.33Re^{0.5} Sc^{0.33} \quad (18)$$

where:

$$Re = \frac{v\rho d_h}{\eta}, Sc = \frac{\eta}{\rho D_\infty}, Sh = \frac{K_m d_h}{D_\infty} \quad (19)$$

where v is the superficial velocity through the feed-brine channel, ρ is the solvent density, η is the solvent viscosity, K_m is the mass transfer coefficient, and D_∞ is the bulk diffusivity of the solute. The feed-brine channel and spacer of a membrane was characterized to calculate the hydraulic diameter (d_h) and the channel area (A_c). The hydraulic diameter (d_h) has been calculated by assuming the channel has a variable cross section due to the spacer (20):

$$d_h = \frac{4\varepsilon}{\frac{2}{h} + (1-\varepsilon)S_{V,SP}} \quad (20)$$

where ε is the spacer porosity, h is the channel height and $S_{V,SP}$ is the specific surface of the spacer. The porosity of spacer can be calculated using the average filament thickness and mesh size of the spacer according to:

$$\varepsilon = \frac{1 - V_{SP}}{V_{TOT}} \quad (21)$$

where V_{SP} is the volume of the spacer, and V_{TOT} is the volume occupied by the spacer. The specific surface of the spacer ($S_{V,SP}$) is calculated by:

$$S_{V,SP} = \frac{4}{\text{spacer_thickness}} \quad (22)$$

Once K_m is known, the concentration at the membrane surface (c_m) can be calculated by the film layer model:

$$c_m = c_p + [c_f - c_p] e^{\frac{J_v}{K_m}} \quad (23)$$

Molecular Size

There has been considerable amount of work performed to determine the most appropriate molecular size parameter to describe the rejection of organic solutes. A recent review by Zheng et al. (29) reported that membrane researchers have employed molecular weight, molecular length, molecular width, molecular diameter, Stokes radius (r_s), calculated molecular diameter and calculated mean size (d_{CMS}) as descriptors for a solute's size, with varying degrees of success. Based on our past research (37), r_s was found to be an effective and convenient method to represent the size of an organic solute. The r_s is determined using the Stokes-Einstein equation:

$$r_s = \frac{kT}{6\pi\eta D_s} \quad (24)$$

where r_s is the molecular radius or Stokes radii (m), D_w is the diffusion coefficient of the organic compound in water ($m^2\text{-sec}^{-1}$), k is the Boltzman constant ($J\text{-K}^{-1}$), T is the absolute temperature (K), and η is viscosity of water ($N\text{-sec}\text{-m}^{-2}$). The diffusion coefficient can be calculated by the Wilke-Chang correlation given by:

$$D_s = \frac{7.4 \times 10^{-8} (\varphi M)^{0.5} T}{\eta_b V_s^{0.6}} \quad (25)$$

where D_s is the diffusion coefficient, φ is an association factor for hydrogen bonding (set at 2.26 for water as the solvent), M is the molecular weight of the solvent (g/mol), T is the temperature, η is kinematic viscosity, and V_s is the molar volume of the solute. The molar volume of a solute was calculated using the LaBas molar volume method (38). Other researchers have been reluctant to use r_s as a solute descriptor because it treats a molecule as a rigid sphere that neglects a molecule's true geometry (29, 39). The theory of hindered transport in liquid filled pores, however, is based on treating a solute as a rigid sphere (13).

Zheng et al. (29) concluded that their newly evaluated descriptor, calculated mean size (d_{CMS}), was superior to all other descriptors evaluated for correlating the rejection of organic compounds to solute size. A solute's d_{CMS} was determined through a characterization of a geometrically optimized structure considering water solvation effects (using molecular modeling software Gaussian 03) and is defined as the cube root of the smallest volume occupied by a molecule. Although Gaussian 03 was not available to us, a similar computation was performed using Hyperchem (Hypercube, Gainesville, FL) so that d_{CMS} could be assessed.

Materials and Methods

Membrane Testing Unit

Flat-sheet membrane experiments were performed using a GE/Osmonics SEPA cell set-up (130 cm² membrane area, 31 mil channel height). For rejection experiments, a 50 L feed solution was fed to the SEPA cells via an adjustable speed motor (Dayton®, Motor Technology, Inc., Dayton, OH, USA) with a diaphragm pump head (Hydra-Cell, Wanner Engineering, Inc., Minneapolis, MN, USA). A SCADA system was employed to collect output signals from flow, pressure, conductivity, and temperature sensors and control and maintain flow rates and pressure set-points. A digital analytical balance was employed to measure the permeate flow rate.

Analytical Methodologies

Chemical analysis was performed by high performance liquid chromatography (HPLC) with either diode array detection or refractive index detection depending on the solute. The detection and quantification of the TOrC was performed using a Hewlett Packard 1050 HPLC equipped with an ultraviolet diode array detector (UV-DAD), C-18 reversed phase column, and an acetonitrile/water eluent. The detection and quantification of the characterization compounds consisting of sugars and alcohols was performed using a Hewlett Packard 1050 HPLC equipped with a 1047A refractive index detector (RID) with deionized water employed as the eluent.

Membrane Experiments

Feed water for membrane experiments was prepared by spiking deionized water with single solutes at nominal feed water concentrations of 700 µg/L (for HPLC-DAD) and 2 mg/L (for HPLC-RID) and adjusting the pH to 6.5. A new membrane coupon was used for each experiment and flushed with 20 liters of deionized water at 1200 kPa psi before rejection experiments to clean off any impurities. Membrane experiments for each solute were conducted by increasing the pressure from approximately 170 kPa to 1700 kPa in increments of 170 kPa. Experiments were conducted at a constant feed water temperature of 18.5°C. At least 250 mL of permeate was collected before a sample was taken at any pressure. Replicate feed and permeate samples were collected in HPLC vials for each pressure evaluated.

Membrane

The membrane chosen for model development was the NF membrane manufactured by Dow/Filmtec (Minneapolis, MN). The membrane is characterized as a softening NF membrane with good rejection for organic solutes with molecular weights greater than 200 Dalton (Dow/Filmtec). Select properties of the NF membrane are presented in Table I.

Table I. Select NF-4040 Properties

Membrane	Material	Est. MWCO*	Zeta Potential [†]	Contact Angle [‡]	Salt Rejection [§]	Lp**
-	-	Daltons	mV	degrees	%	m ³ /m ² -s-Pa
NF-4040	Polypiperazine	180	-33	30	99	1.80E-11

*Based on Bellona (27) [†]Streaming potential at pH 7 with 0.01 NaCl electrolyte solution [‡]Using goniometer [§]Based on Dow/Filmtec spec. sheet (40) **Average value from experiments

Table II. Select properties of solutes used during this study

Compound	CAS Number	MW (g/mol)	Log K _{ow}	Stokes radius (nm)	Diffusion coefficient (m ² /s)	Length (nm)	Width (nm)	Depth (nm)	E _{osm} (Eb)
Characterization Compounds									
1,3-butanediol	107-88-0	90.1	-0.3	0.262	3.18E-10	0.519	0.389	0.277	-0.284
Ethanol	64-17-5	46.1	-0.3	0.180	1.19E-09	0.405	0.212	0.184	-0.281
Glucose	50-99-7	180.1	-3.2	0.334	6.42E-10	0.832	0.425	0.288	-0.279
Glycerin	56-81-5	92.1	-1.8	0.240	3.92E-10	0.573	0.368	0.284	-0.285
Methanol	67-56-1	32.0	-0.8	0.135	1.58E-09	0.213	0.184	0.183	-0.284
MtBE	1634-04-4	86.2	0.9	0.285	7.53E-10	0.534	0.432	0.364	-0.268
Sucrose	57-50-1	342.3	-3.7	0.476	4.57E-10	0.962	0.624	0.634	-0.269
Tetraethylene glycol	112-60-7	194.2	-2.0	0.406	5.28E-10	1.563	0.245	0.213	-0.276
Urea	57-13-6	60.1	-2.1	0.177	1.23E-09	0.293	0.003	0.410	-0.271
TOCs									
Acetaminophen	103-90-2	151.2	0.5	0.335	6.40E-10	0.896	0.451	0.174	-0.220
Atrazine	1912-24-9	215.7	2.6	0.427	5.02E-10	0.792	0.778	0.424	-0.248
Biaphenol-A	80-05-7	228.3	3.3	0.442	4.84E-10	0.964	0.536	0.445	-0.250
Caffeine	58-08-2	194.2	-0.1	0.380	5.65E-10	0.742	0.624	0.200	-0.236
Carbamazepine	298-46-4	236.3	2.5	0.428	5.01E-10	0.925	0.584	0.401	-0.231
DIBET	134-62-3	191.3	2.2	0.421	5.99E-10	0.965	0.605	0.261	-0.230
Phenacetin	62-44-2	179.2	1.6	0.344	5.58E-10	1.176	0.457	0.199	-0.220
Primidone	125-33-7	218.3	0.9	0.368	5.18E-10	0.859	0.522	0.261	-0.254
Propylbenzene	479-92-5	230.3	1.9	0.455	4.71E-10	0.996	0.611	0.428	-0.218
Validation TOCs									
Benzyl alcohol	100-51-6	108.1	1.1	0.282	7.60E-10	0.685	0.435	0.193	-0.259
Cofiline	486-35-6	176.2	0.1	0.374	5.73E-10	0.916	0.644	0.554	-0.238
Butyl paraben	94-26-8	194.2	3.6	0.404	5.30E-10	1.261	0.479	0.238	-0.245
Pentoxifylline	6493-05-6	278.3	0.3	0.481	4.45E-10	1.348	0.708	0.419	-0.236

Organic Compounds

The model organic solutes employed in this study were selected to span a large range of properties relevant to membrane separations, such as size, functional groups, solubility, hydrophobicity, and polarity. A list of the compounds employed in the study is presented in Table II. The compounds termed ‘characterization compounds’ were selected for membrane characterization purposes and are composed of alcohols, sugars, urea, and MtBE in configurations characterized as straight chain aliphatics, branched aliphatics, and ringed aliphatic structures (glucose and sucrose).

Molecular Modeling

The Schrödinger LLC (New York, NY) molecular modeling platform and Hyperchem 7.5 (Hypercube, Gainesville, FL) were used to calculate molecular properties used to develop quantitative structure property relationships (QSPR). For both programs, two dimensional “mol” files (obtained from the National Institute of Standards and Technology) were used to begin calculations. For Schrödinger calculations, LigPrep was used to build three-dimensional optimized structures, QikProp was used to calculate non-quantum mechanic molecular descriptors, and Jaguar was used to calculate quantum mechanic descriptors (using density functional theory and a 6-31 G (pd) basis set). Hyperchem was used

to characterize the dimensions of solutes. Molecular geometry was optimized in the presence of water molecules using molecular mechanic calculations (MM+ force field) and the Polak-Ribiere algorithm. The dimensions of a periodic box surrounding the optimized molecule were used as the length, width, and depth of a molecule.

Results and Discussion

Rejection of TORC by NF Membrane

The rejection of the TORC employed in the study at permeate flux of 20 and 120 LMH for the NF membrane is presented in Figure 1. With the exception of acetaminophen, phenacetin, and bisphenol-A, rejection at the limiting flux (120 LMH) was equal to or greater than 90 percent. For water reuse applications, however, membrane systems are generally operated at an average permeate flux rate of 20 LMH (4). At 20 LMH, both diffusive and convective mass transport contribute to the overall mass transport, and as a result, rejection was significantly lower than under the limiting flux conditions, particularly for acetaminophen and phenacetin.

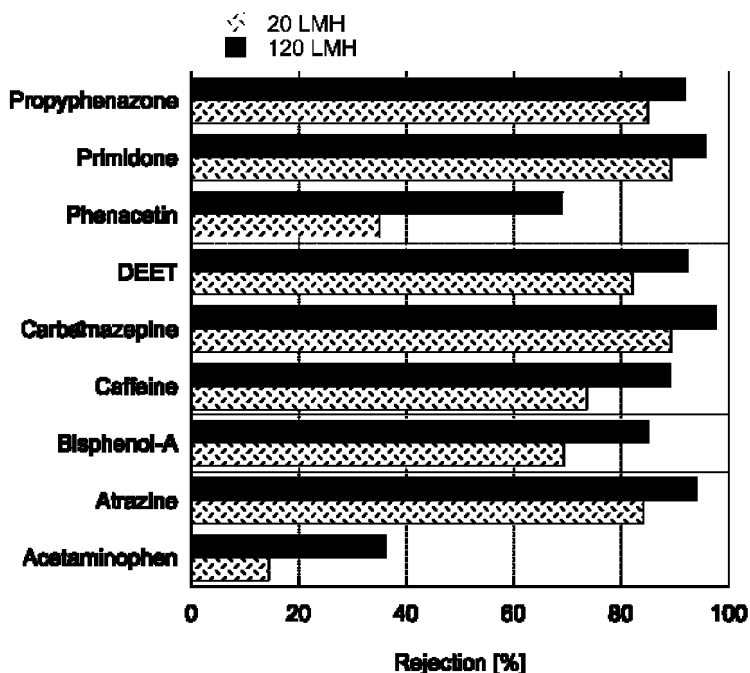


Figure 1. Rejection of TORC employed in study at 20 LMH ($5.6E-6$ m/s) and 120 LMH ($3.3E-5$ m/s) permeate flux (NF membrane, Dow/Filmtec).

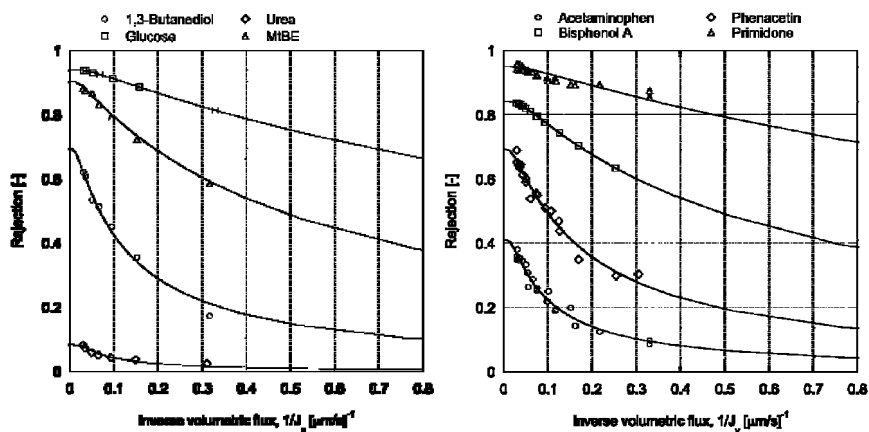


Figure 2. Estimation of phenomenological transport coefficients for select characterization compounds (left) and select TOrc (right) (NF membrane).

Phenomenological Model Development

Rejection results from the characterization compound and TOrc membrane experiments were fit with the phenomenological model by manipulating the model parameters, reflection coefficient (σ) and solute permeability (P). The Solver fitting algorithm in Excel™ was used to minimize the sum of the squared error between model output and experimental rejection data. An example of experimental rejection results and phenomenological fits for characterization compounds and TOrc is presented in Figure 2. As has been previously reported, by plotting rejection as a function of inverse permeate flux, the y-axis intercept corresponds to asymptotic rejection, which is described by the reflection coefficient (σ , (17)). In general, the phenomenological model fit the rejection data satisfactorily for all solutes tested.

Reflection coefficients, as determined through phenomenological model fitting, were used to determine the effective pore size distribution (PSD) of the membrane using the log-normal cumulative density function (Eq. 6). Both r_s and d_{CMS} were evaluated as molecular size parameters to describe rejection and the results are presented in Figure 3. For the characterization compounds, r_s was found to be a good descriptor for correlating the reflection coefficient with solute size according to the log-normal cumulative density function (R^2 value of 0.97). Tetraethylene glycol, a relatively long and narrow molecule, however, was not well described by r_s . Using r_s , the NF membrane was characterized with an effective pore size of 0.221 nm with standard deviation of 0.216 nm. The calculated mean size parameter yielded a better model fit ($R^2 > 0.99$) than the Stokes radius (especially for tetraethylene glycol), however, urea was not included in the fitting exercise as the fact that it is a trigonal planar molecule, which yielded an extremely small height value when measured with Hyperchem, and as a result, a very small and unrealistic d_{CMS} .

A comparison between reflection coefficients determined for TORC and the characterization compounds is presented in Figure 4. Reflection coefficients for TORC did not fit the PSD determined for the characterization compounds using r_s as molecular size descriptor. A separate PSD was developed for the TORC, resulting in an average effective pore size of 0.322 nm and standard deviation of 0.274 nm. Two possible explanations for this discrepancy are hypothesized: either r_s does not adequately characterize the true size of the TORC or solute properties other than size influence the removal of these compounds. While the former explanation is plausible, r_s of acetaminophen would need to be 40 percent smaller to fit the PSD obtained for the characterization compounds. An attempt to use d_{CMS} yielded a similar result and did not improve the fit of the reflection coefficient data (Figure 4). It is worth noting that although Zheng et al. (29) showed that the calculated mean size was better correlated with rejection data, the researchers did not list the dimensions or the d_{CMS} for any of the solutes. By inferring from the researcher's figures, it appears that their calculated mean size is significantly larger than the one calculated during this study (e.g., methanol is 0.375 nm versus our measurement value of 0.193 nm). Whatever the reason, d_{CMS} did not improve the correlation between the reflection coefficient and solute size.

The PSD results indicate that, when size is considered, aliphatic compounds were better removed by the NF membrane than the aromatic compounds. When using the effective PSD approach with the phenomenological model, it may be necessary to characterize the membrane with both aliphatic and aromatic solutes.

The solute permeability coefficient (P) calculated for each characterization compound is presented in Figure 5 as a function of solute size. As was reported in Van der Bruggen and Vandecasteele (23), an attempt was made to calculate solute permeability coefficients (P) using Eq. (5). The permeability coefficient for 1,3-butanediol was used to calculate ρ , as the size and permeability coefficient (P) of 1,3-butanediol represented median values for both parameters. However, this approach was found to poorly correlate with experimentally determined permeability coefficient (P) values for both the r_s and d_{CMS} ($R^2 < 0.5$, Figure 5). An exponential regression equation, however, significantly increased the correlation between the solute permeability coefficient (P) and solute size (Figure 5). Kargol (30) reported that the permeability coefficient (P) is difficult to determine for a solute and that experimentally determined permeability and reflection coefficients are strongly correlated with one another. Kargol (30) subsequently developed a formulation of the phenomenological model with two parameters: membrane solvent permeability constant (L_p) and the reflection coefficient (σ). It is worth noting that a correlation between the permeability (P) and reflection coefficient (σ) for the solutes used during this study yielded a R^2 value of 0.75. Based on these findings, one disadvantage to the phenomenological model is calculating the permeability coefficient (P) as there is no theoretical reasoning why the permeability coefficient (P) decreases exponentially with increasing solute size.

As was the case for the TORC PSD, the d_{CMS} did not improve the correlation between the solute permeability constant and solute size and therefore was not used in any further analysis. With the exception of acetaminophen and phenacetin, TORC permeability coefficients (P) were found to be similar to sucrose, glucose, MtBE, and tetraethylene glycol (Figure 6).

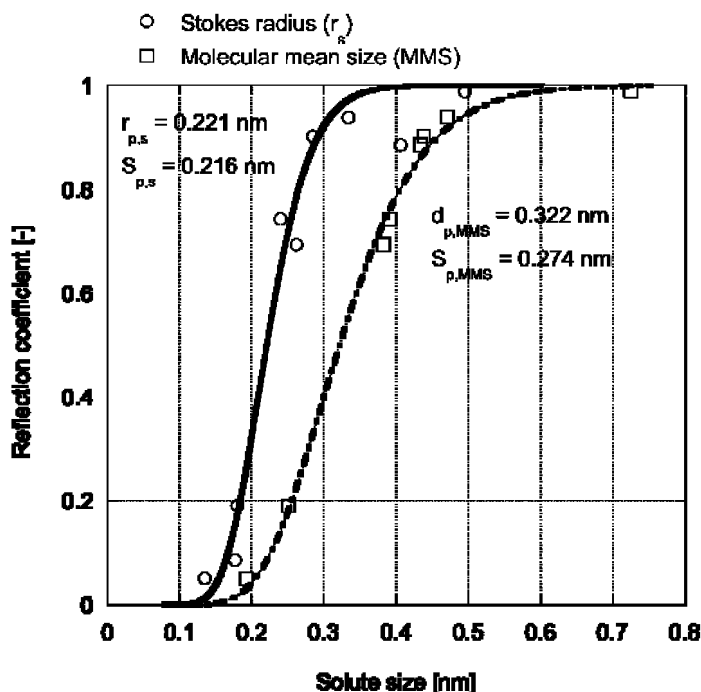


Figure 3. Log-normal cumulative density function fits for characterization compound reflection coefficients using both the Stokes radius (r_s) and calculated mean size (d_{CMS}).

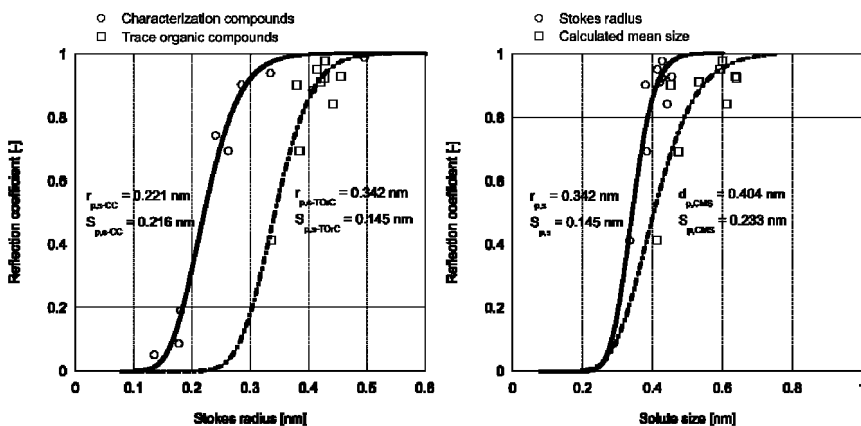


Figure 4. Pore size distributions obtained for characterization compounds and TorC using Stokes radius (left) and TorC using Stokes radius (r_s , left) and calculated mean size (d_{CMS} , right).

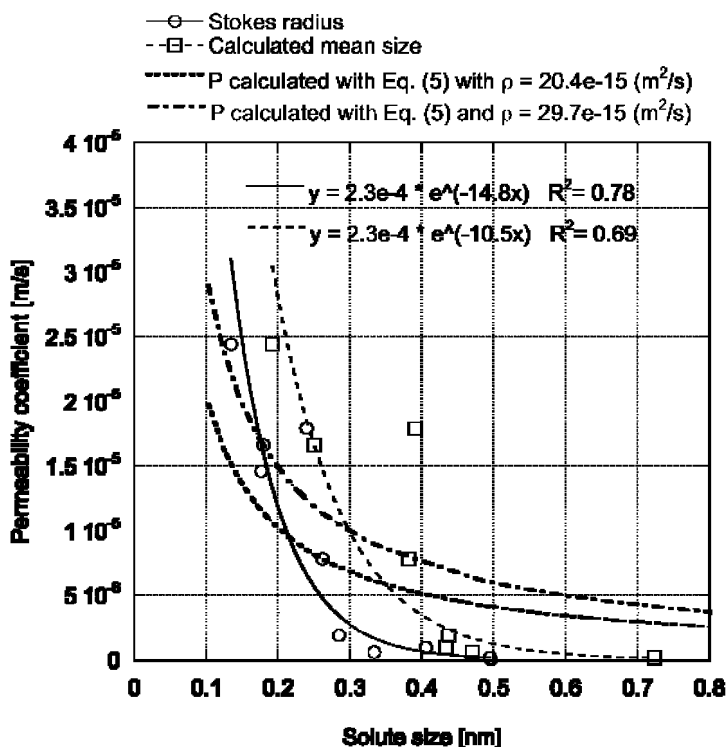


Figure 5. Solute permeability and model fits for characterization compounds using both the Stokes radius (r_s) and calculated mean size (d_{CMS}).

Hydrodynamic Model Development

Characterization compound rejection data was used to calculate the effective pore radius of the NF membrane by fitting the hydrodynamic model to experimental rejection data. Similar to the phenomenological model, minimizing the sum of the square error between model output and experimental rejection values by manipulating the pore radius yielded satisfactory model fits for all solutes. Effective pore radii as determined by the hydrodynamic model are presented in Figure 7. It is worth noting that Bowen and Welfoot (41) introduced a truncated PSD formulation into the hydrodynamic model, however, the determination of the PSD was relatively difficult and its inclusion had a negligible effect (<10 percent) effect on the rejection of uncharged solutes. Hydrodynamic model fits with the characterization compounds yielded an average pore size of 0.454 nm with a standard deviation of 0.075 nm. The average pore radius calculated using the hydrodynamic model ($r_p = 0.454$ nm) was over twice as large as the one calculated with the PSD-phenomenological model (0.221 nm). This result is due to the fact that the hydrodynamic model is based on hindered convection and diffusion as a function of solute geometry in a membrane pore, while the PSD approach assumes that any molecule smaller than a membrane pore

is free to permeate the membrane at infinite permeate flux. For the hydrodynamic model, even molecules much smaller than the membrane pore are sterically hindered (13).

By plotting limiting rejection ($1-\Phi K_c$) versus λ (r_s/r_p), it can be seen that by using a pore radius of 0.454 nm, the hydrodynamic model fit the characterization compound rejection data reasonably well (Figure 8). As was the case for the phenomenological model, tetraethylene glycol rejection was over-predicted by the hydrodynamic model. As was found during phenomenological model development, TORC limiting rejection is not well predicted by the hydrodynamic model calibrated with the characterization compounds and the model over-predicts the rejection of all TORC. The average pore radius of the NF membrane using TORC data was determined to be 0.628 nm with a standard deviation of 0.093 nm, significantly larger than the pore radius determined for the characterization compounds.

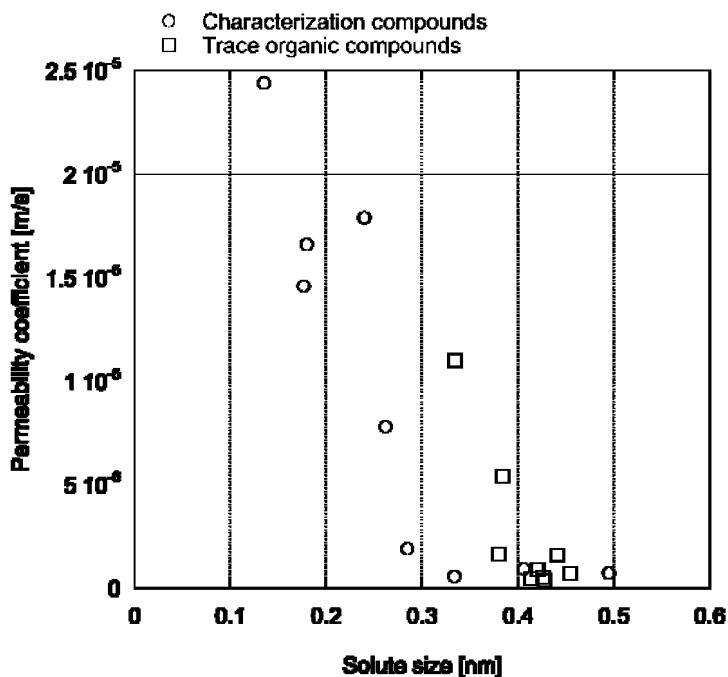


Figure 6. Solute permeability coefficients for characterization compounds and TORC as a function of Stokes radius (r_s).

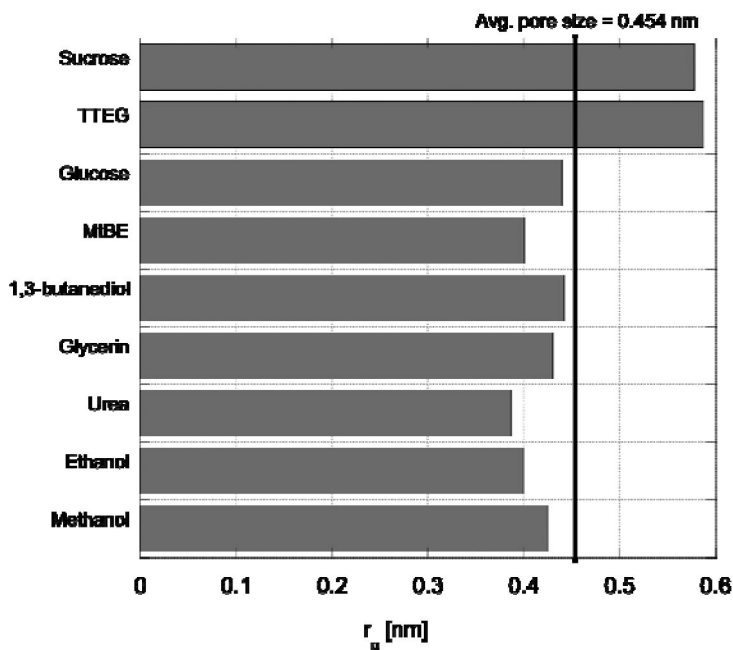


Figure 7. Pore radius obtained for each characterization compound. Solute Stokes radius given in Table II.

Geometric-Hydrodynamic QSPR Model

Santos et al. (32) developed an approach to change a molecule's radius as a function of a solute's angle of orientation to a membrane for inclusion into the hydrodynamic model. While the existence of an explicit orientation angle for a given molecule is unproven, the approach of Santos et al. (32) is of interest since it allows for an additional degree of freedom in the prediction of the rejection of an uncharged solute, where size is the main factor. In this work, the orientation angle of a solute is regarded as a fitting parameter that enables the size of a solute to be manipulated to fit the rejection data. A larger angle means that the solute size is made smaller and vice versa.

The orientation angle fitting exercise required that the characterized effective average pore radius of the membrane allowed all solutes investigated to have an orientation angle between 0 and 90 degrees given their molecular dimensions (i.e., length, width and depth) as calculated by Hyperchem. The pore radius was determined by fitting model to methanol rejection data, which yielded a pore radius of 0.414 nm. Methanol was chosen as it was the closest of investigated solutes to being spherical (i.e., length, depth and width are almost equal) and was, therefore, left out of orientation angle calculations (as was MtBE due to difficulties fitting the model to rejection data). A summary of orientation angles obtained for characterization compounds and ToRC is presented in Figure 9. In general, ToRC required a larger orientation angle to fit the model to the rejection

data. Further analysis was performed to determine if the orientation angle is a function of physicochemical properties of the organic solutes. Multiple linear regression were evaluated using various solute descriptors as predictors for the orientation angle. Statistically significant correlations were found between solute length ($R^2 = 0.66$), highest occupied molecular orbital energy (E_{HOMO} , $R^2 = 0.55$), and the orientation angle. The multiple linear regression QSPR equation presented in Figure 10 using length and E_{HOMO} had an R^2 value of 0.85, a F-value of 34, and a p-value less than 0.001 indicating that the relationship between the predictors and the orientation angle was statistically significant. No other statistically significant correlations were found between other descriptors evaluated (e.g., parameters for hydrophobicity, polarity, shape, etc.). Based on the regression, solutes with greater length and smaller E_{HOMO} energy values have larger orientation angles. This indicates that for longer molecules, molecular geometry is better characterized by width and depth. E_{HOMO} is considered to be a relative measure of the ability of a molecule to donate electrons (42, 43). Compared to the characterization compounds, the TOrc had larger E_{HOMO} values due to their aromatic structure and delocalized electrons. The energy gap between E_{HOMO} and E_{LUMO} of reactants is often used to quantify the strength of interaction between the two (44). One possible explanation for the correlation between E_{HOMO} and the orientation angle is that solutes that interact with membrane materials tend to be rejected to a lower degree than would be expected based on size.

Model Validation

Three of the models developed during this study were used to *a priori* predict the rejection of four nonionic solutes not included in model development: benzyl alcohol, butyl paraben, cotinine, and pentoxifylline. For the phenomenological model, the PSD determined for the TOrc and characterization compounds (Figure 4) were both used with the regression equation to calculate P (Figure 5). The hydrodynamic model (using a pore radius of 0.454 nm) and the modified hydrodynamic QSPR model were also employed. The model outputs for the four nonionic solutes are presented in Figure 11. Pentoxifylline and cotinine were well rejected by the NF membrane and both solutes were well described using the PSD obtained using the characterization compounds. The modified hydrodynamic QSPR model was found to provide good model fits for the rejection of cotinine, pentoxifylline, and isobutyl paraben. Benzyl alcohol exhibited significantly lower rejection than expected based on size and its reflection coefficient was better described by the TOrc PSD. Using the exponential regression to calculate the permeability coefficient, however, yielded a model output that fit the data poorly. The other models significantly over-predicted the rejection of benzyl alcohol. Past research on the rejection of phenols by RO and NF membranes have demonstrated that they often exhibit poor rejection due to solute-membrane interactions, presumably due to hydrogen bonding (26, 45). Other compounds utilized in this study with aromatic phenolic moieties, include acetaminophen and bisphenol-A, both compounds exhibiting lower rejection than expected based on their size.

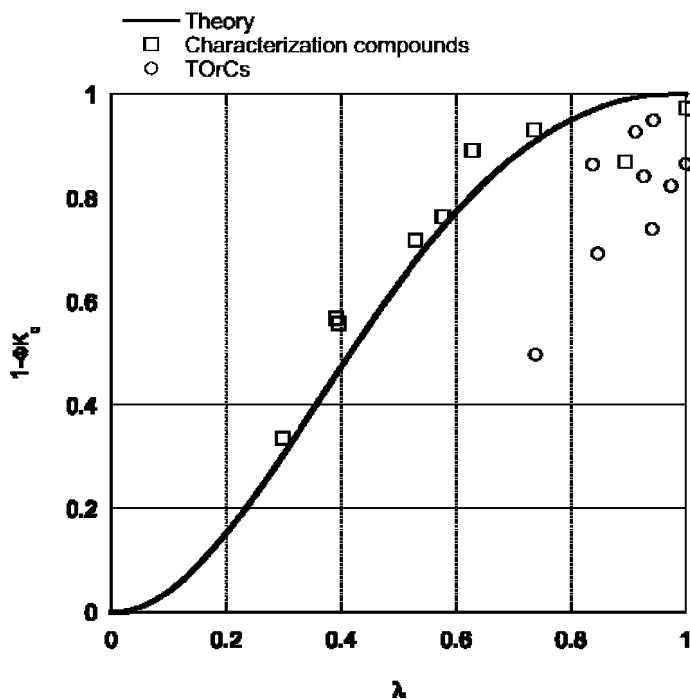


Figure 8. Limiting rejection of characterization compounds as a function of $\lambda = r_s/r_p$.

In terms of implementation, the hydrodynamic model requires the least effort as the average effective pore radius of a membrane can be characterized with rejection data for one compound such as glucose (Figure 7). The hydrodynamic model calibrated with sugars and alcohols, however, was not very effective for describing the rejection of a wide variety of TOrc. Phenomenological model development suggested that TOrc rejection data could be better described by calibrating the model with TOrc rejection data instead of characterization compounds. It is worth noting that the rejection of a few compounds, such as caffeine, carbamazepine and primidone, however, could be satisfactorily described using phenomenological models calibrated with the characterization compounds. Certain compounds, such as acetaminophen, phenacetin and bisphenol-A, exhibited much lower rejection than would be expected based on r_s and models developed using the characterization compounds greatly over-estimated their removal. Alternative molecular size parameters did not improve model fits and other solute descriptors, such as $\log K_{ow}$, were not correlated with rejection or model parameters (i.e., σ or P) as some researchers have suggested (24, 29).

The QSPR model developed to calculate a solute's orientation angle suggests that solutes with greater lengths are not well characterized by r_s , which has been theorized by other researchers (29). Additionally, the statistically significant

inclusion of E_{HOMO} into the QSPR model suggests that the observed difference in rejection for the characterization compounds versus the TOC is related to ionization potential of organic solutes and the degree in which they interact with the membrane polymer. As a result, the rejection of compounds with greater E_{HOMO} values were better described using a solute size significantly smaller (i.e., larger orientation angle) than the one calculated by either r_s or d_{CMS} .

Both modeling approaches examined herein assume a unimodal pore size distribution log-normally distributed for the phenomenological model approach and an average pore size (with no size deviation) in the case of the hydrodynamic model. Past researchers, however, have characterized certain RO and NF membrane as having both bi-modal and non-Gaussian (or non-Galton in the case for the log-normal approach) distributed pore size distributions (46, 47). The possible existence of a bi-modal pore size distribution may impact the usefulness of the modeling approaches evaluated during this study as they are not designed to describe the existence of distinctly different pore sizes. The purpose of this study, however, was not to elucidate the nature of a hypothetical pore structure, but to develop methods to predict the rejection of organic solutes. It is worth noting that a recent study by Kosutic et al. (47) evaluated the pore size distribution of the NF-270 membrane, which, is very similar to the membrane evaluated during this study. The researchers determined the pore size with the highest incidence was 0.9 nm, which, was almost equal to the average pore diameter found in this study using the hydrodynamic model (0.91 nm). Additionally, the membrane was found to have a relatively small incidence of pore sizes in the 1.5 nm range.

While the developed QSPR-hydrodynamic model satisfactorily described three of the four validation compounds, benzyl alcohol was not well described by the developed models, presumably due to strong solute-membrane interactions, which have been reported for phenols and other similar compounds (26, 45). It has been proposed in past studies that organic solutes can interact with membrane materials through hydrophobic interactions (non-specific) and interactions with active sites in membrane materials designed to absorb water molecules (specific) or both. Matsuura and Sourirajan (45) demonstrated that the separation of organic with acidic non-dissociated moieties such as hydroxyl groups by cellulose acetate RO membranes depended on the hydrogen donating ability of the hydroxyl moiety on the molecule, which could be quantified using Taft and Hammett constants. The low separation of benzyl alcohol and phenolic compounds was theorized to be a result of specific solute/membrane interactions. More recently, Williams et al. (26) demonstrated that specific interactions between substituted phenols and polyamide RO and NF membranes lead to adsorption to the membrane's active layer and a decrease in permeate flux. Because the investigated models do not include a mechanistic representation of strong solute-membrane interactions, alternative modeling approaches may be necessary, such as the surface force pore flow model (25) or the modified solution diffusion model (26).

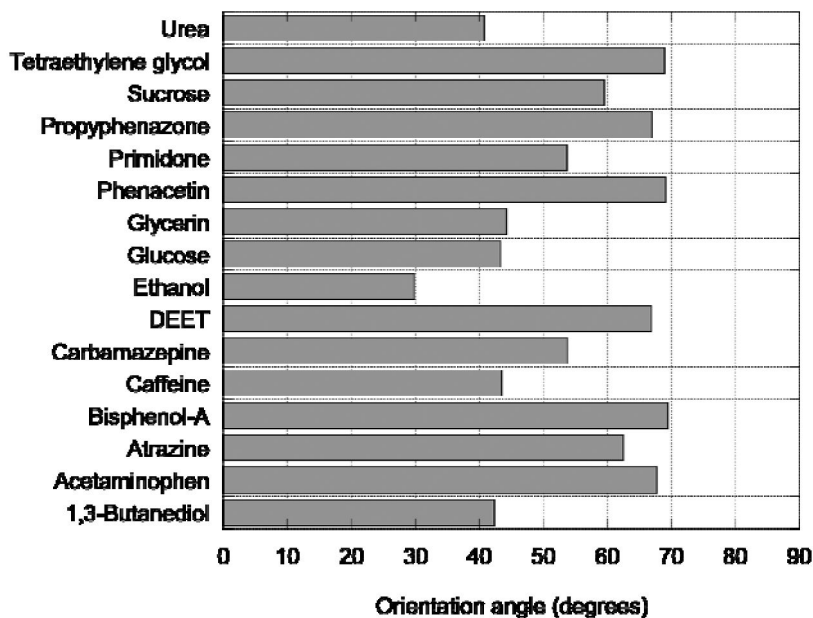


Figure 9. Orientation angles determined for characterization compounds and TORC (methanol and MtBE are excluded).

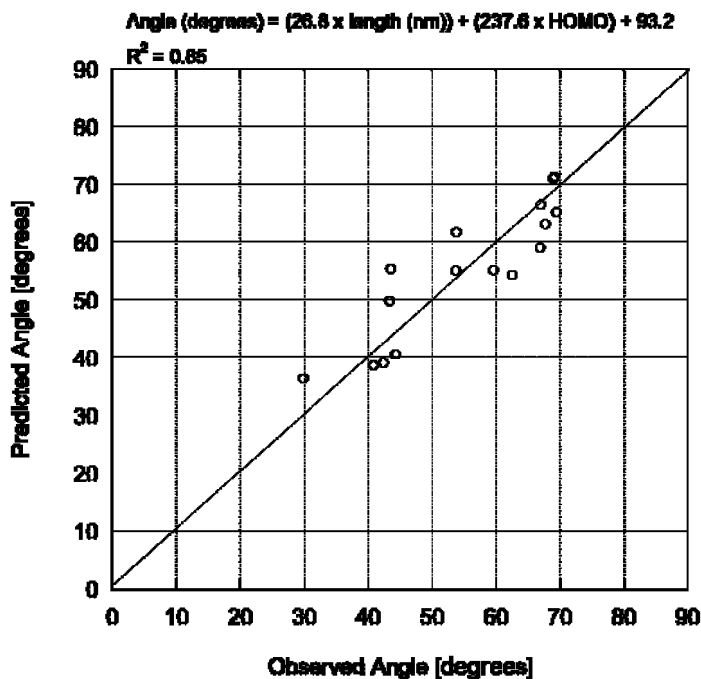


Figure 10. Predicted versus observed (i.e., best fit to rejection data) orientation angle for characterization compounds and TORC.

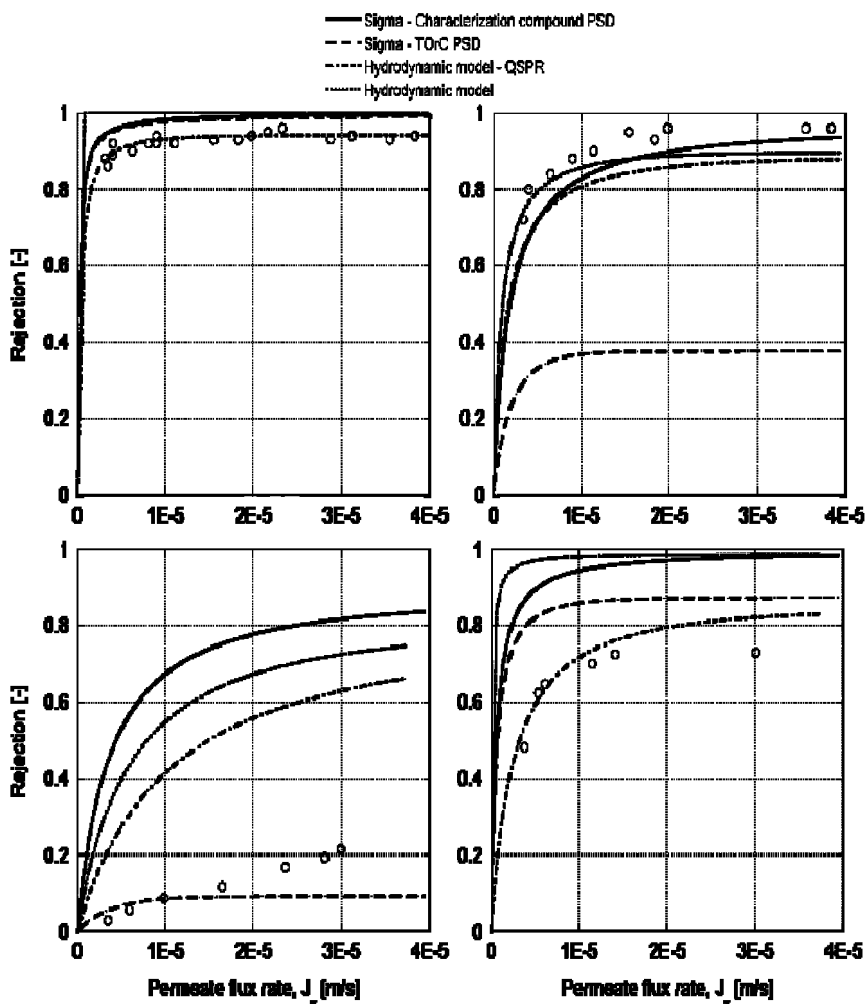


Figure 11. Rejection and model fits for pentoxifylline (top left), cotinine (top right), benzyl alcohol (bottom left), and isobutyl paraben (bottom right).

For compounds not expected to display strong membrane interactions, however, the modeling approaches examined during this study can provide a good estimation of rejection as a function of permeate flux. To *a priori* describe the rejection of nonionic TORC by a NF membrane with the least amount of membrane characterization work, it is recommended that the phenomenological model coefficients be determined for a few aliphatic compounds (e.g., glycerin, glucose, urea) and aromatic TORC (e.g., acetaminophen, caffeine, carbamazepine) spanning a range of molecular size. Once determined, the relationships presented in Figures 4 and 5 can be developed, which, can be used with Eqns. (3 and 4) to describe rejection as a function of permeate flux. Although no ionic organic compounds were examined in this study, a similar approach to the one presented here could be used to describe the rejection of ionic TORC by NF membranes.

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Chapter 21

Concentrations of Hydrophobic Organic Pollutants in U.S. Wastewater Treatment Plants and in Receiving Surface Waters Modeled from EPA Biosolids Monitoring Data

Alex Weir,¹ William E. Moiles,¹ Brian Brockman,¹
Carolyn S. Mattick,¹ Kristin McClellan,^{1,2} Lisa Gerwe,¹
Randhir P. Deo,² and Rolf U. Halden^{*,1,2}

¹School of Sustainable Engineering and the Built Environment,
Arizona State University, Tempe, AZ 85287

²Center for Environmental Biotechnology, The Biodesign Institute at
Arizona State University, Tempe, AZ 85287

*Corresponding author: The Biodesign Institute at Arizona State University,
1001 South McAllister Avenue, P.O. Box 875701, Tempe, AZ 85287-5701.
E-mail: halden@asu.edu.

Organic microcontaminants such as pharmaceuticals and personal care products (PPCPs) are currently not regulated with regards to wastewater treatment removal. To provide a basis for risk assessment, the U.S. Environmental Protection Agency (EPA) conducted a nationwide sampling campaign at seventy-four publicly owned treatment works, to assess contamination of biosolids with 145 different pollutants. However, a similar nationwide study of PPCPs contained in treated effluent of such a large number of wastewater treatment plants has never been conducted.

In this study, a published empirical model was modified, and applied, to estimate from the biosolids concentrations reported by the EPA, the concentrations in raw and treated wastewater of pharmaceuticals and other organic contaminants. Target chemicals included eight organic compounds: (benzo(a)pyrene, beta-estradiol-3-benzoate, fluoranthene, miconazole, norgestimate, pyrene, triclocarban and triclosan.

These compounds were selected based on the hydrophobicity range for which the model previously had been validated.

The results of the mass loading estimations are compared to measured concentrations in treatment plant influent and effluent and also are put in relation to contaminant concentrations found in effluent receiving U.S. surface waters. Potential impacts on sensitive environmental receptors as well as potentially effective treatment methods for wastewater are identified and discussed. The removal efficiencies predicted by the model ranged from $\geq 13\%$ for triclosan to $\geq 82\%$ for benzo(a)pyrene. Modeled contaminant concentrations in treatment plant influent ranged from 0.025 to 12 $\mu\text{g/L}$ whereas modeled contaminant concentrations in effluent ranged from 0.0062 to 10 $\mu\text{g/L}$. A comparison of predicted and observed removal efficiencies for triclosan and triclocarban indicated that the model predictions are conservative in nature and comparable to actual measurements made at sewage treatment plants. This study produced the first concentration estimates for beta-estradiol-3-benzoate, miconazole, and norgestimate in surface waters and identified important information gaps concerning ambient concentrations of microcontaminants and associated ecotoxicological effects.

Introduction

Several studies have shown that secondary treatment of wastewater, with activated sludge processes, results in the incomplete removal of pharmaceuticals and personal care products (PPCPs), as well as other organic chemicals (1–6). Trace concentrations of biologically active contaminants are known to enter ground and surface water supplies mainly through effluent discharge from wastewater treatment plants (WWTPs) (7, 8). Partly because no regulation exists to control these contaminants, the spotlight has recently turned toward the issue of microcontaminants and potential associated risks posed to public health and the environment.

Municipalities typically collect wastewater from residences and convey it in sewerage systems to WWTPs for treatment. Effluent water, having met regulatory standards, is then discharged to surface water. In the United States, secondary wastewater treatment, or better, is applied to over 92% of domestic sewage (9). Secondary treatment relying on activated sludge unit operations is the process of choice due to its greater efficiency as compared to other treatment methods. Activated sludge treatment produces excess biological mass referred to as (municipal) sludge. The amount of sludge produced depends on the composition of the influent as well as the design and operation of the treatment facility. The term ‘biosolids’ specifically refers to sewage sludge that has undergone treatment to meet federal and state standards for beneficial use, thereby allowing for its

application on (agricultural) land as a soil amendment; alternatively, unwanted municipal sludge is incinerated or disposed of in landfills (10, 13).

According to the Toxic Substances Control Act (TSCA), approximately 84,000 inventoried chemicals are manufactured, imported, or processed in the United States (<http://epw.senate.gov/tsca.pdf>). Numerous chemical pollutants can therefore potentially be present in biosolids and aqueous discharge from WWTPs with little to no monitoring or regulation. Since biosolids and reclaimed water are often put to beneficial use, there is a risk of contaminants reaching the general public and negatively impacting the environment. In January 2009, to provide a basis for risk assessment of this issue, the U.S. Environmental Protection Agency (EPA) published the Targeted National Sewage Sludge Survey (TNSSS) (11) that evaluated the level of 145 contaminants found in biosolids from WWTPs across the United States. The report presents statistical methodology and evaluations related to the data collected. It provides estimates of contaminant concentrations in biosolids that are representative of the nation's largest 3,337 WWTPs, all of which use secondary activated sludge treatment or better and combined treat approximately 94% of the wastewater generated in the U.S. (11).

While researchers have published many models predicting the fate of contaminants in WWTPs, none have used the tool to estimate influent and effluent concentrations of WWTPs on a national scale. The goal of this study was to estimate concentrations of select PPCPs and hydrophobic organics in treated wastewater based on the biosolids concentrations reported by the USEPA. In 2009, a simple, yet robust empirical model was introduced allowing for the estimation of levels of hydrophobic organics in biosolids based on influent and effluent concentrations using the pH dependent *n*-octanol-water partitioning coefficient (D_{OW}) as the only input requirement (12). The model identified sorption as the main removal mechanism for hydrophobic organic compounds. Whether wastewater constituents persist in either biosolids or effluent discharge depends to a large degree on the chemicals' hydrophobicity, a property represented by the D_{OW} value (12). In addition, this simplistic model takes into account all removal mechanisms during wastewater treatment through a parameterized value, termed p_{fit} . Of the 145 contaminants measured in the TNSSS, eight were evaluated in this study based on their applicability to the model.

The results of the mass loading estimates were compared to measured contaminant concentrations in surface water reported in the peer-reviewed literature.

Method

The prediction of aqueous concentrations of selected organic pollutants in treated wastewater effluent was made possible by a previously-published empirical model (12) in conjunction with biosolids concentration data by the U.S. Environmental Protection Agency (EPA) published in early 2009 (11).

The model applied in this investigation was derived from empirical data published in the peer-reviewed literature (12). Its previous use was to estimate the fraction of hydrophobic organic compounds (HOCs) that will persist in digested

sewage sludge produced during wastewater treatment ($f_{i(\text{biosolids})}$). This model (12) (see equation 1) uses tabulated pH-corrected values of the *n*-octanol-water partitioning coefficient (i.e., D_{OW} values) to account for partitioning behavior of the HOCs. It approximates the combined effect of all loss mechanisms occurring during treatment, including biodegradation, chemical and physical degradation as well as volatilization, by using a dimensionless fitting parameter, p_{fit} :

$$f_{i(\text{biosolids})} = p_{\text{fit}} \times \frac{D_{i,\text{ow}}}{1 + p_{\text{fit}} \times D_{i,\text{ow}}} \quad (1)$$

For the purposes of this study, D_{OW} values corresponding to pH 7.5 were used, which is the average pH of effluent leaving conventional wastewater treatment plants (12). In addition, a p_{fit} value of 1.76×10^{-6} was applied, based on literature information (12).

In this investigation, because HOC concentrations in biosolids are known but influent concentrations are not, the empirical model is essentially used in reverse: to predict the HOC concentrations entering the WWTP based on the concentrations in the dry biosolids.

Figure 1 depicts a simple diagram applicable to this study. The value of $f_{i(\text{biosolids})}$ obtained from the empirical model expressed in equation 1 can also be expressed as a relationship between the concentration of a given HOC in the biosolids versus those found in influent:

$$f_{i(\text{biosolids})} = \frac{C_{i(\text{biosolids})} \times Y}{C_{i(\text{influent})}} \quad (2)$$

where $C_{i(\text{influent})}$ is the concentration of analyte *i* arriving in influent in units of mass per liquid volume, $C_{i(\text{biosolids})}$ is the concentration of analyte *i* found in biosolids in units of mass per dry mass of biosolids produced, and *Y* is the yield of biosolids per volume of raw wastewater treated in units of dry mass of biosolids per liquid volume of wastewater treated.

A representative value for *Y* was taken from the literature as 1.296×10^{-4} kg/L or 129.6 mg/L (12). This value is in good agreement with the generally accepted range of 100–300 mg/L also reported in the literature (14) and therefore was adopted for the purposes of this study.

Through means of a mass balance, and assuming constant volume of influent and aqueous effluent stream as well as constant loss to biodegradation and other relevant loss processes, the concentration of HOCs in influent can be calculated. Accordingly, this relationship can be written as follows:

$$C_{i(\text{influent})} = \frac{C_{i(\text{biosolid s})} \times Y}{f_{i(\text{biosolid s})}} \quad (3)$$

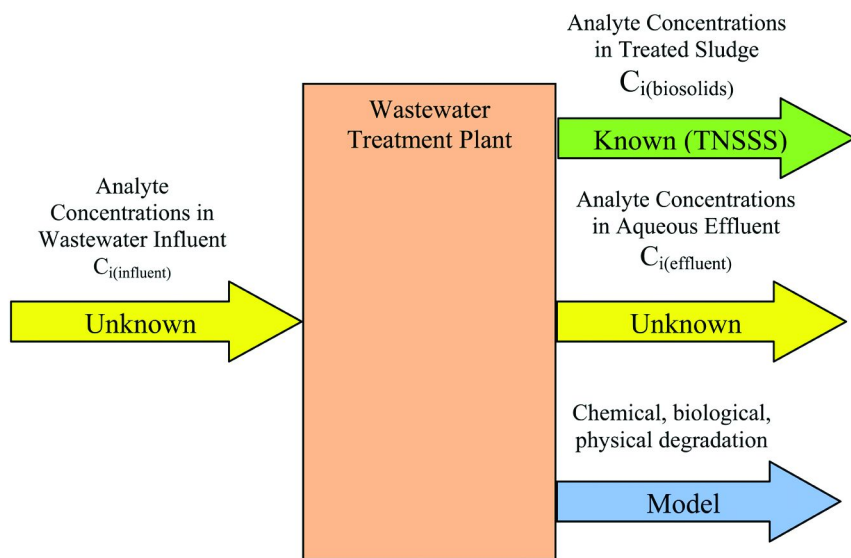


Figure 1. Conceptual diagram showing relevant input, outputs and loss terms in a wastewater treatment plant. (see color insert)

Note that the $f_{i(\text{biosolids})}$ accounts through the p_{fit} value (eq. 1), for losses of the target compound in the treatment plant. Rearrangement of equation 3 yields the final expression for HOC concentrations leaving the WWTP:

$$C_{i(\text{effluent})} = \left[\frac{1}{f_{i(\text{biosolids})}} - 1 \right] C_{i(\text{biosolids})} \times Y \quad (4)$$

Results and Discussion

The model is applicable for HOCs that have D_{OW} values in the range from 4.9 to 6.4 (12). Eight analytes from the EPA study fall within this D_{OW} range and thus were considered in this study. The selected compounds are listed in Table 1 along with their D_{OW} values and information on sources, uses and potential human health and ecological impacts.

Table 1. Overview of organic compounds considered in this study

<i>Analyte</i>	<i>Group</i>	<i>CAS Number</i>	<i>pH adjusted n-Octanol-water partitioning coefficient log (Dow) at pH 7.5</i>	<i>Uses</i>	<i>Sources</i>	<i>Potential Impacts</i>
Benzo(a)pyrene	Organics (PAH)	50-32-8	6.4	No practical use	Incomplete combustion of organics	Probable human carcinogen; mutagenic
Beta-estradiol-3-benzoate	Steroids and Hormones	50-50-0	6.24	Contra-ception	Manufactured (derived from Cholesterol)	Endocrine disruptor
Fluoranthene	Organics (PAH)	206-44-0	5.17	No practical use	Combustion of organic matter	Carcinogen (EPA priority PAH)
Miconazole	Pharmaceuticals	22916-47-8	5.84	Antifungal: Micatin, Monistat, etc	Manufactured	Antifungal Agent with some anti-bacterial properties
Norgestimate	Pharmaceuticals	35189-28-7	5	Treatment of menopause symptoms	Manufactured	Endocrine disruptor
Pyrene	Organics	129-00-0	5.17	Dye-making	Combustion	Toxic to kidneys & liver
Triclocarban	Pharmaceuticals	101-20-2	5.74	Disinfectants, soaps	Manufactured	Anti-fungal, anti-bacterial
Triclosan	Pharmaceuticals	3380-34-5	4.93	Personal Care Products	Manufactured	Anti-fungal, anti-bacterial

Table 2. Summary of modeling results and literature information available for target analytes

<i>Analyte</i>	<i>Concentration in Biosolids^a</i> [g/kg]	<i>log DOW (pH 7.5)^b</i>	<i>Concentration in Influent [g /L]</i>	<i>Concentration in Effluent [g /L]</i>	<i>Calculated Removal Efficiency [%]</i>	<i>Literature Removal Efficiencies [%]</i>
Benzo(a) pyrene	661	6.4	0.1	0.019	82	None Found
Beta-estradiol-3-benzoate	146.9	6.24	0.025	0.006	75	None Found
Fluoranthene	1419	5.17	0.89	0.707	21	None Found
Miconazole	979.7	5.84	0.23	0.104	55	None Found
Norgestimate	27.5	5	0.024	0.02	15	None Found
Pyrene	1646	5.17	1	0.82	21	None Found
Triclocarban	38744	5.74	10	5.192	49	18-100 ^c
Triclosan	12112	4.93	12	10.479	13	50-96 ^c

^a Data taken from (11). ^b Data taken from *SciFinder* online database. ^c Data taken from (2, 15, 16, 28) and references cited therein.

Microcontaminant concentrations measured empirically in biosolids were used to calculate the contaminant levels in wastewater influent and effluent. Results from the empirical model for the select wastewater contaminants are presented in Table 2. Analyte concentrations in raw sewage (WWTP influent) ranged from 0.024 and 0.025 $\mu\text{g/L}$ for norgestimate and beta-estradiol-3-benzoate, respectively, to 10 and 12 $\mu\text{g/L}$ for triclocarban and triclosan, respectively. This is in agreement with a prior study examining the fate of organic wastewater contaminants that found triclosan and triclocarban influent concentrations to be among the highest, whereas those of hormones such as beta-estradiol-3-benzoate were among the lowest influent concentrations (2). Calculated aqueous-phase removal efficiencies ranged from 13 to 82% and were dependent mostly on the hydrophobicity ($\log D_{\text{OW}}$) of the analytes. Triclosan with the lowest D_{OW} value had the lowest calculated removal efficiency while benzo(a)pyrene with the highest D_{OW} value had the highest removal efficiency. This was expected because for hydrophobic compounds, such as the ones included in this study, sorption to organic matter has been identified in previous studies as a master variable governing the removal of organic contaminants during wastewater treatment (2). The calculated aqueous removal efficiency for triclosan of 13% was in the same order of magnitude but lower than the removal efficiencies of 50 - 96% observed in prior studies (2, 16). The aqueous removal efficiency for triclocarban calculated at 49% was found to be similar to values of 18 - 100% reported in the literature (Table 2). Triclosan and triclocarban represent the only analytes from which to judge the accuracy of the model for calculating removal efficiencies. In summary, a comparison of calculated and empirically observed removal efficiencies indicates that the model's estimates reflect either the mid range of actual WWTP performance (Table 2; triclocarban) or they tend to be conservative in nature, i.e., prone to underestimation of the actual removal (Table 2; triclosan).

To evaluate the accuracy of concentrations calculated for influent and effluent, the model output shown in Table 2 was compared to empirical datasets from WWTPs. A study conducted between 2005 and 2008 by the EPA examined influent and effluent data from nine WWTPs featuring several variations of the activated sludge process (15). Samples were analyzed for contaminants of emerging concern, which included triclosan, triclocarban, norgestimate, miconazole, and beta-estradiol-3-benzoate among others. Samples collected for norgestimate, miconazole, and beta-estradiol-3-benzoate had influent or effluent concentrations below the laboratory reporting limit thus their removal efficiencies were not quantified (15).

Furthermore, effluent concentrations calculated here for both triclosan and triclocarban were found to be in the same order of magnitude as empirically observed concentrations made at plants across the U.S. (Figure 2). For the remaining compounds data are lacking.

Upon discharge to surface waters, residual levels of unwanted substances in treated wastewater are further diluted. In this study an average dilution factor of 10 was applied to effluent concentrations to estimate potential impacts on surface waters. Where available, empirical data from waterways were compared with estimated surface water concentrations calculated from the modeled WWTP effluent concentrations shown in Table 2. As can be seen in Figure 3, measured

concentrations correlate well with the surface water concentrations obtained from the model. A nationwide study conducted by the U.S. Geological Survey (USGS) in 1999 and 2000, sampled surface water from 139 streams in the U.S. (7). All samples were analyzed for a range of pharmaceuticals and organic contaminants. Concentrations reported by the USGS are similar to those predicted here. While that study is the only one so far that sampled streams across the U.S., other studies have been conducted to analyze surface waters for concentrations of a smaller number of specific organic contaminants (16–18). One study (16) reports levels of triclosan in experimental wetlands in north Texas from 0.03 to 0.29 $\mu\text{g/L}$. In another study (17), median concentrations of triclocarban in 13 states, measured upstream and downstream of WWTPs, were on the order of 7 and 20 ng/L , respectively. However, maximum concentrations of up to 6,750 ng/L have been reported for urban streams impacted by sewerage leaks (29, 30).

Empirical values were available for five of the eight analytes modeled. Calculated concentrations of the polycyclic aromatic hydrocarbons pyrene and fluoranthene as well as the disinfectants triclosan and triclocarban are within the range of data reported in the literature. The estimated concentration for benzo(a)pyrene is about one order of magnitude below the lowest reported concentration (Figure 3), a fact that may be explained simply by the various additional point and non-point sources of this compound, such as deposition of air pollution. Overall, the observed discrepancies are not unexpected given the average dilution factor of 10 that was applied when estimating treated effluent discharge to surface water. In practice dilution factors can be as small as unity or much greater than 100, depending on the size of the receiving water body. The error bars in Figure 3 show how contaminant concentrations in surface water can vary based on dilution factors ranging from 1 to 100. The occurrence of the contaminants in different parts of the country drives uncertainty as well. The standard deviations for the analyte concentration in the biosolids input data ranged from 1.19 to 2.36 times the mean concentration. The concentrations in the influent and effluent would mimic the variance observed in the biosolids concentration. In addition to uncertainties associated with the dilution and concentration variance, the amount of empirical data available for the contaminants is very limited compared to the large dataset from the sludge survey that was used to estimate this parameter. No empirical information was available from the literature on the concentrations in natural waterways of the remaining three compounds, beta-estradiol-3-benzoate, miconazole, and norgestimate. Thus, this study provides the first recorded concentration estimates for these substances in surface water.

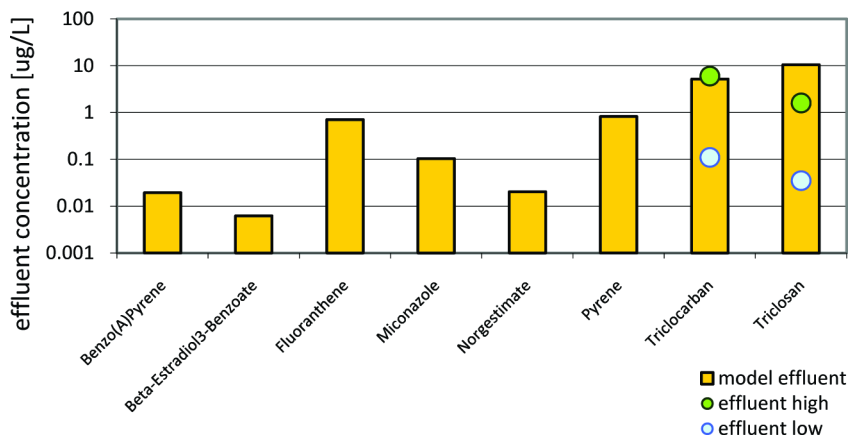


Figure 2. Comparison of modeled effluent concentrations (shown as bars) with effluent concentrations reported for various U.S. wastewater treatment plants. Also shown are the highest reported effluent concentrations as well as the lowest reported effluent concentrations above the detection limit. (see color insert)

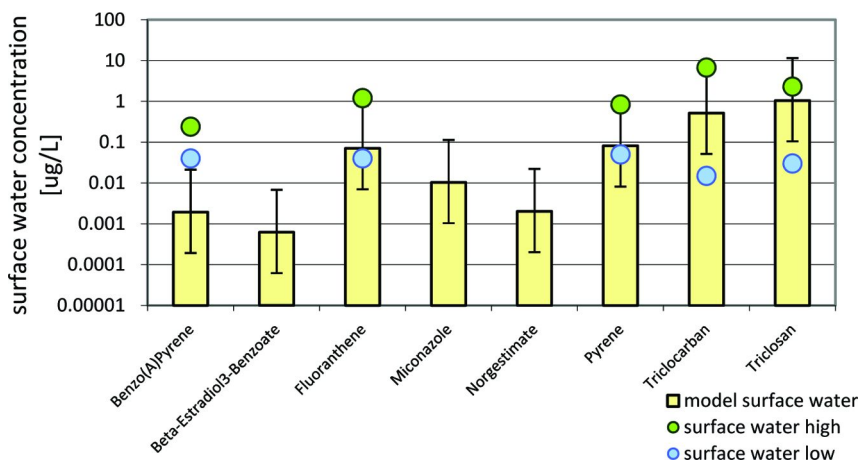


Figure 3. Comparison of surface water concentrations calculated assuming a dilution factor of 10 (data shown as bars) with measured concentrations reported for U.S. streams. Error bars indicate the range of surface water concentrations obtained when using dilution factors ranging from 1 to 100. (see color insert)

Table 3. Overview of toxicity threshold values reported in the literature for the compounds considered in the present study

<i>Chemical Name</i>	<i>Group</i>	<i>CAS Number</i>	<i>Species Affected</i>	<i>End point</i>	<i>Effect Concentration [µg/L]</i>	<i>Reference</i>
Benzo(a) pyrene	PAH	50-32-8	Polychaete Worm	LC50	3.890	(19)
Fluoranthene	PAH	206-44-0	Winter Flounder	LC50	0.100	(20)
Pyrene	PAH	129-00-0	Clam	EC50	0.230	(21)
Triclocarban	PPCP	101-20-2	Opossum Shrimp	EC50	0.209	(22)
Triclosan	PPCP	3380-34-5	Blue Green Algae	EC50	0.700	(23)

Toxicology Analysis

Toxicity data for all analytes were gathered from the EPA ECOTOX database and compared against the estimated surface water concentrations. The lowest reported effect concentration for each analyte is reported in Table 3. The ECOTOX database contained no entries for beta-estradiol-3-benzoate, minconazole, and norgestimate.

LC50 values are the concentration lethal to 50% of the test organisms. EC50 values represent the concentrations affecting 50% of the test organisms or causing 50% inhibition of the measured effect. When compared to toxicity data from the EPA Ecotox database, modeled surface water concentrations of this study's select organic compounds show the possibility of impact on aquatic life (Figure 4).

Modeled surface water concentrations for three of the five compounds with documented toxicity data (fluoranthene, triclocarban, and triclosan) exceed the lower threshold effect concentration for aquatic organisms. Pyrene, while modeled lower than the effect concentration for clams, has already been measured in surface waters at levels exceeding this threshold. Note that the LC₅₀ and EC₅₀ concentrations are for acute toxicity of the contaminants, implying exposure of the organism to such doses for short periods of time. Chronic toxicity values representative of exposure to a lower dose for a longer period of time ideally should be considered but were unavailable. As mentioned previously, for the purposes of this study, a surface water to effluent dilution factor of 10 was used to predict the likelihood of toxicological effects in susceptible receptor organisms. It should be noted, that in some situations across the United States (e.g., the arid southwest) surface waters can be dominated by treated effluent, implying dilution factors of near one. Under these conditions, any potential effects on aquatic species would be exacerbated.

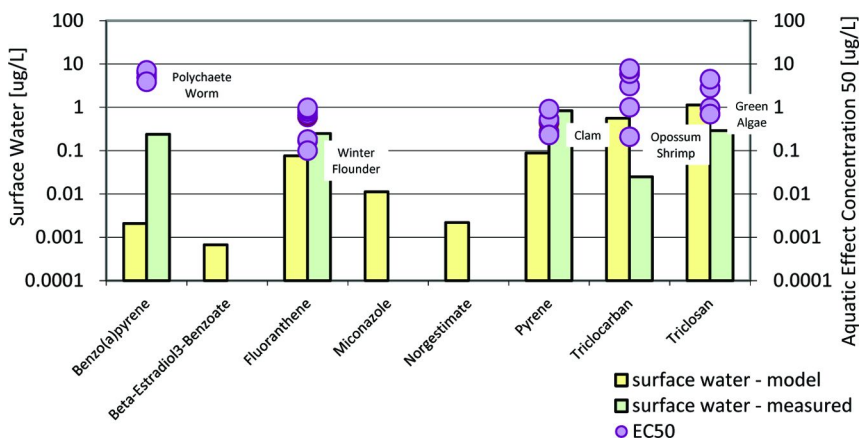


Figure 4. Comparison of modeled and measured surface water concentrations (shown as bars) with ecotoxicological effect concentrations (EC50 values) for aquatic organisms. (see color insert)

Most intriguing is the lack of both surface water concentration data and toxicity data for the same three organic compounds within this study's limited focus. Beta-estradiol-3-benzoate, miconazole, and norgestimate all have modeled surface water concentrations ranging from 0.6-10 ng/L when assuming a dilution factor of 10:1.

Even though no toxicity studies have been conducted for beta-estradiol-3-benzoate or norgestimate, endocrine disrupting effects have been found for hormones similar in structure and function. As an example, the Clark County Water Reclamation District, the Southern Nevada Water Authority and the USGS have all extensively researched possible effects of endocrine disrupters on aquatic life in the lower Colorado River basin. A 1996 USGS study (24) found endocrine disruption by hormones and other water contaminants, including organochlorines, in male and female feral carp in Lake Mead, Nevada. Another study (25) later confirmed some of these findings and correlated gonad underdevelopment to exposure to wastewater treatment plant effluent.

This study's model output shows that hormones are likely present in treated wastewater across the United States, including arid areas like the lower Colorado River basin which may be more susceptible to negative aquatic effects from hormones due to dilution factors of treated wastewater approaching unity. If existing levels are found to be problematic, opportunities exist for municipalities and water authorities to explore more extensive treatment of wastewater to prevent endocrine disruption of resident aquatic species.

Treatment Options

A number of techniques exist to remove PPCPs and other problematic organic contaminants from water, including common methods currently employed by many drinking water authorities. Nanofiltration, reverse-osmosis, and activated

carbon filtration methods have all been shown capable of removing over 90% of most PPCPs and related organic wastewater compounds (26).

Alternatively, ozone treatment has been shown to be over 90% effective for PPCP compounds, including the majority of those typically present in wastewater effluent (27). While this method is cost intensive due to high energy consumption, the oxidation of chemicals alleviates some of the brine disposal concerns associated with filtration techniques and may serve as the best treatment method for wastewater effluent.

Conclusions

The results show that modeled concentrations of microcontaminants were generally in good agreement with reported literature concentrations in the wastewater influent and effluent. However, for many of the contaminants analyzed, there was no reported empirical concentration data or toxicology data available. Further research needs to be conducted in both the area of surface water monitoring and toxicology of PPCPs. In the specific categories of hormones and antibiotics/antifungal agents, there appears to be a stark deficiency in empirical data for both ambient concentrations and toxicological threshold values. In addition, the effects of chronic exposure to these types of contaminants on aquatic life as well as on humans are largely unknown. High research costs may influence the lack of study concerning these types of products, but the growing evidence of endocrine disruption of exposed aquatic species may justify the costly examinations. These studies are needed for environmental and water agencies to evaluate risk and assess the need for the establishment of total maximum daily loads of PPCPs and other microcontaminants where applicable. For WWTP effluent dominated U.S. surface waters, the obtained data suggest that there may be a need for advanced treatment of effluent to achieve residual concentrations of organic wastewater compounds below the toxicity threshold values of susceptible aquatic species.

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Chapter 22

Assessment of the Aquatic Release and Relevance of Selected Endogenous Chemicals: Androgens, Thyroids and Their *in Vivo* Metabolites

Usman Khan and Jim Nicell*

Department of Civil Engineering and Applied Mechanics, McGill University,
817 Sherbrooke Street West, Montreal, Quebec H3A 2K6

*E-mail: jim.nicell@mcgill.ca.

Endogenous chemicals released through anthropogenic excretions enter the environment on a continuous basis. Therefore, it is important to establish their rate of introduction into the environment. An approach was developed to establish such environmental loads for any given endogenous chemical that is likely to be released in anthropogenic excretions. The approach developed was used to quantify influent, effluent and surface water loads of 25 endogenous chemicals including androgens, thyroids and their *in vivo* metabolites, using the United States as an illustrative case. The predicted surface water concentrations matched up well with the limited monitoring data presently available. The environmental relevance of the surface water presence could only be assessed for 5 of the 25 endogenous chemicals due to limited availability of eco-toxicological data. Data available thus far suggest that testosterone, dihydrotestosterone, thyroxine, triiodothyronine are unlikely to pose a risk to the receiving aquatic ecosystem when considered individually. However, androstenedione is predicted to pose a potential risk to the receiving environment in situations where the surface water dilution of the effluents is expected to be low.

Introduction

The detectable presence of pharmaceutically active compounds (PhACs) in the aquatic environment has garnered increasing attention from the scientific community as is evident from the relevant literature compilation maintained by the United States Environmental Protection Agency (1), which on its last update cited 8,882 publications related to the subject of pharmaceuticals and personal care products. (PhACs is defined here to include pharmaceuticals, endogenous hormones and their metabolites generated *in vivo*). Such scientific interest and activity is also gradually leading to the establishment of regulatory guidelines for a number of such contaminants in the form of environmental quality standards (2, 3), water quality standards (4), and acceptable daily intakes for human exposure (5).

Among PhACs that are likely to be present in the environment, special attention is paid to those that are likely to interact with the endocrine system. This largely stems from the eco-toxicological evidence available to date which suggests that endocrine-modulating or disrupting PhACs are likely to manifest significant effects at environmentally relevant concentrations (6). Such contaminants are typically released in human urinary and fecal excretions (7). In the present day wastewater treatment context, anthropogenic excretions are diluted by large amounts of grey water; i.e., water that is partially contaminated. This relatively dilute and complex mixture is then transferred to sewage treatment plants where such contaminants, after undergoing varying levels of treatment, are ultimately discharged into the receiving environment.

Endocrine-disrupting PhACs that are eventually released into the environment can be broadly categorized into two classes:

- (1) Endogenously-produced hormones that are naturally produced by and are present in living organisms and therefore logically have high potential for interactions with the endocrine system; and
- (2) Exogenously-consumed hormones/pharmaceuticals, that are typically not naturally produced and therefore are not naturally present but can nevertheless interact with the endocrine system in either expected or unexpected manners.

Perhaps the greatest distinction between the two categories from an environmental standpoint would be that if an exogenous compound is deemed to be detrimental to the receiving ecosystems, its release could be mitigated by switching to a “greener” alternative or an alternative may be developed if none is available. However, no such alternatives are available with respect to the release of endogenous chemicals, which are a fixed characteristic of our human biology. Given this, the presence of endogenous chemicals in anthropogenic excretions, and therefore wastewater treatment effluents and hence aquatic ecosystems, can be considered to be omnipresent. Therefore, it is imperative that one makes an attempt to quantify the environmental release and the environmental relevance of such contaminants.

Attempts have been made by others to precisely address such concerns by developing models for the environmental release of endogenous estrogens (8–10). Considerably less attention has been paid to the release and eventual fate of endogenous androgens, thyroid hormones and their *in vivo* metabolites. In vertebrates, androgens modulate sexual characteristics by interacting with the androgen receptor (11, 12). Similarly, thyroid hormones regulate a wide range of physiological endpoints including, reproductive ones, by interacting with the thyroid receptor (13–16). The presence of such receptors with a high degree of homology to the respective human receptors has been confirmed in eco-toxicologically relevant aquatic taxons such as fish (12, 17, 18). Therefore, from an environmental perspective, the question is not whether excreted endogenous androgens and thyroid hormones making their way into the environment interact with associated receptors in relevant aquatic taxa but, rather, whether the interactions are expected to be significant from an eco-toxicological standpoint. Furthermore, anthropogenic excretions are not only likely to contain active hormones but also their *in vivo* metabolites. As these *in vivo* metabolites will also make their way into the receiving environment their release loads and environmental relevance is also worth investigating.

In order to address such concerns the first logical step is to develop a model for the environmental release of endogenous chemicals (androgens and thyroids and their *in vivo* metabolites) originating from anthropogenic excretions. Therefore, the overall objectives of the research presented herein were to:

- (1) Develop a model for estimating the quantities (i.e., load and concentrations) of the endogenous chemicals of interest here in the influents and effluents of wastewater treatment plants;
- (2) Where possible to assess the environmental relevance of such contaminants making their way to the environment; and
- (3) Highlight knowledge gaps that need to be addressed before the next logical iterative refinement of the developed model is undertaken.

The application of the proposed methodology will be demonstrated using data corresponding to the Unites States, as an illustrative case.

Model Development

The mass balance model suggested by Johnson and Williams (8) for quantifying the load of a particular contaminant expected to reach the influent of a treatment plant can be logically extended to establish such loads for any given endogenous chemical that is likely to be present in anthropogenic excretions. Therefore, the general form of their model is adapted here and modified, where necessary. Appropriate simplifications and enumerations of the various parameters of the general form that is relevant to the chemicals being considered here are included in the model development.

Estimating Influent Loads of Endogenous Chemicals

Following modification of the general form of the model of Johnson and William (8), the total amount of an endogenous chemical expected to arrive in the influent of a wastewater treatment plant ($H_{T\text{-inf}}$, in kg/d) can be expressed as:

$$H_{T\text{-inf}} = [(1 - k_d)P \cdot C(U_t + F_t) + G + X] \quad (1)$$

where k_d is the fraction of the endogenous chemical that is degraded during sewer transit; P is the total population of the community; C is the fraction of the total population that is connected to the wastewater treatment and collection train; U_t is the amount of the endogenous hormone that is excreted in the urine of population P on a per capita basis ($\mu\text{g}/\text{cap}\cdot\text{d}$); F_t is the amount of the endogenous chemical that is excreted in the fecal matter of population P on a per capita basis ($\mu\text{g}/\text{d}$); G is the potential rate of generation of the endogenous chemical arising from the degradation of another contaminant ($\mu\text{g}/\text{d}$); and X is the contribution to the influent load due to exogenous consumption of the endogenous chemical itself or the consumption of an another chemical/pharmaceutical preparation that may be metabolized by humans to the endogenous chemical being considered ($\mu\text{g}/\text{d}$).

Furthermore, U_t can be represented as a summation of the endogenous chemical excreted by relevant human population cohorts:

$$U_t = \sum_{i=1}^I f_i U_i \quad (2)$$

where: I is the number of population cohorts excreting different amounts of the compound of interest; f_i is the fraction of population P that belongs to cohort i ; and U_i is the amount of the endogenous chemical that is excreted in urine by cohort i on a per capita basis ($\mu\text{g}/\text{cap}\cdot\text{d}$).

The amount of a given endogenous chemical that is excreted by a given cohort i in urine on a per capita basis is likely to be composed of three distinct components (11, 19–23): namely, a fraction that is excreted as the free endogenous chemical itself (F_u), a further fraction that is excreted conjugated to glucuronic acid (G_u), and an additional fraction that is excreted as sulphate conjugates (S_u). Considering this, equation (2) can be expanded as follows:

$$U_t = \sum_{i=1}^I f_i (F_u + G_u + S_u) U_i \quad (3)$$

where, by definition, the sum of the fractions, $F_u + G_u + S_u$, must be equal to one. Similar development can lead to the expansion of the F_t term in equation (1):

$$F_t = \sum_{i=1}^I f_i (F_f + G_f + S_f) F_i \quad (4)$$

where: F_i is the amount of the endogenous chemical that is excreted in fecal matter by cohort i on a per capita basis ($\mu\text{g}/\text{cap}\cdot\text{d}$); F_f is the fraction of F_i that is excreted as the free endogenous chemical itself; G_f is a further fraction of F_i that is excreted conjugated to glucuronic acid; and S_f is an additional fraction of F_i that is excreted as sulfate conjugates.

However, data available to-date regarding fecal excretions of androgens (24) and thyroids (25, 26) suggest that such endogenous chemicals are primarily excreted in the form of free chemical in fecal matter. This is largely a consequence of the intestinal flora acting on conjugated fractions to liberate free chemicals (24, 25). Therefore, equation (4) can be simplified to the following:

$$F_t = \sum_{i=1}^I f_i F_f F_i \quad (5)$$

Further, recognizing that $F_f \approx 1$, equation (5) can be simplified to:

$$F_t = \sum_{i=1}^I f_i F_i \quad (6)$$

The load, X , added due to exogenous use of the chemical itself or another chemical/pharmaceutical-preparation that may be metabolized to the endogenous chemical being considered can be calculated as follows:

$$X = N \cdot C \cdot P(X_u + X_f) \cdot (Mw_{eh} / Mw_p) \quad (7)$$

where: N is the total per capita daily consumption of the pharmaceutical preparation ($\mu\text{g}/\text{cap}\cdot\text{d}$); X_u is the fraction of the administered daily dose of the preparation that is excreted in urine as the endogenous chemical of interest; X_f is the fraction of the administered daily dose that is excreted in fecal matter as the endogenous chemical of interest; Mw_{eh} is the molecular weight of the endogenous chemical being considered; and Mw_p is the molecular weight of the pharmaceutical preparation that is metabolized to the endogenous chemical of interest. Note that the pharmaceutical preparation could be the exogenous form of the endogenous chemical of interest and, in such cases; the molecular weight adjustment (Mw_{eh}/Mw_p) would be equal to 1.

Substitution of equations (7), (6) and (3) into equation (1) leads to the following equation that can be used to estimate the amount of a given endogenous androgen or a thyroid chemical that is expected to reach treatment plants in their influents:

$$H_{T-\text{inf}} = (1 - k_d)P \cdot C \left(\sum_{i=1}^I f_i (F_u + G_u + S_u) U_i + \sum_{i=1}^I f_i F_i^f \right) + G + N \cdot P \cdot C(X_u + X_f) \cdot (Mw_{eh} / Mw_p) \quad (8)$$

Influent Loads

In order to quantify influent loads of a given endogenous chemical (H_{T-inf}), the various daily excretions parameters (i.e., values of U_i and F_i) of equation (8) were quantified. This was accomplished by performing a comprehensive literature search for all studies reporting urinary and fecal excretion profiles of endogenous chemicals of interest here (androgens, thyroids and their *in vivo* metabolites) by mining the following literature databases: MEDLINE, EMBASE, SCOPUS and Scifinder Scholar. No language restrictions were applied in the retrieval of relevant literature and search keywords were kept very generic so as to capture as diverse a body of relevant literature as possible.

In the initial phase, all studies that demonstrated one or more of the following criteria were excluded: (1) excreta to be analyzed had been collected for durations of less than 24 hours; and (2) results reported were normalized to creatinine content of the sample without stating the adjustment creatinine level that was used. The former data was excluded because collection durations of less than 24 hours or analysis of spot urine are unlikely to yield accurate estimates of the daily excretion of the endogenous chemical being considered. Additionally, the use of creatinine-adjusted results, where such adjustment levels were not stated in the original reference, would entail that one multiply reported results (i.e., $\mu\text{g}/\text{mg}$ of creatinine) by the typical daily creatinine excretion (i.e., $\text{mg}/24 \text{ hr}$) thereby, adding an additional degree of variability to the daily excretion values of the endogenous chemical being considered. The validity of applying such a correction to data can further be questioned considering the wide range that constitutes “typical” daily excretion of creatinine (27).

After initial screening of the studies, the estimated per capita daily excretions of the various chemicals ($\mu\text{g}/\text{cap-d}$) from the remaining studies were compiled. The compiled dataset was used to identify the number of population cohorts excreting different amounts of the compound of interest, I , for each endogenous chemical. Identification of the cohorts allowed one to further refine the collection of reported data. At this level of screening, studies were excluded that reported data encompassing more than one relevant cohort. The screening at this stage is best illustrated with the aid of an example: that is, it is known that testosterone levels in women decreases with age (28), therefore a study like Doberne and New (29) which presented mean testosterone excretion for 14 women whose ages were between 22 and 67 is too broad as it combines many different excreting cohorts into one.

From all remaining studies, means and standard deviations (σ 's) were extracted from the reported data. In cases where data was only presented in graphical form, the images were digitized and the dimensions of the extracted images was subsequently used using DigitizeIt™ (30) to estimate means and standard deviations of the reported data. Often, studies reported either the standard error or the range of data rather than the desired σ . In cases where standard error was reported, it was converted to standard deviation by multiplying

it with the square root of the number of observations. For those cases where the range was reported, σ was approximated by dividing the range by four as described by Massart et al. (31). Means from different studies within a relevant population cohort, i , were combined by weighting each mean by the number of subjects monitored in the study. Standard deviations were also combined by the number of subjects, specifically according to the pooled standard deviation method suggested by Massart et al. (31).

Population Parameters

Since the case study reported here had the objective of estimate the release of such contaminants to United States waterways, the national population of the United States was used for P . The base simulation year used here is 2005, because it is the latest year for which certain population cohorts of interest have been enumerated (i.e., number of pregnancies in a given year). The fraction of the national population that is connected to the wastewater treatment and collection trains, C , was reported to be 98.1% (32). Once the appropriate cohorts were identified for the various endogenous chemicals of interest, the fraction of population corresponding to each cohort, f_i , were derived from United States Census Bureau (33) data for the simulation base year of 2005.

Degradation and Sewer Transit Parameters

Typical sewer transit times reported in literature are on the order of a few hours (34, 35). Furthermore, considering that anaerobic conditions set in fairly early on during the transit process (36), it is reasonable to assume that the contaminants considered here won't be significantly transformed en-route to the treatment plant. Hence, it is conservatively assumed here that the total daily loads of endogenous androgens, thyroids and their *in vivo* metabolites released in human urinary and fecal excretions are transmitted to the influents of wastewater treatment plants unchanged (i.e., k_d and $G \approx 0$).

However, the conjugated fraction of the total loads is expected to change during the sewer transit process. Research primarily with conjugated fractions of endogenous estrogens suggests that the glucuronide fraction will be essentially deconjugated to the free form (i.e., $G_u \rightarrow F_u$), while the sulphate fraction will survive the transmission (35, 37). These assertions are assumed to hold true here for the excreted conjugated fractions of the endogenous chemicals being considered here.

Exogenous Chemical Parameters

Besides being an endogenous hormone that is present in anthropogenic excretions, testosterone is also used exogenously by the human population, primarily by those undergoing treatment for hypogonadism (38). Current

consumption rates of exogenous testosterone were not available for the United States. However, consumption data from Canada (39) and Sweden (40) suggests that the population-normalized consumption of exogenous testosterone is between 73 and 120 $\mu\text{g}/\text{cap}\cdot\text{d}$. The mean of this range ($\approx 96 \mu\text{g}/\text{cap}\cdot\text{d}$) is used here as N . Canadian data further suggests that (39) on a mass basis testosterone is predominately applied topically or transdermally, with intravenous administration amounting to a minor fraction of the total administered mass. Further, considering that only 10% of topical administered testosterone is absorbed (41) and that up to 98% of a transdermal dose may remain as residue in a used transdermal patch (42), it is conservatively assumed here that the exogenous mass of testosterone utilized enters the sewer unmetabolized in the free form (i.e., $X_u + X_f = 1$).

Levothyroxine, the synthetic form of the endogenous hormone thyroxine, is among the top 10 prescribed drugs in the United States (43) and is primarily used for the treatment of hypothyroidism (38). Again, actual consumption data with respect to the use of Levothyroxine was not available for the United States. However, population-normalized Canadian (39) and French data (44) suggests that the consumption of levothyroxine in those countries ranged between 2.6 and 4.6, $\mu\text{g}/\text{cap}\cdot\text{d}$. The mean of this range (3.6 $\mu\text{g}/\text{cap}\cdot\text{d}$) is assumed here for N . Reports on disposition of levothyroxine suggest that on average 30% of the administered dose is excreted in fecal matter as thyroxine ($X_f = 0.3$ for thyroxine) while on average 9% of the administered dose is excreted in urine as thyronine ($X_u = 0$ for thyroxine and $X_u = 0.09$ for thyronine) (45, 46).

Effluent Loads of Endogenous Chemicals

The effluent load of a particular endogenous chemical that is likely to be released to the receiving environment, $H_{T\text{-eff}}$ (kg/d), can be calculated from $H_{T\text{-inf}}$ by multiplying it by one minus the removal efficiency expected during the treatment process (Eff):

$$H_{T\text{-eff}} = (1 - Eff) H_{T\text{-inf}} \quad (9)$$

Based on considerations discussed in the preceding section, the influent load ($H_{T\text{-inf}}$) arriving at the influent of the wastewater treatment facility will be composed of endogenous chemicals in free form (which includes the excreted glucuronide fraction that has been assumed to be fully cleaved to the free form) and the sulphate conjugates of the endogenous chemical. Evidence reported to date in literature regarding sulphate conjugates of endogenous estrogens (8, 37) suggests that this fraction is likely to survive the treatment process. Hence, it is assumed here that this will be the case with the sulphate conjugates of endogenous chemicals being considered here. Incorporation of such an assumption leads to the following modification of equation (9):

$$H_{T\text{-eff}} = (1 - Eff) \cdot (H_{T\text{-inf}} - H_{T\text{-infs}}) + H_{T\text{-infs}} \quad (10)$$

where $H_{T\text{-infs}}$ is the total load of the endogenous chemical that is conjugated to sulphate ions and can be stated as:

$$H_{T\text{-infs}} = P \cdot C \sum_{i=1}^I f_i S_u U_i \quad (11)$$

Since measurements of removal efficiencies (*Eff*) from full-scale treatment plants entails that specific monitoring studies measuring both influent and effluent concentrations of the contaminant of interest, such data is rare and rarer still for the compounds being considered here, as will become evident below. Therefore, in cases where such data is not available or where the data available is limited to a small number of samples, *Eff* was estimated using the STPWIN™ software of the USEPA (47).

Predicting Surface Water Load and Concentrations of Endogenous Chemicals

The expected load of a given endogenous chemical in surface water immediately downstream of wastewater treatment plants, $H_{T\text{-sw}}$, can be estimated by dividing $H_{T\text{-eff}}$ by the dilution expected of the effluent in the receiving surface water:

$$H_{T\text{-sw}} = \frac{H_{T\text{-eff}}}{DF} \quad (12)$$

where: *DF* is the dilution factor and is indicative of the number of times a typical wastewater effluent is diluted in the receiving surface water. Based on the work of Rieses et al. (48), Lyndall et al (32) suggested *DF* values of between 4 and 130 for environmental exposure assessment purposes for United States waterways. A *DF* of 4 was suggested to represent a “high-end” exposure scenario while the value of 130 was considered to represent a “typical” exposure scenario. These suggested values of *DF* were used here.

The concentration of a particular endogenous chemical in surface waters immediately downstream of wastewater treatment plants, $C_{T\text{-sw}}$ (ng/L), can then be derived from $H_{T\text{-sw}}$ using the following relationship:

$$C_{T\text{-sw}} = \frac{H_{T\text{-sw}}}{P \cdot W \cdot C} = \frac{H_{T\text{-eff}}}{DF \cdot P \cdot W \cdot C} \quad (13)$$

where *W* is the average daily water use per capita in the United States, which was estimated to be 677 L/cap·d based on the work by Hutson et al. (49).

Environmental Relevance

The environmental relevance of any contaminant making its way into the aquatic environment can be assessed by deriving the contaminant’s Predicted No-Effect Concentration (*PNEC*). A contaminant-specific *PNEC* is typically derived from the lowest available eco-toxicological endpoint by dividing it by an assessment factor (*AF*). The magnitude of *AF* is dependent on the nature of

the lowest available eco-toxicological endpoint (i.e. whether it was obtained after an acute or chronic exposure) and the overall diversity of the eco-toxicological data when chronic eco-toxicological data is available. When the lowest available eco-toxicological endpoint was derived after an acute exposure, a value of 1000 is suggested for *AF* (50, 51). However, in cases where the lowest eco-toxicological endpoint was established after chronic exposure, the magnitude of *AF* depends on the number of chronic endpoints available for different taxa. The typical value used for *AF* when chronic endpoints are available from only one taxon is a 100, and in cases where chronic endpoints are available from two or three taxa the suggested values are 50 and 10, respectively (50, 51).

The derived *PNEC* of a given contaminant could subsequently be compared to its predicted surface water concentration (C_{T-sw}) to derive an environmental risk quotient (*RQ*):

$$RQ = \frac{C_{T-sw}}{PNEC} \quad (14)$$

A risk quotient greater than or equal to one would indicate unacceptable risk of the contaminant being considered, while a value of less than one is indicative of no risk in light of the eco-toxicological data used to derive respective the contaminant's *PNEC*.

Results and Discussion

Influent Loads

Per Capita Release of Androgens and Their in Vivo Metabolites

Through the data mining strategy employed here, 19 endogenous androgens and their *in vivo* metabolites were identified for which varying degrees of information were available with respect to their anthropogenic excretions. The methodology used to quantify influent loads for these is clarified below by illustrating the methodology as applied to three of these compounds. These three represent typical cases of the diversity that were encountered in terms of availability of relevant excretion data. The process for the remaining compound was similar to the three cases illustrated here and has been summarized in tables presented in Appendix A (located in the back of this book).

Case 1 – Example for Which Extensive Data Was Available for All Relevant Cohorts - Testosterone

The body of literature reporting the urinary excretion of testosterone indicated that the urinary excretion of testosterone is dependent on the age the subject (52); gender of the subject (53) and the trimester of pregnancy for pregnant women (54), while the variation due to the day of the menstrual cycle and the onset of menopausal state is likely to be insignificant when compared to observed

inter-individual variation (56, 57). The urinary excretion of testosterone for both sexes was reported to rise during puberty, peaking in the late-teens to mid-twenties and then decline steadily thereafter (52, 53). It was shown that females excrete considerably lesser amounts throughout their life cycle than males (53). The effect of pregnancy was suggested to be significant only during the third trimester, with significantly higher amounts being excreted during the third trimester while excretions profile during the first two trimesters was suggested to be similar to those of normal (i.e. non-pregnant) women (54). Considering these excretion trends and the resolution of data available, the relevant cohort's (*i*'s) for the excretion of testosterone were defined to be: gender, age cohorts within each of the gender cohorts, and pregnant women in the third trimester cohort within the cohort of women that are likely to be pregnant (i.e., assumed to be 15-40 year old females (58)). Urinary excretion (U_i 's) data for each of these cohorts is summarized in Table I. U_i for the whole population was obtained by multiplying U_i values with their respective f_i 's (from (33)) and subsequently summing these for all cohorts (*i*'s), as indicated in equation (2). The analysis (Table I) suggests that, on a demographic normalized basis, the daily urinary excretion of testosterone on a per capita basis is expected to be 28 $\mu\text{g}/\text{cap}\cdot\text{d}$ (U_i). Of this total per capita load, 20 $\mu\text{g}/\text{cap}\cdot\text{d}$ is expected to be conjugated to the glucuronide moiety while the majority of the remaining is expected to be conjugated to sulphate ions (i.e., 7.9 $\mu\text{g}/\text{cap}\cdot\text{d}$).

In contrast to the vast body of literature reporting urinary excretions of testosterone, the fecal excretions of endogenous androgens is rarely studied. To date, only one study has analyzed human fecal excretions for their androgen content (24). In that study, upon analysis of 18 fecal samples of young males, aged 21-36, it was suggested that the average testosterone content of the analyzed human fecal samples was 10 $\mu\text{g}/\text{cap}\cdot\text{d}$ (24). The average urinary excretion of testosterone for such individuals based on our analysis is expected to be between 66 and 85 $\mu\text{g}/\text{cap}\cdot\text{d}$ (Table I). If one were to use the mean of this range ($\approx 75 \mu\text{g}/\text{d}$) as the likely urinary excretion for such individuals, their reported fecal excretion of 10 $\mu\text{g}/\text{cap}\cdot\text{d}$ amounts to 13% of the urinary excretions. Since no other data is available regarding fecal excretions, it was assumed here that the fecal excretions for all cohorts of interest amounted to 13% of the respective urinary excretions. Such an approximation allows one to estimate F_i as 3.6 $\mu\text{g}/\text{cap}\cdot\text{d}$; this fecal load is expected to be in free form in light of previously mentioned considerations. Therefore, the total per capita daily load of testosterone released by humans in fecal and urinary excretions (i.e., $U_i + F_i$) amounts to 31.6 $\mu\text{g}/\text{cap}\cdot\text{d}$. Recalling that the fraction conjugated to the glucuronide moiety is expected to revert to the free form during sewer transit, 23.8 $\mu\text{g}/\text{cap}\cdot\text{d}$ of this load is expected to arrive in the influents of wastewater treatment plants in the free form and therefore would be available for treatment. The remaining 7.9 $\mu\text{g}/\text{cap}\cdot\text{d}$, would arrive at the treatment plant "as-is" in the form of sulphate conjugates and, as assumed earlier, would not be available for treatment.

Table I. Total per capita daily urinary excretion of testosterone (U_t) and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$) (see Appendix B for larger version of table)

Androgen	U_t					Conjugated fraction of U_t					
	Quantifying U_t				$f_i^{(b)}$	$U_t \cdot f_i$ ($\bar{x} \pm \sigma$) ($\mu\text{g}/\text{cap-d}$)	F_u (%)	G_u (%)	S_u (%)	Reference	
	Reference	Age (yr)/status	# of samples	n							U_t ($\bar{x} \pm \sigma$) ($\mu\text{g}/\text{cap-d}$)
Testosterone	Male	(59-60)	0-5	23	1.4 ± 1.1	0.04	0.1 ± 0.0	0.3	70	30	(74)
		(59-60)	5-10	88	5.6 ± 4.4	0.03	0.2 ± 0.1				
		(72,79,61,62,64,65)	10-15	298	20 ± 21	0.04	0.7 ± 0.8				
		(72,53,56,62-71)	15-20	205	85 ± 34	0.11	9.1 ± 3.6				
		(53, 53, 53, 59, 63, 64, 67, 69)	30-40	74	66 ± 33	0.07	4.6 ± 2.3				
		(52, 53, 59, 64, 65-69)	40-50	63	49 ± 12	0.08	3.7 ± 0.9				
	(52, 53, 59, 64, 69)	50+	57	31 ± 15	0.13	4.1 ± 2.0					
	Female	(60)	0-5	16	1.2 ± 1.6	0.07	0.1 ± 0.1	4.0	75	21	(74)
		(55, 60, 61, 63)	5-10	7	8.1 ± 3.0	0.06	0.5 ± 0.2				
		(60, 63, 71)	10-20	39	6.1 ± 3.2	0.14	0.8 ± 0.4				
		(53, 63, 64, 67, 71, 72)	20-40	79	6.5 ± 3.5	0.27	1.7 ± 0.9				
		(51, 63, 67, 71, 73)	40+	56	4.6 ± 2.5	0.47	2.1 ± 1.1				
		(54)	3 rd trimester	32	17 ± 6.9	0.01 ^(d)	0.2 ± 0.1				
Total ($\mu\text{g}/\text{cap-d}$)		$U_t = \sum U_t \cdot f_i$ (n=1037) ^(a)			28 ± 5.1	$F_u \cdot U_t^{(b,c)} = 0.3$ $G_u \cdot U_t^{(b,c)} = 20$ $S_u \cdot U_t^{(b,c)} = 7.9$					

(a) Based on demographic data for the simulation base year 2005 (33); (b) Total per capita daily load of testosterone excreted in human urine ($\mu\text{g}/\text{cap-d}$); (c) Total per capita daily load of testosterone excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of testosterone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of testosterone excreted in human urine conjugated to sulfate ions ($\mu\text{g}/\text{cap-d}$); (f) Based on the number of pregnancies in the year 2005 (38).

$$\begin{aligned}
 * F_u \cdot U_t &= (F_u \sum U_t \cdot f_i)_{\text{males}} + (F_u \sum U_t \cdot f_i)_{\text{females}} \\
 \dagger G_u \cdot U_t &= (G_u \sum U_t \cdot f_i)_{\text{males}} + (G_u \sum U_t \cdot f_i)_{\text{females}} \\
 \S S_u \cdot U_t &= (S_u \sum U_t \cdot f_i)_{\text{males}} + (S_u \sum U_t \cdot f_i)_{\text{females}}
 \end{aligned}$$

Case 2 – Example for Which a Reasonable Data Set Was Available, but with Information Lacking on Certain Cohorts - Androstenedione

As with the excretion of testosterone, the literature suggested that daily urinary excretion of androstenedione was dependent on the age of the subject (69); gender of the subject (69, 76) and the trimester of pregnancy for pregnant women (54). Therefore the relevant cohorts (i 's) for androstenedione were defined to be: gender, age cohorts within each of the gender cohorts, and women in the third trimester within the cohort of women likely to be pregnant. Sufficient data was available to quantify each of the age cohorts for the males; however, this was not possible for all the female cohorts (Table II). For females, limited data was available; the three identified studies reported daily urinary excretion of androstenedione by adult women (20-40 age cohort) (75, 76) and pregnant women in their third trimester (54). The approach taken here to estimate excretion by women of other age cohorts than the 20 to <40 year olds is best illustrated with the aid of an example. For example, data presented in Table II suggest that males in the age cohort of 10 to <15 years excrete approximately 30% of the levels excreted by those males in the 20 to <40 year cohort; therefore, the approximation made here was to multiply the female 20 to <40 year cohort reported data (75, 76) by 30% to generate the excretion profile for the female age cohort of 10 to <15 years. A similar approach was used to quantify all female age cohorts for which no data was available.

Table II. Total per capita daily urinary excretion (U_t) of androstenedione and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$) (see Appendix B for larger version of table)

Androgen	U_t					Conjugated fraction of U_t					
	Quantifying U_t			$f^{(b)}$	$U_t \cdot f_t$	F_u	G_u	S_u			
	Reference	Age (yr)/status	n	U_t ($\mu\text{g}/\text{cap}\cdot\text{d}$)	($\pm \sigma$)	(%)	(%)	(%)	Reference		
Androstenedione	Male	(82)	<10	5	0.8 ± 0.0	0.07	0.8 ± 0.6				
		(62)	10-15	16	6.2 ± 9.4	0.04	0.2 ± 0.3				
		(82)	15-20	11	16.6 ± 5.3	0.04	0.6 ± 0.3				
		(69)	25-30	6	27.4 ± 5.4	0.07	4.0 ± 0.4				
		(69)	35-40	4	47.4 ± 4.2	0.07	3.3 ± 0.3	3.7	23	73	(76)
		(69)	40-50	8	38.6 ± 1.6	0.08	2.9 ± 0.4				
		(69)	50-60	9	28 ± 2.2	0.06	1.7 ± 0.1				
		(69)	61+	7	20 ± 2.3	0.07	1.4 ± 0.2				
		(69)	61+	7	20 ± 2.3	0.07	1.4 ± 0.2				
	Female	Approx ^(a)	0-10	-	0.0 ± 0.0	0.07	0.0 ± 0.0				
		Approx ^(a)	10-15	-	2.8 ± 0.6	0.03	0.1 ± 0.0				
		Approx ^(a)	15-20	-	5.3 ± 1.7	0.03	0.2 ± 0.1				
		(71, 76)	25-40	29	18.7 ± 4.7	0.12	2.1 ± 0.6				
		Approx ^(a)	45-50	-	13.3 ± 3.5	0.08	1.8 ± 0.3	2.0	22	76	(76)
		Approx ^(a)	55-60	-	9.8 ± 2.5	0.06	0.6 ± 0.2				
Approx ^(a)	61+	-	6.3 ± 1.8	0.10	0.6 ± 0.2						
(74)	3 rd trimester	32	6.4 ± 3.0	0.01 ^c	0.1 ± 0.0						
Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)					$U_t = 22U_t \cdot f_t$ ($n=106$) ^(b)	19 ± 1.1	$F_u \cdot U_t^{(61)(7)} = 0.6$	$G_u \cdot U_t^{(61)(7)} = 4.3$	$S_u \cdot U_t^{(61)(7)} = 14$		

(a) Based on demographic data for the simulation base year 2005 (33); (b) Total per capita daily load of androstenedione excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (c) Total per capita daily load of androstenedione excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of androstenedione excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Approximated by relative ratio from associated male cohorts (see text for details); (f) Total per capita daily load of androstenedione excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$); (g) Based on the number of pregnancies in the year 2005 (54).

$$* F_u \cdot U_t = (F_u \sum U_t \cdot f_t)_{\text{males}} + (F_u \sum U_t \cdot f_t)_{\text{females}}$$

$$\dagger G_u \cdot U_t = (G_u \sum U_t \cdot f_t)_{\text{males}} + (G_u \sum U_t \cdot f_t)_{\text{females}}$$

$$\S S_u \cdot U_t = (S_u \sum U_t \cdot f_t)_{\text{males}} + (S_u \sum U_t \cdot f_t)_{\text{females}}$$

The analysis presented in Table II suggests that total daily urinary excretion of androstenedione (U_t) on a per capita basis is expected to be approximately 19 $\mu\text{g}/\text{cap}\cdot\text{d}$. Of this total per capita urinary load, 4.3 $\mu\text{g}/\text{cap}\cdot\text{d}$ is likely to be present in urinary excretions in the form of glucuronide conjugates, 14 $\mu\text{g}/\text{cap}\cdot\text{d}$ as the sulphate conjugates, and the minor amount of 0.6 $\mu\text{g}/\text{cap}\cdot\text{d}$ as free androstenedione.

Due to the paucity of data on the fecal disposition of endogenous chemicals, it remains unclear what levels of such chemicals are eliminated in fecal excretions. However, it is possible to suggest whether fecal elimination is likely to be a major elimination route for a given chemical of interest. In particular, Yang et al. (90) suggested that the molecular weight of a chemical was a major determinant of the likelihood of a chemical ending up in biliary excretions rather than being eliminated in urinary excretions. Specifically, a threshold 475 Da was suggested, where chemicals having a molecular weight greater than this threshold were likely to appear in biliary excretions. Further, recognizing that fecal excretions originate in biliary excretions, the importance of fecal disposition of an endogenous chemical can be assessed by comparing the molecular weight of the endogenous chemical to the threshold of 475 Da. The molecular weight of all androgens and their *in vivo* metabolites of interest here was well below this threshold and therefore the fecal excretion of such chemicals is expected to be low compared to their elimination in urine. This is consistent with the earlier derived fecal-to-urine ratio of 13% for testosterone, the only androgen which has been quantified in human fecal excretions.

Considering this, and given the lack of data reporting the fecal excretion of androstenedione, the fecal load was assumed to be equal to 13% of the total urinary load based on the ratio of fecal to urinary excretion of testosterone. Hence, F_t was assumed to be 13% of androstenedione's U_t and is therefore equal to 2.5 $\mu\text{g}/\text{cap}\cdot\text{d}$. This suggests that the total load (i.e., $U_t + F_t$) of androstenedione arriving in

the influents of the wastewater treatment plants is likely to be 21.5 $\mu\text{g}/\text{cap}\cdot\text{d}$ and, of this, 7.4 $\mu\text{g}/\text{cap}\cdot\text{d}$ will be in the free form ready to undergo treatment and the remainder will be present as the treatment-resistant sulphate conjugated fraction.

Case 3 – Example with Limited Data Availability, but with Information Available for Adult Male and Female Cohorts - Dihydrotestosterone

Since, dihydrotestosterone is produced upon *in vivo* metabolism of endogenously produced testosterone (38), one would expect its urinary excretion to be distinctly different depending on the age of the subject, gender of the subject, and the trimester of pregnancy for pregnant women. However, daily urinary loads of dihydrotestosterone are rarely reported in literature. In total, two studies were identified reporting daily urinary excretions of dihydrotestosterone in human urinary excretions, one for adult males (77), while the other one reporting on excretions of adult females (78). Given the lack of data, no option is available except to assume all the males in the population excrete as adult males and all the females as adult females. Obviously, the estimate loads generated with such assumptions will be an overestimate, but from an environmental relevance perspective the estimate would be a conservative one.

In light of the assumption that all males and females within the population excrete dihydrotestosterone as adults, the total daily urinary excretion of dihydrotestosterone on a per capita basis (U_i) is expected to be 19 $\mu\text{g}/\text{cap}\cdot\text{d}$ (Table III). Again, data on the fecal disposition of endogenous dihydrotestosterone was lacking and was approximated to amount to 13% of U_i , as was used above, allowing one to estimate F_i as 2.3 $\mu\text{g}/\text{cap}\cdot\text{d}$. Enumeration of U_i and F_i further allows one to estimate the total load of dihydrotestosterone expected to arrive in influents of wastewater treatment plants as 21.3 $\mu\text{g}/\text{cap}\cdot\text{d}$, where all of this is expected to be available for treatment since the fraction of urinary dihydrotestosterone bound to sulphate ions is expected to be negligibly small (79).

Per Capita Release of Thyroids and Their in Vivo Metabolites

The literature survey identified 6 different endogenous thyroids and their *in vivo* metabolites for which varying degrees of information were available with respect to their anthropogenic excretion rates. In general, excretions of endogenous thyroids have not been extensively studied. The process to calculate influent loads is detailed below for the two biologically active thyroids, thyroxine and triiodothyronine, while the process is summarized for the remaining four *in vivo* metabolites in Appendix A (see Table A.XVII of Appendix A, located in the back of this book) and briefly discussed below to highlight some key assumptions.

Table III. Total per capita daily urinary excretion (U_t) of dihydrotestosterone and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$) (see Appendix B for larger version of table)

Androgen		U_t					Conjugated fraction of U_t				
		Quantifying U_t		n	U_t (x.a.e.) ($\mu\text{g}/\text{cap}\cdot\text{d}$)	$f_u^{(a)}$	$U_t \cdot f_u$ ($\mu\text{g}/\text{cap}\cdot\text{d}$)	F_u (%)	G_u (%)	S_u (%)	Reference
		Reference	Age (yr)/Status								
Dihydrotestosterone	Males	(77)	Adults	14	32 = 20	0.5 ^(b)	16 = 9.9	minor	100	0	(20), (79)
	Females	(78)	Adults	5	5.1	0.5 ^(b)	2.5	minor	100	0	(20), (79) ^(c)
	Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)				$U_t = \sum U_t \cdot f_u (n=19)^{(d)}$		19 = 9.9	$F_u \cdot U_t^{(e)} \approx 0$		$G_u \cdot U_t^{(e)} \approx 19$	$S_u \cdot U_t^{(e)} \approx 0$

(a) Based on demographic data for the simulation base year 2005 (53); (b) Due to lack of data it is conservatively assumed here that the whole male and female populations excrete dihydrotestosterone as adults; (c) Total per capita daily load of dihydrotestosterone excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of dihydrotestosterone excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of dihydrotestosterone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Total per capita daily load of dihydrotestosterone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$); (g) Assumed to be equal to the conjugated and unconjugated proportions of male urinary excretions.

$$* F_u \cdot U_t = (f_u \sum U_t \cdot f_u)_{\text{males}} + (f_u \sum U_t \cdot f_u)_{\text{females}}$$

$$\dagger G_u \cdot U_t = (G_u \sum U_t \cdot f_u)_{\text{males}} + (G_u \sum U_t \cdot f_u)_{\text{females}}$$

$$\S S_u \cdot U_t = (S_u \sum U_t \cdot f_u)_{\text{males}} + (S_u \sum U_t \cdot f_u)_{\text{females}}$$

Triiodothyronine and Thyroxine

Urinary excretion of triiodothyronine was shown to vary significantly with gender (80) and age of the subject (80, 81). While urinary excretion of thyroxine was shown to vary significantly with gender only, the reported age effect was statistically insignificant (80); indicating that inter-individual variation was more pronounced than any age induced effect. Onset of pregnancy and the day of the menstrual cycle were shown to be not significant factors with respect to urinary excretions of either thyroxine or triiodothyronine (82, 83). Therefore, the relevant cohorts (*i s*) for triiodothyronine were defined to be gender and age cohorts within each of the gender cohorts, and gender only for thyroxine. The urinary excretions (U_i) data for each of these cohorts along with the subsequently derived daily urinary loads (U_i) are summarized in Tables IV and V.

The analysis for triiodothyronine (Table IV) suggests that its net daily urinary load (U_i) is expected to be 2.2 $\mu\text{g}/\text{cap}\cdot\text{d}$, and all of this is expected to be available for treatment as the fraction of triiodothyronine excreted as sulphate conjugates has been reported to be negligibly small (21). As was the case with fecal disposition of endogenous androgens, the endogenous thyroid content of fecal samples is rarely measured. The lone study quantifying daily fecal content of triiodothyronine (25), reported analyses of daily feces samples from children and found that the daily load present in their fecal excretions amounted to 10.6 $\mu\text{g}/\text{cap}\cdot\text{d}$. Iversen and Pedersen (80) measured the daily load of free triiodothyronine in children's urinary excretions and suggested that this amounts to 1.55 $\mu\text{g}/\text{cap}\cdot\text{d}$. Further, recognizing that urinary loads not only include the free fraction but also the two conjugated fractions, this daily urinary load of free triiodothyronine must be corrected for the conjugated fraction. Literature suggests that approximately 32.4% (i.e., a sample weight mean of data from three sources: (21, 84) and (85)) of the total urinary load of triiodothyronine is present as conjugated triiodothyronine. Using this value along with the free daily load measured by Iversen and Pedersen (80), the total

daily load of triiodothyronine in children's urinary excretion can be estimated to be 2.28 $\mu\text{g}/\text{cap}\cdot\text{d}$. This, along with the reported fecal content 10.6 $\mu\text{g}/\text{cap}\cdot\text{d}$ (25), suggests that the daily fecal load of triiodothyronine (F_t) amounts to be 4.4 times higher than the net urinary load (U_t). Therefore, F_t can be estimated to be 9.7 $\mu\text{g}/\text{cap}\cdot\text{d}$. This along with U_t allows one to estimate that the total load of triiodothyronine in anthropogenic excretions to be 11.9 $\mu\text{g}/\text{cap}\cdot\text{d}$.

Table IV. Total per capita daily urinary excretion (U_t) of triiodothyronine and its conjugated and unconjugated fractions ($F_u\cdot U_t$, $G_u\cdot U_t$, $S_u\cdot U_t$) (see Appendix B for larger version of table)

Thyroid		U_t					Conjugated fraction of U_t			
		Quantifying U_t			$f^{(a)}$	$U_t \cdot f_t$ ($\mu\text{g}/\text{cap}\cdot\text{d}$)	F_u (%)	G_u (%)	S_u (%)	Reference
		Reference	Age (yr)/status	n # of samples						
Triiodothyronine	Male	(80)	<50	61	2.5 \pm 0.9	0.42	1.0 \pm 0.4			
		(80)	61+	11	1.8 \pm 0.8	0.07	0.1 \pm 0.1	68.1	31.9	\approx 0 (21,84,85)
	Female	(80)	<50	68	2.1 \pm 0.8	0.35	0.7 \pm 0.3			
		(80)	51+	15	1.7 \pm 0.4	0.16	0.3 \pm 0.1	68.1	31.9	\approx 0 (21,84,85)
Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)					$U_t = \Sigma U_t \cdot f_t$ (n=155) ^(d)	2.2 \pm 0.5	$F_u \cdot U_t^{(e,f)}$ = 1.5	$G_u \cdot U_t^{(e,f)}$ = 0.7	$S_u \cdot U_t^{(e,f)}$ = 0	

(a) Based on demographic data for the simulation base year 2005 (33); (b) Total urinary loads calculated from reported free fraction (80) and sample weighted correction for level of conjugation from (21,84,85); (c) Total per capita daily load of triiodothyronine excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of triiodothyronine excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of triiodothyronine excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Total per capita daily load of a triiodothyronine excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$).

$$* F_u \cdot U_t = (F_u \sum U_t \cdot f_t)_{\text{males}} + (F_u \sum U_t \cdot f_t)_{\text{females}}$$

$$\dagger G_u \cdot U_t = (G_u \sum U_t \cdot f_t)_{\text{males}} + (G_u \sum U_t \cdot f_t)_{\text{females}}$$

$$\S S_u \cdot U_t = (S_u \sum U_t \cdot f_t)_{\text{males}} + (S_u \sum U_t \cdot f_t)_{\text{females}}$$

Table V. Total per capita daily urinary (U_t) excretion of thyroxine and its conjugated and unconjugated fractions ($F_u\cdot U_t$, $G_u\cdot U_t$, $S_u\cdot U_t$) (see Appendix B for larger version of table)

Thyroid		U_t					Conjugated fraction of U_t			
		Quantifying U_t			$f^{(a)}$	$U_t \cdot f_t$ ($\mu\text{g}/\text{cap}\cdot\text{d}$)	F_u (%)	G_u (%)	S_u (%)	Reference
		Reference	Age (yr)/status	n # of samples						
Thyroxine	Male	(80)	All	61	2.5 \pm 0.9	0.5	1.2 \pm 0.5			
		(80)	All	68	2.1 \pm 0.8	0.5	1.1 \pm 0.4	70	30	\approx 0 (21,84,86)
	Female	(80)	All	68	2.1 \pm 0.8	0.5	1.1 \pm 0.4			
		(80)	All	68	2.1 \pm 0.8	0.5	1.1 \pm 0.4	70	30	\approx 0 (21,84,86)
Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)					$U_t = \Sigma U_t \cdot f_t$ (n=127) ^(d)	2.3 \pm 0.6	$F_u \cdot U_t^{(e,f)}$ = 1.7	$G_u \cdot U_t^{(e,f)}$ = 0.7	$S_u \cdot U_t^{(e,f)}$ \approx 0	

(a) Based on demographic data for the simulation base year 2005 (33); (b) Total urinary loads calculated from reported free fraction (80) and sample weighted correction for level of conjugation from (21,84,86); (c) Total per capita daily load of thyroxine excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of thyroxine excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of thyroxine excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Total per capita daily load of a thyroxine excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$).

$$* F_u \cdot U_t = (F_u \sum U_t \cdot f_t)_{\text{males}} + (F_u \sum U_t \cdot f_t)_{\text{females}}$$

$$\dagger G_u \cdot U_t = (G_u \sum U_t \cdot f_t)_{\text{males}} + (G_u \sum U_t \cdot f_t)_{\text{females}}$$

$$\S S_u \cdot U_t = (S_u \sum U_t \cdot f_t)_{\text{males}} + (S_u \sum U_t \cdot f_t)_{\text{females}}$$

The analysis for thyroxine (Table V) suggests that its daily urinary load (U_i) is expected to be 2.3 $\mu\text{g}/\text{cap}\cdot\text{d}$. Again, recognizing the limitation that the one study (25) reporting fecal content for thyroxine involved the analysis of samples from children only, the fecal to urinary load ratio for children was used here to estimate net fecal loads of thyroxine (F_i) from the determined net urinary loads (U_i). Using an analysis similar to that used to establish fecal-to-urinary load ratio for triiodothyronine explained in the preceding section, one can estimate the net fecal loads of thyroxine to be 4 times the net urinary loads of thyroxine (21, 25, 84, 86). Hence, F_i can be estimated to be 9.2 $\mu\text{g}/\text{cap}\cdot\text{d}$ and the total load due to anthropogenic excretion ($U_i + F_i$) for thyroxine can be approximated as 11.5 $\mu\text{g}/\text{cap}\cdot\text{d}$.

In Vivo Metabolites

The evaluation of the urinary loads for the remaining four *in vivo* metabolites is summarized in Appendix A (see Table A.XVII of Appendix A, located in the back of this book). As is evident from the tabulated data, these chemicals have not been extensively studied. Since these compounds originate from *in vivo* metabolism of thyroxine and triiodothyronine (91), one might expect a gender and possibly an age dependency. But no data exists to support or negate such a hypothesis. Given the lack of data, it is assumed here that the limited data available and presented in Appendix A (see Table A.XVII of Appendix A, located in the back of this book) are representative excretion profiles for the whole population. Considering that this data largely stems from studying adult excretions, the excretion values generated in this manner might be overestimated, but from an environmental relevance perspective these estimates are expected to be conservative.

As with other compounds of interest, data on the fecal disposition of these chemicals was missing. Recognizing that reverse triiodothyronine, 3',5'-diiodo-L-thyronine, 3,3'-diiodothyronine have molecular weights above the biliary excretion threshold as is the case with triiodothyronine and thyroxine, the daily fecal disposition (F_i) for these three was assumed to be equal to 4.2 times their respective daily urinary loads (U_i), where 4.2 is the mean of the thyroxine and triiodothyronine fecal-to-urine load ratio derived earlier. Thyronine's molecular weight is well below the threshold and one would expect its fecal excretion to be of minor importance compared to its urinary excretion and definitely not as high as the values derived earlier for triiodothyronine and thyroxine. Hence, it is conservatively assumed here that the F_i for thyronine is equal to its U_i .

Influent Loads

The influent loads of the various endogenous chemicals released in anthropogenic excretions in the United States were estimated by enumerating the various parameters of equation (8). The U.S. nationwide influent loads for endogenous androgens and their *in vivo* metabolites are summarized in Table

VI, ranging from 6 kg/d (epitestosterone) to 763 kg/d (5 α -androstanedione). The U.S. nationwide influent loads of the various endogenous thyroids and their *in vivo* metabolites were considerably lower and have been summarized in Table VII. The estimated loads varied from 0.5 kg/d (3', 5'-diiodo-L-thyronine) to 3.8 kg/d (thyroxine).

Three of the twenty five endogenous chemicals (i.e., testosterone, thyroxine and thyronine) also had exogenous contributions to their total influent loads. Exogenous consumption of relevant pharmaceutical preparations amounted to approximately 75%, 8% and 1.4% of the total influents loads of testosterone, thyroxine and thyronine, respectively.

The minor contribution of exogenous use of levothyroxine to the influent loads of thyroxine and thyronine suggests that when the developed approach is applied to different geographical locations, it is not necessary to ascertain the use of levothyroxine in the local population. Typical values, as used here, may suffice since the consumption of levothyroxine is not expected to vary drastically among developed countries. Even if one considers the consumption of the levothyroxine to be variable, an error of 100% in the exogenous consumption of levothyroxine will only manifest itself as an 8% and 1.4% error in the influent loads of thyroxine and thyronine, respectively.

Conversely, the analysis suggests that exogenous testosterone will likely be a major contributor to the influent load of testosterone, where an increase of a 100% in the exogenous load of testosterone manifests itself as an increase of 75% in the total influent load of testosterone. Therefore, where the interest is in predicting the influent loads of testosterone, estimation of the consumption of exogenous testosterone in the local population is desirable.

Effluent Loads

The effluent loads for the various thyroids and androgens were calculated using equation (10). As justified earlier, the sulfate conjugate fractions of the influent loads were assumed to be unavailable for treatment. Literature reporting removal efficiencies (*Eff*) at full-scale treatment plants were only available for three of the endogenous androgens (i.e., testosterone (87), andorsterone (86, 87) and etiocholanolone (86, 87)) and none of the thyroids. Such data gaps were addressed by modeling *Eff* for the remaining compounds of interest using the USEPA's STPWIN™ software (47). The modeled and reported (when available) *Eff*'s along with the subsequently derived effluent loads are summarized in Tables VI and VII.

It is worth noting that even though a 100% increase in the use of exogenous testosterone manifests as a 75% increase in the influent load, the resultant increase in the effluent load would approximately amount to 10%. This is largely due to the assumption that all the exogenously-applied testosterone was available for treatment due to its mode of administration, the high removal efficiency that has been reported for its removal during sewage treatment, and the assumption that the sulphate conjugates are not available for treatment.

Surface Water Concentrations

Surface water concentrations were estimated from the derived effluent loads with the aid of equation (13). Two scenarios could be modeled using this equation and incorporating the range of dilution factors (*DF*) suggested by Lyndall et al. (32). Estimated surface water concentrations calculated using a *DF* of 4 were likely to be encountered in a “high-end” exposure scenario, while an evaluation with a *DF* of 130 would generate concentrations likely to be relevant in a typical exposure scenario. By evaluating the surface water concentrations for these two scenarios one can get a better grasp of the range of concentrations that are likely to be encountered upon conducting an extensive monitoring campaign.

Table VI. Summary of the modeled influent loads, effluent loads and surface water concentrations of endogenous androgens and their *in vivo* metabolites in U. S. waterways (see Appendix B for larger version of table)

Androgens and their <i>in vivo</i> metabolites	CAS	Parameters for $H_{T,est}$			Influent			Effluent		Surface Water	
		$U_i^{(a)}$	$F_i^{(b)}$	N	$H_{T,est}^{(c)}$	$H_{T,est}^{(d)}$	$H_{T,est}^{(e)}$	$E_{ff}^{(g)}$	$H_{T,est}^{(h)}$	$C_{T,sw-high}^{(i)}$	$C_{T,sw-typical}^{(j)}$
		(μg/cmp-d)			(kg/d)			(kg/d)		(ng/L)	
5α-Androstane-3-one	846-46-8	2329	303		763	238	525	0.29	613	781	6.0
Androst-4-ene-3,6,17-trione	2243-06-3	1332	173		436	363	73	0.12	428	545	4.2
Androstane-3,17-dione, (5β)	1229-12-5	1577	205		517	124	393	0.29	405	515	4.0
11β-Hydroxyandrostane	57-61-4	710	92		233	0	233	0.46	125	159	1.2
Dehydroepiandrosterone	53-43-0	395	51		129	106	23	0.35	121	154	1.2
5βAndrostane-3-11-17-trione	1429-06-7	351	46		115	64	51	0.46	92	117	0.9
Androstane-3,11,17-trione, (3α)	1482-70-8	303	39		99	39	60	0.46	72	92	0.7
Androstetriol	4159-30-5	393	51		129	0	129	0.48	67	85	0.7
11-Ketotestosterone	739-27-5	365	47		120	0	120	0.46	65	82	0.6
11βHydroxydehydroepiandrosterone	739-26-4	336	44		110	0	110	0.46	59	75	0.6
11-ketoadrostane	1231-82-9	333	43		109	0	109	0.46	59	75	0.6
5βAndrostane-3α17βdiol	1851-23-6	296	51		130	43	87	0.88	53	68	0.5
Androstene	53-41-8	1463	190		479	30	450	0.99 ^(g)	34	43	0.3
Ethiochololone	53-42-9	1492	194		489	22	467	0.99 ^(g)	26	34	0.3
5α-Androstane-3α17βdiol	1852-53-5	100	13		33	14	19	0.89	16	20	0.2
Androstenedione	63-05-8	19	2		6	4	2	0.12	6	7.5	0.1
Epitestosterone	481-30-1	17	2		5	1	5	0.38	4	4.7	0.0
Testosterone	58-22-4	28	4	96 ⁽ⁱ⁾	37	2	35	0.99 ^(h)	3	3.4	0.0
Dihydrotestosterone	521-18-6	19	2		6	0	6	0.70	2	2.4	0.0

(a) From tables I-III in main text and tables A.1-XVII in appendix A; (b) Estimated to be 13% of the respective U_i (from testosterone arises to fecal load ratio for adult males- see text for details); (c) Exogenous testosterone consumption estimated from Canadian and Swedish consumption data (see text for details); (d) From equation (8); (e) Using equation (11); (f) Estimated using STPWIN (47); (g) From (87,88); (h) From (87); (i) Equation (10); (j) Equation (13) with a *DF* of 4; (k) Equation (13) with a *DF* of 130.

Table VII. Summary of the modeled influent loads, effluent loads and surface water concentrations of endogenous thyroids and their *in vivo* products in U.S. waterways (see Appendix B for larger version of table)

Thyroids and their <i>in vivo</i> metabolites	CAS	Parameters for $H_{T,est}$			Influent			Effluent		Surface Water	
		$U_i^{(a)}$	F_i	N	$H_{T,est}^{(c)}$	$H_{T,est}^{(d)}$	$H_{T,est}^{(e)}$	$E_{ff}^{(g)}$	$H_{T,est}^{(h)}$	$C_{T,sw-high}^{(i)}$	$C_{T,sw-typical}^{(j)}$
		(μg/cmp-d)			(kg/d)			(kg/d)		(ng/L)	
Triiodothyronine	6893-02-3	2.2	9.7		3.4	0.0	3.4	0.14	2.96	3.8	0.03
Thyroxine	51-48-9	2.3	9.2	3.6 ^(k)	3.6	0.0	3.6	0.37	2.30	2.9	0.02
3,3'-Diiodothyronine	70-40-6	1.3	5.5 ^(k)		2.0	0.2	1.9	0.23	1.59	2.0	0.02
Thyronine	1596-67-4	4.1	4.1 ^(k)	3.6 ^(k)	2.4	0.0	2.4	0.75	0.60	0.8	0.01
Reverse triiodothyronine	5817-39-0	0.5	2.1 ^(k)		0.8	0.1	0.7	0.14	0.67	0.9	0.01
3,5'-Diiodo-L-thyronine	4192-14-7	0.4	1.5 ^(k)		0.5	0.1	0.5	0.26	0.42	0.5	0.00

(a) From Tables IV-V in main text and Tables A.XVIII in appendix A; (b) Estimated to be 4.2x the respective U_i (see text for details); (c) Estimated to be 1.0x the respective U_i (see text for details); (d) Due to exogenous triiodothyronine consumption estimated from Canadian and French consumption data (see text for details); (e) From equation (8); (f) Using equation (11); (g) Estimated using STPWIN (47); (h) Equation (10); (i) Equation (13) with a *DF* of 4; (j) Equation (13) with a *DF* of 130.

The modeled surface water concentrations for endogenous androgens and their *in vivo* metabolites evaluated for a “high-end” exposure scenario (Table VI) ranged from a few ng/L to almost 800 ng/L. In contrast, when evaluated for a typical exposure scenario (DF = 130), the range in surface water is expected to range from less than 0.1 ng/L to a maximum of 6 ng/L. Of the 19 endogenous androgens and their *in vivo* metabolites whose surface water concentrations have been estimated here, only 2 have been actually monitored in United States surface waters; testosterone and androsterone (89). The range predicted here for testosterone covering the two scenarios was from less than 0.1 ng/L to 3.4 ng/L. In comparison, in a surface water monitoring study conducted all across the United States (89), 70 samples were analyzed and, of these, only 2 had testosterone concentrations above the detection limit of 5 ng/L. This suggests that the concentration in the majority of the samples was less than 5 ng/L. For androsterone, the predicted range here encompassing the two scenarios was 43 ng/L to 0.7 ng/L. In the same study mentioned above (89), 10 positive detections were reported from the analysis of 70 samples for androsterone, with a median for the detected samples of 17 ng/L and with a detection limit of 5 ng/L. Both comparisons suggest that the estimates generated here for surface water concentrations are reasonable surrogates for actual field measurements.

The modeled surface water concentrations for endogenous thyroids evaluated for a “high-end” exposure scenario (Table VII) ranged from less than 1 ng/L to almost 4 ng/L, while for a typical exposure scenario all the predicted concentration were less than 0.1 ng/L. To date, it appears that no attempts have been made in the United States or elsewhere to quantify the environmental concentrations of endogenous thyroids.

Environmental Relevance

Before one can assess the environmental relevance of the endogenous chemicals being considered here, their *PNECs* must be established. In order to establish contaminant-specific *PNECs*, eco-toxicological data was sought for each of the 25 endogenous chemicals. However, a literature survey indicated that relevant data was available for only 5 of the 25 endogenous chemicals of interest. The data used to derive *PNECs* along with the derived *PNECs* for these five chemicals is summarized in Table VIII. The derived *PNECs* for testosterone, androstenedione and dihydrotestosterone are considered to be more robust than those derived for thyroxine and triiodothyronine, as the *PNECs* for the androgens were derived from classical eco-toxicological endpoints. A conservative application factor of 1000 was used to derive the *PNECs* for the two thyroids since the lowest reported endpoints still yielded a response in the test fish.

The environmental relevance for those chemicals for which *PNEC* values could be established was assessed for the two exposure scenarios using equation (14). The resultant risk quotients (*RQ*) are presented in Table VIII. The *RQs* derived indicate that under a typical exposure scenario none of the five hormones are likely to pose a risk; however, when assessments were conducted for a high exposure assessment scenario, only androstenedione was found to pose unacceptably high risk to the receiving aquatic ecosystem. This suggests that

androstenedione is likely to pose a risk to the receiving aquatic ecosystem in only those locations where the dilution of wastewater effluents is low.

The lack of relevant eco-toxicological data for the remaining chemicals prevents one from estimating their *PNECs*. However, *in vivo* and *in vitro* potency data can be used to indicate the potential of the remaining chemicals to act as endocrine disrupting chemicals. The *in vivo* and *in vitro* potency data suggests that the majority of the remaining chemicals are weak modulators of the appropriate receptors (99–110). However, three important exceptions to this generalization were identified: androsterone, which was shown to be 2.6 times more potent than testosterone in inducing secondary male characteristics in exposed *O. Latipes* (99); androstane-3,11,17-trione, which was 5 times more potent than dihydrotestosterone in binding to the androgen receptor extracted from the ovaries of *M. undulates* (105); and 5 α -androstenedione, which was found to be twenty times more potent than dihydrotestosterone in binding to the androgen receptor extracted from the brains of *M. undulates* (105). Therefore, there exists real potential for these chemicals to modulate the androgen receptor in fish, particularly so in the case of androsterone, since effects were demonstrated *in vivo*. Therefore, these three chemicals should be prioritized for eco-toxicological testing.

For the remaining chemicals (not including the five known hormones for which *PNECs* were derived and the three that are likely modulators) the likely relevance is less certain. It is known that most of them are weak modulators based on *in vitro* binding affinity to appropriate receptors (100–110) but what such weak binding to the receptors means in terms of toxic potential is less certain.

Table VIII. Predicted No–Effect Concentrations (*PNECs*) and Risk Quotients (*RQs*) for endogenous hormones for which eco-toxicological data was available (see Appendix B for larger version of table)

Hormones	Endpoint	Taxon	Species	Duration (d)	Effect	Concentration ($\mu\text{g/L}$)	Reference	AF	<i>PNEC</i> (ng/L)	<i>RQ</i>	
										high	typical
Testosterone	NOEC	Fish	<i>O. tshawytscha</i>	29	Sex differentiation	1	(92)	10 ^(a)	100	3.4x10 ⁻²	2.6x10 ⁻⁴
	NOEC	Algae	<i>S. subspicatus</i>	3	Growth	335	(93)				
	NOEC	Crustacean	<i>B. calyciflorus</i>	4	Females fertilized	1	(94)				
Androstenedione	NOEC	Fish	<i>G. affinis</i>	24	Masculinization	0.04	(95)	10 ^(b)	4	1.9x10 ⁸	1.44x10 ²
	NOEC	Crustacean	<i>D. magna</i>	21	Abnormalities	2291	(96)				
Dihydrotestosterone	NOEC	Fish	<i>G. aculeatus</i>	18-35	Spiggin induction	2	(97)	100 ^(c)	20	1.2x10 ¹	8.8x10 ⁻⁴
Thyroxine	LOEC	Fish	<i>C. auratus</i>	15d	No. of fins	10	(98)	1000 ^(d)	10	2.9x10 ⁻¹	2.2x10 ⁻¹
Triiodothyronine	LOEC	Fish	<i>C. auratus</i>	40d	Tail fin length	10	(98)	1000 ^(d)	10	3.8x10 ⁻¹	2.9x10 ⁻²

(a) AF=10 is used, considering 3 chronic endpoints from three different taxa were available; (b) AF=10 is used, even though only data from two taxa was available because the chronic endpoint for algae is unlikely to be below the one reported for fish; (c) AF=100 is used, since only one chronic endpoint was available; (d) AF=1000 is used conservatively, as the endpoint available was an LOEC.

Overall Perspective

Very little attention has been paid to the environmental release and relevance of endogenous androgens, thyroids and their *in vivo* metabolites. The intent here was to develop a systematic approach that allows one to conservatively quantify the loads of such chemicals released in anthropogenic excretions. The compiled data set made it possible to generate mean loads of such compounds in urinary and fecal excretions (i.e., U_i and F_i) of various population cohorts of interest. These loads should be applicable to any situation where the desire is to quantify net loads of such contaminants. Considering that population demographics in most instances are not expected to vary drastically between communities, the derived population-normalized net urinary and fecal loads (U_i and F_i) could even be used on an individual treatment plant level to conservatively estimate the likely loads of such contaminants in the influents of treatment plants. Such predicted loads could also be coupled to influent monitoring data to understand the sewer behavior of such contaminants. If the predicted loads are found to be consistently higher than the measured loads, degradation/transformation of the excreted load is a likely cause. In this case, it is suggested that a comparison of the monitored and predicted values should be made on a daily load basis. That being said, it is important to understand that if the interest lies in predicting the maximum risks posed or the environmental relevance of such contaminants, then the assumption that the excreted loads are conserved during sewer transit is a conservative one.

In order to calculate effluent loads, it was assumed that the sulphate conjugates of the endogenous chemicals are likely to be resistant to treatment in sewage plants. It is worth investigating the effect of this assumption on the predicted effluent loads. If one were to assume the converse, i.e., that the sulphate conjugates are completely available for treatment, the effluent loads for a number of the endogenous chemicals will be significantly reduced, while for others minimal to no reduction in effluent loads would be observed (see Figure A.1 of Appendix A, located in the back of this book). From an environmental relevance perspective, the assumption that the sulphate conjugates are not available for treatment is a conservative one. Therefore, if the interest lies in risk assessment and environmental relevance of such contaminants such an assumption may suffice at the preliminary assessment stage until readily useable data is available indicating otherwise.

Overall, from an environmental assessment perspective, these assumptions are only worth investigating in further detail for those compounds that pose a significant risk according to the analysis described above. In this way, impact assessments could be conducted more efficiently by only focusing efforts on investigating compounds that have a reasonable possibility of triggering important eco-toxicological impacts.

Conclusions

In the strategy employed here to translate the derived excretion loads from anthropogenic excretions to the environment, justifiable but conservative assumptions were made where data was found lacking. Therefore, the predicted

surface water concentrations should represent conservative estimates of their likely presence in the environment. The estimates were subsequently used to assess the environmental relevance of the chemicals for which *PNECs* could be derived. The conservative analysis suggests that all of the endogenous chemicals for which *PNECs* could be derived are unlikely to pose a risk to receiving ecosystems when evaluated for a typical exposure scenario. When the relevance was evaluated even more conservatively by considering a high exposure scenario, exposure to only androstenedione raises concern. Therefore, it is suggested that the conservative nature of the assumptions used here be investigated for androstenedione only, in the event that its predicted surface water concentrations are overestimates and the resultant risk is overstated.

Overall, it is suggested that the strategy developed here could be used to conduct a preliminary risk assessments of any endogenous chemicals in order to identify compounds of concerns. Those compounds that are identified as having a potential risk could then be investigated in further detail to assess their impacts in greater detail and with greater accuracy. An additional utility of the generated surface water estimates produced here is that they can serve as detection limit bench marks for analytical chemists or regulatory bodies that are interested in detecting or monitoring such chemical.

Suggestions for Further Development

The focus here has been to establish a nationwide release inventory of all major endogenous androgens, thyroid hormones and their *in vivo* metabolites, as they make their way to receiving environment through the wastewater treatment and collection train. However, other potential sources that may be important on a regional scale including the contributions of surface runoffs from feedlots and sludge application sites, slaughterhouse facilities discharging endogenous chemical-laden wastes, and/or industrial effluents that themselves may have significant androgenic or thyroidal content.

Further, the analysis here was limited to a range of dilution factors that are relevant for a nationwide assessment. However, the combination of factors yielding surface water concentrations on a local level may be very different. In certain instances, a combination of factors could lead to a situation where a dilution factor of less than the lower limit of 4 used here may be appropriate under low flow conditions (i.e. 7Q10). In other cases no secondary treatment may be available for a wastewater stream being discharged into the environment. Furthermore, each of the parameters used to estimate surface water concentrations has variability associated with it, which is not accounted for in a deterministic risk assessment. Therefore, the next logical step of the strategy developed here would be to move from the national deterministic realm to the probabilistic geo-referenced regional realm by incorporating probabilistic considerations into geographical information system (GIS) contaminant fate models such as GREAT-ER (*III*) or PhATE (*II*2). Such an analysis will allow one to go beyond a national assessment and identify regional hot spots, where contaminants

identified in a nationally assessment as low risk may indeed pose a risk to the local environment.

Lastly, the interest here has been on risk posed by individual chemicals and, as such, the net androgenic and thyroidal potential of mixtures of such chemicals hasn't been considered. Work with mixtures of endogenous estrogens suggests that the concept of concentration addition can be used to reasonably predict the worst case impact of chemical mixtures that manifest their effects by interacting with a receptor (113). Since endogenous thyroids and androgens also act through similar receptor-mediated modes of action as endogenous estrogens, it is plausible that mixtures of such chemicals would also act in a concentration-additive manner. Ideally, sufficient toxicity data or *in vivo* effect data would be available for each of the endogenous chemicals so that one can establish environmental relevance of the mixture being discharged. Unfortunately, such data are rare for the chemicals of interest here. In such cases where enough toxicity data or *in vivo* effect data are not readily available, an *in vitro* surrogate typically used is the receptor binding potency of the chemical (114). Therein lays the limitation of using such an approach as it implicitly assumes that the biological response of a chemical can be predicted by considering the chemical's binding potential. The limited *in vivo* data available for such chemicals further raises questions about exclusively relying on *in vitro* binding data. For example, work by Sperry and Thomas (105) suggested that for the androgen receptors of *M. undulatus* the binding potency of testosterone was 37 to 625 times higher than the binding potency of androsterone. However, *in vivo* data reported by Kawamoto (99) suggests that it is actually androsterone that is 2.6 times more potent than testosterone in inducing secondary male characteristics in exposed *O. latipes*. Considering such dramatic discrepancies between *in vitro* and *in vivo* data and the limited availability of *in vivo* data, it is perhaps too premature to robustly establish the ecotoxicological relevance of the mixtures of interest here. However, as relevant data becomes available such mixture effects could be, and should be, considered.

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Chapter 23

Assessment of the Contribution of Triclosan to Dioxin Emissions from Sludge Incineration in the U.S. Using a Mathematical Model

K. D. Doudrick,^{1,†} D. B. Jones,^{1,†} T. Kalinowski,^{2,†} E. M. Hartmann,^{2,†}
and R. U. Halden^{*,1,2}

¹School of Sustainable Engineering & the Built Environment,
Arizona State University, 1001 South McAllister Avenue, P.O. Box 875701,
Tempe, AZ 85287

²The Biodesign Institute, Arizona State University, P.O. Box 875001,
Tempe, AZ 85287

†These authors contributed equally.

* Corresponding author: School of Sustainable Engineering and the Built Environment, Arizona State University, 1001 South McAllister Avenue, P.O. Box 875701, Tempe, AZ 85287-5701. E-mail: halden@asu.edu.

Triclosan, a household antimicrobial, is present in raw and treated wastewater and in digested sewage sludge (biosolids) across the U.S. It was demonstrated to be converted to various dioxins upon chlorination and combustion, common processes in sewage treatment and biosolids disposal. In this study, a mathematical model was developed to assess triclosan's contribution to dioxin emissions resulting from sewage sludge incineration. Triclosan transformation rates were identified from the literature. Sludge incineration was identified as a probable pathway leading to dioxin formation because triclosan is exposed to chlorine during drinking water and wastewater treatment and accumulates in biosolids. Representative concentrations and transformation rates of triclosan, chlorinated triclosan, and dioxins were utilized to populate a mathematical model predicting the mass of dioxins formed from triclosan combustion. Analyses considered representative tetrachlorodibenzo-*p*-dioxin (TCDD) concentrations and toxic equivalency quantities (TEQ) based

on established and theoretical (TEQ*) dioxin toxicity data. The model suggests that triclosan conversion to dioxins accounts for a significant fraction of the TCDD and TEQ burden from sludge incineration. Depending on triclosan concentrations in sludge, annual dioxin mass loads range were predicted to range from 2 to 168 g total TCDD/yr or 0.3 to 32 g I-TEQ*/yr, suggesting that triclosan contributes significantly to the total dioxin emissions in the U.S. This study is the first to quantitatively link triclosan-enriched sludge incineration to toxic dioxins in the U.S. Future work should seek to verify the modeling results obtained here.

Introduction

Since it was first patented and used in the medical field beginning in 1964, triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether, TCS) has become an ingredient in many household products. This broad spectrum antimicrobial is commonly found in various personal care products, including liquid hand soaps and toothpastes. Consequently, triclosan is also found in wastewater treatment plants across the country at a loading rate of 620 to 2490 $\mu\text{g}/\text{person}/\text{day}$, where as much as 98% is removed through conventional treatment processes (1, 2). Of this, 31 to 69% is removed and concentrated in sludge (1, 2). Triclosan has also been identified as an unwanted contaminant in surface waters (3–5), aquatic sediments (3, 6, 7), agricultural soils (8), dust (9), aquatic and terrestrial biota (10, 11), as well as in human urine (12) and breast milk (4).

Triclosan (Figure 1a) is a pre-dioxin, meaning that it can be transformed into a dioxin-like compound, one of a family of compounds generally referred to as dioxins (Figure 1b). Dioxins are known human carcinogens as well as persistent organic pollutants (13). The term “dioxin-like compound” refers to three separate groups of chlorinated aromatic compounds that share similar structural features and dioxin-like toxicity. These are polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and co-planar polychlorinated biphenyls (PCBs). PCDDs are commonly referred to as dioxins and have a total of 75 isomers. PCDFs, also called furans, have a total of 135 isomers, and there are a total of 209 PCB isomers. Toxic equivalency factors (TEFs) are assigned to each dioxin-like compound to normalize each congener's toxicity to that of the most toxic one, *i.e.*, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Both 2,3,7,8-TCDD and 2,3,7,8-TCDF are assigned a TEF value of 1, and all other compounds are considered less toxic than these compounds. A TEF value of 0 is assigned to compounds considered to be non-toxic relative to TCDD. Of the 419 total dioxin-like congeners, there are currently 29 that are considered toxic with TEF values greater than 0 (7 PCDDs, 10 PCDFs, and 12 PCBs) (14). PCDD/Fs are considered toxic when they carry four or more chlorines of which four need to be present in the lateral 2-, 3-, 7-, and 8- positions; PCBs are considered dioxin-like toxicants when they feature four or more chlorines in lateral positions with only one or no chlorine substituents in one of the four available *ortho* positions. Because these dioxin-like

compounds are often found together in the environment, a total toxic equivalency quantity (TEQ) is used to represent the toxicity of all compounds. Its value is calculated as the sum of the products of the compound concentrations and their respective individual TEFs. There are currently two TEQ classification systems in use: the International TEQ (I-TEQ) system formulated in 1989 is used by the U.S. Environmental Protection Agency (USEPA), and represents only dioxins and furans, and the World Health Organization TEQ (WHO-TEQ) system that was updated in 2006 (15) and that represents dioxins and furans as well as dioxin-like polychlorinated biphenyls. Currently, the I-TEQ is used to define USEPA dioxin emission standards. For simplicity, all dioxin-like compounds will be referred to as dioxins in this chapter.

When triclosan is present, dioxins can be formed in surface waters irradiated with ultraviolet (UV) rays from sunlight (16), during UV disinfection of drinking water and wastewater (17, 18), and through combustion processes (19, 20). Triclosan has frequently been demonstrated to transform through UV and heat into a dichlorinated dioxin congener that is not considered to exhibit dioxin-like toxicity, based on current TEF values (14). However, triclosan can become further chlorinated in situations where free chlorine is available, such as during chlorination of drinking water and wastewater, and during combustion in incinerators (21). Some of these chlorinated triclosan compounds, most notably 2',3,4,4',5-pentachloro-2-hydroxydiphenyl ether (referred to as TCS(V) in this chapter), have been demonstrated to lead to higher chlorinated dioxins, such as congeners of TCDD, when heated (19) or exposed to UV light (22).

Triclosan contains trace amounts of toxic dioxins as production impurities (23, 24). Previous work suggests that triclosan does not contain 2,3,7,8-TCDD (23, 25). However, advances in gas chromatography-mass spectrometry have shown that 2,3,7,8-TCDD is indeed present at trace concentrations in technical grade triclosan (24). Previous studies considering the major sources of dioxins in the environment suggested that the production of triclosan was an important contributor of dioxins due to the ubiquitous use of triclosan containing trace concentrations of dioxin (26, 27).

Great strides have been made in recent years to decrease the human health risks associated with dioxins, but anthropogenic dioxins continue to accumulate in the environment (13). A global mass balance of PCDD/Fs performed in 1999 found that atmospheric deposition rates still exceed known emission sources by "well over a factor of two" (28). Because of triclosan's ubiquity and its demonstrated propensity to become a toxic, persistent pollutant, an evaluation is important to estimate how much of the triclosan in the environment can be potentially transformed into dioxins. This information is critical for a safety assessment of triclosan and to determine if triclosan production should be regulated or restricted.

In this study, the potential transformation of triclosan into dioxin from chlorination and combustion is evaluated. The focus is on the combustion of municipal biosolids containing triclosan and chlorinated derivatives of triclosan. This is justified because triclosan present in soap or other consumer products is exposed to free chlorine when combined with chlorinated drinking water and during conveyance of domestic sewage to wastewater plants. The documented

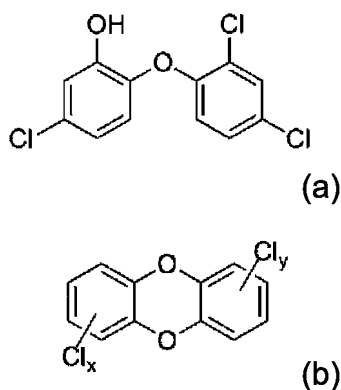


Figure 1. Chemical structure of triclosan (a) and generic polychlorinated dibenzo-*p*-dioxin (b).

sequestration of triclosan and its higher chlorinated congeners in municipal sewage sludge opens a pathway for dioxin formation from triclosan-derived chemical precursors during sludge incineration. The goal of the present study was to estimate triclosan-related dioxins to emissions from sludge incineration and to compare this environmental mass loading to toxic inputs resulting from dioxin impurities in manufactured triclosan.

Methods

Through a literature review, transformation pathways for triclosan to dioxin were identified, compiled, and used to define the established and proposed environmental pathways. To evaluate the contribution of triclosan to dioxin emissions from the combustion of municipal biosolids, a mathematical model was developed using the representative constant values extracted from the literature. The model was used to determine the concentration of dioxin in air emissions from biosolids incineration on a total TCDD and I-TEQ basis. Total TCDD is the sum of all 22 congeners, and I-TEQ was chosen because the USEPA emission standards are based on this classification.

The mathematical model generated to determine the estimated concentration of dioxin emitted from the combustion of sludge containing triclosan derivatives (C_{DEST}) is shown below in equation 1.

$$\text{Eq. 1} \quad C_{\text{DEST}} = (C_{\text{TCS-DS}}) (\eta_{\text{TCS(V)/TCS}}) (\xi_{\text{TCDD/TCS(V)}}) (\omega)$$

where C_{DEST} is the concentration of dioxin emissions attributable to triclosan conversion during incineration of municipal sludge (ng/kg biosolids); $C_{\text{TCS-DS}}$ is the triclosan concentration in biosolids (ng/kg biosolids); $\eta_{\text{TCS(V)/TCS}}$ is the ratio of TCS(V) mass per TCS mass in biosolids (dimensionless); $\xi_{\text{TCDD/TCS(V)}}$ is the ratio of TCDD mass generated per TCS(V) mass combusted (dimensionless); and ω is the percentage of the dioxin mass passing through the flume to the atmosphere (%).

Results

The literature review conducted served to identify mechanisms of dioxin formation from triclosan as a precursor and to parameterize the dioxin emission model.

Triclosan Transformation Mechanisms

Triclosan may be converted to dioxins under a variety of conditions (Figure 2). If triclosan is exposed to heat (i.e., combusted), it is known to transform to dichlorodibenzo-*p*-dioxin (DCDD) (14). In the presence of chlorine-containing chemicals such as polyvinyl chloride, DCDD can become further chlorinated (21), ultimately leading to toxic dioxin-like congeners. In addition, triclosan can be converted to DCDD when released to surface waters and exposed to sunlight or when exposed to UV disinfection during water treatment (16–18). In aqueous environments and in the presence of hypochlorite ions from bleach, for example, the 2,7- and 2,8-substituted DCDD congeners can become further chlorinated by electrophilic aromatic halogenation. Triclosan can also be further substituted in chlorinated tap water (19). When the more highly chlorinated triclosan is then discharged to wastewater treatment plants, it concentrates in sludge (1) whose combustion can lead to the formation of the 1,2,3,8-TCDD congener and could possibly form trace concentrations of the toxic 2,3,7,8-TCDD congener (19). This latter process of TCDD formation from sludge incineration is the focus of the mathematical model presented in this study.

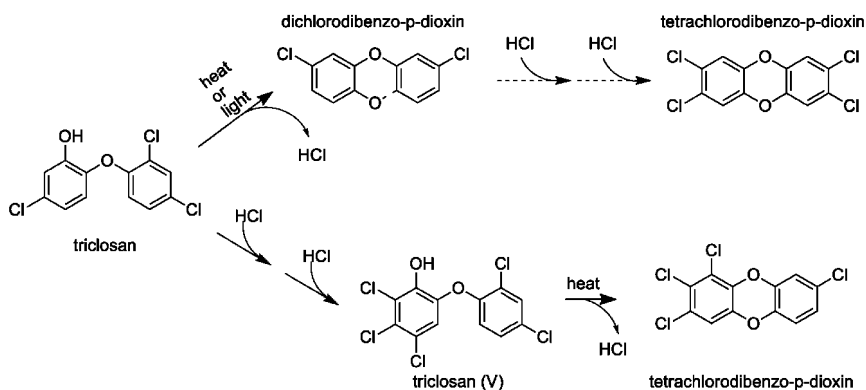


Figure 2. Mechanisms of triclosan conversion leading to the formation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (top) and 1,2,3,8-tetrachlorodibenzo-*p*-dioxin (bottom).

Table I. Input Parameters Extracted from the Literature to Parameterize the Triclosan Combustion Model Presented in this Study

<i>Parameter</i>	<i>Value</i>	<i>Literature Source</i>
Total production of sludge in U.S.	6.51x10 ⁹ kg/yr	31
TCS mass / sludge mass, mean (minimum) [90 th percentile]	16 mg/kg (0.33 mg/kg) [34 mg/kg]	32
Mass ratio of TCS(V) to TCS	0.51%	29
Percentage of sludge combusted	19%	14
TCS(V) conversion efficiency to 1,2,3,8-TCDD	7.9%	19
Current TEF for 1,2,3,8-TCDD	0	14
Proposed TEF* for 1,2,3,8-TCDD	0.19	33
Percent of dioxin escaping flume	1%	30
Measured I-TEQ emission from biosolids combustion	6.87 ng I-TEQ/ kg sludge	14
Measured total TCDD emissions from biosolids combustion	37.81 ng total TCDD/kg sludge	14
Exhaust volume per mass of sludge	8 m ³ exhaust/kg sludge burned	34

Input Parameters for the Mathematical Model

The input parameters gathered from the literature are shown in Table I. The total production of sludge in the United States and triclosan sludge concentrations were obtained from results of the *National Biosolids Regulation, Quality, End Use and Disposal Survey* conducted by the Northeast Biosolids and Residuals Association (NEBRA) in 2007 (31). McAvoy and co-workers (29) conducted a survey on the mass balance of triclosan and its chlorinated derivatives in wastewater treatment plants. These data were used to estimate the TCS(V) concentration in biosolids relative to the triclosan concentration. An early study from Japan (19) had demonstrated that TCDDs were formed from the combustion of triclosan. When the air volume was small, the chlorinated derivatives of triclosan formed 2,3,7,8-TCDD, as determined by gas chromatography-mass spectrometry. However, when a larger and more realistic combustion chamber was used, 2,3,7,8-TCDD was not detected in high concentrations. Rather, 1,2,3,8/1,2,3,7-TCDD was detected from combustion of TCS(V). However, 2,3,7,8-TCDD could have been present in low concentrations, but because of the mass spectrometric analytical techniques available at the time, it could not be distinguished from the 1,2,3,8/1,2,3,7-TCDD congener peak. Because no other TCDD congeners were reported, these data were reported as total TCDD. The amount of TCDD produced from combustion of TCS(V) was utilized to calculate

the conversion efficiency for TCS(V) to 1,2,3,8-TCDD (or total TCDD) from the combustion of sludge.

Based on a sewage sludge incineration mass balance, approximately 1% of dioxins formed during the combustion process escape into the air (30). Although this model only considers the mass of dioxins from combustion of triclosan, it assumes that only 1% of these dioxins escape to the atmosphere. Since the USEPA does not regulate dioxins from sludge combustion, the dioxin emission limit for hazardous waste combustion was used for comparison.

Results for the Mathematical Model

The mathematical model was applied for three types of dioxin measurements, total TCDD, I-TEQ, and I-TEQ*. Currently, the TEF value for 1,2,3,8-TCDD is considered 0 for regulatory purposes; however, a more plausible alternative TEF value of 0.19 for 1,2,3,8-TCDD was suggested recently that is based on a quantitative spectrometric data-activity relationship (QSDAR) model (33). Modeling results for total TCDD and I-TEQ* were compared to the measured concentrations of dioxin from biosolids incineration. The results from the mathematical model are shown in Table II and Table III.

When dioxin emissions were calculated on a total TCDD basis, the concentrations of dioxins attributed to triclosan combustion were on the same order of magnitude as the measured dioxin values, with the ratio ranging from 0.036 to 3.6. This suggests that a sizable proportion of dioxin emissions from sludge incineration result from the combustion of triclosan: at least 3.6% and possibly as much as the theoretical maximum of 100%. Similar percentages of 3.7 to 100% were obtained from investigating emission concentrations on an I-TEQ* basis, which includes a TEF value of 0.19 for 1,2,3,8-TCDD. However, because the concentration of 1,2,3,8-TCDD was not reported for the measured emission, a TEF value of greater than the appropriate factor of 0.19 was used for the group of TCDD congeners detected; therefore, the I-TEQ* method was prone to overestimation of toxicity in this particular instance. The I-TEQ measured emission concentrations were much lower than the total TCDD concentrations which means that the dioxins emitted were a mixture of all or some of the 22 TCDD congeners rather than just the toxic form, 2,3,7,8-TCDD. Therefore, the total TCDD method is assumed to account for all TCDD congeners for both the measured emissions from sludge incineration and measured emission from combustion of triclosan. The estimated dioxin emissions from triclosan are 0 ng I-TEQ/kg sludge when using the EPA/WHO TEF values because 1,2,3,8-TCDD is considered non-toxic by this convention.

Table II. Mathematical Model Results for Triclosan Conversion to Total TCDD from Sludge Incineration

<i>Triclosan Biosolids Concentration (mg/kg sludge)^a</i>	<i>Incineration Emission Concentration (ng total TCDD/kg sludge)</i>	<i>Percentage of Total Dioxin Emissions Attributable to the Incineration of Triclosan</i>	<i>Mass of total TCDD Emitted from TCS(V) Combustion (g/yr)</i>
0.33	1.3	3.6%	2
16	64	170%	80
34	136	360%	168

^a Values represent the minimum, average, and 90th percentile concentrations of triclosan found in biosolids in the U.S. (32)

Table III. Mathematical Model Results for Triclosan Conversion to I-TEQ*^a from Sludge Incineration

<i>Triclosan Biosolids Concentration (mg/kg sludge)^b</i>	<i>Incineration Emission Concentration (ng I-TEQ*/kg sludge)</i>	<i>Percentage of Total Dioxin Emissions Attributable to the Incineration of Triclosan</i>	<i>Mass of Dioxins Emitted from TCS(V) Combustion (g I-TEQ* /yr)</i>
0.33	0.3	3.7%	0.3
16	12	180%	15
34	26	370%	32

^a I-TEQ* represents the international toxic equivalency quantity (I-TEQ) plus the theoretical toxicity attributable to the 1,2,3,8-TCDD congener (33). ^b Values represent the minimum, average, and 90th percentile concentrations of triclosan found in biosolids in the U.S. (32)

Dioxin Contamination from Triclosan Production

The chemical industry (24) routinely performs monitoring of TCDD in bulk triclosan and detected 2,3,7,8-TCDD in six samples of triclosan produced in India and China in 2002. The concentrations ranged from 17.2 to 1712 pg of 2,3,7,8-TCDD per gram of triclosan (pg/g) with a mean concentration of 333 pg/g, which is equivalent to 333 parts-per-trillion_(w/w). Based on the mean TCDD concentration and an annual U.S. triclosan consumption of 600,000 kg (35), the mass of dioxins formed annually from production of triclosan for the U.S. market is 0.2 g of 2,3,7,8-TCDD per year. This amount is small compared to the calculated dioxin mass derived from the combustion of triclosan in biosolids, which is estimated at 15 g/yr with lower and upper bounds of 0.3 and 32 g/yr, respectively, based on the adjusted I-TEQ* value.

Dioxin Emission Standards

Currently, there is no dioxin emission standard for sewage sludge incinerators according to the USEPA Code of Federal Regulations 40 503 (36). However, in the U.S., medical, municipal, and hazardous waste incinerators all have dioxin regulatory standards. Depending on the type, size, and date of plant construction, the standard ranges from 0.2 to 2.3 ng I-TEQ/dscm at 7% O₂, where dscm at 7% O₂ stands for dry standard cubic meter corrected to the standard oxygen level (14). Based on the average exhaust gas volume formed from sludge incineration of 8 m³/kg of sludge (34), the average dioxin emission concentration is 0.86 ng I-TEQ/m³ or approximately four times the lowest emission standard of 0.2 ng I-TEQ/dscm for new hazardous waste incinerators. Refining this calculated value by correcting for the actual oxygen content during incineration is desirable, but unfortunately the required parameters were unavailable for this dataset.

Additional critical data needs were identified in this study. The progeny and speciation of triclosan-derived dioxins requires further study. There may be additional congeners that are formed during the combustion of triclosan in the presence of sludge. To date, only a single study has investigated the formation of dioxin from triclosan combustion (19). Because that work investigated the combustion of bleached clothing material and the by-products produced, additional investigations concentrating on dioxin emissions from the combustion of triclosan-laced sludge are still needed. Specifically, controlled studies on sludge incineration are needed to better parameterize the conversion rate of triclosan to dioxin. This would help to reduce the considerable range of model output reported in this study. Furthermore, additional work is necessary to better understand and quantify the relative toxicities of various dioxin congeners. Of the 419 dioxin-like congeners, only 29 are assigned TEF values by the WHO, despite the fact that recent developments indicate toxic activities of many other congeners (33). Finally, more work is needed to produce consensus values for calibration of the model presented here. For example, the values for conversion rate of TCS(V) to 1,2,7,8-TCDD and concentrations of dioxins present as impurities in triclosan containing products are each based on only a single study.

In addition, contributions of dioxins to the environment from alternate mechanisms, such as dioxins formed from triclosan photolysis in surface waters, and waste products formed during manufacture of triclosan need to be evaluated and quantified as well. This information will lead to a more comprehensive qualitative and quantitative understanding of triclosan's contribution to dioxin loading to the environment.

Although dioxin emissions from sludge incineration are not currently regulated under USEPA rule, dioxin emission data collected by the USEPA show that sewage sludge incinerators should be regulated on the same level as other waste incinerators in the U.S. Furthermore, the model for dioxin emissions caused by combustion of municipal sludge contaminated with triclosan and its chlorinated derivatives shows that triclosan could be a major contributor to the measured dioxin emissions breaching other waste incinerator standards.

Conclusions

This study asserts that the incineration of sludge contaminated with triclosan and its derivatives could be a major contributor to dioxin emissions in the U.S. Based on varying triclosan concentrations in digested sludge, modeling results suggest that triclosan may account for at least 3.6% and possibly for as much as 100% of measured dioxins emitted from sewage sludge incinerators. A similar range of 3.7 to 100% was calculated when utilizing the I-TEQ* method. Some of the calculated contribution ratios were greater than unity or 100% (e.g. the value of 1.7 resulting from using mean triclosan concentrations contained in U.S. sewage sludge), which reflects an existing uncertainty in the parameters utilized to run the dioxin contribution model.

The results of this model also suggest that combustion of triclosan-laced sludge is an important contributor of dioxins to the environment. Therefore, when considering triclosan as a precursor of dioxins to the environment, combustion of sludge should also be accounted for in addition to the dioxins formed during triclosan production.

While numerous studies have quantified individual transformation pathways for triclosan to dioxins, this study is the first to compile all the known data and to quantify on a national scale for the U.S., the amount of triclosan that may be converted to dioxin through sludge incineration. If triclosan usage continues to increase, the total annual dioxin emissions will likely continue to rise as well. Future work should seek to verify the modeling results obtained here.

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Chapter 24

Literature Review of Meta-Analyses and Pooled Analyses of Disinfection By-Products in Drinking Water and Cancer and Reproductive Health Outcomes

Mark J. Nieuwenhuijsen,^{*1,2} James Grellier,² Nina Iszatt,²
David Martinez,¹ Md Bayzidur Rahman,³ and Cristina M. Villanueva¹

¹CREAL, Barcelona, Spain

²Imperial College London, United Kingdom

³The University of New South Wales, Australia

* Address correspondence to: Mark J. Nieuwenhuijsen, Ph.D., Research Professor in Environmental Epidemiology, Center for Research in Environmental Epidemiology (CREAL), Parc de Recerca Biomèdica de Barcelona - PRBB (office 183.05) C. Doctor Aiguader, 88, 08003 Barcelona, Spain. Tel.: direct (+34) 93 2147337. Assistant: Anna Sillero: (++34) 93 2147390. Fax: (++34) 93 2147302. Email: mnieuwenhuijsen@creal.cat.
Web pages: <http://www.creal.cat/> <http://www.prbb.org/>.

Disinfection of drinking water has led to major improvements in public health in developed countries since its introduction in the first half of the 20th century. Chlorination disinfection by-products (DBPs) are formed when water is chlorinated and organic matter in the water reacts with chlorine.

A number of meta-analyses and pooled analyses of exposure to DBPs and various health outcomes have been published and here we review these studies to provide an overall overview of the evidence of disinfection by-products and health effects.

This review showed that various meta-analyses and pooled analyses have found statistically significant excess risk for some indicator of exposure to chlorinated water or trihalomethanes and bladder and colorectal cancer, small for gestational age, still birth, all congenital anomalies combined and ventricular septal defects, but no statistical significant excess risk for many other

congenital anomalies. The excess risk was generally small, but robust, with little sensitivity to the results of individual studies or evidence of publication bias.

Acronyms

OR:	Odds Ratio. It is a measure of the probability of having a disease provided a certain level of exposure compared to a referent category. OR is used in case-control and cross-sectional studies and interpretation is similar to RR
RR:	Relative Risk. It is a measure of the probability of having a disease provided a certain level of exposure compared to a referent category. RR is used in cohort studies and interpretation is similar to OR.
CI:	Confidence Interval, usually given as 95% CI. Provides information on the statistical significance of a point risk estimate (odds ratio or relative risk).
LBW:	low birth weight, generally defined as birth weight < 2500 g
TLBW:	term low birth weight, generally defined as birth weight < 2500 g at week 37 or older
PTD:	preterm delivery, generally defined as a birth with gestational age < 37 weeks
SGA:	small for gestational age, generally defined as birth weight below the 10%ile at a given gestational age
IUGR:	intra uterine growth retardation, generally defined as birth weight below the 10%ile at a given gestational age
THM:	Trihalomethane
TTHM:	total trihalomethanes, the sum of chloroform, chlorodibromomethane, bromodichloromethane and bromoform
DBPs:	disinfection by-products
Meta-analysis:	statistical method to combine the results of different studies, usually which have been peer reviewed, using the original risk estimates (e.g. RR, OR) of the individual studies to obtain an overall summary estimate(s) of exposure and disease
Pooled analysis:	statistical method to combine the results of different studies, usually which have been peer reviewed, using subject level data of the different studies to obtain an overall summary estimate(s) of exposure and disease

Introduction

Disinfection of drinking water has led to major improvements in public health in developed countries since its introduction in the first half of the 20th century. It has now been more than 30 years since the discovery that by-products can be formed in small quantities as part of the chlorination process (1). Chlorination disinfection by-products (DBPs) are formed when water is chlorinated and organic matter in the water reacts with chlorine. Up to 600 DBPs have been identified (2, 3), and these chemicals differ considerably in their physicochemical properties, including volatility. Their formation and occurrence depend on many factors, including disinfectant dose, type of treatment, pH, temperature, residence time, water source, and bromine levels (4, 5). Different mixtures of by-products may exist in different locations depending on the various factors mentioned above, making it more difficult to ascertain the risk, if any, of health effects in relation to *specific* DBPs and mixtures of DBPs, as well as to compare findings from different epidemiological studies.

The most frequently used disinfectants are chlorine and other chlorine-based chemicals (chlorine dioxide, chloramine, etc.). Other disinfectants used in drinking water are ozone, potassium permanganate, UV radiation, hydrogen peroxide, etc. All these are highly reactive chemical that produces a mixture of by-products after reacting with organic matter. The trihalomethanes (THMs) are the most commonly formed group of byproducts. Concentrations of four THMs are routinely monitored in many countries and have therefore been used most frequently as an indicator of exposure to DBPs. THMs are volatile and individuals may be exposed not only through ingestion but also through inhalation and dermal absorption, during activities such as showering, bathing, and swimming. Recent modelling of THM uptake suggest that dermal and inhalation exposure may lead to the highest levels in blood (6). For non-volatile DBPs, such as the haloacetic acids (HAAs), ingestion is thought to be the main route of exposure (4).

Several reviews of associations between exposure to DBPs and several health outcomes have been conducted. Overall these reviews have concluded that relationships between DBP exposure and health outcomes remain unclear (5, 7–12). However, most of these reviews have been qualitative rather than quantitative, and have not attempted to provide overall summary estimates for DBP exposure and health effects. The reviews have not taken into account the size of individual studies, nor provided systematic analyses of methodological heterogeneities between the studies. Meta-analyses and pooled analyses systematically combine data from a number of studies to estimate overall summary measures of association, and permit the investigation of heterogeneities between studies. A number of meta-analyses and pooled analyses of exposure to DBPs and various health outcomes have been published and here we review these studies to provide an overall overview of the evidence of disinfection by-products and health effects, as well as discussing some of the limitations of the studies. Since meta-analyses and pooled analyses can only be conducted when a number of studies have been carried out, we focus on outcomes that have been more frequently studied and ignore the less investigated outcomes such as time to pregnancy, semen quality, and cancers of the kidney, lung and skin.

Meta-Analyses and Pooled Analyses of Health Effects Related to Exposure to Chlorination Disinfection By-Products

Cancers

The first meta-analysis of the association between chlorinated drinking water and by-products and cancer was conducted by Morris et al. (13) and included ten studies on various types of cancer. It included 7 studies on bladder cancer, 7 studies on colon cancer, and 6 studies on rectal cancer. Consumption of chlorinated water, surface water, or water with high levels of chloroform was used as a surrogate for exposure to chlorination by-products. A meta-analysis of all cancer sites yielded a relative risk estimate for exposure to chlorination by-products of 1.15 (95% CI 1.09 to 1.20). Summary relative risk estimates for organ-specific cancers were 1.21 (95% CI 1.09 to 1.34) for bladder cancer, 1.38 (95% CI 1.01 to 1.87) for rectal cancer and 1.11 (95% CI 0.91 to 1.37) for colon cancer.

Villanueva et al. (14) included six case-control studies (6084 incident bladder cancer cases and 10,816 controls) and two cohort studies (124 incident bladder cancer cases) in their meta-analysis of bladder cancer. "Ever consumed" (vs. "never consumed") chlorinated drinking water was associated with an statistically significant increased risk of bladder cancer in men (combined odds ratio [OR]=1.4, 95% CI 1.1 to 1.9) and a statistically significant increased risk in women (combined OR=1.2, 95% CI 0.7 to 1.8). The combined OR for mid-term exposure for both sexes was 1.1 (95% CI 1.0 to 1.2) and for long-term exposure 1.4 (95% CI 1.2 to 1.7). The combined estimate of the slope for a linear increase in risk was 1.13 (95% CI 1.08 to 1.20) for 20 years and 1.27 (95% CI 1.15 to 1.43) for 40 years of exposure for both sexes.

Table I. Pooled analysis of bladder cancer and THM

<i>THM Exposure level ($\mu\text{g/l}$)</i>	<i>Men OR* (95%CI)</i>	<i>Women OR* (95%CI)</i>
0-1 ‡	1.00	1.00
>1-5	1.10 (0.92-1.31)	0.99 (0.72-1.36)
>25-50	1.25 (1.04-1.50)	1.04 (0.76-1.43)
>50	1.44 (1.20-1.73)	0.93 (0.67-1.28)

* OR (95%CI) = Odds ratio (95% confidence interval).

In a pooled analysis, Villanueva et al. (15) included 2806 cases and 5254 controls, for which measures of exposure were known for at least 70% of the exposure window of 40 years, specifically, 45 to 5 years before the interview. Cumulative exposure to THMs was estimated by combining individual year-by-year average THM level and daily tap water consumption. The resulting adjusted OR for men exposed to an average of more than 1 $\mu\text{g/L}$ THMs compared with those who had lower or no exposure was 1.24 (95% CI 1.09 to 1.41). Estimated relative risks increased with increasing exposure, with an OR of 1.44 (95% CI 1.20 to 1.73) for exposure higher than 50 $\mu\text{g/L}$. Similar results were found with other indices of THM exposure. Among women, THM exposure was not associated with bladder cancer risk (OR = 0.95; 95% CI 0.76 to 1.20) (Table I).

After Villanueva et al. (15) Rahman et al. (16) conducted a meta-analysis of colorectal cancer with 13 studies (3 cohort and 10 case-control studies). In this meta-analysis the effect measures were pooled by using random effects methods comparing the highest exposure category with the lowest one. For colon cancer, the pooled relative risk (RR) from cohort studies was 1.11 (95% CI: 0.73 to 1.70), the odds ratio (OR) from case-control studies was 1.33 (95% CI: 1.12 to 1.57), and the pooled estimate combining both study types, assuming OR as a close estimate of RR, was 1.27 (95% CI: 1.08 to 1.50). For rectal cancer, pooled RR was 0.88 (95% CI: 0.57 to 1.35), pooled OR was 1.40 (95% CI: 1.15 to 1.70), and the pooled RR combining both study types was 1.30 (95% CI: 1.06 to 1.59).

Reproductive Outcomes

Two meta-analyses by Hwang and Jaakkola (17) and Hwang et al. (18) reported evidence for an effect of exposure to chlorination by-products on the risk of neural tube, urinary system and ventricular septal defects, but risks for other anomalies were considered heterogeneous and inconclusive. However, the meta-analysis by Hwang and Jaakkola. (17) only included five studies and did not include more recent studies, while the study by Hwang et al. (18) generally focused on a few subcategories of anomalies, and also did not include the most up-to-date studies available. Nieuwenhuijsen et al. (19) conducted meta-analyses including all studies of DBPs and congenital anomalies. For all congenital anomalies combined, the meta-analysis presented evidence of an excess risk for high versus low exposure to water chlorination or TTHM (OR = 1.17, 95% CI 1.02 to 1.34), based on a small number of studies. The meta-analysis also suggested a statistically significant excess risk for ventricular septal defects (OR = 1.59 95% CI 1.21 to 2.07), but this was based on only three studies and there was little evidence of an exposure-response relationship. Other congenital anomalies did not show statistically significant excess risk. (Tables II and III).

Table II. Meta-analyses disinfection by-product exposure and congenital anomalies: highest versus lowest exposure

<i>Congenital anomalies</i>	<i>N*</i>	<i>OR** (95%CI)</i>
All	5	1.17 (1.02-1.34)
Neural tube	8	1.06 (0.89-1.26)
Major Cardiac	8	1.16 (0.98-1.37)
Ventricular septal	3	1.59 (1.21-2.07)
Respiratory	4	1.12 (0.91-1.37)
Cleft lip/palate	7	0.98 (0.88-1.08)
Urinary tract	4	1.33 (0.92-1.92)
Hypospadias	4	1.03 (0.84-1.28)

* N= number of studies. ** = highest versus lowest exposure category.

Table III. Meta-analyses of disinfection by-product exposure and congenital anomalies: total trihalomethane exposure

<i>Congenital anomalies</i>	<i>N*</i>	<i>OR** (95%CI)</i>
Neural tube	7	1.006 (0.950-1.065)
Major cardiac	5	0.993 (0.947-1.041)
Cleft lip/palate	5	1.003 (0.960-1.048)

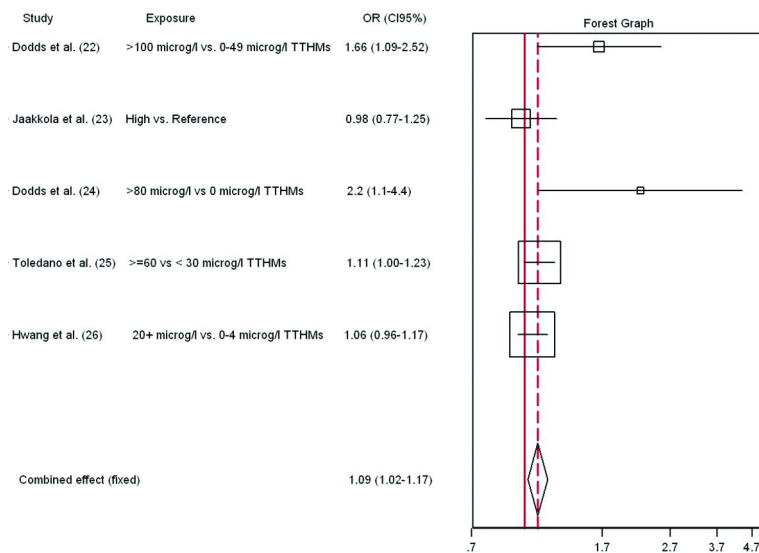
* N= number of studies. ** Per 10 µg/L increase in Total trihalomethanes.

After Nieuwenhuijsen et al. (19) Grellier et al. (20) conducted meta-analyses of total trihalomethane concentration and low birth weight (LBW), term low birth weight (TLBW), preterm delivery (PTD) and small for gestational age (SGA) (including intra uterine growth retardation (IUGR)). They found little or no evidence for associations between third trimester TTHM exposure and LBW (OR per 10 µg TTHM/L increase = 0.9999, 95% CI 0.9735 to 1.0270), TLBW (OR per 10 µg TTHM/L increase = 1.0337, 95% CI 0.9272 to 1.1525) or PTD (OR per 10 µg TTHM/L increase = 0.9896, 95% CI 0.9781 to 1.0013). They did find, however, evidence for an association with SGA (OR per 10 µg TTHM/L increase = 1.0100 95% CI 1.0006, 1.0194) (Table IV).

Table IV. Meta analyses of trihalomethane exposure and pre-term delivery, low birth weight and small for gestational age

<i>Exposure agent indicator</i>	<i>Exposure timing</i>	<i>Health outcome</i>	<i>Number of studies included</i>	<i>OR slope per 10µg/L</i>	<i>Lower CI</i>	<i>Upper CI</i>	<i>p-value</i>
Total THM	Third trimester	LBW	4	0.9999	0.9735	1.0270	0.9876
		PTD	6	0.9896	0.9781	1.0013	0.0814
		SGA	6	1.0100	1.0006	1.0194	0.0361
	Any exposure timing	LBW	5	1.0013	0.9747	1.0286	0.9267
		PTD	8	0.9894	0.9777	1.0007	0.0653
		SGA	8	1.0096	1.0009	1.0184	0.0309
	Entire pregnancy only	SGA	4	1.0105	0.9712	1.0514	0.6059

Table V. Meta-analyses of disinfection by-product exposure and stillbirth



After Grellier et al. (20) A meta-analysis of exposure to chlorination by-products and stillbirth conducted as part of this review chapter and presented in Table V showed a small excess risk of stillbirth (9%, 95%CI 2% to 17%) when comparing the highest with the lowest exposure groups in the studies. The study by Aschengrau et al. (21) was left out of this meta-analysis because it compared chlorination with chloramination, and including the study resulted in statistically significant heterogeneity. Including the study increased the summary estimate somewhat (15%, 95%CI 0-33%).

Aschengrau et al. (21) was excluded from the analyses because the reference group was chloraminated.

In Jaakkola et al. (23) the exposures are chlorination yes/no and colour (low, medium high)

Discussion

This review showed that various meta-analyses and pooled analyses have found statistically significant excess risk for some indicator of exposure to chlorinated water or trihalomethanes and bladder and colorectal cancer, small for gestational age, still birth, all congenital anomalies combined and ventricular septal defects, but no statistical significant excess risk for many other congenital anomalies. The excess risk was generally small, but robust, with little sensitivity to the results of individual studies or evidence of publication bias.

Epidemiological studies investigating exposure to disinfection by-products and health outcomes have been limited by a number of factors including the relative crude exposure assessment (perhaps with the exception of some of the more recent studies), small samples sizes, heterogeneity in some outcomes, and, to a lesser extent, potential for bias and confounding. The factors limiting the available meta-analyses and pooled analyses of this evidence base have been the lack studies on DBPs other than THMs and (explicit) inclusion of various exposure routes such as inhalation and dermal absorption. Furthermore, the number of epidemiological studies available has often been small which limits the possibility in meta-analysis of stratifying by study characteristics or carrying out meta-regression. These factors together may explain some of the mixed results, and possibly the lack of associations for some of the congenital anomalies. Furthermore, combined with the small number of studies included in the meta-analyses, these factors also reduce the strength of any conclusions that can be drawn from meta-analyses and pooled analyses. These analyses are therefore not meant to provide definitive conclusions on the subject, but instead serve as a useful tool to evaluate the current status of this growing body of research and to offer guidance for the way forward.

Crude exposure assessment leading to exposure misclassification or measurement error may bias the measures of effect towards the null. The use of ecologic water supply zone estimates as an exposure index may result in exposure misclassification (27). Also, cancer studies involve retrospective exposure assessment and have to go back many years in time, which makes it more difficult to get good quality exposure data. Furthermore, whilst ingestion has

generally been the primary exposure route of interest, uptake through showering, bathing and swimming could be considerable, specifically for THMs due to their volatility, but these routes have only been considered in a few studies, and not in the meta-analyses and pooled analyses. In addition, exposure estimates in many reproductive studies have been based primarily on maternal residence at birth. This ignores any exposure which occurs outside the home, e.g. in the workplace (28), and also ignores the possibility e.g. that a mother may have moved her household during her pregnancy (29, 30). Exposure assessment based on maternal residence at birth may, therefore, result in exposure misclassification.

On the whole, epidemiological studies have used THMs as a proxy for total DBP load, but THMs are not necessarily a good proxy measure. Some recent studies have therefore investigated other DBPs such as HAAs and mutagen X (MX) but for the meta-analyses and pooled analyses only THMs were available. The metabolism of different DBP species varies (5), so it is insufficient to analyse DBPs as a whole, or to use TTHM as a proxy. Investigation of the relation between non-THM by-products and the outcomes is required in order to help elucidate the specific DBP driving the associations observed. A detailed assessment of the DBP mixture is necessary to explain any observed epidemiological results.

In addition, studies from some countries, including Scandinavia, have generally shown low levels of DBPs with a small range, making the assessment of risks more difficult due to both a higher probability of exposure misclassification and a smaller difference in exposure between dose groups. Where seasonal variability in DBPs has not been taken into account further errors in the exposure assessment are likely.

Sample sizes have often been insufficient to produce robust results, specifically for congenital anomalies and, to a lesser extent, for stillbirth, and other outcomes. However, there are exceptions, for example, studies on SGA/IUGR, which provided sufficiently large numbers of cases to create various exposure categories with more robust risk estimates, which should improve the overall assessment of risk.

Some outcomes such as congenital anomalies have not been well defined and/or are difficult to study. Congenital anomalies have often been analysed either as one group or in main categories e.g. neural tube, major heart and abdominal defects, due to the small number of cases in each study. These anomalies, however, are generally heterogeneous with respect to both phenotype and presumed aetiology. Nieuwenhuijsen et al. (31) showed that focusing on isolated subcategories may produce different findings. Furthermore, in some countries, registration of congenital anomalies may occur up to one year after the birth (e.g. in Taiwan), which will improve the completeness of the registry, by including cases that are more difficult to identify at birth such as hypospadias.

The retrospective and registry based nature of many of the reproductive studies has meant that information on potential confounders, and other risk factors for birth outcomes, such as maternal smoking and alcohol consumption have often been lacking.

Investigation of gene-environment interaction and/or the effects on susceptible groups has been limited (e.g. (32–34)). Preliminary studies suggest that certain groups may be more susceptible to the influence of DBPs (35), and

thus these effects may be masked in studies which only look at the population in general.

A limitation of the presented meta-analyses and pooled analyses is the relatively small number of studies, and therefore the need to conduct relatively simple analyses, comparing high versus low exposure categories and combining what could be regarded as different and/or inconsistent estimates of exposure (e.g. high vs. low TTHM concentration and chlorination vs. non-chlorination). However, what in one study is a high level of THMs may be low in another study. While it is assumed that the meta-analysis is statistically accurate, the biological basis for comparing studies with this degree of heterogeneity in the definition of exposure is more problematic and this should be taken into account when interpreting the results. Ideally, all the exposure categories have the same cutoffs but in practice this is often impossible because of the different local conditions. The analyses of TTHM exposure-response relationships combined more comparable exposure levels and are therefore probably more informative, but could only be conducted for a few endpoints because of the lack of a sufficient number of studies. The question is here though whether TTHM may be the putative agent or a (not so good) marker for something else.

The Way Forward

Given the many studies that have been conducted and the limited evidence for a strong association between chlorination by-products and cancer and reproductive outcomes, we might ask whether there is a need to conduct more studies, and if so, what should they look at? Disinfection of drinking water is an important part of public health and many people are potentially exposed to chlorination by-products; ongoing surveillance of any possible adverse health effects is therefore warranted, even though the relative risks may be small. As mentioned above, the mixture of the by-products may differ by geographical area and by time, for example because of changes in water treatment methods. However, generally only indicator substances such as TTHM have been used to examine the health risks. It is important that we understand the underlying mixture of the by-products, both of existing and new studies, and where possible examine any possible health risks of specific DBPs or mixtures. There is a need to study any possible effects in more specific places, for example where we can examine the potential effects of certain mixtures such as brominated species. Places such as Perth, Australia or Barcelona, Spain may be suitable locations because of the high levels of brominated compounds in their drinking water.

Furthermore, it would be worthwhile examining the various exposure pathways and routes other than ingestion in more detail, specifically for volatile by-products such as THMs, since the level of exposure and metabolism may be different, and the measures for exposure prevention are likely to be different. The exposure assessment should also take into account the effect of post tap water processing methods (boiling, filtering, storing etc) on DBP concentration because filtering and boiling can substantially reduce some of the DBP concentration. For studies of cancer and congenital anomalies, this can probably only be done in case-control studies but these would have to be able to estimate exposure

during the most critical (early) periods of pregnancy, which may prove difficult. Prospective exposure assessment through a cohort design would be more ideal, but practical and financial constraints preclude such a study, as the size of the cohort would need to be extremely large to study rare diseases.

Regarding the outcomes, the focus of future studies should be on subcategories of congenital anomalies, rather than on the whole group, and should focus on anomalies for which the ascertainment is reasonably complete and consistent if registry-based designs were used. Findings for ventricular septal defects should be followed up, preferably in well-designed case-control studies. Furthermore, the study by Nieuwenhuijsen et al. (31) showed an excess risk for bromoform and gastroschisis and this may worth examining in more detail, and in a different population. One of the problems in this study was the low levels of bromoform in England and Wales, and therefore it should be examined in places where bromoform levels are higher (such as Perth).

Further work is needed on the relation between potential confounders such as smoking and alcohol intake and the relation with the by-products in the water and personal behavioural characteristics such as tap water ingestion (instead of bottled water), showering and bathing to examine to what extent confounding may explain findings for registry based studies where this information is missing.

Mechanisms of action of DBPs are not clearly understood. There is some suggestion that some chlorination by-products may interfere with folate metabolism, and this and other potential mechanisms such as oxidative stress and genotoxicity, could be examined with biomarkers in pregnant women to assess to what extent this may be possible. Furthermore, genotyping may identify susceptible populations (for example according to variants of DBP metabolising enzymes such as CYP2E1, GSTT1, etc.).

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Chapter 25

Impact of Point-of-Use Water Softening on Sustainable Water Reclamation: Case Study of the Greater Phoenix Area

E. N. Daugherty,¹ A. V. Ontiveros-Valencia,² J. S. Rice,¹ M. J. Wiest,¹
and R. U. Halden^{*,1,3}

¹School of Sustainable Engineering and the Built Environment,
Arizona State University, Tempe, AZ 85283, USA

²School of Sustainability, Arizona State University, Tempe, AZ 85283, USA

³Center for Environmental Biotechnology at the Biodesign Institute,
Arizona State University, Tempe, AZ 85283, USA

*Corresponding author: School of Sustainable Engineering and the Built
Environment, Arizona State University, 1001 South McAllister Avenue,
P.O. Box 875701, Tempe, AZ 85287-5701. E-mail: halden@asu.edu.

Point-of-use (POU) water treatment is becoming more widespread due to concerns over the quality of municipal drinking water, specifically the presence of pharmaceuticals and personal care products (PPCPs), organohalogen pesticides, excessive hardness and excessive concentrations of total dissolved solids (TDS) also known as salinity. This report investigated the unintended consequence of POU water softening - the issue of salt accumulation in sewer systems across the nation, which forces regulatory authorities to examine possible salinity management tactics to preserve future water supplies. In the Greater Phoenix Metropolitan Area, increasing salt concentrations are already a concern for many municipal water supplies. To assess current salinity conditions, the Central Arizona Salinity Study (CASS) was initiated to establish a salt balance within the Phoenix area and provide potential management solutions. The results of the CASS showed that the second largest contributor to the salt residual in Phoenix is society, which includes residential, commercial, and industrial sectors. A common and well known

way society contributes salt loading to wastewater is through POU ion-exchange water softeners. By expanding upon the research done by the CASS and narrowing the focus to a typical residential home, this report was able to quantify the amount of salts contributed by residential POU water softeners to wastewater. Salt residual and population data taken from the CASS were extrapolated to find the time period when the buildout condition for the Greater Phoenix area would occur. According to the CASS assumption, buildout would occur when the metropolitan population reached a maximum of 12 million people, as forecasted by the Maricopa Association of Governments (MAG). Assuming that salt residuals follow population growth trends, the buildout year was calculated to occur between 2065 and 2085, depending on population growth dynamics. Based on the known amount of salt released by POU water softeners and the calculated number of households with a water softeners, the annual salt contribution was calculated. The calculations predict annual salt loadings to wastewater from water softening to be approximately 45,500 tons in the year 2000; 142,200 tons in the year 2040; and 192,300 tons at buildout of the City of Phoenix. For the year 2000, the amount of salts contributed by water softeners alone accounted for 38.5% of society's salt contribution and 3% of the total salts entering Phoenix. For the year 2040, the amount of salts from water softeners are projected to account for 49% of society's contribution and 8% of the total salts entering Phoenix. At buildout, 41% of society's contribution and 8.6% of the total salt loading will come from water softeners. These calculated amounts of salt from water softeners are due only to the population increase. In actuality, these numbers will be even larger as they do not account for additional water softeners purchased for pre-existing homes that will be used to treat the increasing concentrations of TDS.

Keywords: salinity; sustainability; TDS; total suspended solids; water reuse

Introduction

The effects of increasing salinity levels in the soil and groundwater in the arid southwest has been well documented over the past 30 to 40 years. Today, increasing total dissolved solids (TDS) or salinity levels across the United States are not only affecting agricultural productivity but the quality of future surface water and groundwater supplies as well. Studies performed in the northeastern region of the U.S. have established a positive relationship between the amount of impervious surfaces to increasing salinity levels caused

by de-icers that are used to prevent ice formation (1). In the southeastern U.S., salt accumulation in river estuaries has negatively impacted the development of blue crab populations (2). Such research has shown that increasing TDS concentrations across the nation are creating significant problems to ecosystems and potential, future, potable water supplies. Furthermore, the issue of emerging contaminants such as pharmaceuticals and personal care products (PPCPs), has lead the general public to behavioral changes favoring POU water treatment systems for removing undesirable water contaminants. While popular and heavily marketed, water softeners do not treat emerging contaminants such as PPCPs or organohalogen pesticides and only contribute additional salts to local sewer systems. Although the issues related with salt addition have been identified, many local governments are still struggling to find effective and economically feasible solutions that can be implemented into the existing water system. One example is California's Assembly Bill 1366 that would allow the local government to control the amount of salts released by self-regenerating water softeners (3). Local water and wastewater managers agree with the bill stating that their plants were not made to remove salts and that the amounts released by water softeners increases operational costs and pollutes downstream water sources (3). To further investigate the effects of salt addition to wastewater, this paper will examine the contribution of point-of-use (POU) water softening systems to the increasing TDS levels and degradation of reclaimed water using Phoenix, Arizona as a case study.

In December of 1948, Central Arizona Project (CAP) officials recognized the problem of salt accumulation in the Phoenix metropolitan area and stated that, "...eventually it will be necessary to release salt-laden water from the Maricopa and Pinal units to maintain a salt balance in those areas" (4). However, it was not until recently when city officials began to notice a significant increase in salinity levels in groundwater and surface water that salinity became an important consideration in municipal water supply and infrastructure planning. To evaluate the salinity issues in Central Arizona, the Central Arizona Salinity Study (CASS) was initiated by the United States Bureau of Reclamation (USBR) and the Sub-Regional Operating Group (SROG) consisting of the cities of Phoenix, Mesa, Scottsdale, Glendale, and Tempe. Together, these cities jointly operate the 91st Avenue Wastewater Treatment Plant located in Phoenix, Arizona, where SROG recently confirmed the increasing trend in TDS concentrations of the treatment plant's effluent. The goal of the ongoing CASS study is to evaluate and understand the issue of salinity in order to develop options that will provide a quality, cost-effective, sustainable, and reliable water supply (5). The information provided by the CASS is the basis for the calculations performed in this report.

According to a salt balance performed by the CASS on the Phoenix metropolitan area, the primary source of imported salts is the Central Arizona Project (CAP) and natural waterways such as the Salt River Project (SRP) and other rivers which collectively introduced 1.32 million tons of salt for the year 2000 (5). The second largest source for salt accumulation in Phoenix is TDS disposal into the sewer system by society. The category of society as defined by the CASS includes residential, commercial, and industrial entities. The numbers for the society category are based on population data provided by the Maricopa Association of Governments. For the purpose of this report, only the residential

sector will be examined. Residential homes can contribute to TDS in numerous ways including the disposal of food waste, cleaning chemicals, paints, detergents, degreasers, and through appliances such as water softeners (6). After society, agriculture is the next largest source but contributes very little to the overall amount of salt. The salt balance found that only a small fraction of this salt is removed naturally via the Gila River, resulting in 1.1 million tons of salt being retained in Phoenix each year. This information adapted from the CASS along with the percent contribution is presented in Table I below.

Table I. Salt Balance on Phoenix, AZ in Year 2000. Adapted from CASS (5)

<i>Entering Phoenix Metro^a</i>	<i>TDS^a (mg/L)</i>	<i>Salt (tons)^a</i>	<i>Percent Contribution</i>
Groundwater	680	34,218	2.34%
Salt River Project (SRP)	480	528,768	36.14%
Central Arizona Project (CAP)	650	664,768	45.44%
Gila River	550	67,320	4.60%
Agua Fria River	400	27,200	1.86%
Society	300	118,320	8.09%
Agricultural Fertilizer		17,800	1.22%
Turf Fertilizer		4,700	0.32%
TOTAL		1,463,094	
<i>Exiting Phoenix Metro</i>	<i>TDS (mg/L)</i>	<i>Salt (tons)</i>	<i>Percent Contribution</i>
Groundwater	1,100	41,888	11.50%
Gila River	2,370	322,320	88.50%
TOTAL		364,208	
Residual Salt Load		1,098,886	

^a Values taken from the CASS Study (5).

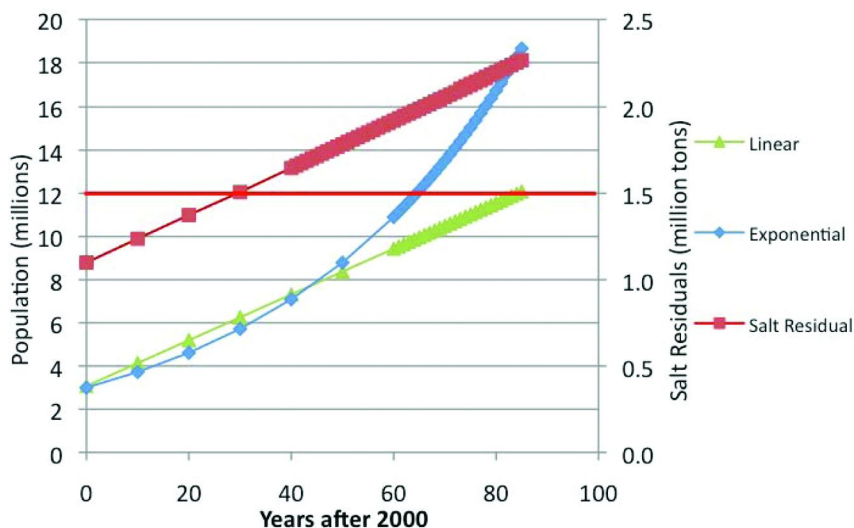


Figure 1. Graph of projected population trends and salt residual levels over time in the Phoenix area. (see color insert)

The data presented in Table I are for the year 2000 and the CASS predicts that the residual amounts of salt in Phoenix will only increase over time. Projected estimates shown in Figure 1 display salinity residuals in the Phoenix area from the year 2000 until the population reaches its “buildout” capacity at a population of 12 million people. Based on population numbers provided by the Maricopa Association of Governments (MAG) for the years 2000 and 2040 (5), the data were extrapolated in exponential and linear trends to predict buildout to occur in 2065 or 2085, respectively. This approximate range is the result of many factors, including population growth rates, economic factors, and regulated salinity management practices.

Given recent developments in the Southwest economy, the linear population model likely represents the more likely scenario. According to the linear projection, Figure 1 shows that the amount of salt residuals will increase from 2000 to 2040 by 386,844 tons, resulting in an annual increase of about 9,670 tons per year. By the time the buildout occurs, the salt residual will reach a maximum of approximately 2.2 million tons of salt in the year 2085, again based on the linear extrapolation.

For the Phoenix metropolitan area, the effects of salt accumulation can be seen in the comparison of recent TDS levels in CAP and SRP source waters to the reclaimed water from wastewater treatment plants shown below in Figure 2. This graph shows that the range of minimum and maximum TDS concentrations in reclaimed water surpasses those found in source waters.

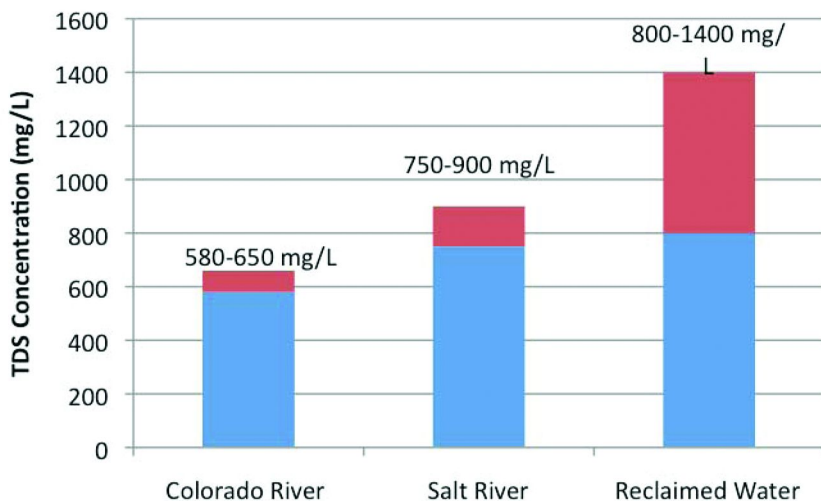


Figure 2. Comparison of recent TDS concentration ranges in select Phoenix-Area sources adapted from CASS (5). Shown are the minimum (blue) and maximum (red) values for the TDS range found in the three water types. (see color insert)

This illustrates that additional salts generated from society within Phoenix are being discharged directly into the sewer system at a markedly high and unregulated rate (5).

In addition to the degradation of source water, other consequences of importing and retaining large amounts of salt can be experienced by all areas of society including residential, commercial, and industrial. Besides the aesthetic effects, source waters with high TDS concentrations can result in high “avoidance costs” for homeowners as well as commercial users (5). These costs include the purchasing of bottled water and water softeners such as under-the-sink reverse osmosis (RO) units. High salinity also reduces the life of water-utilizing home appliances, plumbing, and the efficiency of detergents. Water softening on the commercial scale also increases the costs for goods and services and can discharge anywhere from 1,000 to 500,000 pounds of salt per year depending on the size and type of service building (6). Golf courses that depend on reclaimed water also incur the consequences and costs of treating high salinity effluent from wastewater treatment plants. Such water with higher sodium levels creates a semi-impermeable layer within the soil resulting in poor infiltration. Maintenance of golf courses has been achieved, but only at the cost of increased chemical use and labor (5). Other serious effects of high TDS levels includes the degradation of environmental habitats from the discharge of wastewater treatment plant effluent; the reduced efficiency of industrial processes leading to increased energy consumption; and the decreased productivity of agriculture due to increased salt loading; and the possibility of rendering groundwater unusable for urban expansion (5). Although the current impacts of increased salinity levels are subtle, they are expected to increase exponentially with the population in the near future due to increased salt contribution.

The Phase II of the CASS Report (7) explored various strategies for salinity management in central Arizona. The Planning Subcommittee was assigned the task to see where salinity management strategies could be applied the most effectively and economically. The results of the subcommittee were as follows:

- Continued funding for the Colorado River Basin Salinity Control Program (CRBSCP) which reduces salinity in the Lower Colorado River Basin States and Mexico.
- Large scale desalination plants along the CAP and SRP or at existing water treatment plants.
- Desalination or other “advanced water treatment” of wastewater effluent and/or brackish groundwater (7).

While these options are all possible, the high operating costs and brine management from desalination plants must be considered.

Another proposed idea was to limit the amount of salt entering the water system and establish a public outreach program that would host salinity awareness and water softener efficiency campaigns. These campaigns will focus on educating residents on how salinity introduced by water softeners can limit water resources and offer suggestions on how to have a more efficient water softener (7). This initiative, which is similar to what is being done in California, show that much consideration is being placed on the amount of salts released by water softeners (3). To further examine the impacts of water softeners, the following section will briefly describe the mechanisms and chemistry behind ion exchange water softeners.

A. Ion-Exchange Water Softeners

Arizona is one of the states with high levels of water hardness (8) which can reach 20 grains per gallon (gpg) (1gpg = 17.1 mg/L) (9). High concentrations of calcium (Ca^{2+}) and magnesium (Mg^{2+}) in heated household water can generate problems such as calcium carbonate scaling in appliances. This leads to the decrease of effectiveness and performance of water appliances, and produces undesirable economic costs. Hard water can also make cleaning challenging since it reduces the ability to remove dirt and grime. Therefore, the ion exchange water softening offers a solution to treat hard water.

Chemistry behind Ion Exchange Water Softeners

Water softeners are mainly constituted by two tanks: a mineral and a brine tank (Figure 3). The ion exchange resins (also called zeolites) are placed in the mineral tank. These resins are made of polymers with specific chemical functional groups that carry negative charges that will attract positive charges. The resin is typically coated with positive ions such as sodium (Na^+) or potassium (K^+) prior to the softening process. The brine tank contains the brine solution which is composed of the Na^+ or K^+ and water. Although the Na^+ and K^+ ions have a

positive charge similar to calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions that causes them to be attracted to the exchange resins, the strength of the Na^+ or K^+ charges is less than those of Ca^{2+} and Mg^{2+} ions. Therefore, as water containing calcium and magnesium enters the water softener, the Na^+ and K^+ ions on the resin are replaced with the Ca^{2+} and Mg^{2+} and are released into the water that supplies the house (Figure 3).

Once the resins become saturated with Ca^{2+} and Mg^{2+} , the system begins a regeneration process. This process starts with a backwash of the mineral tank to remove dirt. A recharge phase follows the backwash and flushes the brine solution into the mineral tank. The high concentration of Na^+ and K^+ from the brine causes the removal of Ca^{2+} and Mg^{2+} ions from the saturated resins and replaces them with the Na^+ and K^+ ions. The Ca^{2+} and Mg^{2+} ions that were replaced are then disposed of into the sewer system along with the excess brine (9).

The softener's stated capacity is established by grains of hardness that are removed before the resins become saturated. Frequency of the regeneration process is dependent on the water hardness, water flow, and size of water softener equipment and capacity of the resins (10).

Sodium levels in softened water increase approximately 8 mg/L for each grain of hardness removed. For instance, after the removal of 12 grains per gallon of hardness, the treated water's sodium content will have increased 96 mg/L (10).

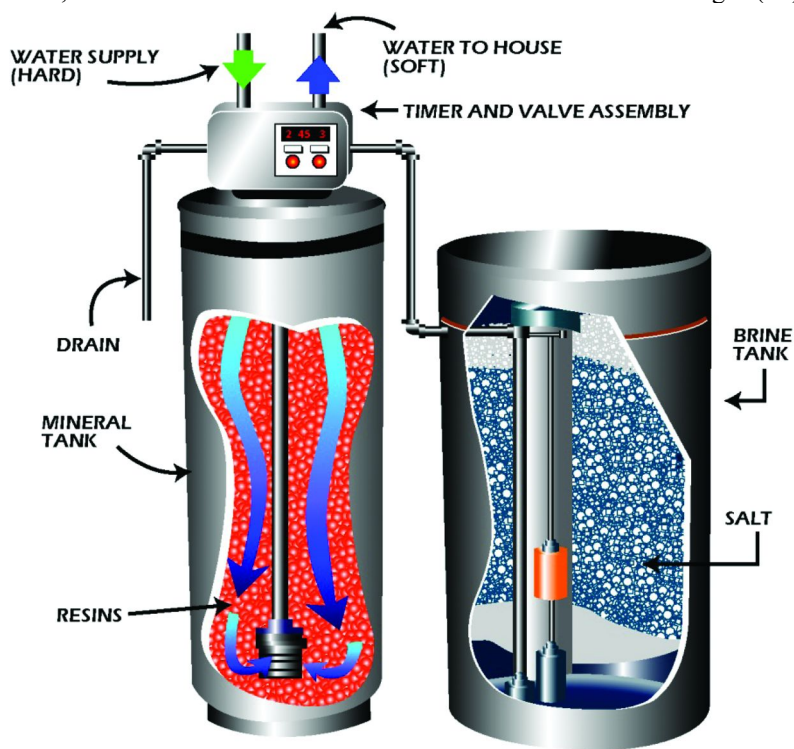


Figure 3. Typical household water softener. Adapted from Phase 2 of the Central Arizona Salinity Study (6). (see color insert)

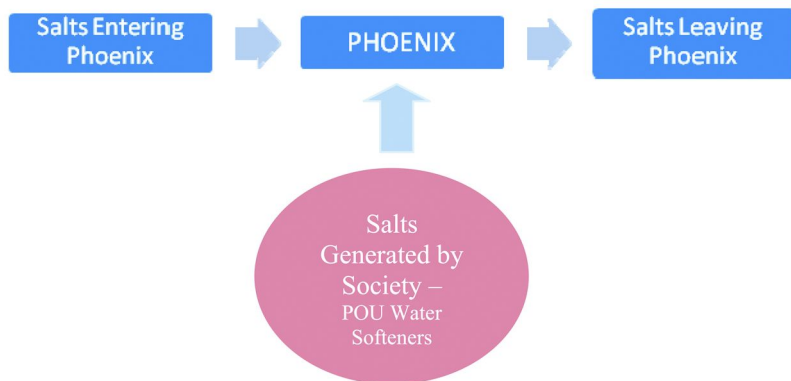


Figure 4. Schematic model of the salt balance in Phoenix. (see color insert)

As mentioned above, POU water softeners directly release large amounts of salt into the sewer systems, thereby increasing the total TDS concentrations in wastewater treatment plant effluent by 300 – 500 mg/L higher than those concentrations of the potable water supply. Furthermore, these large amounts of salt are directly responsible for the degradation of surface and ground water. However, regulations on water softeners should not be made until more is known quantitatively about their salt contribution. In the following, the effects of POU water softening and the amount of salt released as a percentage of society's overall contribution, as presented by the CASS for Phoenix, Arizona, will be investigated.

Methods

This study was based on the findings of the CASS performed in 2003. The CASS provided a base model for the amount of salts entering and leaving Phoenix as shown by the square boxes in Figure 4. The calculations in this study expanded upon the CASS model by determining the amount of the salt residual that is generated by POU water softeners as shown by the circle in Figure 4.

Specifically, the contribution of POU water softeners was quantified to provide insight into how much salt could be eliminated through the regulation of water softening systems. In order to do this, a mass balance was created around a typical POU, ion-exchange water softener. Knowing that TDS is not treated in wastewater treatment plants, the amount of salt released by the water softener was assumed to directly contribute to the amount of TDS leaving the waste water treatment plant.

To calculate the amount of salt (TDS concentrations) that is generated by POU water softeners, population estimates from the Maricopa Association of Governments (MAG) were taken from the CASS study for the years 2000, 2040, and at "buildout" (2065-2085) (5). According to demographic information from the year 2000, the average number of people per household in Phoenix was three (11). This number was then used to determine the number of households in Phoenix by dividing the population for each time period by three. Additional

information from the CASS survey showed that 26% of homes built prior to the year 2000 and 51% of homes built after the year 2000 possess water softeners (6). Taking the amount of households in Phoenix and applying these percentages yielded the total number of water softeners in Phoenix for each specified year.

The next step was determining how much salt a typical, residential POU ion-exchange water softener uses per year. In order to quantify this amount a few assumptions were made. The first assumption was the average regeneration cycle of a water softener which was found to occur every 4000 gallons or 15,140 Liters (13). For each cycle, a common water softener utilizes 2.5 to 8 pounds (1.1 to 3.6 kilograms) of salt per regeneration averaging a salt contribution of 5.25 pounds (2.4 kilograms) per regeneration (14). In addition, the CASS assumed that for the years 2040 and at buildout (2065-2085) the water usage demand would need to be regulated to accommodate the increasing population. This decreased the water usage for 2040 from 238 gallons per capita per day (gpcd) to 200 gpcd, and from 200 gpcd to 150 gpcd at buildout (5, 12). Based on these assumptions, the total salt contribution from POU water softeners for all the households in phoenix was calculated.

Table II. Number of Water Softeners per Household*

<i>Year</i>	<i>2000</i>	<i>2040</i>	<i>Buildout</i>
Population	3,072,149	7,326,000	12,000,000
Households^a	1,024,050	2,442,000	4,000,000
Number of Water Softeners Based on Population Growth	266,253	990,000	1,784,000
Water Demand per Person - L/cap/day (gal/cap/day)	900 (238)	757 (200)	568 (150)
Water Demand per Household - L/home/day (gal/home/day)	2767 (714)	2325 (600)	1747 (450)
Water Demand per Household - L/home/year (gal/home/year)	1,009,864 (260,610)	848,625 (219,000)	636,469 (164,250)
Household Regenerations per Year	65	55	41
Household kilograms of Salt per Year (pounds)	155 (342)	131 (278)	98 (216)
Total Contribution of Household Metric Tons of Salt per Year (Tons)	41,301 (45,536)	129,050 (142,000)	174,412 (192,000)

* See methods section for calculation. ^a Based on 3 people per household.

Results

The values for the number of water softeners with and without considering the growth of sales for each year are summarized below in Table II.

To see how this amount compares with the total salts entering, leaving, and remaining in Phoenix, a summary is presented below in Table III.

Figures 5, 6, and 7 show the percentage of society's salt contribution which is the result of POU water softener usage for the years 2000, 2040, and at buildout. As the figures below indicate, the replacement of water softeners with other alternatives that use less or even no salt would enable the salt contribution to be significantly reduced.

Table III. Mass of Salt Contributed by Water Softeners as Portion of Society

<i>Year</i>	<i>2000</i>	<i>2040</i>	<i>Buildout</i>
Salt Entering Phoenix (tons) ^a	1,463,094	1,849,938	2,229,012
Society's Input (tons) ^a	118,320	290,333	472,226
Input from Water Softeners (tons)	45,536	142,000	192,000
Salt Leaving Phoenix (tons) ^a	364,208	203,048	41,888
Salt Residual (tons) ^a	1,098,886	1,646,890	2,187,124

^a Taken from the CASS.

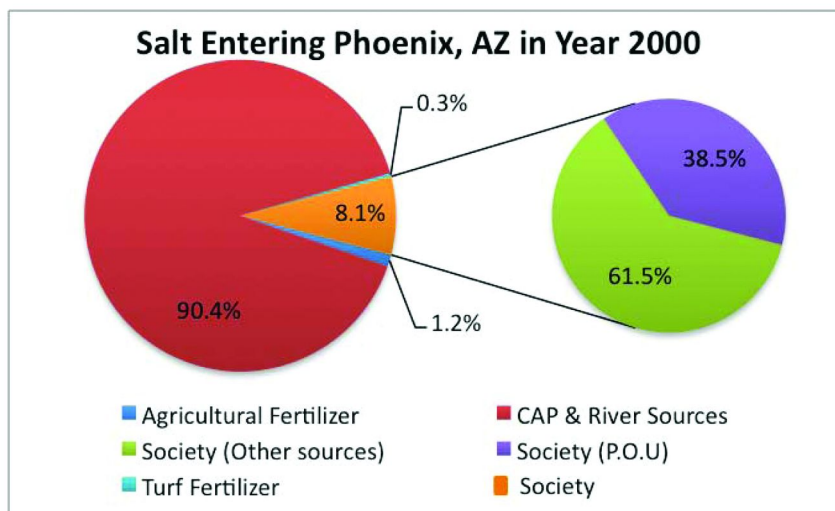


Figure 5. Amount of salts from point-of-use water softeners expressed as a percentage of society's salt contribution for the year 2000. (see color insert)

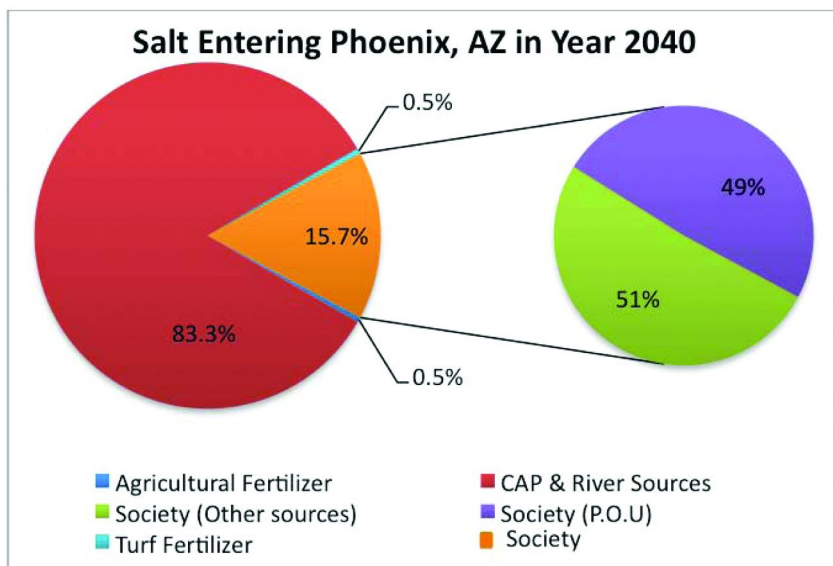


Figure 6. Amount of salts from point-of-use water softeners expressed as a percentage of society's salt contribution for the year 2040. (see color insert)

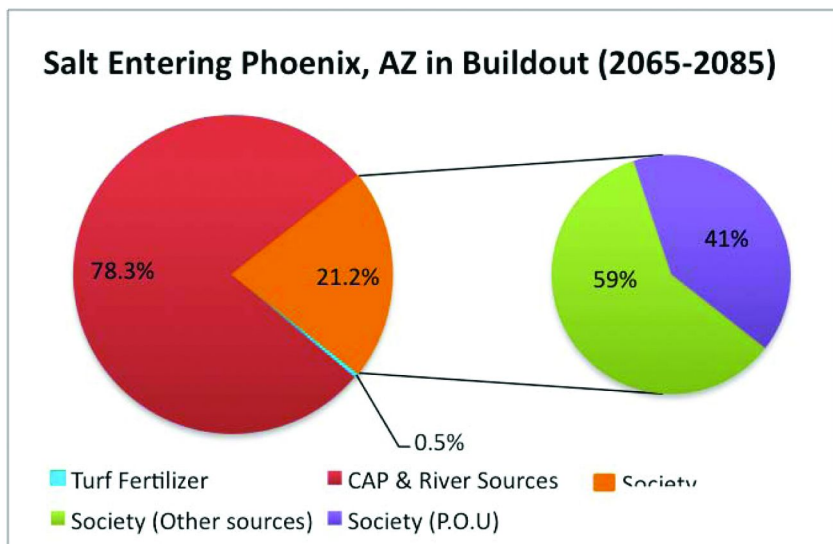


Figure 7. Amount of salts from point-of-use water softeners expressed as a percentage of society's salt contribution at buildout of the City of Phoenix (2065-2085). (see color insert)

Discussion

Water reclamation is an important process for areas such as Phoenix where water resources are scarce. Reclaimed wastewater is utilized for a variety of applications including irrigation, cooling, groundwater recharge, and wildlife habitat restoration. The viability of reclaimed water depends on the quality of the treated wastewater. As the TDS levels continue to increase, wastewater treatment plants will need to consider additional treatment options such as reverse osmosis desalination in order to maintain the quality of reclaimed water for reuse.

As a temporary alternative to centralized desalination, the limitation of TDS disposal into the sewer system by society is another option that can help reduce the amount of TDS released from wastewater treatment plants. As previously mentioned, some communities are currently looking at regulating POU water softeners as a way to decrease salt loading. As shown in the results, the amount of salts that are added by POU ion-exchange water softeners to Phoenix wastewater make up a substantial portion of society's total contribution. In 2000, salts from water softeners made up 38.5% salt from society, and in 2040 the projected amount will increase to 49%, about half of all of society's salt contribution. However, at buildout it was predicted that it would decrease to 41% as a result of regulated decreased water usage by law. In regards to the total amount of salt entering Phoenix, the primary source is still the Central Arizona Project (CAP) and Salt River Project (SRP) source waters. However, the amount of salts from water softeners as a percentage of the total salts in Phoenix is expected to increase over time. In 2000, water softener salts made up approximated 3% of the total salts, and in 2040 and at buildout it was estimated to increase to 7.7% and 8.6%, respectively. Although this amount may seem small, it accounts for millions of tons of salt that is being disposed of into the Phoenix sewer systems. The regulation of ion-exchange water softeners could eliminate the contribution of this salt thereby reducing the costs of additional treatment that will ultimately be funded by tax payers.

As previously noted, these numbers were calculated based on peer-reviewed specific assumptions made by the CASS. For the year 2040, when the population is estimated to be double its current amount, water from the CAP is assumed to be used more for municipal purposes than agricultural, and even more water from CAP will need to be imported or leased from Native American tribes which in turn will increase the amount of salts imported into Phoenix (5). At buildout, it was further assumed that Phoenix would be importing the maximum amount of CAP water (containing additional salt) and that new water sources would also need to be utilized as well. No water would be used for agricultural purposes and no water would be wasted or released via the Gila River (5). These assumptions reveal that the issue of salt accumulation within the Phoenix area will be an increasingly serious issue that will need to be addressed in the near future. While desalination may be a valid option, this report suggests that limiting the amount of salts released by society, specifically through the use of water softeners, would also help reduce the salt loading on reclaimed water.

Phoenix neighbor, City of Scottsdale, has already invested in a reverse osmosis treatment system at the Scottsdale Water Campus for aquifer recharge.

In order for water to be injected in deep wells or directly into the aquifer, the wastewater must be extensively treated to drinking water quality and conditioned to be compatible with native groundwater. The water moves through the soil in the aquifer and is then reused once it reaches an active well. Pre-existing wastewater treatment plants in the Phoenix area, however, do not have the luxury of incorporating reverse osmosis treatment. In cases such as the Cave Creek WWTP, discharged effluent can contain TDS levels as high as 1200 mg/L, 36% of which comes from residential salt addition (5, 6).

The limit of TDS concentrations in reclaimed water that Phoenix can sustain is a function of crop salinity tolerance, groundwater salinity levels, and wildlife adaptability. Wastewater treatment plants in the Phoenix area discharge an average concentration of TDS of 1000 mg/L and the maximum TDS concentration in effluent has gotten as high as 1400 mg/L at the 91st Avenue WWTP (7). These TDS concentrations include an average of 650 mg/L contributed by source water, which shows society's contribution to be an average of 350 mg/L. Most golf courses desire a TDS level of no more than 1000 mg/L to prevent damage to their grass. Turf grasses tolerate no more than 450 mg/L TDS (7, 16). For salt-sensitive plants, the level where TDS causes severe damage is at 1920 mg/L (7). Society currently contributes 300 to 500 mg/L to the wastewater and as the population grows, so will the TDS levels in wastewater discharge. Once it reaches the point at which the water can no longer be considered potable, the water will have to be desalinated before it can be reused.

The annual salt accumulation resulting from residential POU ion-exchange water softeners has both environmental and economical effects. It is gradually inhibiting water reclamation initiatives and costing society money to counteract the effects of high salinity water (5, 7). In order to alleviate environmental and financial setbacks caused by increasing salinity levels, alternative no-salt water conditioning devices can be used, centralized desalination can be implemented, or Phoenix can regulate salinity by establishing a Maximum Contaminant Level (MCL) for TDS. Those that choose not to use alternative softening methods and surpass the MCL can be charged a monetary fine. The money collected could be used toward centralized desalination or other techniques used for mitigating damages done by salt.

Alternative conditioning devices are available that succeed in not adding to the total TDS in wastewater. Some are more established in the mainstream of products such as reverse osmosis membranes while others are emerging technologies that hope to eliminate the impacts of water softeners on increased salinity levels in wastewater. These devices function using one of two possible mechanisms: 1) physical removal of scale-causing minerals using a filter or electrode assembly, 2) induction of bulk precipitation in which the scale-forming minerals precipitate in the bulk solution rather than on the surface of piping, heating elements, or other appliance components.

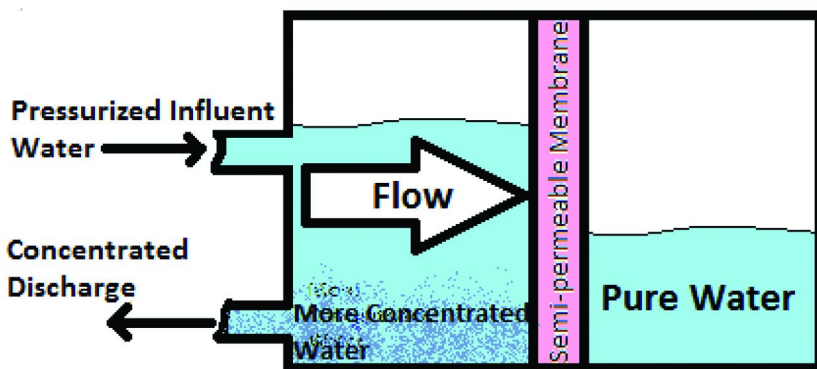


Figure 8. Dynamic cross-section of a reverse osmosis unit adapted from (17).
(see color insert)

A. Reverse Osmosis

Unlike ion-exchange water softeners, reverse osmosis (RO) does not add TDS to the sewer. The water is pressurized through a semi-permeable membrane which does not need regeneration; it is just replaced after a certain volume of water has been treated. The purified water that makes it through the membrane is called permeate or recovered water. The water that does not get through the membrane is very high in salts and other impurities and is called rejectate, concentrate, or brine. The brine is sent down the drain. The brine disposed of by the RO unit is salinity that already existed in the tap water supplied by drinking water treatment plants (17). Figure 8 is a schematic of a reverse osmosis system.

Some POU reverse osmosis systems can waste a lot of water due to their very low recovery rate of 5-15%. For every gallon of recovered water, 8 gallons or more are wasted down the drain (17).

B. Capacitive Deionization Technology

Capacitive deionization is a technology that removes salts and other impurities from water by pumping the water through an electrode assembly. The ions in the water are attracted to the opposite charge and accumulate at the electrode. Regeneration once the electrodes are at capacity is simply performed by reversing the charge and backwashing the impurities down the drain (18). Figures 9 and 10 illustrate how a capacitive deionization system works and how it is regenerated.

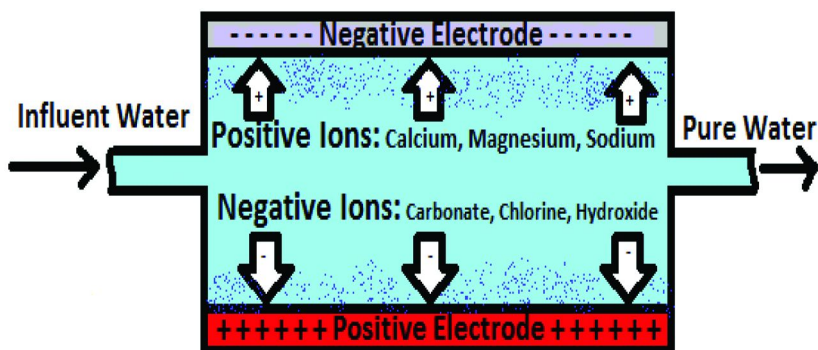


Figure 9. Schematic of a capacitive deionization system during normal operation (modified from (18)). (see color insert)

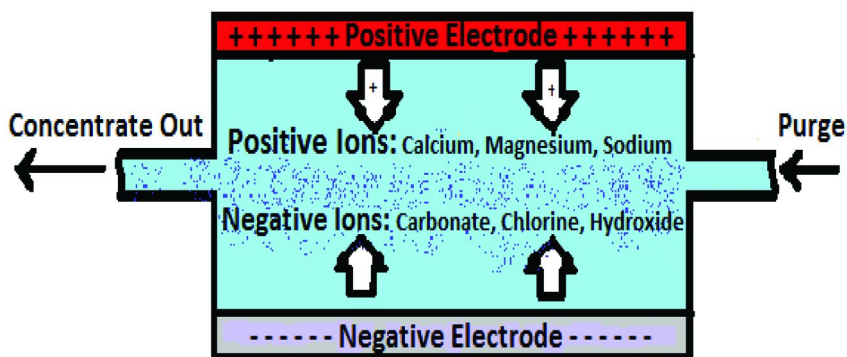


Figure 10. Schematic of a capacitive deionization system during regeneration (modified from (18)). (see color insert)

Similar to reverse osmosis, there is no added salt in the regenerative process and the scale forming minerals are physically removed. This technology uses lower pressures and energy than reverse osmosis but is more effective for lower TDS levels. Reverse osmosis is more effective at TDS levels of above 6000 mg/L which is much higher than the ~200 mg/L that normally comes out of drinking water treatment plants. It is effective for the TDS levels in drinking water in Phoenix (18).

C. Template Assisted Crystallization

Template assisted crystallization uses a template (treated polymer beads) to assist in the crystallization of salts by converting dissolved hardness into microscopic crystals. Once these crystals are of a size that is relatively insoluble they are released into the water (19). Figure 11 shows an illustration of these beads collecting hardness and then releasing the crystals.

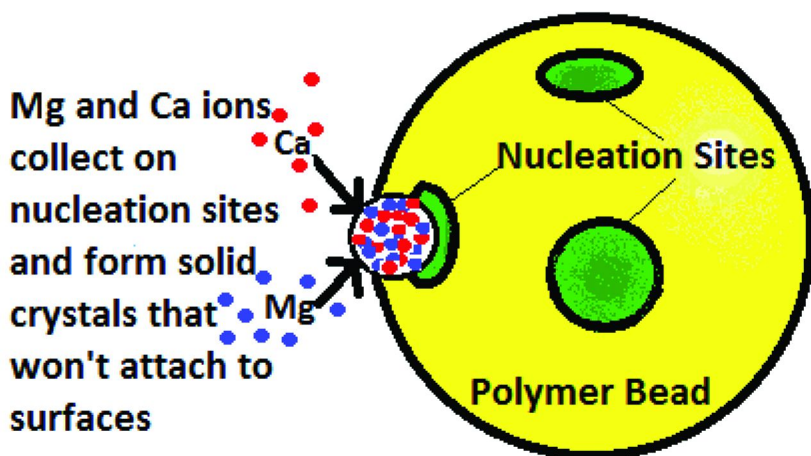


Figure 11. Hardness drawn to nucleation sites in template assisted crystallization creating seed crystals that are then released into the water. Adapted from (19). (see color insert)

Table IV. Comparison of alternative water conditioning methods

<i>Technology</i>	<i>Efficiency</i>	<i>Cost</i>
Reverse Osmosis	50% recovery rate 79% reduction of TDS (20)	Capital: ~\$300 O&M: \$129/year (20)
Capacitive Deionization	80% recovery rate 76% reduction of TDS (21)	Unavailable
Template Assisted Crystallization	100% recovery rate 83% reduction of TSS (Total Suspended Solids) (19)	Capital: ~\$2000 O&M: Media replaced every 3 years (19, 22)

The released crystals do not build up as scale on water boilers and other appliances. The water is not necessarily softened because the hardness is still in the water but the outcome is the same as that of softening. This option induces bulk precipitation and requires no chemicals or backwashing and does not add to the total TDS in wastewater (19).

D. Comparison of Alternative Treatment Devices

The salt-free ion-exchange alternatives can be directly compared technically and economically using existing literature on the performance of these devices. Table IV below is a summary of the average efficiency and cost of each alternative. Reverse osmosis and capacitive deionization are technologies that physically

remove ions from the water, whereas template assisted crystallization aids in bulk precipitation of ions that form scale.

E. Centralized Desalination

Centralized desalination of the effluent water from the wastewater treatment plants gives Phoenix more control over the destination of salt. The concentrated brine can be disposed of rather than spread out over agriculture fields and deposited into underground aquifers. Centralized desalination is currently being used in Scottsdale in their water remediation efforts. The treated wastewater passes through microfiltration and reverse osmosis before being used for irrigation or groundwater aquifer recharge.

Recovery efficiency and brine disposal are the two main problems that need to be dealt with when using centralized water softening. Recovery is the percentage of water filtered versus the total amount of water used. If 85% of the total amount of water is recovered for use, the other 15% is wasted in a highly concentrated brine solution and must be disposed of. Fifteen percent can be a lot of water considering about 700 millions of gallons get treated for municipal use in Phoenix every day (12).

The brine produced by desalination can be beneficial for a variety of uses. These include: irrigation of salt tolerant crops such as cotton or alfalfa; supplements for animal dietary needs, fertilizers (mainly potassium salts); soil conditioners for remediation of sodic and acidic soils; fire retarding and proofing chemicals; manufacture of magnesium oxide and magnesium metal; manufacture of light-weight and fireproofing building products; manufacture of plastics, paint, ink, and sealant products; dust suppression; stabilizers for road base construction and salt for deicing roads; flocculating agents for water/wastewater treatment; and various applications in food and chlor-alkali industries (23).

In order to get a sense of the cost of centralized desalination in Phoenix, consider the centralized desalination plant that was built in Yuma, Arizona in 1992. This plant cost the state \$280 million (24). It was forced to shut down due to floods and design flaws. The cost to put this plant back in to operation is \$30 million to get it back on line and an additional \$30 million per year. This plant has the capacity to produce 25 million gallons of desalted water per year, or 0.07MGD, a small fraction of Phoenix's six water treatment plants with a combined production capacity of over 700 million gallons per day (12). The high production cost for a significant desalted water production is the main reason the plant has not been put back into operation. Note that this plant desalts the water using a thermal process which consumes a high amount of energy. To desalt the water using reverse osmosis, the cost would be \$1.50 to 2.50 per 1,000 gallons (3,785 Liters) treated (25). To reduce TDS in drinking water before it is supplied to homes in order to eliminate the need for water softeners and ensure the reusability of the wastewater with reverse osmosis, 20% could be treated then mixed. This would result in an approximate 20% reduction of TDS. The cost would be \$0.38 million per day or \$141.8 million per year considering the fact that Phoenix treats 777 million gallons per day of drinking water (12). Centralized desalination has a very high

cost associated with it but if society continues to degrade source water by raising the TDS levels in wastewater, centralized desalination may be needed.

F. Salinity Regulation

Establishing a maximum contaminant limit (MCL) for TDS might help to alleviate the increase of salt from residential wastewater that has been produced with water softeners. New laws, methods and restrictions on sanitary sewer disposal could keep the salinity concentration in effluent stable (7). In fact, the industry of water softeners is self regulated and there are no federal, state or local laws that control this industry. The National Sanitation Foundation (NSF) and the Water Quality Association (WQA) provide the guidelines to certify these equipments concerning their effectiveness, marketing and performance but further considerations are in order for these devices (10).

Corresponding to drinking water guidelines, the World Health Organization states that “the palatability of water with a TDS level of less than 600 mg/L is generally considered to be good; drinking-water becomes significantly and increasingly unpalatable at TDS levels greater than about 1000 mg/L” (26). On the other hand, the US Environmental Protection Agency has determined a secondary maximum contaminant level (SMCL) of 500 mg/L for TDS, but it must be emphasized that a SMCL is not enforceable (27). Since high concentrations of TDS are not considered to represent a health risk for the population, this might be influencing the lack of an MLC (27).

However, this paper has portrayed that the salt contribution from water softeners to wastewater has negative effects on the treatment of wastewater and reclaiming processes. Hence, the necessity to implement specific limits for water softeners' salt contribution is not in question. Agricultural and drip irrigation uses for wastewater might have to be stopped because high levels of TDS adversely impact the growth and survival of salt intolerant plant species. For instance, a golf course might neglect the use of wastewater with 1000 mg/L of TDS since the turf's growth is more difficult to be accomplished and extra water is needed to flush out the excess of salt from the root zone. Another issue presented by high levels of TDS is the mineral deposit formation on municipal and household pipes and fixtures. The build-up of salts on cooling towers dramatically reduces the number of cycles and potentially damages the systems. There are multiple reasons not only environmental but also economical to control the levels of TDS in wastewater especially for the water that has been softened (9).

The selection criteria from the consumer must include the environmental impact that water softeners might represent because of their salt contribution to wastewater. Water softener companies have generated profitable gains in the Phoenix area and might see an increase in the demand of their products with projected metropolitan growth. However, these companies are currently providing unsustainable technologies that solve a problem (water hardness) while creating another of greater magnitude: high TDS concentrations in residential effluents. Developing an MCL might help to regulate the steady increase of salt from water that has been softened. Establishing a control parameter for TDS

could obligate this industry to fabricate better equipment that would eliminate the need of salt to perform water softening processes (10).

Conclusion

Water sources for inhabitants of metropolitan Phoenix are saline-rich by nature. The Salt River water is the natural water source with the highest concentration of salt for people living in Arizona. The CASS report performed a mass balance on the salinity in the Phoenix area and identified the issues associated with the increase of salinity in the Phoenix area. The salinity retained in the Phoenix area is distributed in the soil, in the wastewater treatment plants, on household appliances, cooling towers and evaporation ponds. As the TDS levels rise in wastewater, the ability to reuse it decreases. Phoenix relies heavily on reclaimed wastewater for irrigation, cooling and groundwater recharge. However, there is public concern about emerging contaminants found in wastewater which are resistant to traditional treatment methods, and has led people to install point-of-use water treatment systems including water softeners. Some examples of these emerging contaminants of public concern are EDC's (endocrine disrupting compounds) found in pharmaceuticals and personal care products (PPCP's) and PBDE's (polybrominated diphenyl ethers) found in flame retardants (15). Although water softeners are not a way to remove such contaminants, they are often packaged with additional POU water treatment systems such as activated carbon filters designed to remove organic trace contaminants. The residential use of POU ion-exchange water softeners now and in the future will exacerbate the issue of salt accumulation in Phoenix.

The report finds that the contribution of salt from water softeners is 45,536 tons of salt for the year 2000. The projections for 2040 and for the build-out capacity of Phoenix of 12 million people showed a linear relationship between the steady residential reliance on water softeners and rising TDS concentrations in wastewater. Changes must be implemented in order to sustain water reuse and reduce expenses incurred due to rising salinity levels in wastewater.

Recommendations include increased support and awareness of no-salt POU water conditioning devices, centralized desalination, and enforceable salinity regulations.

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Chapter 26

Bioassay Guided Fractionation (Toxicity Identification and Evaluation) for the Determination of Estrogenic Agents in Environmental Samples

Daryl Bulloch, Ramon Lavado, and Daniel Schlenk*

Department of Environmental Sciences, University of California Riverside,
CA 92521, USA

*Corresponding Author: Daniel.schlenk@ucr.edu

Toxicity Identification Evaluations (TIEs) have been an integral component of environmental toxicology for the determination of causal factors impacting biological responses in environmental matrices. Modifications of the “bioassay-guided” fractionation scheme have allowed environmental toxicologists to target specific biological responses in an attempt to determine “cause”. This particular review focuses on the biological response of feminization or estrogenic activity resulting from exposure to environmental estrogens in aqueous media. While the approach has shown some promise with regard to specific estrogen receptor ligands, a more challenging aspect has been determining causative agents for the *in vivo* process of feminization, which is of more ecological relevance. Feminization is a complex biological response with multiple targets for xenobiotics to impair. Consequently, a whole animal approach calibrating vitellogenin to estradiol equivalents in environmental media will allow a more relevant assay for estrogenic activity that incorporates multiple mechanisms of action.

1. History of Bioassays for Toxicity Identification

The aquatic bioassay concept was developed in the early 20th century in an attempt to determine whether aqueous media harbored chemical agents that could impair aquatic ecosystems (1). Bioassays are typically used in concert with analytical chemistry to estimate ecological risks within specific areas. In contrast to analytical chemistry, which requires a validated method and analytical standard to measure specific agents within a matrix, bioassays integrate responses of an entire mixture of components and stressors.

If significant toxicity is observed within a sample, "Toxicity Identification Evaluations" (TIEs) are used to "identify" the putative cause(s) of the toxicity in the sample (2, 3). Based on an understanding of physicochemical properties of the potential toxicant (i.e. polarity), or the mechanism or mode of action, various treatments and manipulations of the environmental sample can be carried out to enhance or diminish the toxicity and provide insight as to the potential cause.

The sample is typically fractionated to discretely isolate specific components of the mixture such as metals or organic compounds of specific chemical properties. Most studies employ an extraction of the sample using a solid-phase matrix such as C18 (Figure 1) (4, 5). In some cases, passive sampling devices (e.g. Semi Permeable Membrane Devices) have been placed in various locations to sample waterways over a period of time and then subsequently eluted with a solvent (6, 7). In addition, the pH of a collected water sample can also be adjusted to isolate chemicals of varied polarity, or solvent/water mixtures can be used sequentially to obtain fractions of increasing hydrophobicity characteristics (8). In some cases, treatment of toxic samples with enzymes or enzyme inhibitors can be used to determine whether specific pesticides such as organophosphates or pyrethroid insecticides may be the cause of the toxicity (9). In this way, the biological response "guides" the investigator to the chemical identity of the toxic agent(s).

While TIEs have historically used acute whole animal lethality, other sublethal biological responses can also be used to identify specific chemical agents. The pharmaceutical industry routinely employs bioassay-guided evaluations of synthetic chemicals and/or extracts of natural products in the drug development process (10). Many of these bioassays are cell-based *in vitro* responses that use specific receptors important in the desired mode of action of the therapeutic agent. For example, if estrogen-like pharmaceutical agents are desired for drug development, an estrogen receptor-based bioassay can be used in cell-lines or other biological vectors (i.e. yeast) that are easily manipulated and provide rapid results in a screening approach. Whole animal *in vivo* responses of estrogenic activity can also be used to identify active agents. Thus, if estrogenic substances are present within an environmental matrix, then the same bioassays used for drug "discovery" can also be used for "identification" of the substance within the matrix.

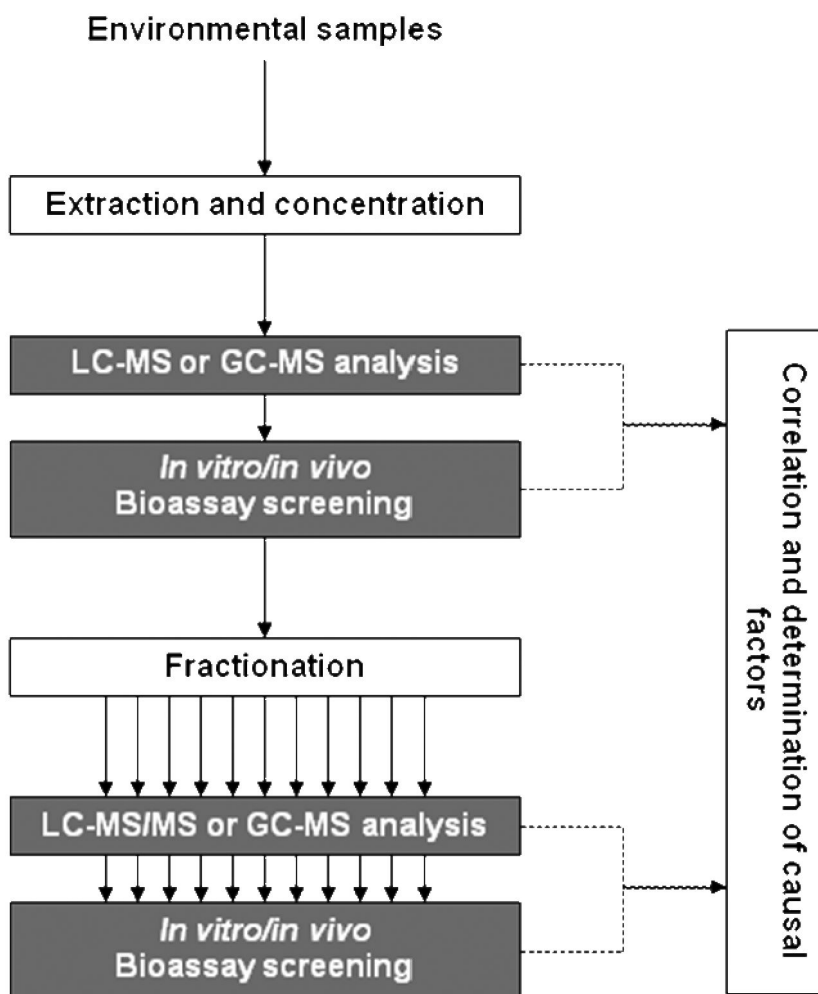


Figure 1. Toxicity Identification Evaluation (TIE) scheme used for estrogenic analysis of environmental samples.

2. Mode of Action/Receptor-Driven Responses

Incorporation of mode of action into the risk assessment process has been recommended by a recent NRC panel and implemented by the USEPA (11). Receptor-driven responses have been utilized in several mode-of-action based risk assessments. Activation of the aryl-hydrocarbon receptor has been shown to initiate multiple adverse outcome pathways that lead to severe biological responses such as cardiac abnormalities in development, immune dysfunction and/or carcinogenesis (12–14). The risk of aryl-hydrocarbon receptor agonists such as planar polychlorinated biphenyls and other structurally similar compounds has been estimated using toxicity equivalent factor (TEF) methodology. Since 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most potent ligand for the receptor, toxicities of other AhR ligands can be calculated based upon the relative

affinities of the ligand to TCDD equivalents (15). For example, impairment of fish reproduction occurs in salmonids at a TCDD equivalent concentration of 30 pg/g eggs. If a compound has a 10-fold lower affinity for AhR, then 300 pg/g of the compound would be needed to cause the same toxicity.

A similar approach can be used for nuclear receptors such as estrogen, androgen and thyroid receptors. Activities can be expressed as equivalents for each natural ligand. For example, for the estrogen receptor, affinities relative to 17 β -estradiol (E2) can be used to calculate estradiol equivalent factors (EEQs) to determine overall estrogenic activity of a chemical or a mixture of chemicals having that activity (16). In this way, the biological activity of any sample can be calculated for use in exposure and effects assessments in an ecological risk assessment paradigm. This chapter will focus specifically on bioassays that provide estrogenic signals.

3. Bioassays

Multiple *in vitro* assays are available for the assessment of estrogenic responses for chemical testing. Receptor binding assays for the estrogen receptor (ER) have been approved for the endocrine disrupter screening program (EDSP) (17). In contrast to receptor binding assays that often require radiolabeled material, reporter and/or transcriptional activation assays in genetically modified organisms as well as estrogen-responsive immortal cell-lines require much less expertise and can be carried out without significant investments in laboratory instrumentation or reagents. The Yeast Estrogen receptor assay (YES) is one of the most widely used *in vitro* bioassay using the human ER- α and its corresponding response element linked to a reporter gene construct that produces a colorimetric response when the ER is bound and activated (18). This assay has been used in numerous studies evaluating the estrogenic activity of environmental media. The MCF-7 cell line (E-screen) is also widely used and is an immortal cell-line that proliferates upon interaction with estrogens (19). The YES and MCF-7 assays have been through “round-robin” testing and have been shown to have relatively low interlaboratory variability compared to several other *in vitro* bioassays (20). In Europe, the Estrogen-Receptor-CALUX system has been widely used and shown to provide greater sensitivity than YES (21–23). Each of these *in vitro* assays can be calibrated against E2 to provide estradiol equivalents of any matrix allowing temporal and spatial comparisons between samples.

While *in vitro* systems are relatively simple to use and can provide rapid results for estrogen receptor ligands, there are several disadvantages that are associated with their use. First of all, activation of the ER requires normal cell viability. If the environmental sample is acutely toxic to cells, then it is not possible to obtain an accurate evaluation of the estrogenic activity of the sample. If the assays are colorimetric, then extracts or media must not possess a conflictive color that quenches the response. In addition, many of the cell-lines or microorganisms used in these systems lack biotransformation pathways that are present in other tissues. Consequently, compounds that require metabolic activation to ER ligands tend not to be active with many of these systems.

One way to address this issue is to utilize cells that possess biotransformation enzymes such as isolated hepatocytes, which can be isolated from fish (24). Not only does the hepatocyte possess enzymes for biotransformation, the cells also possess ER-regulated transcripts that can be used to evaluate estrogenic activity. One of the most common ER-regulated transcripts (mRNA) is the egg-yolk precursor lipoprotein, vitellogenin. Measurements of this transcript or the protein by enzyme-linked immunosorbent assay (ELISA) or Western immunoblots not only provides a sensitive measure of estrogenic activity; it is directly linked to a primary estrogenic effect in the whole animal.

Measurement of vitellogenin or other egg-envelope proteins under estrogen control is a robust indicator of estrogen receptor activation and feminization within oviparous animals such as fish, birds and amphibians. The presence of these proteins in the blood or livers of male or sexually immature animals has been utilized as a powerful *in vivo* bioassay for estrogenic activity (25). Unfortunately, many studies fail to calibrate the response to estradiol equivalents and also express the protein in a mass/volume manner usually in the plasma/serum. If the osmolality of fish remained constant, then a mass/volume metric would be sufficient. However, plasma protein concentrations and osmolality can be dramatically altered by numerous stressors and normal environmental factors (26, 27). Consequently, as with measurements of proteins in any other tissue, the mass should be normalized to total protein within that tissue even if the "tissue" is blood. To enhance sensitivity, measurement of the vitellogenin transcript (mRNA) through quantitative polymerase chain reaction (qPCR) methods has shown great promise (28).

The great advantage of using vitellogenin measurements in the whole animal as an indicator of estrogenic activity is that the integration of the complete endocrine system within the animal allows expansion to all modes or mechanisms of action for the estrogenic or feminization response. If a compound enhances E2 synthesis through increasing gonadotropin release or induction of aromatase, its estrogenic activity would not likely have been observed *in vitro* and the response of the sample would be underestimated. Consequently, the *in vivo* approach provides greater sensitivity and more biological relevance than *in vitro* assays.

4. The Use of TIEs for Estrogenic Evaluations

TIEs combining chemical identification assays with *in vitro* and *in vivo* bioassays have been used to identify causative agents in various water systems. A "typical" TIE process for the analysis of complex sewage treatment works (STW) effluent and the identification of the agents responsible for observed activity is shown in Figure I.

One of the first evaluations occurred in the UK where wastewater effluent was extracted and concentrated using a C18 solid-phase extraction (SPE) cartridge and eluted with a series of methanol/water mixtures of increasing polarity (29, 30). Fractions were analyzed by an *in vitro* bioassay (YES), yielding estrogenic activity in the 50-85% methanol fractions. The active fractions were then further separated and analyzed by reversed-phase LC-MS, though this did not yield

valuable results. GC-MS was then used to identify the estrogenic compounds, where α -terpinoids along with approximately 20 unknowns were the major components. Standards of terpinols proved non-estrogenic, so a shallower HPLC gradient was run on the active fraction to further separate the estrogenic compounds for identification. The *in vitro* bioassay identified more resolved active fractions, which were hypothesized to be potent estrogens based on their estrogenic activity. Standards of the contraceptive 17 α -ethynylestradiol (EE2) were run on the same shallow HPLC gradient, and were found to elute in the same fraction as the observed estrogenic activity found with the STW effluent extract. This was confirmed by running a standard of estrone (E1), E2, and EE2 on the same GC-MS method previously used. Larger volumes of the STW effluent were extracted to increase the concentrations of the steroids purported to be present, where analysis then confirmed their presence and allowed quantification. A similar approach was used in Lake Mead, Nevada USA, but the MCF-7 cell line was used and only estrone and estradiol were identified (31).

5. *In Vitro* Bioassays vs. Chemical EEQs

As can be seen in Figure II, there is a strong linear correlation between most *in vitro* bioassays and the associated chemical assay activity. This plot compiles many studies conducted around the world over the last decade where estrogenic activity was measured in a variety of waters, including river surface water, stream surface water, and wastewater treatment plant influent and effluent. The estrogenicities of the different waters were calculated in terms of their EEQs, expressed in nanograms of E2 per liter. Chemical measurements were made mainly by LC-MS, GC-MS, and less often by commercially available ELISA kits. Bioassay measurements made *in vitro* were performed by a number of different assays, including YES assay, ER-CALUX assay, E-screen assay and vitellogenin (VTG) induction in primary hepatocytes of various fish species. Binding studies have shown the binding pocket of the ER to be quite large - approximately twice the volume required to bind E2 (32). This allows a wide variety of compounds to bind to the ER, though their binding affinities are many orders of magnitude lower than the E2-ER affinity. The binding promiscuity of the ER is often used to explain part or all of the observed *in vitro* estrogenicity in a sample which goes unaccounted for by chemically measured estrogenic activity attributed to natural and synthetic steroids.

However a handful of studies failed to see correlations between the chemically measured estrogenic activity and the *in vitro* estrogenic activity. In one example, the *in vitro* estrogenicity of seawater from 5 locations around Suruga Bay, Japan determined by the yeast two-hybrid system was compared with the activity estimated by chemical analysis (33). Samples of the seawater were first acidified, then extracted with Empore C18 solid-phase disks and eluted as a single fraction with acetone and dichloromethane. The chemical analysis used GC-MS to quantify the amount of 25 known estrogenic compounds present in the water. Standards of these compounds were subsequently used to measure their estrogenic potency in the same yeast two-hybrid system for the *in*

vitro estrogenicity calculations. Concentration data combined with estrogenic potency then allowed the estimation of the chemical estrogenicity by addition of the activities of the compounds (sometimes referred to as Concentration Addition). The chemically estimated estrogenicities overestimated the bioassay determined estrogenicities at all sampling locations. E1 was the major contributor to estrogenicity at the two sites near sewage treatment plants which received influent from industrial and municipal sources. At the other three sites, however, steroids were not detected despite estrogenic activity ranging from 0.04-1.3 ng/L EEQs. The causative agents at these three sites were solely attributed to the alkylphenols (octylphenol, nonylphenol) and bisphenol A (BPA). Only one of the two sites near sewage treatment plants out of all 5 sites elicited an estrogenic response in the bioassay, although the chemical estrogenicity for this site was nearly equivalent to that of another site where BPA and alkylphenols were the causative agents. Since the yeast two-hybrid bioassay produces an estrogenic response from direct estrogen receptor binding, it can be inferred that the sample with E1 elicited a response because of the potent binding between E1 and the estrogen receptor. The authors offered several explanations for the discrepancy between the bioassay and the analytical chemistry. Since the bioassay uses yeast cells, compounds which are very lipophilic yet estrogenic may not cross the cell membrane to induce a response. Also, organotin compounds along with other known estrogen receptor antagonists may have been present in the seawater.

In another example, *in vitro* ER binding assays yielded activity an order of magnitude greater than the chemically determined activity when studying wastewater treatment plant influent on the Meuse and Rhine rivers, Netherlands (23). In this study three different *in vitro* bioassays were compared for sensitivity towards estrogenicity near several wastewater treatment plants receiving both municipal and industrial effluent and two estuaries near the North Sea. Water samples were extracted for hormone analysis with SDB-XC (reversed-phase) SPE disks and eluted with methanol. Eluates were then run in an ER-CALUX assay, a YES assay, and an ER competitive binding assay to optimize sample preparation techniques and assay running conditions. Estrogenicity of the eluates was also characterized by chemical analysis (instrumentation unreported), with the percentage of calculated estrogenicity attributable to steroid hormones also reported. The disagreement between *in vitro* EEQs and calculated EEQs was attributed to ER activation by “unknowns” present in the influent and effluent, which may have been transformed by the wastewater treatment process into more bioactive metabolites. Calculations of chemical activity also did not take into account synergism and antagonism between the substances present, which may have further contributed to the discrepancies.

In summary, in contrast to analytical chemistry methods, *in vitro* bioassays are excellent indicators of overall ER ligand occurrence and allow the rapid determination of “unknown” as well as “known” ER-ligands within various environmental media. Since the assays have fairly rapid through-put capabilities, they may be used in combination with analytical chemistry to identify unknown ER-ligands through TIE or fractionation manipulations.

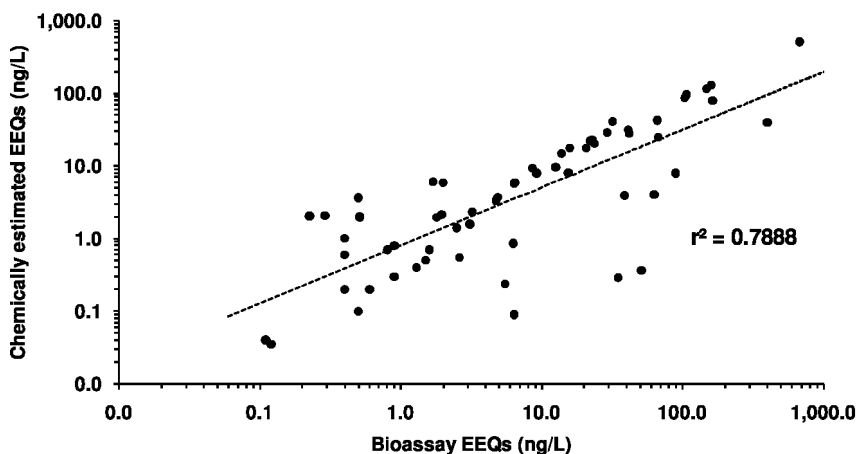


Figure II. Relationship between *in vitro* bioassay EEQs and chemically estimated EEQs in various studies (30, 33, 36, 38, 39, 64–67).

6. *In Vitro* Bioassays and TIEs

As mentioned above, initial TIE studies with STW effluents focused on the identification of ER-ligands and identified steroid hormones as causal factors. However, as more TIEs have been carried out over recent years with different environmental media including sediments and surface water, more studies indicated significant discrepancies between the occurrence of steroid estrogens and estrogenic activities. For example, in a study from Japan, genistein was identified as the predominant estrogenic compound from a surface water sample in Osaka that was active in an *in vitro* ER assay without the detection of steroid estrogens (34). Water samples were collected from 13 localities along the Lake Biwa-Yodo River. The YES assay activities ranged between non-detect to 19.6 ng/L EEQs. Two of the samples with the highest *in vitro* activity were fractionated by HPLC and evaluated by YES and UV-spectroscopy. Analysis of a sample collected just downstream of a sewage-treatment plant indicated YES activity in fractions where E2 and E1 co-eluted. However, E2 and E1 were not measured in the sample. The activity in the other sample, which was obtained from a tributary river in a primarily residential area with some industrial development (i.e. Osaka City), occurred in fractions that did not correspond to E2, E1, or known synthetic estrogens. Subsequent evaluation by LC-MS identified and quantified the phytoestrogen genistein in the active fraction. However, the calculated EEQs were significantly less than the YES EEQs.

In a study evaluating sediment cores from several rivers in the UK (Mersey, Tees, Thames, Tyne, and Southampton Water) that receive pollution from domestic and industrial effluents, samples were centrifuged to separate solids from pore water, which was then extracted with C8 SPE, and eluted with methanol (35). The solids were extracted several times with dichloromethane and extracts were combined and evaporated. Sediment samples were fractionated by normal phase SPE on a silica column, and extracted sequentially by hexane, dichloromethane, acetone, and methanol to give 4 fractions of increasing polarity. These fractions

were then assayed for estrogenicity using the YES assay, where positive fractions were further fractionated using HPLC. Acetone and pore water extracts were separated by reversed-phase chromatography, while dichloromethane extracts were separated by normal phase chromatography. Estrogenic activity in the resulting fractions was characterized using the same *in vitro* bioassay, where positive fractions were then analyzed by GC-EI-MS. Nearly all pore water and sediment samples demonstrated estrogenic activity, ranging from 2-68 ng/L EEQs for the pore waters and 0.2-13 ng/g EEQs for the sediment. Similarities in fractionation profiles for the pore waters led the authors to suggest that causative agents responsible for the estrogenicity observed in the bioassay were similar, and that steroid estrogens were likely responsible for the activity. Interestingly, analytical confirmation failed to detect the presence of steroid estrogens, so it was suggested the activity resulted from compounds of similar polarity to steroid estrogens. Chemical analyses of the sediment extracts were probed with an ER quantitative structure-activity relationship model, identifying three compounds responsible for estrogenic activity. Nonylphenol, cinnzarizine, and cholesta-4,6-dien-3-one were identified as estrogenic. However, EEQs calculated from the analytical chemistry measurements accounted for less than 1% of the observed estrogenic activity determined from the bioassay.

In Canada, 4 wastewater plants receiving domestic influent as well as a bleached kraft pulp mill were sampled and analyzed for estrogenic activity (36). Whole wastewater was first extracted several times with dichloromethane, then combined, dried down, and analyzed by high-resolution GC-MS. In this portion of the experiment, steroid hormones E1, E2, EE2, and estriol (E3) were detected though %RSD (Relative Standard Deviation) for E2 and E3 was 100% and 66%, respectively. The whole wastewater extracts were also assayed for estrogenicity *in vitro* with the YES assay. Oasis HLB SPE cartridges were used for a TIE and produced 5 fractions of decreasing polarity. The 100% methanol SPE eluate from each site was determined the most estrogenic, so it was further fractionated by normal phase High Pressure Liquid Chromatography (HPLC). Estrogenic fractions were collected and analyzed by GC-MS, where analysis in selective ion monitoring mode failed to identify any steroidal estrogens. The fractions which previously contained steroid estrogens in the whole wastewater analysis tested positive for the sterols β -sitosterol, stigmasterol, 3β -stigmasta-5,25-dien-3-ol, as well as galacturonic acid, binaphthylsulphone, and several fatty acids. It was hypothesized that steroid estrogens were not detected in the TIE fractions due to masking by fatty acids and other natural products.

In another UK study, effluent, water, and sediment up and downstream of two major STWs on rivers where intersex fish were found were characterized by the YES assay and chemical measurement (37). Effluent and water samples were acidified and extracted using Oasis HLB SPE cartridges, and eluted sequentially with methanol, ethyl acetate, and hexane. The fractions were combined and evaporated, then divided for analysis by the YES assay and fractionation by reversed-phase HPLC in a TIE design. Sediment cores were extracted three times by sonication with methanol, with organic fractions then being combined and evaporated and submitted to assays as were the water SPE eluates. Eluates subject to HPLC fractionation were separated on a C18 column into about 100

fractions of increasing polarity, all of which were evaluated for estrogenicity. Fractions eliciting an estrogenic response were analyzed by GC-MS for compound identification and quantitation. The majority of sites exhibited a good correlation between *in vitro* EEQs and chemically estimated EEQs, with the majority of chemical estrogenic activity being accounted for by steroidal estrogens. In sediment samples downstream of the Horsham STW effluent, however, no steroidal estrogens were detected by GC-MS in fractions predicted by standards. Some E2 was detected in this sample, though at a GC-MS retention time 1-2 minutes different from the E2 standard. Synthetic estrogens, androsterone, and known xenoestrogens were all ruled out, leaving the identity of causative agents at this site unknown.

A 24-hour composite wastewater effluent sample was collected from a municipal wastewater sewage treatment plant in the city of Turku/Åbo, Finland (38). Samples were acidified and then extracted using Oasis HLB SPE cartridges which were washed with 40% methanol followed by 2% NH₄OH in water, and finally eluted in 10% methanol in methyl tert-butyl ether (MTBE) and evaporated. Wastewater was also fractionated by reversed phase HPLC into 6 fractions of decreasing polarity using an acetonitrile/water gradient without prior extraction or treatment, and subsequently analyzed by the YES bioassay. Quantitative chemical analysis was then performed on the SPE eluate by reversed-phase LC-MS/MS. The estrogenicity by chemical measurement of the whole SPE eluate was 6.6 ng/L EEQs, while the HPLC fractions submitted to the YES bioassay added up to 136.5 ng/L EEQs (both values are averages of two identically treated samples). The fractions in the bioassay had a significantly higher estrogenic activity than the whole water sample, explained by the presence of hydrophilic “unknowns” responsible for estrogenic inhibition in the original wastewater sample. This hypothesis was tested by adding E2 to the individual HPLC fractions and re-assaying with the YES bioassay. Estrogenic activity was found to be suppressed 20-30% in the first two HPLC fractions, supporting the hypothesis that hydrophilic antagonists were at least in part responsible for the inhibition.

Beck *et al.* characterized estrogenicity of selected German Baltic Sea waters using the YES bioassay and LC-MS for chemical identification (39). Surface water samples were collected at 5 locations in north-eastern Germany in 2003 and 2004, three in inner coastal waters and two farther out at an outer bay and peninsula as reference sites. The surface water samples were extracted with Oasis HLB self-packed SPE cartridges and eluted with acetone/methanol 80:20. The extracts were then purified on silica columns. The purified extracts were then analyzed by reversed-phase LC-MS and the YES assay. EEQs calculated from the analytical chemistry did not correlate well when plotted against *in vitro* estrogenicity, with the correlation coefficient (r^2) in 2003=0.39 and in 2004=0.34. Higher values in the bioassay indicated “unknown” estrogenic compounds which were not identified in the chemical analysis, and anti-estrogenic activities could have impaired bioassay activities.

The effluent from 7 sewage treatment plants in and around Madrid (Spain) reported an inability to identify substantially causative agents for observed estrogenic activity due to a lack of information on a majority (about 70%) of the detected compounds (40). Surface waters from 7 STPs on rivers in and around

Madrid receiving both urban and industrial waste influents were sampled in June and July in 2000 at 2-hour intervals for 24 hours. Water samples were extracted with 5 g and 2 g C18 SPE cartridges arranged in series, and eluted into two extracts of decreasing polarity with 70% and 100% methanol (fraction 1 and 2, respectively) from the 5 g cartridge and an extract of 100% methanol (fraction 3) from the 2 g cartridge. Fraction 2 was analyzed by GC-MS for chemical identification, where steroidal estrogens and phenolic estrogens were quantified. Estrogenic activity was only found in 5 of the sample effluents from 4 of the sampling sites, and no causative agents were definitively identified.

A TIE procedure to study estrogenic compounds in surface water and sediment pore water samples from the Tyne and Tees estuaries in the United Kingdom, was carried out using a YES assay and LC-MS for estrogenicity characterization (30). Surface water samples were collected up and downstream as well as at the point discharge location for the Dabholm Gut STW on the Tees estuary and the Howdon STW on the Tyne estuary during the summer of 1998. Both STW received effluent from domestic and industrial sources, where Dabholm Gut received some untreated and secondary treated effluent while Howdon received primary treated effluent. Water samples were extracted with three different SPE cartridges stacked in series, in the order C8, ENV+ and nonporous carbon to retain non-polar, mid-polar, and leftover compounds, respectively. Eluates were collected with methanol and evaporated to dryness. Sediment samples were centrifuged, and the pore water was drawn off and SPE extracted identically to the surface water samples. Whole SPE extracts were assayed for estrogenicity with the YES assay, and samples exhibiting activity were subsequently fractionated by reversed-phase HPLC into 35 x 5 mL fractions of decreasing polarity. The HPLC fractions were analyzed by the YES assay, where active fractions were further analyzed by GC-MS to quantify chemical activity. The majority of the estrogenic activity in fractions from all sites resulted from the C8 SPE eluates, corresponding to compounds in the mid- to non-polar range (\log octanol/water partition coefficient- K_{ow} >3). Much of the activity in the surface water C8 extracts was attributed to E2, with a small contribution from several xenoestrogens, including androsterone, nonylphenol, bis(2-ethylhexyl)phthalate, and an unidentified agent. Only the pore water extract from Dabholm Gut demonstrated estrogenicity, estimated at 7 ng/L EEQs. Active fractions from Dabholm Gut pore water were pooled and underwent HPLC separation on a shallower gradient and subsequently analyzed by GC-MS, but no steroid estrogens were detected. Sediment samples left over from pore water extraction were extracted with dichloromethane and analyzed for estrogenic activity, yielding YES activities of 4.7 ng/g EEQs dry sediment at the Howdown site and 0.9 ng/g EEQs dry sediment at the Dabholm Gut site. Chemical analysis on these samples failed to identify steroidal estrogens.

7. *In Vivo* Bioassays vs. Chemical Identification

While *in vitro* estrogenic activity generally correlates well with chemically measured activity, *in vitro* and *in vivo* activities rarely correlate within specific studies. The biosynthesis and catabolism of estrogens is a complex and dynamic process with multiple targets for impairment by xenobiotics. Thus, a xenobiotic may cause feminization via mechanisms outside of direct interactions with the ER and not be detected through *in vitro* bioassays. Since most studies present *in vivo* activity as vitellogenin protein in either mass/volume plasma or as percent control (qPCR) rather than EEQs, study-to-study comparisons are difficult to conduct. However, linear regressions comparing vitellogenin and chemically-derived EEQs can be carried out for individual studies. As seen in Table I, linear correlations (*r*-squared values) between chemically-derived EEQs and *in vivo* estrogenic activity are generally poor and range from nearly non-existent to 0.765.

As an example, wastewater effluent from several sites around New York City was evaluated using YES and compared to *in vivo* vitellogenin (EEQs ng/L) derived from fish exposed to the effluent and eluted fractions from an Empore C18 extract (41). *In vitro* activity significantly underestimated the *in vivo* estrogenic activity of the effluent and eluted in different fractions relative to *in vivo* activity. Upon HPLC fractionation, additional discrepancies were observed with *in vivo* activity observed in more polar fractions and in other less polar fractions, but not co-eluting with steroid hormones (42). In fact, YES EEQs co-eluted with steroid hormones and were relatively consistent with chemically derived EEQs in these specific fractions (43). A TIE was carried out on effluent from a WTP which had the highest *in vitro* activity. Potential causative agents identified in active *in vivo* HPLC fractions were a mixture of alkylphenols, bisphenols, triclosan, oxybenzone, galaxolide, and two phthalates (42). Calculated EEQs from the analytical chemistry and the *in vitro* activity significantly underestimated the *in vivo* EEQs.

Similarly, in a study evaluating sediment extracts from the Southern California bight, *in vivo* estrogenic activity (vitellogenin) was measured in male California halibut injected with the extract and compared to the occurrence of E2, E1 and several nonylphenol ethoxylates (NPEOs) and nonylphenols (NPs) (44). Sediment was sampled at 4 locations along the bight, and included samples from the outfall for the Los Angeles County Sanitation District (LACSD), the outfall for the Orange County Sanitation District (OCSD), the outfall for the City of San Diego (SD), and a reference location (OCT-11). Sediment samples were separated from pore water, extracted three times with a methanol/acetone 1:1 mixture, centrifuged, and supernatants were combined and dried down. Intraperitoneal injections in the fish were performed on days 1 and 3 of 7-day experiments, with doses of sediment extract reconstituted in ethanol of 0.05 mL/100 g of fish. On day 7, plasma samples were collected and vitellogenin levels determined by ELISA. Steroidal analysis of the sediment eluates was carried out by immunoaffinity extraction coupled with LC-MS. NPEOs and their metabolites were analyzed first by purifying the SPE eluates by reverse-phase HPLC, with fractions dried down and reconstituted in methanol. The concentration of E2 in sediment extracts ranged from 0.16-0.45 ng/g, with the OCSD site having the highest E2 concentrations. E1 was observed only at the LACSD site. The OCSD

site had the highest NP and NPEO concentrations, at 3200 ng/g and 330-3900 ng/g, respectively. The *in vivo* activity of LACSD sediment extracts was the highest, at 6x greater than SD extracts and 90x higher than OCSD extracts. A comparison of chemically calculated EEQ and *in vivo* plasma vitellogenin levels in the male halibut correlated very poorly, with an r^2 of 0.0024.

TIEs were carried out on the LACSD sediment extracts using juvenile rainbow trout to accommodate a larger number of samples. Fractions of 0.1 mL were injected into the trout, and serum vitellogenin was measured after 7 days. Fractions were obtained by sequential elution of a C18 SPE cartridge after sediment extraction, eluting with ethanol/water solutions of 10, 20, 50, 75, and 100% ethanol. Each fraction was evaluated using 3-4 animals. Fractions were reduced in volume and separated by HPLC, with fractions collected every 3 minutes. The resulting fractions were analyzed as before for vitellogenin expression in trout, using 3-4 animals per HPLC fraction. The HPLC fractions were analyzed for steroids by GC-MS/MS. Thirty-two compounds were targeted for analysis by LC-MS/MS, with an additional 30 compounds analyzed and quantified by GC-MS/MS. The 75% ethanol eluate was responsible for the highest nominal vitellogenin expression despite E2 and other steroids eluting in the 50% ethanol fraction. Chemical analysis for 62 compounds of the active HPLC fractions only yielded a positive identification for oxybenzone in three fractions at 0.36, 0.86, and 0.38 ng/g sediment concentrations. In addition, at least one unknown compound with a mass fragmentation profile of m/z 70 to m/z 51 was observed in one of the active fractions. Steroid hormones were not detected in any of the biologically active fractions. Subsequent concentration-response studies with oxybenzone indicated estrogenic activity, but at effective concentrations an order of magnitude greater than that observed in the environmental samples (45).

More recently, water samples from agriculturally impacted waterways in California's Central Valley were subjected to chemical estrogen analysis as well as *in vitro* and *in vivo* vitellogenin measurements in rainbow trout (*Oncorhynchus mykiss*) (46). Surface water grab samples were collected on six dates during 2006-2007 along the Sacramento-San Joaquin River system. Water was extracted by Empore C18 SPE disks, eluted with methanol, evaporated to dryness, and reconstituted in acetonitrile and derivatized for GC-MS analysis for estrogenic compounds. The estrogenicity of the SPE eluates was evaluated by the induction of vitellogenin expression *in vitro* in rainbow trout primary hepatocytes and *in vivo* estrogenic activity was quantified by measuring serum vitellogenin protein levels by commercially available ELISA after exposing the trout by intraperitoneal injection of extracts on days 1 and 3 of a 7-day exposure. Bioassay-guided fractionation was carried out in a TIE design for two sites with high *in vitro* and *in vivo* activity by eluting their surface water extracts from the C18 SPE disks with 20, 40, 60, and 100% methanol. The *in vitro* and *in vivo* estrogenicity of these fractions were determined by the same assays mentioned above. In addition, chemical analysis by LC-MS was carried out to identify causative agents in fractions demonstrating elevated estrogenic activity. Estrogenic activity was detected at 6 of the 16 total sites, with the highest *in vitro* and *in vivo* activities at the Sacramento River Delta and Napa River. The Each site was selected for fractionation and subsequent TIE analysis as described

Table I. Correlation between *in vivo* bioassay estrogenicity and EEQs estimated from chemical analysis detected in waters from different studies

<i>Author</i>	<i>Year</i>	<i>Fish species</i>	<i>r</i> ²	<i>Reference</i>
Schlenk <i>et al.</i>	2005	California Halibut (<i>P. californicus</i>)	0.002	(44)
Aerni <i>et al.</i>	2004	Rainbow trout (<i>O. mykiss</i>)	0.007	(52)
Rodgers-Gray <i>et al.</i>	2000	Roach (<i>R. rutilus</i>)	0.036	(53)
Houtman <i>et al.</i>	2006	Bream (<i>A. brama</i>)	0.043	(51)
Lavado <i>et al.</i>	2009	Rainbow trout (<i>O. mykiss</i>)	0.052	(46)
Fawell <i>et al.</i>	2001	Rainbow trout (<i>O. mykiss</i>)	0.055	(54)
Pawlowski <i>et al.</i>	2003	Rainbow trout (<i>O. mykiss</i>)	0.062	(55)
Ohkubo <i>et al.</i>	2003	Japanese common goby (<i>A. flavimanus</i>)	0.069	(56)
Björkblom <i>et al.</i>	2009	Three-spined stickleback (<i>G. aculeatus</i>)	0.098	(57)
Tilton <i>et al.</i>	2002	Channel catfish (<i>I. punctatus</i>)	0.120	(58)
Hemming <i>et al.</i>	2001	Fathead minnows (<i>P. promelas</i>)	0.202	(59)
Harries <i>et al.</i>	1997	Rainbow trout (<i>O. mykiss</i>)	0.506	(60)
Todorov <i>et al.</i>	2002	Sunshine bass (<i>M. saxatilis</i>)	0.603	(43)
Petrovic <i>et al.</i>	2002	Common carp (<i>C. carpio</i>)	0.608	(61)
Vermeirssen <i>et al.</i>	2005	Brown trout (<i>S. trutta</i>)	0.621	(62)
Sheahan <i>et al.</i>	2002	Rainbow trout (<i>O. mykiss</i>)	0.765	(63)

above. For the Sacramento River Delta most of the estrogenic activity *in vitro* and *in vivo* eluted in the 60% methanol fraction. Chemical analysis of these fractions contained low concentrations of herbicides (2.5 ng/L diuron and 0.2 ng/L 2-hydroxyatrazine) and a mixture of nonylphenol ethoxylates (421 ng/L). For the Napa River site, most of the estrogenic activity *in vitro* and *in vivo* eluted in the more nonpolar 80 and 100% methanol fractions. Chemical analysis of the 80% methanol fraction contained 6.2 ng/L diuron, 4.1 ng/L simazine, and 2.8 ng/L 2-hydroxyatrazine. The 60% methanol fraction from the Sacramento River Delta and the 80% methanol fraction from the Napa River, along with a 60% methanol fraction from a 17 β -estradiol positive control sample, were subjected to HPLC fractionation and subsequent *in vitro* and *in vivo* bioassay analysis as before. Recovery of *in vitro* activity was around 80%, but recovery of the *in vivo* activity was over 300% indicating potential antagonism within non-fractionated extracts. For all sites, chemically calculated EEQs (usually <1 ng/L EEQs) greatly underestimated bioassay EEQs, and steroid estrogens were not detected. The poor correlation between *in vitro* and *in vivo* responses ($r^2=0.052$) was explained by the possibility that the causative agent(s) were site-specific and likely had different modes of action.

8. Biological Fluid TIEs

Several groups have employed novel TIE methods to fractionate various biological fluids from fish exposed to wastewater effluents and contaminated environments. Gibson *et al.* have shown that multiple natural and synthetic steroid estrogens as well as alkylphenols can be identified in the bile of fish such as the rainbow trout and roach (47, 48). EE2 concentrations as high as 20 ng/mL within bile have been reported within fish exposed for 10 days indicating significant uptake within a rapid period. This seems to be contrary to recent reports indicating limited exposure to waste-water derived-EE2 which has recently been reported to occur at concentrations less than 1 ng/L (49) as well as the pharmacokinetics of EE2 in salmonids, which indicates a fairly rapid 20 h half-life (50).

Using this approach, water and sediment extracts from several rivers and estuaries receiving municipal sewage treatment plant effluents in the Netherlands was investigated using *in vitro* (ER-calux) and *in vivo* (vitellogenin) assays in male bream (*Abramis brama*) (51). Sediment samples from 5 sites were collected and extracted by accelerated solvent extraction and cleaned with gel permeation prior to *in vitro* analysis by ER-CALUX. Average vitellogenin concentrations at non-reference locations were significantly higher than at reference locations. *In vitro* activity of the sediment samples ranged between 0.22 ng E2/g and 0.96 ng E2/g dry weight, but the correlation coefficient between sediment EEQ derived from ER-CALUX and vitellogenin expression between the sites was less than 0.05. However, when the investigators also evaluated the *in vitro* estrogenic activity of the gut contents, they found a very high correlation with vitellogenin ($r^2 = 0.8$) and conducted a subsequent TIE analysis of the gut contents. The contents were first homogenated, extracted, cleaned of fats, and then further cleaned on a gel permeation column where it was eluted with dichloromethane. The remaining extract was fractionated by reversed-phase HPLC, where fractions were divided up for bioassay activity testing using the ER-CALUX assay as well as chemical analysis by GC-MS with an ion trap in MS/MS mode and GC-MS with a mass selective detector. The majority of estrogenic activity in fractions corresponded qualitatively to steroid estrogens including 17 α - and β -E2, E1, and BPA. BPA was detected in high concentrations (up to 3263 ng/g lipid) in animals from contaminated sites, but not from reference locations. EEQs derived from the analytical chemistry accounted for 50-100% of the observed *in vitro* estrogenicity. Several exogenous compounds including triclosan, chlorophene, 2,7-dibenzo-*p*-dioxin, hexa- and heptachlorinated biphenyls, polycyclic aromatic hydrocarbons along with the endogenous compound cholesterol (and cholesterol derivatives) and the plant sterol campesterol were detected. The investigators concluded that diet may be an important pathway of xenoestrogen exposure. However, it should be noted that E2 is normally metabolized through gut biliary elimination and re-absorbed through enterohepatic circulation. Consequently, it is unclear whether the compounds measured in the gut were actually anthropogenic/xenobiotic or endogenous E2. Gut content TIEs represent a novel qualitative method for identifying bioavailable xenoestrogens, but care must be given when using an *in vitro* assay to quantify estrogens in the gut. While steroid

concentrations or *in vitro* activity may be higher in the gut from contaminated sites due to dietary accumulation, this may be a result of altered steroid metabolism (e.g. aromatase induction) in fish from the contaminated sites. Consequently, care should be given with the interpretation of results from these studies.

9. Conclusion

The toxicity identity evaluation (TIE), employing a gamut of *in vitro* and *in vivo* bioassays in concert with chemical analysis, is a powerful tool to discern causative agents responsible for observed biological effects such as ER activation. When *in vitro* bioassays are designed with variants of the human ER, it is unsurprising that steroidal hormones account for most of the bioactivity as they bind the receptor many orders of magnitude above xenoestrogens. It is also unsurprising that estrogenic waters receiving domestic influent owe much of their observed estrogenicity to steroid hormones, as they are commonly excreted from humans and “pseudo-persistent” due to their constant occurrence particularly in wastewater dominated surface waters. However, chemical analysis as well as *in vitro* bioassay results often correlate poorly with observed *in vivo* effects, as either measurement cannot account for the complex biochemical cascades inherent in intact organisms. This depicts xenobiotic-induced feminization as a much more complicated process than direct binding of the xenobiotic to the ER. Other mechanisms including the perturbation of the hypothalamus-gonad-pituitary axis, steroid biosynthesis, steroid catabolism, or synergy and potentiation between xenoestrogens may be responsible for estrogenic effects observed in samples lacking steroid estrogens. It is concluded then that causal relationships between environmental pollutants as identified by TIEs requires additional research to shed light into the mechanistic complexities underlying the *in vivo* effects of environmental estrogens.

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Chapter 27

Fluorinated Chemicals and the Impacts of Anthropogenic Use

D. E. C. Adams¹ and R. U. Halden^{*,1,2}

¹School of Sustainable Engineering and the Built Environment,
Arizona State University, 1001 South McAllister Avenue, Room A210,
Tempe, AZ 85287-5701

²Center for Environmental Biotechnology at the Biodesign Institute,
Arizona State University, Tempe, AZ 85283

*Corresponding author: School of Sustainable Engineering and the Built
Environment, Arizona State University, 1001 South McAllister Avenue,
P.O. Box 875701, Tempe, AZ 85287-5701. E-mail: halden@asu.edu.

This literature review provides insights into the natural occurrence of fluorinated organic compounds, their chemical properties, their history and applications, associated risks and benefits, and policy approaches for sustainable and safe uses in the future.

Owing to their unique chemical properties and widespread uses, organofluorines are indispensable in modern society. However, their environmental persistence, toxicity and bioaccumulation potential also pose considerable risks to the environment and human populations. Less than a dozen organofluorines are known to occur naturally whereas manmade organofluorines figure in the hundreds. This implies that the large-scale environmental release of manmade organofluorines is an ongoing experiment the consequences and implications of which are as of yet poorly understood. However, available data indicate organofluorines to be more persistent and more toxic than their non-fluorinated counterparts.

Reviewed in detail are the history of production and regulation of chlorofluorocarbons (CFCs), perfluorooctane sulfonate (PFOS), and perfluorooctanoic acid (PFOA). Unforeseen risks to the environment and human health are discussed and shown, at least for some organofluorines, to

outweigh their respective economic benefits. In 1989, the Montreal Protocol on Ozone Depleting Substances banned the use of CFCs by international agreement. In 2006, the 2010/15 PFOA Stewardship Program began, with the intention of reducing PFOA emissions by 95% by 2010 and achieving a complete phase-out of PFOA by 2015. In May of 2009, the Stockholm Convention on persistent organic pollutants (POPs) recognized PFOS as a POP and restricted its use to specific industrial applications. These three compounds represent only a small number of the organofluorines in production, however. Further investigations are warranted into other members of this family of persistent chemicals.

The bioaccumulation potential, capacity for long-range transport, toxicity and persistence of organofluorines challenge human society to carefully manage chemical production and releases. To stem the ongoing accumulation of anthropogenic fluorinated compounds in the environment, the chemical industry should be incentivized to develop organofluorine replacements that resemble naturally occurring compounds and for which natural degradation pathways exist. Implementing this and similar more sustainable practices, derived from the precautionary principle, will aid in eliminating or significantly reducing unwanted ecological and human health risks.

Introduction

Fluorinated organic compounds or, in shorthand, organofluorines, play an important role in modern society. Their uses include refrigerants, stain repellants, paper coatings (1), agrichemicals, pharmaceuticals (2), non-stick coatings and materials made from Teflon[®], chlorofluorocarbons (CFCs), and hydrofluorocarbons (HFCs).

Organofluorines are used so widely because of the unique properties derived from their chemical structure. They have short, strong bonds between carbon and fluorine atoms, which limit reactivity and increase durability in the environment. In contrast to their prominence in a modern society, organofluorine compounds are produced in nature only rarely and in small quantities (3). The fact that most organofluorines are anthropogenic in origin and lack a large-scale natural degradation pathway makes them an important topic to analyze from an environmental health and sustainability perspective.

This literature review provides an overview of organofluorine compounds in the context of environmental and human health effects. It discusses natural occurrences of organofluorines, the significance of the carbon-fluorine bond in manmade products of daily use, degradation pathways, as well as the environmental and health effects posed by these anthropogenic compounds. It concludes with a cost-benefit analysis of the uses of organofluorines and a discussion of the sustainability of organofluorine production.

Natural Organofluorines

In nature, organofluorines occur in only a few locations, namely in certain species of plants. These organofluorines are produced in the form of fluoroacetate, which discourages herbivores from consuming a plant. Fluoroacetates are found in approximately 40 plant species across Australia, Brazil, and Africa (4). Given that there are between 250,000 and 350,000 identified plant species (5), only a very small fraction of the plant life on the planet produces organofluorines. Similarly, of all the microorganisms on earth, only two species are known to produce fluoroacetate, one of which is *Streptomyces cattleya* (6).

The rarity of organofluorines in nature is surprising and noteworthy. Living organisms, including plants, fungi, and microbes, are extremely versatile with respect to the number of compounds they synthesize as secondary metabolites. Thousands of biogenic compounds have been found on and within the earth's crust. Secondary metabolites of micro- and macro-organisms are known to frequently contain bromine, chlorine, and to a lesser degree iodine. In contrast, carbon-bound fluorine has been identified in less than a dozen discrete fluorinated natural products (3). Among these, the two of greatest interest are fluoroacetate, which occurs in several plant species, and 4-fluorothreonine in *S. cattleya*. Fluoroacetate was the first naturally occurring organofluorine to be identified in 1943, and the most recent is 4-fluorothreonine identified in 1986 (7). This discovery was of particular significance because the biosynthesis of fluoroacetate and 4-fluorothreonine in the bacterium *S. cattleya* is the most studied system of natural organofluorine production to date (8). The rapid lifecycle of bacteria relative to plants makes bacterial biosynthesis a comparatively more convenient system for examining biological fluorination.

A wide variety of plants produce fluoroacetate at low levels, including agronomic crops like soya bean (*Glycine max*) and crested wheat grass (*Agropyron cristatus*), with concentrations up to 4 parts-per-million (ppm) on a dry weight basis (3). In contrast, only a handful of plants produce fluoroacetate at toxic levels. The leaves of the South African shrub *Dichapetalum cymosum* have been reported to contain fluoroacetate at levels of up to 250 ppm (3). Farmers in this geographic region customarily fence off the plant in the early spring to prevent their livestock from eating and dying from the poisonous plant materials. In central Africa, other members of the *Dichapetalum* genus are found which produce fluoroacetate. *D. braunii* has the highest recorded level of fluoroacetate in its seeds: concentrations of up to 8000 ppm have been reported (9). In Australia, particularly the southwestern portion of Western Australia, 35 species of fluoroacetate-accumulators have been identified (3). *Oxylobium parviflorum* (box poison) and *Gastrolobium bilobum* (heart leaf poison) are the two most common species, and can contain up to 2600 ppm in their leaves and 6500 ppm in their seeds (10). Species occurring in northern Australia are less toxic, with leaves of *Acacia georginae* containing up to 25 ppm of fluoroacetate, whereas *Gastrolobium grandiflorum* was reported to contain up to 185 ppm of the organofluorine compound (10). Finally, the South American plant *Palicourea marcgravii* has been found to accumulate levels of up to 500 ppm in its seeds and flower stalks (10). Table I gives a brief summary of plants showing significant

fluoroacetate accumulation, their reported concentration levels, and general geographic location where the plant species can be found.

Fluoroacetate production likely is a defense mechanism in plants, a theory supported by the fact that fluoroacetate concentrations vary within the plant depending on the tissue under consideration. Tissues that are the most essential and vulnerable to plants – flowers, seeds, and young leaves – have the highest concentrations of fluoroacetate. This is consistent with chemical defense strategies in other plants featuring poisonous compounds, where the plants are protecting the parts that are most important to survival and reproduction (11).

Countering the biological adaptation of a poisonous defense mechanism in plants of these regions, tolerance to fluoroacetate shows a marked increase in foraging animals in areas with fluoroacetate-producing plants, particularly in seed eaters and herbivores which are directly exposed to the toxin. The Emu (*Dromaius novaehollandiae*), a seed eating bird from the region of Australia where *O. parviflorum* and *G. bilobum* are endemic, has a very high level of resistance to fluoroacetate. The mean lethal dose (LD₅₀) for an emu ranges from 100–200 mg/kg of body weight compared to an LD₅₀ range of 0.2–20 mg/kg for seed eating birds living outside the range of fluoroacetate-producing plants (12). Similarly, in mammals, the brush-tailed possum *Trichosurus vulpecula* is much more tolerant of fluoroacetate in southwestern Australia than members of the same species in eastern Australia – it is up to 150 times more resistant to fluoroacetate poisoning (13). Insects, in addition to birds and mammals, show advanced fluoroacetate tolerance in regions where the producers are present. Species that feed on plants that do not produce fluoroacetate are 40 to 150 times more susceptible to fluoroacetate poisoning (3).

The high level of toxicity of fluoroacetate, and its relation to anthropogenically produced organofluorines, makes it a chemical of interest when discussing the prevalence of these chemicals in our society. Similar to anthropogenic organofluorines, the carbon-fluorine bond (to be discussed in the next section) in fluoroacetate transforms the readily biodegradable compound acetate into a very persistent chemical derivative. Because of the chemical's persistence and high toxicity, it is important to consider the tolerance mechanism in species that can live with high concentrations of fluoroacetate. One of the most studied tolerance mechanisms is in bacteria. As discussed later on, a small number of prokaryotic organisms are capable of targeted degradation and utilization of fluoroacetate (14, 15).

This biodegradation pathway can perhaps be applied to artificial organofluorines in the future, thereby presenting a mechanism for limiting organofluorines' persistence in the environment. This would be an important step towards sustainability in the global chemical industry by greatly reducing the amount of persistent chemicals that make their way into the environment. Understanding the fate of organofluorines in the biosphere and their effects on biota is essential given the rarity of fluorinated compounds in nature, their relative stability and persistence, their lack of large-scale natural degradation processes, and the increasing anthropogenic production and usage of this class of predominantly anthropogenic compounds.

Table I. Identity and Distribution of Plants Featuring Significant Fluoroacetate Accumulation

<i>Name</i>	<i>Location</i>	<i>Fluoroacetate concentration</i>	
		<i>Leaf (ppm)</i>	<i>Seed (ppm)</i>
<i>Glycine max</i> (soya bean) ^a	Worldwide	4	-
<i>Agropyron cristatus</i> (crested golden wheat) ^a	Worldwide	4	-
<i>Dichapetalum cymosum</i> ^a	South Africa	250	-
<i>Dichapetalum braunii</i> ^b	Central Africa	-	8000
<i>Oxylobium parviflorum</i> ^c	Western Australia	2600	6500
<i>Gastrolobium bilobum</i> ^c	Western Australia	2600	6500
<i>Acacia georginae</i> ^c	Northern Australia	25	-
<i>Gastrolobium grandiflorum</i> ^c	Northern Australia	185	-
<i>Palicourea marcgravii</i> ^c	South America	500	500

^a Taken from reference (3). ^b Taken from reference (9). ^c Taken from reference (10). - Not present or not determined.

Properties of Organofluorines

An organofluorine is defined as an organic molecule with one or more of its hydrogen atoms replaced by a fluorine atom. This change in the atomic composition of these organic molecules can drastically change their properties (16), making them useful in a wide number of industrial, pharmaceutical, and agrochemical applications, as well as in consumer products. Because of the abundance of uses for organofluorines in modern society, they are of significant economic value.

The importance of organofluorines lies in their carbon-fluorine (C-F) bond, the strongest single bond in organic chemistry (2). The widespread usage of organofluorines stems from the C-F bond and its implications to molecular bond strength, persistence, reactivity, and durability. However, these properties, which make organofluorines desirable from an economic standpoint, have the opposite effect when considered from a health and environmental standpoint. While degradation pathways do exist for organofluorines on small scales, there is no rapid large-scale process for breakdown of these chemicals, thereby implying their persistence in the environment and potential accumulation in animals and humans.

A. The Carbon-Fluorine Bond

Of all the elements in the periodic table, fluorine is the most electronegative. When a C-F bond is formed, it is the strongest single bond in organic chemistry, making fluorine substitution desirable for pharmaceutical development as well as

material sciences (2). The C-F bond gets its stability from electrostatic attraction between the polarized, positively charged carbon atom ($C \delta^+$) and the negatively charged fluorine atom ($F \delta^-$) (2). The partially ionic nature of the bond causes the distance between the two atoms to be short, essentially increasing the persistence and durability of the compound, while reducing the reactivity. As more fluorine atoms are added to a given carbon atom of a chemical compound, the bond strengthens and shortens (2). Hence, carbons with four fluorine atoms attached to them (known as fluoroalkanes such as tetrafluoromethane or carbon tetrafluoride) are some of the most unreactive organic compounds. This low reactivity and short bond length means that these compounds are durable against degradation, and persistent in the environment. Therefore, accumulation of these chemicals becomes an issue as the anthropogenic use of them keeps increasing.

B. Degradation Pathways

Despite their strong chemical bonds and the scarcity of organofluorines in nature, degradation processes do exist for these compounds. However, the processes that have been identified are not currently capable of being scaled-up to deal with the high level of anthropogenic production and use of organofluorines.

One degradation process is the photochemical reaction that chlorofluorocarbons (CFCs) undergo in the stratosphere. These chemicals (as the name indicates) consist of chlorine, fluorine, and carbon atoms. Because of the C-F bond, CFCs are very stable in the lower atmosphere. This stability allows them to move all the way into the upper atmosphere without degrading, and once they reach high altitudes they are exposed to much more solar radiation. This increased radiation results in photolysis of the carbon-chlorine bond, releasing a chlorine atom into the atmosphere (17). This radical (or free) chlorine atom is long lived in the upper atmosphere and acts as a catalyst for the conversion of ozone (O_3) into O_2 . Ozone absorbs ultraviolet radiation much better than O_2 does, so the addition of CFCs to the atmosphere causes more UV radiation to reach the earth's surface, causing many detrimental effects. It should be noted that the photodegradation does not act on the C-F bond but instead on the weaker C-Cl bond in the chemical. In this case, the low reactivity of CFCs and the strength of the C-F bond allow these organofluorines to find their way into the upper atmosphere, causing serious damage to the ozone layer and everything this layer protects from the sun's harmful rays.

Biodegradation is an alternative breakdown pathway for organofluorines that, unlike the photolysis of CFCs, can work on the C-F bond to effect defluorination. Several examples of this process exist naturally in microorganisms, though they are not common. Several microorganisms found in the soils of New Zealand have tolerance to naturally occurring fluoroacetate and are capable of growing on and utilizing fluoroacetate as a sole source of carbon. The bacterium *Pseudomonas* sp. and the fungus *Fusarium solani* produce enzymes which are capable of defluorinating fluoroacetate (14). In addition to these two, the microorganism *Moraxella* sp. breaks down fluoroacetate, displacing the fluoride atom and forming an enzyme-bound ester intermediate (15). Microorganisms known to feature biodegradation capabilities for organofluorines come from the limited

geographic regions where these rare compounds occur naturally. In the future, with better understanding of the natural processes at work, the biodegradation process can perhaps be optimized and scaled-up to reduce the environmental persistence of organofluorines.

C. Persistence of Organofluorines and Health Effects

Persistent organic pollutants (POPs) are defined as organic substances that are able to bioaccumulate, can be transported long distances, pose potential risks to animal and human health, and persist in the environment (18). The C-F bond that all organofluorines share makes them persistent in the environment. Acetate is a non-toxic and readily biodegradable compound that becomes highly toxic and persistent when subjected to a single fluorine substitution. Unlike traditional POPs, manmade perfluorinated organic compounds can display polar properties and be highly mobile. Examples include perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). Both are found in humans and wildlife globally, even in remote locations such as the Arctic and Antarctic (18). On May 9, 2009, PFOS, its salts, and perfluorooctane sulfonyl fluoride (PFOSF) were added to Annex B of the Stockholm Convention on POPs, restricting the acceptable uses for these chemicals to specialized industrial purposes (photographic processes, aviation, semiconductors, medical devices, metal plating, and pest control for certain situations) (19). The POP characteristics that PFOS and its related compounds demonstrate are extreme persistence, substantial bioaccumulating and biomagnifying properties, capacity for long-range transport, and considerable toxicity. Unlike typical POPs with bioaccumulation potential, PFOS binds to proteins in the blood and liver (19). Thus, the bioaccumulation of perfluorinated compounds (PFCs) is mechanistically different from that of traditional POPs. This implies that established quantitative structure activity relationship (QSAR) models are ill-equipped to describe and predict partitioning into fat (lipophilicity) and other biologically relevant chemical behaviors.

The recognition of PFOS and its related compounds as POPs and their addition to the Stockholm Convention was due to several contributing factors. A body of research showing the global distribution of organofluorines was important to demonstrate the long-range transport of these compounds. In one study, high volume air samples were gathered by research vessels in the Atlantic and Southern Oceans, as well as in the Baltic Sea and one land-based site near Hamburg, Germany. Taken in 2007 and 2008, these samples were analyzed for organofluorines. Findings lent credence to the model of atmospheric long-range transport of organofluorines to remote locations, such as the earth's polar regions (20). Concentrations of organofluorines were found in urban environments and were higher than in remote locations, as could be expected near production facilities. However, the presence of organofluorines in remote locations thousands of miles away from production facilities indicates that global transport of these chemicals is occurring (20). In addition to this study, other studies have been conducted on animals in both developed areas (the North American Great Lakes region, for instance) and remote areas (the Arctic and Antarctic). One study demonstrated the occurrence of organofluorines at detectable concentrations in

animals globally and provided additional evidence for their long-range transport. Measured levels of PFOS typically are expressed in ng/g wet weight in liver tissue and in ng/mL in blood plasma. In the Arctic Circle, concentrations of up to 680 ng/g were found in polar bear livers, while concentrations of up to 230 ng/mL were found in the blood plasma of the ringed seal in the Baltic Sea. In the US, river otters were found to have concentrations of up to 990 ng/g, while mink were found to have concentrations of up to 3680 ng/g. Bald eagles were found to contain concentrations of up to 2570 ng/mL, while the common loon and brown pelican exhibit concentrations of up to 690 ng/g and 620 ng/g, respectively. Carp were found to be the fish with the highest observed levels of PFOS, with concentrations of up to 300 ng/g in the muscle tissue from fish caught in Saginaw Bay, Michigan. In Mississippi, the yellow-blotched map turtle was found to have concentrations of up to 700 ng/g in its liver. In the Mediterranean Sea, India, Korea, Japan, and the North Pacific, dolphins, birds, tuna, and albatrosses were also studied, and liver tissue and blood plasma samples were taken. Similarly, samples from birds and seals in Antarctica were also included in the study (21). This global study on the concentrations of PFOS in various species included species of different trophic levels to provide information on bioaccumulation potential, and tested for four organofluorines, including PFOS and PFOA. Two studies focusing on the Common Guillemot (*Uria aalge*) in the Baltic Sea were conducted concerning organofluorine concentrations in egg, liver, kidney, and muscle tissue of the birds (22, 23). A study on PFOS and its related compounds in Herring Gull eggs in the Laurentian Great Lakes of North America showed that organofluorine concentrations were highest in eggs from colonies close to highly urbanized or industrial sites in Lakes Erie and Ontario (24). A study on Lake Trout in the Great Lakes corroborated the findings that organofluorines are present even away from industrial and urban centers, but can be found in higher concentrations near areas of production (25). The majority of studies into global organofluorine distribution and transport have focused on the Northern Hemisphere, as most production and usage of these compounds occurs here. For this reason, the far reaches of the Southern Hemisphere are some of the most remote in that they lie the farthest from sources of organofluorines, and are therefore important to the study of long-range transport. A study of animal samples across the Southern Ocean and Antarctic was conducted to test for 10 different PFCs during 1995-2005. The samples included livers from albatross, blood from elephant seals, and blood and eggs from penguins and polar skua (26). Findings showed that even in these highly remote locations, PFOS and PFOA could be found in low, but detectable, levels. All these studies which show the presence of organofluorines, and particularly PFOS, clearly display the capacity for long-range distribution of these compounds, a property associated with POPs.

Another contributing factor to the addition of PFOS to the Stockholm Convention on POPs was research showing bioaccumulation in fish, birds, and Arctic and Antarctic animals. This demonstration of bioaccumulation in addition to results of possible detrimental health effects in animals fulfilled two more conditions of the requirements for consideration as a POP. The study on globally distributed PFOS in wildlife obtained results on organofluorine concentrations in a wide range of species globally, and at differing trophic levels (21). These

findings showed higher concentrations in apex predators (river otter, polar bear, mink, bald eagle) than in species from lower trophic levels, thus suggesting bioaccumulation of PFOS. The fish from the study with the highest concentration of PFOS were carp from Saginaw Bay, Michigan. Under laboratory conditions, American Minks (*Mustela vison*) were fed these carp and the bioconcentration factor in the liver of the minks was determined to be approximately 22 (21). This is a direct demonstration of the bioaccumulation potential of these organofluorine compounds as they move up the food chain. Another study in the Canadian Arctic showed biomagnification of PFOS in marine food webs (27). A study of Polar Bears (*Ursus maritimus*) substantiated these findings of bioaccumulation of PFCs. Samples from 1984–2006 were tested for PFCs and showed annual increases from 2.3% to 27.4% (28). According to trends developed in the study, bioaccumulation could lead to possibly toxic levels of PFCs in polar bears by 2014–2024 (28). Exposure to PFOS and PFOA has been observed to have negative developmental effects on rats and mice, resulting in cardiac abnormalities, cleft palate, placental edema, delayed skeletal ossification, reduction in fetal weight, and increased pup mortality (29). Additionally, the U.S. Environmental Protection Agency (US EPA) has classified PFOA as a carcinogen in animals (30).

The third contributing factor was research into health concerns for human exposure to organofluorine compounds. Several studies showed that PFCs are present in global human populations by sampling and testing blood and breast milk (31–34). Furthermore, concerns about PFOA are that it may have significant effects on the nervous, endocrine, and immune systems, as well as the capacity to be transferred to the fetus through the umbilical cord (35). PFOA in some cases can also cause cancer of the liver, testes, pancreas, and mammary glands (36), and can result in developmental diseases and embryonic deformities (37). Given these serious possible correlations between PFOA, PFOS, and PFCs in general, the precautionary principle should be employed. Principle 15 of the Rio Declaration states:

“In order to protect the environment, the precautionary approach shall be widely applied by States according to their capabilities. Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation” (38).

This is a main principle included in the Stockholm Convention, and it is consistently applied to the classification of POPs. The inclusion of PFOS as a POP was an important step in the regulation and restriction of a chemical that is persistent, lacks a large-scale degradation pathway, bioaccumulates, is capable of long-range transport, and has possible toxic effects on humans and the biosphere. Whereas regulatory interventions against release of PFOS and PFOA already have and continue to be contemplated, the problem of organofluorines is much broader. Essentially, mankind has embarked on the mass production of organic chemicals that will stay in the chemosphere and biosphere for decades and centuries, if not millennia.

An Organofluorine Timeline

The first reported organofluorine synthesis occurred in 1835, and was that of methyl fluoride from dimethyl sulfate by Dumas *et al.* (39). Elemental fluorine, however, was not isolated for the first time until 1886 by Henri Moissan (40). This isolation, while an important step in the progress of organofluorine chemistry, did not provide a safe way to control the reaction of elemental fluorine with hydrocarbon-based substrates. For almost 40 years after Moissan's discovery studies on elemental fluorine were rare, due to the risk of deadly reactions involving flames and detonation (41).

In 1928, the first chlorofluorocarbon (CFC) was developed by Midgley, McNary and Henne. Until CFCs were developed, refrigerants used were sulfur dioxide and ammonia, chemicals that are flammable, toxic, and corrosive (41). The synthesis of CFCs provided a nonflammable, low-toxicity, inert alternative for use as a refrigerant. This research by Midgley *et al.* was sponsored by General Motors (GM) Corporation, a manufacturer of refrigerators in the late 1920s. After the discovery of CFCs, GM approached DuPont as a manufacturer for these compounds, and in 1930, the two companies started Kinetic Chemicals Inc., a joint corporation (42). In 1931, Kinetic Chemicals trademarked Freon[®]-12 and began the expansion of its facilities. Over the next several years, Kinetic Chemicals expanded their chemical line to Freon[®]-11, Freon[®]-113, Freon[®]-114, and Freon[®]-22 (42). In 1938, a chemist working for DuPont discovered polytetrafluoroethylene (PTFE), which DuPont trademarked as Teflon[®] in 1945. During World War II, further development of fluorine chemistry was undertaken.

The Manhattan Project utilized uranium hexafluoride (UF₆) in a gas-diffusion process to separate uranium isotopes ²³⁵U and ²³⁸U. However, UF₆ is almost as reactive as elemental fluorine, so in order to use it, a number of materials had to be developed that would not react with UF₆. Fluoropolymers were the material of choice for this, and a range of coolants, lubricants, and polymers that could be fabricated into components were developed (42).

In 1951, the manufacturing company 3M opened the first commercial plant for fluorocarbon production (42). The large scale of production and their continued research into organofluorines led to the development of water and oil repellent agents such as 3M's Scotchgard[®], and other textile finishes. PTFE and related organofluorines have been, and continue to be used in many consumer products. Teflon[®] is used as a coating in cookware because of its non-stick properties, and in fabrics and textiles as a soil and stain repellent. Gore-Tex[®] is a breathable, waterproof fabric used in many outdoor clothing applications (i.e., windbreakers, rain parkas, snow clothing, etc.), and has a layer of waterproof laminate made out of PTFE. In a similar fashion, fluorocarbon-based paints are used to weatherproof many external surfaces on buildings and other structures (43).

The most recent advancements in organofluorine chemistry have been in the fields of medicine and agriculture. Pharmaceuticals and agrochemicals are two of the main branches of organofluorine research, but other important uses exist. Perfluorocarbon (PFC) fluids are used as a contrast agent for ultrasound imaging in cardiology (44), and anesthetics have been improved from ether and chloroform to safer alternatives like halothane (CF₃-CHClBr) (43). Many current

pharmaceuticals are fluorinated, and touted as antibiotic, antifungal, anticancer, and antiviral drugs. Much of their bioactivity is owed to the fluorine atoms in their structure. The antidepressant drug Prozac[®] for example, has a trifluoromethyl group (CF₃) in it, which allows the drug to easily cross the blood-brain barrier where it acts (43). 5-Fluorouracil (5-FU), an anticancer drug, is the fluorinated version of uracil, which itself is a naturally occurring derivative of pyrimidine, a compound common in nature (45). Both these compounds are colorless, crystalline solids with high melting points, but the fluorinated version is much more biologically active (46). 5-FU is frequently used to treat solid tumors such as liver, gastric tract, and colorectal carcinomas. Unfortunately, medical applications of this organofluorine are limited because of its strong intestinal toxicity, short plasma half-life, and poor tumor affinity (45). Agrochemicals with fluorine atoms in their structure are used as fungicides, herbicides, and insecticides. Examples include the herbicides Treflan[®] and Fusilade[®], the insecticides fipronil and lambda-cyhalothrin, and the fungicides epoxiconazole and trifloxystrobin (43, 47).

Organofluorine compounds are critical in the way they support the current standard of living, and the multitude of uses for them ensures that they have permeated our culture. In 1992, the estimated value of all commercially available, fluorine containing products was estimated to be around \$50 billion per year (48). In the 18 years since this estimate, the value of the organofluorine industry has surely increased.

A. Concerning CFCs, HCFCs, and HFCs

As mentioned briefly above, CFCs were discovered in 1928, and production of these compounds began. At the time, they provided an ideal alternative to the use of sulfur dioxide and ammonia as refrigerants. CFCs have low toxicity, are nonflammable, and very stable. The first CFC developed was CFC-12 (CF₂Cl₂) by Midgley *et al.*, and the desirable properties of CFC-12 launched an investigation into other possible CFCs. As new compounds were discovered, they were applied to many new areas such as aerosol propellants, foam blowing agents, and solvents. Their usage became more widespread through World War II, and the CFC market showed growth all the way into the 1970s (41). By the early 1970s, CFCs were being produced in many countries around the world, while at the same time scientists were starting to identify and communicate the role CFCs were playing in the depletion of the ozone layer (49).

When it was discovered that CFCs were causing ozone depletion, a combination of governmental regulation and innovation on the part of the chemical industry allowed for the phase-out and replacement of CFCs. Similar to the way CFCs replaced sulfur dioxide and ammonia, compounds were developed during the phase-out of CFCs to provide low toxicity substitutes with similar performance properties. Compared to CFCs, hydrochlorofluorocarbons (HCFCs) have a reduced ozone depletion potential and hydrofluorocarbons (HFCs) have almost zero ozone depletion potential. From a structural chemistry perspective, this is because the chlorine atom that was released during the photochemical reaction of CFCs in the upper atmosphere is no longer present in the compound.

This chlorine atom, which was the catalyst for ozone depletion, is not a problem anymore, and the desirable stability and persistence of the compound is maintained by the still present C-F bond. The legal framework that prompted this change is a shining example of global cooperation.

In December of 1978, a ban was passed on CFCs for use as aerosol propellants in North America. Globally however, CFC use continued to rise into the late 1980s. In 1987, the *Montreal Protocol on Substances That Deplete the Ozone Layer* was opened for signature, but was not entered into force until January 1, 1989 (50). The Montreal Protocol is designed to protect the ozone layer by halting the production of a number of substances (CFCs among them) that have been recognized as being harmful to the ozone layer. Given the global scale that ozone depletion was acting on, cooperation between countries around the world and the widespread implementation of the Montreal Protocol were necessary to affect any measurable results. The success of this protocol has been recognized as one of the most significant international efforts from both a policy and an environmental standpoint. Kofi Annan, Secretary-General of the United Nations from 1997-2007 and 2001 Nobel Peace Prize winner, has said, "Perhaps the single most successful international agreement to date has been the Montreal Protocol" (50).

This governmental agreement is both promising and impressive. A chemical that was originally designed as an inert, non-toxic improvement was determined to have negative global impacts on humans and the surrounding environment. As a result, international agreements were made to halt the chemicals' production. These agreements have been adhered to, and the ozone layer is expected to return to its pre-industrial age levels between the years 2060-2075 (50).

The balance between health and environmental costs and economic and technological advancement is a complicated issue when dealing with organofluorines. CFCs, for example, played a very important role in making modern life what it currently is. Beginning with refrigeration, CFCs replaced noxious, flammable, unstable gases with an alternative not exhibiting any of the previous weaknesses. Unforeseen however, was the global impact that CFCs would have on the ozone layer. Their stability, the very trait that made them desirable, also allowed them to persist in the environment causing a major global problem. At the time, CFCs represented a marked improvement in refrigeration and air-conditioning technology and safety. As they became mainstream, approaching 2.5 billion pounds produced annually in 1986 (41), the global implications of their use became apparent. CFCs are the number one compound that comes to mind when discussing ozone depletion, and due to their long atmospheric lifetime, they are considered probable contributors to global warming. Fortunately, regulations and a small concentration compared to other gases in the atmosphere mean that CFCs are not of serious concern as green house gases (41).

B. Concerning PTFE, PFOS, and PFOA

Polytetrafluoroethylene (PTFE) and its related compounds are some of the most common organofluorines used today. PTFE is used in surface coatings for cookware, clothing, building materials, and upholstery and textiles.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are important to many specialized industrial purposes including aviation, the manufacture of semiconductors, medical devices, metal plating, specific pest control, lubricants, and fire suppressants and retardants (51). These chemicals are extremely persistent and, as discussed in a previous section, have been found in measurable levels in animals and humans worldwide. Of these chemicals, two of them are now regulated by governmental authority. Whereas PFOS is now recognized by the Stockholm Convention as a POP, restrictions on the production of persistent PTFE are not currently being considered.

Similar to the restrictions on PFOS, though on a less global scale than the Stockholm Convention, the US Environmental Protection Agency (US EPA) has identified PFOA as an organofluorine of concern to public and environmental health. The EPA describes PFOA as a synthetic chemical that does “not occur naturally in the environment” and is environmentally persistent, occurs at low levels in the environment and in the blood of the general U.S. population, remains in people for a very long time, and causes developmental and other adverse effects in laboratory animals (30). According to the EPA, “In 2006, EPA and the eight major companies in the industry entered into the 2010/15 PFOA Stewardship Program, in which the companies committed to reduce global facility emissions and product content of PFOA and related chemicals by 95 percent by 2010, and to work toward eliminating emissions and product content by 2015” (30).

In the past, significant changes have been ushered in when knowledge of dangerous health effects became public knowledge and triggered a large public outcry. CFCs and PFOS represent two examples for this genesis. The cooperative program to regulate PFOA marks an opportunity for the chemical industry to begin to shoulder the responsibility of developing and producing chemicals that (contrary to current use PFCs) have natural counterparts and thus likely will have established degradation mechanisms and pose fewer health risks. Such a proactive behavior would represent a significant change compared to previous actions by major companies that often have appeared reactive, merely seeking to exculpate themselves from costly lawsuits and settlements such as those that occurred in 2004-2005.

In mid-2004, blood serum was collected from residents of towns near DuPont’s Teflon[®] production facility in Parkersburg, West Virginia. Results from these tests came back in 2005 with PFOA levels “60-75 times higher than in the general population,” and “[Du Pont] began offering bottled water to all residents being serviced in the Little Hocking Water District within days” (52). This swift action by the manufacturer is applaudable and may have been triggered in part by the company’s concerns over obtaining a potentially tarnished public image. By 2001, there were reports in the scientific literature of PFOA studies on animals showing that the compound produced multiple tumor types, had adverse reproductive and developmental effects and was a liver toxicant (53, 54). These results in 2001 should have given the manufacturer ample time, opportunity, and reason to be concerned about high serum PFOA levels in the towns surrounding their Teflon plant. Yet, it took until October of 2005 for the results to be made publically available to the residents, and for the manufacturer to take action (52).

In addition to the 2005 West Virginia incident, cases of PFOA contamination occurred in Alabama and New Jersey in 2008 (1). The serum half-life of PFOA in humans is 4.4 years, much longer than the chemical's half-life in animals that have undergone testing (54). Additionally, the health concerns that have been uncovered in animal tests and the global distribution of PFOA make it a chemical of serious concern. The 2008 case of PFOA contamination in Decatur, Alabama, triggered an EPA drinking-water advisory, with similar warnings being issued in New Jersey (1). Epidemiologic studies from the U.S. and Denmark published in 2007 showed that detectable levels of PFOS and PFOA can be found in the general population and that these exposures show a negative association with developmental health outcomes, including decreases in birthweight, ponderal index, and head circumference of newborns (55).

Pre-emptive action on the part of major chemical companies would be a welcome change of pace from previous industrial practices, and would demonstrate significant corporate responsibility and environmental stewardship. The 2010/15 PFOA Stewardship Program could prove to be a step in the right direction towards self-governance by the chemical industry. Optimistically, it is possible that the program will be as successful in limiting PFOA as the Montreal Protocol was in limiting CFCs, and will demonstrate the kind of cooperation that is needed within the industry to move away from the use of persistent organic pollutants, no matter how useful they are. Such a stewardship program may mark the beginning of a paradigm shift towards self-regulation by chemical developers and manufacturers and in the future may not require the guiding hand of the EPA. Ideally, organic chemicals for which no effective natural degradation processes are known should not be produced in the first place. From an industry perspective, manufacturing of chemicals with a pre-determined end-of-life scenario may appear counter-intuitive, since many manufactured chemicals require durability and persistence. Yet, excessive environmental persistence can have severe human health and economical implications, as the case studies of PFOS and PFOA illustrate. Developing a family of durable, yet biodegradable chemicals that do not have at least some negative impacts on human health and the environment is a daunting task that nevertheless should be pursued with great rigor and adequate funding.

Toward Sustainable Use of Organofluorines

In order to gain a more complete understanding of the role organofluorines may play in the future, both the risks and benefits associated with these compounds should be considered. This will allow for an assessment of the utility of organofluorines in modern society, as well as the present and future health and environmental risks that some of these compounds pose due to their prolonged persistence. Table II presents an amassed list of the uses for organofluorines, and Table III presents the potential risks posed by organofluorines. Many of the risks listed in Table III, such as persistence and the potential for long-range transport, apply to a wide range of organofluorines; however, some risks are compound-specific, e.g., the link between ozone depletion and CFCs. While

some chemicals may have multiple uses or risks associated with them, not all do. Similarly, not all uses and risks associated with organofluorines could be included here.

Table II. Selected Major Uses of Organofluorines

Commercial	<ul style="list-style-type: none"> • Refrigerants – inert, low toxicity, nonflammable • Aerosols/propellants • Air conditioners for cars • Non-stick cookware • Fabric/Textile coating – dirt proof and waterproof • Weatherproof paint • Photographic processing • Pest control for specific situations
Industry & Manufacturing	<ul style="list-style-type: none"> • Lubricants • Semiconductors • Uranium enrichment • Fire suppressants and retardants • Aviation • Metal plating
Medical	<ul style="list-style-type: none"> • Ultrasound imaging • Anesthetics
Pharmaceuticals	<ul style="list-style-type: none"> • Antibacterial • Antifungal • Anticancer • Antiviral • Antidepressant
Agricultural	<ul style="list-style-type: none"> • Insecticides • Herbicides • Fungicides

Table III. Selected Major Risks of Organofluorines

- Extremely persistent
- Ozone depletion potential
- High global warming potential
- Long-range transport
- Toxic degradates (e.g., formation of PFOA)
- Bioaccumulation
- Potential toxicity
- Carcinogenic
- Endocrine disruption
- Liver toxicant
- Developmental and reproductive problems
- Decrease in birthweight
- Decrease in head size at birth
- Others

It is important to note that formulating generalizations for an entire family of chemicals rarely hold true for each and every member of the group. Whereas the persistence of organofluorines and their accumulation in the biosphere are two common traits of this family of chemicals, not all organofluorines may exhibit these adverse properties and not all will need to be regulated. It is conceivable that restrictions in the use of compounds may have to be made on a case-by-case basis, with careful consideration of the associated risks and benefits of chemical use or abstention thereof. Further study of specific compounds may result in the identification of significant risks, while many compounds in the organofluorine family may have only minimal risks associated with them. While a discussion of options for regulating organofluorines is beyond the scope of this chapter, one has to assume that the potential for adverse effects and thus the need for regulation of organofluorine likely will increase with increasing fluorine content. Therefore, perfluorinated compounds are prime candidates for studies on potential adverse effects and the need for regulations.

The large number and wide range of uses for organofluorines shows how integrated they have become into modern society. A cost-benefit analysis on the compounds is difficult to perform, as the health risks to humans and the environment must be weighed against the social and economic benefits these chemicals provide. They are crucial in many ways, but unfortunately also pose multiple health risks to humans and the environment, which potentially can make the currently unregulated use of certain organofluorines unacceptable. In the past, CFCs, PFOS, and PFOA have all been deemed too dangerous for unregulated use – for the depletion of the ozone layer in the case of CFCs, and for their classification as persistent organic pollutants in the case of PFOS and PFOA.

While not all organofluorines share adverse characteristics – such as representing ozone depleting agents, acting as carcinogens or endocrine disruptors – they all do share one common trait, their pronounced persistence. This persistence, and the ever-increasing usage and production of these chemicals, ensures that compounds which do not have any negative effect at low concentrations are increasing in the environment and potentially can accumulate in the chemosphere and bioaccumulate in biota. Additionally, the youth of this branch of chemistry (<100 years) and the lack of organofluorines in nature imply that comprehensive knowledge of the effects of these chemicals on a global scale is evolving only slowly.

Replacement chemicals also are not necessarily safe. As discussed earlier, CFCs were introduced to replace other hazardous refrigerants (41). When the ozone depletion potential of CFCs became apparent, restrictions were put in place to limit their use (50). Today, potential restrictions are pondered for HCFCs and HFCs, the compounds that replaced CFCs. Concerns are based on their persistence and global warming potential as greenhouse gases.

The organofluorine industry is based on chemicals whose reactivity and interaction with the natural world is poorly understood due to lack of historical data. As consumption continues and increases, health concerns related to these compounds likely will continue to surface at increasing frequency and severity. This scenario is likely as these compounds continue to accumulate with no rapid, large-scale degradation process existing to break them down. As a precautionary measure, the substitution of organofluorines with less persistent compounds is desirable, if applicable. Preferred candidate compounds serving as replacements ideally should be identical to or resemble the structure of naturally occurring compounds. This would assure that natural degradation mechanisms for these substances already exist, which will prevent them from accumulation in the bio- and chemosphere. Naturally occurring compounds offer the benefit that degradation strategies and detoxification mechanisms typically already have evolved over thousands of years of exposure to and interactions with the biosphere. However, matching the desirable properties of organofluorines with bio-compatible replacements presents a significant challenge.

Conclusions

The scarcity of organofluorines in nature stands in stark contrast to the large-scale production and environmental release of fluorinated and perfluorinated compounds by mankind. Due to the strength of the fluorine-carbon bond and widespread lack of degradation and detoxification mechanisms, organofluorines tend to meet the requirements for classification as persistent organic pollutants, i.e., long-range transfer, bioaccumulation, toxicity, and persistence. While a small number of organofluorines already are or soon will be regulated by national and international bodies of governance, a comprehensive approach for managing organofluorines is lacking. Indeed, the current regulatory framework in the U.S. and abroad allows for the mass manufacturing of persistent compounds (including organofluorines) for which no known large-scale degradation processes

are known or likely exist. Thus, current chemical manufacturing processes set mankind on a trajectory of continued production and accumulation of persistent and potentially harmful compounds in the chemosphere and biosphere. Unless a course correction in chemical management occurs, harmful threshold values will be reached at some point in the future that could lead to adverse effects locally and globally. These effects may be limited to specific susceptible species or they may be systemic, with broad impacts on the ecosystem and human populations, triggered by, for example, the destruction of the ozone layer and global warming.

Precautionary measures could be taken today to prevent long-term pollution and prevent the need for expensive mitigation measures in the future. Practical steps toward this goal include the removal of potentially problematic compounds from production, replacement of xenobiotic chemistry with biogenic analogs that are compatible with biology and for which degradation mechanisms are known and established, as well as an integration of concepts in sustainability and earth systems engineering and management (56) in the design of chemicals intended for mass production.

Adoption of precautionary measures does not imply that the production of organofluorines has to cease altogether. Instead, society should strive to limit organofluorine uses to only the essential ones. These should be limited in scale to enable physical, chemical and biological degradation processes to keep pace, thereby preventing a net accumulation of organofluorines in the environment. Essential uses will include the use of organofluorines in medicine and select technical processes. Non-essential uses of persistent organofluorines, such as for stain protection and mass produced consumer products of daily use, should be discontinued either voluntarily or by regulatory action. A guiding principle to consider when weighing the risks and benefits of organofluorines is that of the precautionary principle:

“When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically. In this context the proponent of an activity, rather than the public, should bear the burden of proof. The process of applying the precautionary principle must be open, informed and democratic and must include potentially affected parties. It must also involve an examination of the full range of alternatives, including no action” (57).

Currently, the risks associated with some organofluorines are not within reason, are not tolerable to the average human, and are not greatly outweighed by the technological and economic benefits of these chemicals. While the specific organofluorines that are of most concern have been greatly restricted, the continued use and development of chemicals that have no end-of-life scenario will surely lead to other problems in the future. The best way to avoid these problems is to move away from persistent, anthropogenic chemicals and to develop chemicals that can be integrated into natural chemical cycles which will ensure perpetual detoxification and removal of potentially harmful substances from the biosphere.

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APPENDIX A

Assessment of the Aquatic Release and Relevance of Selected Endogenous Chemicals: Androgens, Thyroids, and Their *in Vivo* Metabolites

Usman Khan and Jim Nicell*

Department of Civil Engineering and Applied Mechanics, McGill University,
817 Sherbrooke Street West, Montreal, Quebec H3A 2K6

*E-mail: jim.nicell@mcgill.ca

Table A.I. Total per capita daily urinary (U_i) excretion of 5 α -androstane-3 α 17 β diol and its conjugated and unconjugated fractions ($F_u \bullet U_i$, $G_u \bullet U_i$, $S_u \bullet U_i$)

Chemical		U_i					Conjugated fraction of U_i				
		Quantifying U_i			$f_i^{(a)}$	$U_i \cdot f_i$	F_u	G_u	S_u	Reference	
		Reference	I	n	U_i ($\bar{x} \pm \sigma$)	$\bar{x} \pm \sigma$	(%)	(%)	(%)		
	Age (yr)/status	# of samples	($\mu\text{g}/\text{cap-d}$)	($\mu\text{g}/\text{cap-d}$)							
5 α -Androstane-3 α 17 β diol	Male	(1-3)	Adults	49	155 \pm 61	0.5 ^(b)	76 \pm 30	minor	53.5	46.5	(8)
	Female	(2-6)	Adults	64	47 \pm 37	0.5 ^(b)	24 \pm 19	minor	53.5	46.5	(8) ^(g)
	Total ($\mu\text{g}/\text{cap-d}$)					$U_i = \sum U_i \cdot f_i$ (n=113) ^(c)	100 \pm 35	$F_u \cdot U_i^{(d),(*)} \approx 0$		$G_u \cdot U_i^{(e),(f)} = 54$	$S_u \cdot U_i^{(d),(g)} = 47$

(a) Based on demographic data for the simulation base year 2005 (7); (b) Due to the lack of data it is conservatively assumed here that the whole male and female populations excrete 5 α -androstane-3 α 17 β diol as adults; (c) Total per capita daily load of 5 α -androstane-3 α 17 β diol excreted in human urine ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of 5 α -androstane-3 α 17 β diol excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of 5 α -androstane-3 α 17 β diol excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (f) Total per capita daily load of 5 α -androstane-3 α 17 β diol excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$); (g) Assumed to be equal to the conjugated and unconjugated proportions of male urinary excretions.

$$* F_u U_i = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_i = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\S S_u U_i = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table A.II. Total per capita daily urinary excretion (U_i) of etiocholanolone and its conjugated and unconjugated fractions ($F_u \cdot U_i$, $G_u \cdot U_i$, $S_u \cdot U_i$)

Chemical	U_i						Conjugated fraction of U_i			
	Quantifying U_i			$f_i^{(a)}$	$U_i \cdot f_i$		F_u	G_u	S_u	Reference
	Reference	I	n		$x \pm \sigma$					
		Age (m/yr)/status	# of samples	$(\mu\text{g}/\text{cap}\cdot\text{d})$		$(\mu\text{g}/\text{cap}\cdot\text{d})$	(%)	(%)	(%)	
Etiocholanolone	Male	(9)	0-<3m	24	87 ± 45		Minor	95	5	(28)
		(9,11)	3m - <12	46	447 ± 334					
		(9,11)	12-<17	92	1379 ± 748					
		(9,10,12-17,20,27)	17-50	136	2495 ± 1336					
		(9,19,22)	50-<70	91	1883 ± 755					
		(9,19-21)	70+	66	1453 ± 657					
	Female	(9)	0-<3m	24	73 ± 37		Minor	95	5	(28) ^(f)
		(9,11)	3m - <12	42	265 ± 148					
		(9,11)	12-<17	49	1175 ± 637					
		(4,6,9,12,14,16-18,20,23-26)	17-50	290	1578 ± 797					
		(9,18,24,26)	50-<70	97	1073 ± 544					
		(9,20)	70+	32	577 ± 255					
	Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)		$U_i = \sum U_i \cdot f_i$ (n=989) ^(b)				1492 ± 384	$F_u \cdot U_i^{(c),(f)} \approx 0$ $G_u \cdot U_i^{(d),(f)} = 1417$ $S_u \cdot U_i^{(e),(f)} = 75$		

(a) Based on demographic data for the simulation base year 2005 (7); (b) Total per capita daily load of etiocholanolone excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (c) Total per capita daily load of etiocholanolone excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of etiocholanolone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of etiocholanolone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Assumed to be equal to the conjugated and unconjugated proportions of male urinary excretions.

$$* F_u U_i = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_i = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\S S_u U_i = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table A.III. Total per capita daily urinary excretion (U_t) of epitestosterone and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Chemical		U_t						Conjugated fraction of U_t			
		Quantifying U_t				$f_i^{(a)}$	$U_t \cdot f_i$	F_u	G_u	S_u	Reference
		Reference	Age (yr)/status	n	U_t ($\bar{x} \pm \sigma$)						
Epitestosterone	Male	(29-31)	0-10	14	2.2 ± 1.9	0.07	0.2 ± 0.1	0.3	81.3	18.3	(40)
		(29,30,32,33)	10-15	114	9.0 ± 6.0	0.04	0.3 ± 0.2				
		(30,31,33-39)	15-30	125	51 ± 39	0.11	5.5 ± 4.1				
		(27,31,32,34-37)	30-50	94	38 ± 15	0.15	5.5 ± 2.2				
		(32,35,37)	50+	46	23 ± 9	0.13	3.1 ± 1.1				
	Female	(29,31)	0-10	5	3.0 ± 2.7	0.07	0.2 ± 0.2	2.0	94.3	3.7	(40)
		(29,31,39)	10-20	11	8.8 ± 3.1	0.07	0.6 ± 0.2				
		(4,31,32,34,35,38,39,41,42)	20-40	114	6.0 ± 3.6	0.14	0.8 ± 0.5				
		(31,34,35,39)	40+	44	2.6 ± 2.4	0.24	0.6 ± 0.6				
	Total ($\mu\text{g}/\text{cap-d}$)		$U_t = \sum U_t \cdot f_i$ (n=567) ^(b)				16.7 ± 4.9	$F_u \cdot U_t^{(c),(f)} = 0.1$ $G_u \cdot U_t^{(d),(f)} = 13.9$ $S_u \cdot U_t^{(e),(g)} = 2.7$			

(a) Based on demophaic data for the simulation base year 2005 (?); (b) Total per capita daily load of epitestosterone excreted in human urine ($\mu\text{g}/\text{cap-d}$); (c) Total per capita daily load of epitestosterone excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of epitestosterone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of epitestosterone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$).

$$* F_u U_t = (F_u \sum U_i \cdot f_i)_{males} + (F_u \sum U_i \cdot f_i)_{females}$$

$$\dagger G_u U_t = (G_u \sum U_i \cdot f_i)_{males} + (G_u \sum U_i \cdot f_i)_{females}$$

$$\S S_u U_t = (S_u \sum U_i \cdot f_i)_{males} + (S_u \sum U_i \cdot f_i)_{females}$$

Table A.IV. Total per capita daily urinary excretion (U_i) of dehydroepiandrosterone and its conjugated and unconjugated fractions ($F_u \cdot U_i$, $G_u \cdot U_i$, $S_u \cdot U_i$)

Chemical	U_i					Conjugated fraction of U_i				
	Quantifying U_i			$f_i^{(a)}$	$\frac{U_i \cdot f_i}{x \pm \sigma}$	F_u	G_u	S_u	Reference	
	Reference	Age (m/yr)/status	# of samples							U_i ($x \pm \sigma$) ($\mu\text{g}/\text{cap-d}$)
Dehydroepiandrosterone	Male	(9)	0-3m	24	58 ± 29	0.00	Minor	7.0	93.0	(8)
		(9-11)	3m - <12	46	78 ± 63	0.08				
		(9-11)	12-<17	92	157 ± 109	0.04				
		(9,10,12,14-17)	17-50	84	909 ± 375	0.24				
		(9,19)	50-<70	72	303 ± 361	0.10				
		(9,19)	70+	37	207 ± 113	0.04				
	Female	(9)	0-3m	24	43 ± 22	0.00	Minor	7.0	93.0	(8) ^(f)
		(9,11)	3m - <12	42	96 ± 61	0.08				
		(9,11)	12-<17	49	197 ± 139	0.03				
		(6,9,12,14,16-18,23-25,43)	17-50	259	360 ± 416	0.23				
		(9,22,24,43)	50-<70	92	243 ± 45	0.11				
		(9)	70+	24	87 ± 29	0.05				
	Total ($\mu\text{g}/\text{cap-d}$)		$U_i = \sum U_i \cdot f_i$ (845) ^(b)			395 ± 137	$F_u \cdot U_i^{(c)(*)} = 0$ $G_u \cdot U_i^{(d)(\dagger)} = 28$ $S_u \cdot U_i^{(e)(\S)} = 367$			

(a) Based on demographic data for the simulation base year 2005 (7); (b) Total per capita daily load of dehydroepiandrosterone excreted in human urine ($\mu\text{g}/\text{cap-d}$); (c) Total per capita daily load of dehydroepiandrosterone excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of dehydroepiandrosterone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of dehydroepiandrosterone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$); (f) Assumed to be equal to the conjugated and unconjugated proportions of male urinary excretions.

$$* F_u U_i = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_i = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\S S_u U_i = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table A.V. Total per capita daily urinary excretion (U_t) of androstane-3,11,17-trione, (5.α) and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Chemical		U_t					Conjugated fraction of U_t				
		Quantifying U_t			$f_i^{(a)}$	$U_t \cdot f_i$	F_u	G_u	S_u	Reference	
		Reference	Age (yr)/status	n	U_t ($\bar{x} \pm \sigma$) ($\mu\text{g}/\text{cap}\cdot\text{d}$)	$x \pm \sigma$ ($\mu\text{g}/\text{cap}\cdot\text{d}$)	(%)	(%)	(%)		
Androstane-3,11,17-trione, (5.α)	Male	(44)	Adults	9	299 ± 47	0.5 ^(b)	149 ± 23	Minor	55.0	45.0	(44)
	Female	(44)	Adults	6	305 ± 55	0.5 ^(b)	153 ± 28	Minor	55.9	44.1	(44)
	Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)					$U_t = \sum U_t \cdot f_i$ (n=15) ^(c)	302 ± 36	$F_u \cdot U_t^{(d)(*)} = 0.0$ $G_u \cdot U_t^{(e)(\dagger)} = 168$ $S_u \cdot U_t^{(f)(§)} = 135$			

(a) US Census Bureau (7) for the simulation year 2005; (b) Due to lack of data is conservatively assumed here that the whole male and female populations excrete androstane-3,11,17-trione, (5.α) as adults; (c) Total per capita daily load of androstane-3,11,17-trione, (5.α) excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of androstane-3,11,17-trione, (5.α) excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of androstane-3,11,17-trione, (5.α) excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Total per capita daily load of androstane-3,11,17-trione, (5.α) excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$).

$$* F_u U_t = (F_u \sum U_t \cdot f_i)_{\text{males}} + (F_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_t = (G_u \sum U_t \cdot f_i)_{\text{males}} + (G_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\S S_u U_t = (S_u \sum U_t \cdot f_i)_{\text{males}} + (S_u \sum U_t \cdot f_i)_{\text{females}}$$

Table A.VI. Total per capita daily urinary excretion (U_t) of androstenediol and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Chemical	U_t						Conjugated fraction of U_t				
	Quantifying U_t			$f_i^{(a)}$	$U_t \cdot f_i$		F_u (%)	G_u (%)	S_u (%)	Reference	
	Reference	Age (yr)/status	# of samples		U_t ($\bar{x} \pm \sigma$) ($\mu\text{g}/\text{cap-d}$)	$\bar{x} \pm \sigma$ ($\mu\text{g}/\text{cap-d}$)					
Androstenediol	Male	(12)	20-50	16	460 \pm 43	0.5 ^(b)	225 \pm 21	minor	100.00	0.00	Assumed ^(g)
	Female	(6,12,24)	20-50	40	330 \pm 195	0.5 ^(b)	168 \pm 99	minor	100.00	0.00	Assumed ^(g)
	Total ($\mu\text{g}/\text{cap-d}$)					$U_t = \sum U_t \cdot f_i (n=56)^{(c)}$		393 \pm 101	$F_u \cdot U_t^{(d),(e)} \approx 0$ $G_u \cdot U_t^{(e),(f)} = 393$ $S_u \cdot U_t^{(f),(g)} = 0$		

(a) Based on demographic data for the simulation base year 2005 (7); (b) Due to the lack of data it is conservatively assumed here that the whole male and female populations excrete androstenediol as adults; (c) Total per capita daily load of androstenediol excreted in human urine ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of androstenediol excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of androstenediol excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (f) Total per capita daily load of androstenediol excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$); (g) Assumed to be all conjugated to glucuronic acid.

$$\begin{aligned}
 * F_u U_t &= (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}} \\
 \dagger G_u U_t &= (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}} \\
 \S S_u U_t &= (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}
 \end{aligned}$$

Table A.VII. Total per capita daily urinary excretion (U_t) of androstane-3,17-dione, (5β) and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Chemical		U_t					Conjugated fraction of U_t				
		Quantifying U_i			$f_i^{(a)}$	$U_t \cdot f_i$	F_u	G_u	S_u	Reference	
		Reference	Age (yr)/status	n	U_i ($x \pm \sigma$) ($\mu\text{g}/\text{cap}\cdot\text{d}$)	$x \pm \sigma$ ($\mu\text{g}/\text{cap}\cdot\text{d}$)	(%)	(%)	(%)		
Androstane-3,17-dione, (5β)	Male	(44)	Adults	9	1686 \pm 432	0.5 ^(b)	840 \pm 215	Minor	78	23	(44)
	Female	(44)	Adults	6	1468 \pm 322	0.5 ^(b)	737 \pm 162	Minor	69	32	(44)
	Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)					$U_t = \sum U_i \cdot f_i$ (n=15) ^(c)	1577 \pm 269	$F_u \cdot U_t^{(d)(*)} \approx 0$ $G_u \cdot U_t^{(e)(\dagger)} = 1157$ $S_u \cdot U_t^{(f)(\S)} = 419$			

(a) US Census Bureau (7) for the simulation base year 2005; (b) Due to the lack of data it is conservatively assumed here that the whole male and female populations excrete as adults; (c) Total per capita daily load of androstane-3,17-dione, (5β) excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of androstane-3,17-dione, (5β) excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of androstane-3,17-dione, (5β) excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Total per capita daily load of androstane-3,17-dione, (5β) excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$).

$$* F_u U_t = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_t = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\S S_u U_t = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table A.VIII. Total per capita daily urinary excretion (U_t) of 11 β -hydroxyandrosterone and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Chemical	U_t						Conjugated fraction of U_t				
	Quantifying U_t			$f_i^{(a)}$	$U_t \cdot f_i$ $x \pm \sigma$	F_u $(\%)$	G_u $(\%)$	S_u $(\%)$	Reference		
	Reference	Age (m/yr)/status	n # of samples							U_t ($x \pm \sigma$) ($\mu\text{g}/\text{cap}\cdot\text{d}$)	
1 11 β -Hydroxyandrosterone	Male	(9)	0-3m	24	123 \pm 63	0.00	0.2 \pm 0.1	Minor	100	0	Assumed ^(f)
		(9,10)	3m - <12yr	28	523 \pm 251	0.08	43 \pm 21				
		(9, 10)	12-<17	67	815 \pm 383	0.04	30 \pm 14				
		(9,12,14,16,17,20)	17-<50	106	1025 \pm 389	0.24	244 \pm 93				
		(9)	50-<70	24	1456 \pm 429	0.10	143 \pm 42				
		(9,20,21)	70+	53	1101 \pm 418	0.04	40 \pm 15				
	Female	(9)	0-<3m	24	30 \pm 16	0.00	0.1 \pm 0.0	Minor	100	0	Assumed ^(f)
		(9)	3m - <12yr	24	137 \pm 55	0.08	11 \pm 4.2				
		(9)	12-<17	24	304 \pm 109	0.03	11 \pm 3.7				
		(4, 6, 9, 12, 14, 16, 17, 20, 23, 26)	17-<50	132	553 \pm 254	0.23	129 \pm 59				
		(9,26)	50-<70	39	381 \pm 163	0.11	40 \pm 17				
		(9,20)	70+	32	363 \pm 320	0.05	20 \pm 17				
	Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)					$U_t = \sum U_t \cdot f_i$ (577) ^(b)	710 \pm 124	$F_u \cdot U_t^{(c)} = 0.0$ $G_u \cdot U_t^{(d)} = 710$ $S_u \cdot U_t^{(e)} \approx 0.0$			

(a) US Census Bureau (7) for the simulation base year 2005; (b) Total per capita daily load of 11 β -hydroxyandrosterone excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (c) Total per capita daily load of 11 β -hydroxyandrosterone excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of 11 β -hydroxyandrosterone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of 11 β -hydroxyandrosterone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Assumed to be primarily conjugated to glucuronide ions based on observations of (45).

$$* F_u U_t = (F_u \sum U_t \cdot f_i)_{\text{males}} + (F_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_t = (G_u \sum U_t \cdot f_i)_{\text{males}} + (G_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\S S_u U_t = (S_u \sum U_t \cdot f_i)_{\text{males}} + (S_u \sum U_t \cdot f_i)_{\text{females}}$$

Table A.IX. Total per capita daily urinary excretion (U_i) of 11 β -hydroxyetiocholanolone and its conjugated and unconjugated fractions ($F_u \cdot U_i$, $G_u \cdot U_i$, $S_u \cdot U_i$)

Chemical	U_i						Conjugated fraction of U_i				
	Quantifying U_i			$f_i^{(a)}$	$U_i \cdot f_i$		F_u	G_u	S_u	Reference	
	Reference	Age (m/yr)/status	n		U_i ($\bar{x} \pm \sigma$)	$\bar{x} \pm \sigma$					(%)
11 β -Hydroxyetiocholanolone	Male	(9)	0-3m	24	61 \pm 31	0.00	0.1 \pm 0.1	Minor	100	0	Assumed ^(f)
		(9,10)	3m - <12yr	28	282 \pm 126	0.08	23 \pm 10				
		(9, 10)	12-<17	67	410 \pm 201	0.04	15 \pm 7.3				
		(9,12,14,16,17,20)	17-50	106	364 \pm 161	0.24	87 \pm 38				
		(9)	50-70	24	444 \pm 148	0.10	44 \pm 15				
		(9,20,21)	70+	53	490 \pm 281	0.04	18 \pm 10				
	Female	(9)	0-3m	24	30 \pm 16	0.00	0.1 \pm 0.0	Minor	100	0	Assumed ^(f)
		(9)	3m - <12yr	24	152 \pm 62	0.08	12 \pm 4.8				
		(9)	12-<17	24	289 \pm 117	0.03	10 \pm 5.0				
		(4, 6, 9, 12, 14, 16, 17, 20, 23, 26)	17-50	132	328 \pm 184	0.23	77 \pm 43				
		(9,26)	50-70	39	305 \pm 153	0.11	32 \pm 16				
		(9,20)	70+	32	363 \pm 320	0.05	20 \pm 17				
Total ($\mu\text{g}/\text{cap-d}$)		$U_i = \sum U_i \cdot f_i$ (577) ^(b)				336 \pm 66	$F_u \cdot U_i^{(c),(*)} \approx 0$ $G_u \cdot U_i^{(d),(†)} = 336$ $S_u \cdot U_i^{(e),(§)} \approx 0$				

(a) US Census Bureau (7) for the simulation base year 2005; (b) Total per capita daily load of 11 β -hydroxyetiocholanolone excreted in human urine ($\mu\text{g}/\text{cap-d}$); (c) Total per capita daily load of 11 β -hydroxyetiocholanolone excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of 11 β -hydroxyetiocholanolone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of 11 β -hydroxyetiocholanolone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$); (f) Assumed to be primarily conjugated to glucuronide ions based on observations of (45).

$$* F_u U_i = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$† G_u U_i = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$§ S_u U_i = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table A.X. Total per capita daily urinary excretion (U_i) of androsterone and its conjugated and unconjugated fractions ($F_u \cdot U_i$, $G_u \cdot U_i$, $S_u \cdot U_i$)

Chemical	U_i						Conjugated fraction of U_i				
	Quantifying U_i			$f_i^{(a)}$	$U_i \cdot f_i$	$x \pm \sigma$	F_u	G_u	S_u	Reference	
	Reference	I	n								$U_i (x \pm \sigma)$
Androsterone	Male	(9)	0-3m	24	102 ± 52	0.00	0.2 ± 0.1	Minor	93	7	(28)
		(9,11)	3m - <12	46	442 ± 305	0.08	36 ± 25				
		(9,11)	12-<17	92	1397 ± 705	0.04	51 ± 26				
		(9,10,12-17,20,27)	17-50	136	2490 ± 913	0.24	593 ± 217				
		(9,19,22)	50-70	91	2039 ± 919	0.10	201 ± 91				
		(9,19-21)	70+	66	1295 ± 557	0.04	47 ± 20				
	Female	(9)	0-3m	24	44 ± 22	0.00	0.1 ± 0.0	Minor	93	7	(28) ^(f)
		(9,11)	3m - <12	42	383 ± 244	0.08	30 ± 19				
		(9,11)	12-<17	49	1352 ± 733	0.03	47 ± 25				
		(4,6,9,12,14,16-18,20,23-26)	17-50	290	1427 ± 675	0.23	334 ± 158				
		(9,18,24,26)	50-70	97	963 ± 405	0.11	102 ± 43				
		(9,20)	70+	32	447 ± 278	0.05	24 ± 15				
	Total ($\mu\text{g}/\text{cap-d}$)					$U_i = \sum U_i \cdot f_i$ (n=989) ^(b)	1463 ± 292	$F_u \cdot U_i^{(c)(*)} \approx 0.0$ $G_u \cdot U_i^{(d)(†)} = 1361$ $S_u \cdot U_i^{(e)(§)} = 102$			

(a) Based on demographic data for the simulation base year 2005 (7); (b) Total per capita daily load of androsterone excreted in human urine ($\mu\text{g}/\text{cap-d}$); (c) Total per capita daily load of androsterone excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of androsterone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of androsterone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$); (f) Assumed to be equal to the conjugated and unconjugated proportions as for male urinary excretions.

$$* F_u U_i = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$† G_u U_i = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$§ S_u U_i = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table A.XI. Total per capita daily urinary excretion (U_t) of 5 α -androstanedione and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Chemical		U_t					Conjugated fraction of U_t				
		Quantifying U_t			$f_i^{(a)}$	$U_t \cdot f_i$	F_u	G_u	S_u	Reference	
		Reference	I	n	$U_t (x \pm \sigma)$	$x \pm \sigma$	(%)	(%)	(%)		
	Age (yr)/status	# of samples	($\mu\text{g}/\text{cap}\cdot\text{d}$)	($\mu\text{g}/\text{cap}\cdot\text{d}$)							
5 α -Androstanedione	Male	(44)	Adults	9	2780 \pm 500	0.5 ^(b)	1384 \pm 249	Minor	62.5	37.5	(44)
	Female	(44)	Adults	6	1882 \pm 551	0.5 ^(b)	945 \pm 277	Minor	68.0	32.0	(44)
	Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)					$U_t = \sum U_t \cdot f_i$ (n=15) ^(c)		2329 \pm 372	$F_u \cdot U_t^{(d),(e)} \approx 0$ $G_u \cdot U_t^{(e),(f)} = 1508$ $S_u \cdot U_t^{(f),(g)} = 821$		

(a) US Census Bureau (7) for the simulation base year 2005; (b) Due to the lack of data it is conservatively assumed here that the whole male and female populations excrete 5 α -androstanedione as adults; (c) Total per capita daily load of 5 α -androstanedione excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of 5 α -androstanedione excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of 5 α -androstanedione excreted in human urine conjugated to glucurone ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Total per capita daily load of 5 α -androstanedione excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$).

$$* F_u U_t = (F_u \sum U_t \cdot f_i)_{\text{males}} + (F_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_t = (G_u \sum U_t \cdot f_i)_{\text{males}} + (G_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\S S_u U_t = (S_u \sum U_t \cdot f_i)_{\text{males}} + (S_u \sum U_t \cdot f_i)_{\text{females}}$$

Table A.XII. Total per capita daily urinary excretion (U_t) of 11-ketoandrosterone and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Chemical	U_t						Conjugated fraction of U_t				
	Quantifying U_i				$f_i^{(a)}$	$U_i \cdot f_i$	F_u	G_u	S_u	Reference	
	Reference	Age (m/yr)/status	# of samples	U_i ($\bar{x} \pm \sigma$) ($\mu\text{g}/\text{cap-d}$)		$\bar{x} \pm \sigma$ ($\mu\text{g}/\text{cap-d}$)	(%)	(%)	(%)		
11-Ketoandrosterone	Male	(9)	0-3m	24	91 ± 31	0.00	0.2 ± 0.1	Minor	100	0	Assumed ^(f)
		(9,10)	3m - <12yr	28	232 ± 110	0.08	19 ± 8.9				
		(9,10)	12-<17	67	223 ± 127	0.04	8.1 ± 4.6				
		(9,10,17)	17-50	38	405 ± 164	0.24	96 ± 39				
		(9)	50-70	24	380 ± 179	0.10	37 ± 18				
		(9,21)	70+	35	239 ± 128	0.04	8.6 ± 4.6				
	Female	(9)	0-3m	24	30 ± 16	0.00	0.1 ± 0.0	Minor	100	0	Assumed ^(f)
		(9)	3m - <12yr	24	152 ± 62	0.08	12 ± 4.8				
		(9)	12-<17	24	289 ± 116	0.03	10 ± 4.0				
		(6,9,17)	17-50	51	395 ± 200	0.23	92 ± 47				
		(9)	50-70	24	335 ± 124	0.11	36 ± 13				
		(9)	70+	24	259 ± 85	0.05	14 ± 4.6				
	Total ($\mu\text{g}/\text{cap-d}$)		$U_t = \sum U_i \cdot f_i$ (387) ^(b)				333 ± 66	$F_u \cdot U_t^{(c),(e)} \approx 0.0$	$G_u \cdot U_t^{(d),(f)} = 333.0$	$S_u \cdot U_t^{(d),(f)} \approx 0.0$	

(a) US Census Bureau (7) for the year 2005; (b) Total per capita daily load of 11-ketoandrosterone excreted in human urine ($\mu\text{g}/\text{cap-d}$); (c) Total per capita daily load of 11-ketoandrosterone excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of 11-ketoandrosterone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of 11-ketoandrosterone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$); (f) Assumed to be primarily conjugated to glucuronide ions.

$$* F_u U_t = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_t = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\S S_u U_t = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table A.XIII. Total per capita daily urinary excretion (U_i) of 11-ketoetiocholanolone and its conjugated and unconjugated fractions ($F_u \cdot U_i$, $G_u \cdot U_i$, $S_u \cdot U_i$)

Chemical	U_i						Conjugated fraction of U_i				
	Quantifying U_i			$f_i^{(a)}$	$U_i \cdot f_i$	$x \pm \sigma$	F_u	G_u	S_u	Reference	
	Reference	Age (m/yr)/status	# of samples								$U_i (x \pm \sigma)$
11-Ketoetiocholanolone	Male	(9)	0-3m	24	61 ± 31	0.00	Minor	100	0	Assumed ^(f)	
		(9,10)	3m - <12yr	28	236 ± 96	0.08					
		(9,10)	12-<17	67	580 ± 458	0.04					
		(9,10,14,17)	17-<50	45	536 ± 208	0.24					
		(9)	50-<70	24	381 ± 132	0.10					
		(9,21)	70+	35	310 ± 237	0.04					
	Female	(9)	0-3m	24	30 ± 16	0.00	Minor	100	0	Assumed ^(f)	
		(9)	3m - <12yr	24	137 ± 54	0.08					
		(9)	12-<17	24	304 ± 109	0.03					
		(9,14,16,17,23,26)	17-<50	81	361 ± 168	0.23					
		(9,26)	50-<70	39	302 ± 107	0.11					
		(9)	70+	24	198 ± 70	0.05					
	Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)		$U_i = \sum U_i \cdot f_i$ (439) ^(b)				365 ± 69	$F_u \cdot U_i^{(c)(*)} \approx 0.0$ $G_u \cdot U_i^{(d),(f)} = 365$ $S_u \cdot U_i^{(e),(f)} \approx 0.0$			

(a) US Census Bureau (7) for the year 2005; (b) Total per capita daily load of 11-ketoetiocholanolone excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (c) Total per capita daily load of 11-ketoetiocholanolone excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of 11-ketoetiocholanolone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of 11-ketoetiocholanolone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Assumed to be primarily conjugated to glucuronide ions.

$$* F_u U_i = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_i = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\S S_u U_i = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table A.XIV. Total per capita daily urinary excretion (U_t) of 5 β -androsterane-3-11-17-trione and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Chemical		U_t					Conjugated fraction of U_t				
		Quantifying U_t			$f_i^{(a)}$	$U_t \cdot f_i$	F_u	G_u	S_u	Reference	
		Reference	Age (yr)/status	n # of samples	U_t ($\bar{x} \pm \sigma$) $\mu\text{g}/\text{cap}\cdot\text{d}$		$\bar{x} \pm \sigma$ $\mu\text{g}/\text{cap}\cdot\text{d}$	(%)	(%)		(%)
5 β -Androstane-3-11-17-trione	Male	(44)	Adults	9	356 \pm 110	0.5 ^(b)	177 \pm 55	Minor	44.4	55.6	(44)
	Female	(44)	Adults	6	346 \pm 191	0.5 ^(b)	174 \pm 96	Minor	29.2	70.8	(44)
	Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)					$U_t = \sum U_t \cdot f_i$ (n=15) ^(c)	351 \pm 110	$F_u \cdot U_t^{(d)(*)} \approx 0$ $G_u \cdot U_t^{(e)(f)} = 129$ $S_u \cdot U_t^{(g)(\S)} = 222$			

(a) US Census Bureau (7) for the simulation base year 2005; (b) Due to the lack of data it is conservatively assumed here that the whole male and female populations excrete as adults; (c) Total per capita daily load of 5 β -androsterane-3-11-17-trione excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of 5 β -androsterane-3-11-17-trione excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of 5 β -androsterane-3-11-17-trione excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Total per capita daily load of 5 β -androsterane-3-11-17-trione excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$).

$$* F_u U_t = (F_u \sum U_t \cdot f_i)_{\text{males}} + (F_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_t = (G_u \sum U_t \cdot f_i)_{\text{males}} + (G_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\S S_u U_t = (S_u \sum U_t \cdot f_i)_{\text{males}} + (S_u \sum U_t \cdot f_i)_{\text{females}}$$

Table A.XV. Total per capita daily urinary excretion (U_t) of androst-4-ene-3,6,17-trione and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Chemical		U_t						Conjugated fraction of U_t			
		Quantifying U_t				$f_i^{(a)}$	$U_t \cdot f_i$	F_u	G_u	S_u	Reference
		Reference	Age (yr)/status	# of samples	U_t ($\bar{x} \pm \sigma$) ($\mu\text{g}/\text{cap}\cdot\text{d}$)						
Androst-4-ene-3,6,17-trione	Male	(44)	Adults	9	1890 \pm 805	0.5 ^(b)	941 \pm 401	Minor	4.3	95.7	(44)
	Female	(44)	Adults	6	778 \pm 336	0.5 ^(b)	391 \pm 169	Minor	9.7	90.3	(44)
	Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)		$U_t = \sum U_t \cdot f_i$ (n=15) ^(c)					1332 \pm 435	$F_u \cdot U_t^{(d),(e)} \approx 0.0$	$G_u \cdot U_t^{(d),(f)} = 79$	$S_u \cdot U_t^{(d),(g)} = 1253$

(a) Based on demographic data for the simulation base year 2005 (?); (b) Due to the lack of data it is conservatively assumed here that the whole male and female populations excrete androst-4-ene-3,6,17-trione as adults; (c) Total per capita daily load of androst-4-ene-3,6,17-trione excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of androst-4-ene-3,6,17-trione excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of androst-4-ene-3,6,17-trione excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Total per capita daily load of androst-4-ene-3,6,17-trione excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$).

$$* F_u U_t = (F_u \sum U_t \cdot f_i)_{\text{males}} + (F_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_t = (G_u \sum U_t \cdot f_i)_{\text{males}} + (G_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\S S_u U_t = (S_u \sum U_t \cdot f_i)_{\text{males}} + (S_u \sum U_t \cdot f_i)_{\text{females}}$$

Table A.XVI. Total per capita daily urinary excretion (U_t) of 5 β -androsterane-3 α 17 β diol and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Chemical		U_t						Conjugated fraction of U_t			
		Reference		Quantifying U_i		$f_i^{(a)}$	$U_i \cdot f_i$ $\bar{x} \pm \sigma$	F_u (%)	G_u (%)	S_u (%)	Reference
		Age (yr)/status	# of samples	n	U_i ($\bar{x} \pm \sigma$) ($\mu\text{g}/\text{cap}\cdot\text{d}$)						
5 β -Androstane-3 α 17 β diol	Male	(2,3)	Adults	36	617 \pm 171	0.5 ^(b)	304 \pm 84	minor	62.4	37.6	(8)
	Female	(2-4)	Adults	36	182 \pm 119	0.5 ^(b)	92 \pm 60	minor	62.4	37.6	(8) ^(g)
	Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)		$U_t = \Sigma U_i \cdot f_i (n=72)^{(c)}$					396 \pm 104	$F_u \cdot U_t^{(d),(e)} \approx 0$	$G_u \cdot U_t^{(d),(f)} = 247$	$S_u \cdot U_t^{(d),(g)} = 149$

(a) US Census Bureau (7) for the year 2005; (b) Due to the lack of data it is conservatively assumed here that the whole male and female populations excrete 5 β -androsterane-3 α 17 β diol as adults; (c) Total per capita daily load of 5 β -androsterane-3 α 17 β diol excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of 5 β -androsterane-3 α 17 β diol excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of 5 β -androsterane-3 α 17 β diol excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Total per capita daily load of 5 β -androsterane-3 α 17 β diol excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$); (g) Assumed to be equal to the conjugated and unconjugated proportions as reported for male urinary excretion.

$$* F_u U_t = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_t = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\S S_u U_t = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table A.XVII. Total per capita daily urinary excretion (U_i) of reverse triiodothyronine (rT_3), 3',5'-diiodo-L-thyronine (3',5' T_2), 3,3'-diiodothyronine (3',3' T_2) and thyronine (T_0) and its conjugated and unconjugated fractions ($F_u \cdot U_i$, $G_u \cdot U_i$, $S_u \cdot U_i$)

Chemical	U_i					Conjugated fraction of U_i				
	Quantifying U_i				$f_i^{(a)}$	$U_i f_i$	F_u	G_u	S_u	Reference
	Reference	Age (yr)/status	# of samples	$U_i(x \pm \sigma)$ ($\mu\text{g}/\text{cap-d}$)	$x \pm \sigma$ ($\mu\text{g}/\text{cap-d}$)	$x \pm \sigma$ (%)	$x \pm \sigma$ (%)	$x \pm \sigma$ (%)		
rT_3	All (46)	21-82	15-25	0.5 ± 0.1	$1.0^{(b)}$	0.5 ± 0.1	9.7	23.2	67.1	(46)
	Total ($\mu\text{g}/\text{cap-d}$)				$U_i = \sum U_i f_i (n = 15-25)^{(c)}$	0.5 ± 0.1	$F_u \cdot U_i^{(d),(e)} = 0.1 \quad G_u \cdot U_i^{(e),(f)} = 0.1 \quad S_u \cdot U_i^{(e),(f)} = 0.3$			
3',5' T_2	All (46)	21-82	16-34	0.4 ± 0.1	$1.0^{(b)}$	0.4 ± 0.1	1.8	44.6	53.5	(46)
	Total ($\mu\text{g}/\text{cap-d}$)				$U_i = \sum U_i f_i (n = 16-34)^{(c)}$	0.4 ± 0.1	$F_u \cdot U_i^{(d),(e)} = 0.0 \quad G_u \cdot U_i^{(e),(f)} = 0.2 \quad S_u \cdot U_i^{(e),(f)} = 0.2$			
3',3' T_2	All (46)	21-82	16-34	1.3 ± 0.6	$1.0^{(b)}$	1.3 ± 0.6	26.6	33.5	39.9	(46)
	Total ($\mu\text{g}/\text{cap-d}$)				$U_i = \sum U_i f_i (n = 16-34)^{(c)}$	1.3 ± 0.6	$F_u \cdot U_i^{(d),(e)} = 0.4 \quad G_u \cdot U_i^{(e),(f)} = 0.5 \quad S_u \cdot U_i^{(e),(f)} = 0.5$			
T_0	All (47)	Adults?	22	4.1 ± 1.3	$1.0^{(b)}$	4.1 ± 1.3	100	0.0	0.0	(47)
	Total ($\mu\text{g}/\text{cap-d}$)				$U_i = \sum U_i f_i (n = 22)^{(c)}$	4.1 ± 1.3	$F_u \cdot U_i^{(d),(e)} = 4.1 \quad G_u \cdot U_i^{(e),(f)} = 0.0 \quad S_u \cdot U_i^{(e),(f)} = 0.0$			

(a) Based on demographic data for the simulation base year 2005 (7); (b) Age cohorted data for triiodothyronine and thyroxine (Table IV and V) suggests age isn't a huge factor with respect to the excretion of endogenous thyroids therefore it is assumed here that population age cohorts not studied (<20 for reference 46 and non-adults for reference 47) excrete as age cohorts studied; (c) Total per capita daily load of thyroids excreted in human urine ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of thyroids excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of thyroids excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (f) Total per capita daily load of thyroids excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$).

$$* F_u U_i = (F_u \sum U_i \cdot f_i)_{males} + (F_u \sum U_i \cdot f_i)_{females}$$

$$\dagger G_u U_i = (G_u \sum U_i \cdot f_i)_{males} + (G_u \sum U_i \cdot f_i)_{females}$$

$$\S S_u U_i = (S_u \sum U_i \cdot f_i)_{males} + (S_u \sum U_i \cdot f_i)_{females}$$

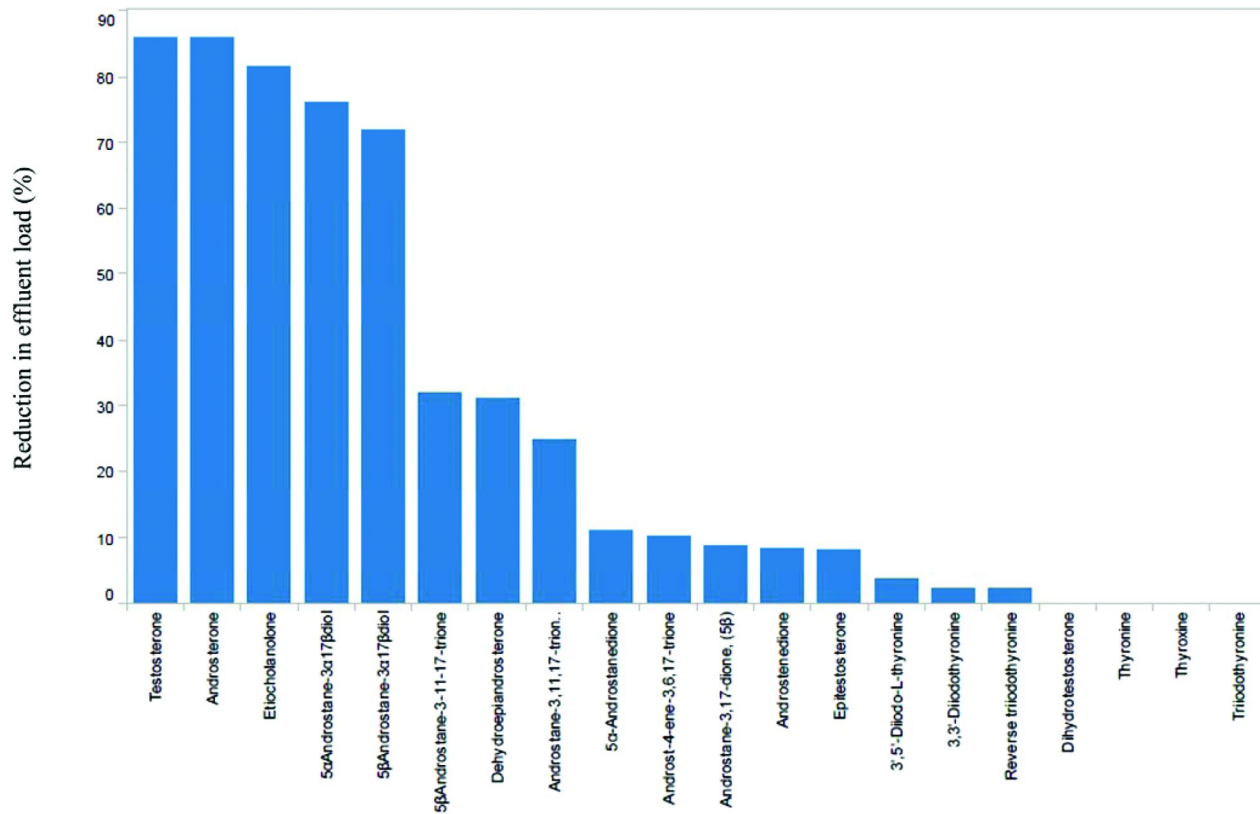


Figure A.1. Percent reduction in effluent loads if sulphate conjugates are made available for treatment.

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APPENDIX B

Assessment of the Aquatic Release and Relevance of Selected Endogenous Chemicals: Androgens, Thyroids, and Their *in Vivo* Metabolites

Usman Khan and Jim Nicell*

Department of Civil Engineering and Applied Mechanics, McGill University,
817 Sherbrooke Street West, Montreal, Quebec H3A 2K6

*E-mail: jim.nicell@mcgill.ca

Table B.I. Total per capita daily urinary excretion of testosterone (U_i) and its conjugated and unconjugated fractions ($F_u \cdot U_i$, $G_u \cdot U_i$, $S_u \cdot U_i$)

Androgen		U_i					Conjugated fraction of U_i				
		Quantifying U_i			$f_i^{(a)}$	$U_i \cdot f_i$ ($\mu\text{g}/\text{cap-d}$)	F_u (%)	G_u (%)	S_u (%)	Reference	
		Reference	Age (yr/status)	n							U_i ($\bar{x} \pm \sigma$) ($\mu\text{g}/\text{cap-d}$)
Testosterone	Male	(39-60)	0-5	23	1.4 ± 1.1	0.04	0.1 ± 0.0				
		(39-63)	5-10	88	5.6 ± 4.4	0.03	0.2 ± 0.1				
		(32, 59, 61, 62, 64, 65)	10-15	298	20 ± 21	0.04	0.7 ± 0.8				
		(52, 53, 59, 62-71)	15-30	205	85 ± 34	0.11	9.1 ± 3.6	0.3	70	30	(74)
		(32, 53, 55, 59, 63, 64, 67-69)	30-40	74	66 ± 33	0.07	4.6 ± 2.3				
		(32, 53, 59, 63, 64, 67-69)	40-50	63	49 ± 12	0.08	3.7 ± 0.9				
	(32, 53, 59, 64, 69)	50+	57	31 ± 15	0.13	4.1 ± 2.0					
	Female	(60)	0-5	16	1.2 ± 1.6	0.07	0.1 ± 0.1				
		(53, 60, 61, 63)	5-10	7	8.1 ± 3.0	0.06	0.5 ± 0.2				
		(60, 63, 71)	10-20	39	6.1 ± 3.2	0.14	0.8 ± 0.4				
		(53, 63, 64, 67, 71, 72)	20-40	79	6.5 ± 3.5	0.27	1.7 ± 0.9	4.0	75	21	(74)
		(53, 63, 67, 71, 73)	40+	56	4.6 ± 2.5	0.47	2.1 ± 1.1				
	(54)	3 rd trimester	32	17 ± 6.9	0.01 ^(f)	0.2 ± 0.1					
Total ($\mu\text{g}/\text{cap-d}$)		$U_i = \sum U_i \cdot f_i$ (n=1037) ^(b)			28 ± 5.1	$F_u \cdot U_i^{(c),(e)} = 0.3$ $G_u \cdot U_i^{(d),(f)} = 20$ $S_u \cdot U_i^{(d),(f)} = 7.9$					

(a) Based on demographic data for the simulation base year 2005 (33); (b) Total per capita daily load of testosterone excreted in human urine ($\mu\text{g}/\text{cap-d}$); (c) Total per capita daily load of testosterone excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of testosterone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of testosterone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$); (f) Based on the number of pregnancies in the year 2005 (58).

$$\begin{aligned}
 * F_u \cdot U_i &= \left(F_u \sum U_i \cdot f_i \right)_{\text{males}} + \left(F_u \sum U_i \cdot f_i \right)_{\text{females}} \\
 \dagger G_u \cdot U_i &= \left(G_u \sum U_i \cdot f_i \right)_{\text{males}} + \left(G_u \sum U_i \cdot f_i \right)_{\text{females}} \\
 \S S_u \cdot U_i &= \left(S_u \sum U_i \cdot f_i \right)_{\text{males}} + \left(S_u \sum U_i \cdot f_i \right)_{\text{females}}
 \end{aligned}$$

Table B.II. Total per capita daily urinary excretion (U_i) of androstenedione and its conjugated and unconjugated fractions ($F_u \cdot U_i$, $G_u \cdot U_i$, $S_u \cdot U_i$)

Androgen		U_i					Conjugated fraction of U_i				
		Quantifying U_i			$f_i^{(a)}$	$U_i \cdot f_i$ ($\bar{x} \pm \sigma$)	F_u	G_u	S_u	Reference	
		Reference	I	n							U_i ($\bar{x} \pm \sigma$)
			Age (yr)/status	# of samples	($\mu\text{g}/\text{cap}\cdot\text{d}$)	($\mu\text{g}/\text{cap}\cdot\text{d}$)					
Androstenedione	Male	(62)	<10	5	0.0 \pm 0.0	0.07	0.0 \pm 0.0	3.7	23	73	(74)
		(62)	10-<15	16	6.2 \pm 9.4	0.04	0.2 \pm 0.3				
		(62)	15-<20	11	16 \pm 9.3	0.04	0.6 \pm 0.3				
		(69)	20-<30	6	57 \pm 5.4	0.07	4.0 \pm 0.4				
		(69)	30-<40	4	47 \pm 4.2	0.07	3.3 \pm 0.3				
		(69)	40-<50	8	38 \pm 4.6	0.08	2.9 \pm 0.4				
		(69)	50-<60	9	28 \pm 2.2	0.06	1.7 \pm 0.1				
		(69)	61+	7	20 \pm 2.3	0.07	1.4 \pm 0.2				
	Female	Approx ^(g)	0-<10	-	0.0 \pm 0.0	0.07	0.0 \pm 0.0	2.0	22	76	(74)
		Approx ^(g)	10-<15	-	2.0 \pm 0.6	0.03	0.1 \pm 0.0				
		Approx ^(g)	15-<20	-	5.3 \pm 1.7	0.03	0.2 \pm 0.1				
		(75, 76)	20-<40	29	16.7 \pm 4.7	0.12	2.1 \pm 0.6				
		Approx ^(g)	40-<50	-	12.3 \pm 3.5	0.08	1.0 \pm 0.3				
		Approx ^(g)	50-<60	-	9.0 \pm 2.5	0.06	0.6 \pm 0.2				
Approx ^(g)	61+	-	6.3 \pm 1.8	0.10	0.6 \pm 0.2						
(54)	3 rd trimester	32	6.4 \pm 3.0	0.01 ^a	0.1 \pm 0.0						
Total ($\mu\text{g}/\text{cap}\cdot\text{d}$)					$U_t = \sum U_i \cdot f_i$ (n=106) ^(b)	19 \pm 1.1	$F_u \cdot U_t^{(c),(f)} = 0.6$ $G_u \cdot U_t^{(d),(f)} = 4.3$ $S_u \cdot U_t^{(e),(g)} = 14$				

(a) Based on demographic data for the simulation base year 2005 (33); (b) Total per capita daily load of androstenedione excreted in human urine ($\mu\text{g}/\text{cap}\cdot\text{d}$); (c) Total per capita daily load of androstenedione excreted in human urine in free form ($\mu\text{g}/\text{cap}\cdot\text{d}$); (d) Total per capita daily load of androstenedione excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap}\cdot\text{d}$); (e) Total per capita daily load of androstenedione excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap}\cdot\text{d}$); (f) Approximated by relative ratio from associated male cohorts (see text for details); (g) Based on the number of pregnancies in the year 2005 (58).

$$* F_u \cdot U_i = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\dagger G_u \cdot U_i = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\S S_u \cdot U_i = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table B.III. Total per capita daily urinary excretion (U_t) of dihydrotestosterone and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Androgen		U_t						Conjugated fraction of U_t			
		Quantifying U_t			$f_i^{(a)}$	$U_t \cdot f_i$ ($\mu\text{g}/\text{cap-d}$)	$U_t \cdot f_i$ ($\mu\text{g}/\text{cap-d}$)	F_u (%)	G_u (%)	S_u (%)	Reference
		Reference	Age (yr)/status	# of samples							
Dihydrotestosterone	Male	(77)	Adults	14	32 ± 20	0.5 ^(b)	16 ± 9.9	minor	100	0	(20), (79)
	Female	(78)	Adults	5	5.1	0.5 ^(b)	2.5	minor	100	0	(20), (79) ^(d)
	Total ($\mu\text{g}/\text{cap-d}$)		$U_t = \sum U_t \cdot f_i$ (n=19) ^(c)					19 ± 9.9	$F_u \cdot U_t^{(d),(e)} \approx 0$	$G_u \cdot U_t^{(e),(f)} = 19$	$S_u \cdot U_t^{(f),(g)} \approx 0$

(a) Based on demographic data for the simulation base year 2005 (33); (b) Due to lack of data it is conservatively assumed here that the whole male and female populations excrete dihydrotestosterone as adults/c; Total per capita daily load of dihydrotestosterone excreted in human urine ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of dihydrotestosterone excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of dihydrotestosterone excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (f) Total per capita daily load of dihydrotestosterone excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$); (g) Assumed to be equal to the conjugated and unconjugated proportions of male urinary excretions.

$$* F_u \cdot U_t = (F_u \sum U_t \cdot f_i)_{\text{males}} + (F_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\dagger G_u \cdot U_t = (G_u \sum U_t \cdot f_i)_{\text{males}} + (G_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\S S_u \cdot U_t = (S_u \sum U_t \cdot f_i)_{\text{males}} + (S_u \sum U_t \cdot f_i)_{\text{females}}$$

Table B.IV. Total per capita daily urinary excretion (U_t) of triiodothyronine and its conjugated and unconjugated fractions ($F_u \cdot U_t$, $G_u \cdot U_t$, $S_u \cdot U_t$)

Thyroid		U_t					Conjugated fraction of U_t					
		Quantifying U_t			$f_i^{(a)}$	$U_t \cdot f_i$ ($\mu\text{g}/\text{cap-d}$)	F_u (%)	G_u (%)	S_u (%)	Reference		
		Reference	1 Age (yr)/status	n # of samples							$U_t^{(b)}$ ($\bar{x} \pm \sigma$) ($\mu\text{g}/\text{cap-d}$)	
Triiodothyronine	Male	(80)	<60	61	2.5 ± 0.9	0.42	1.0 ± 0.4	68.1	31.9	≈ 0	(21,84,85)	
		(80)	61+	11	1.8 ± 0.8	0.07	0.1 ± 0.1					
	Female	(80)	<50	68	2.1 ± 0.8	0.35	0.7 ± 0.3	68.1	31.9	≈ 0	(21,84,85)	
		(80)	51+	15	1.7 ± 0.4	0.16	0.3 ± 0.1					
	Total ($\mu\text{g}/\text{cap-d}$)					$U_t = \sum U_t \cdot f_i$ (n=155) ^(c)		2.2 ± 0.5		$F_u \cdot U_t^{(d), (e)} = 1.5$ $G_u \cdot U_t^{(d), (e)} = 0.7$ $S_u \cdot U_t^{(d), (e)} \approx 0$		

(a) Based on demographic data for the simulation base year 2005 (33); (b) Total urinary loads calculated from reported free fraction θ and sample weighted correction for level of conjugation from (21,84,85); (c) Total per capita daily load of triiodothyronine excreted in human urine ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of triiodothyronine excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of triiodothyronine excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (f) Total per capita daily load of a triiodothyronine excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$).

$$* F_u \cdot U_t = (F_u \sum U_t \cdot f_i)_{\text{males}} + (F_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\dagger G_u \cdot U_t = (G_u \sum U_t \cdot f_i)_{\text{males}} + (G_u \sum U_t \cdot f_i)_{\text{females}}$$

$$\S S_u \cdot U_t = (S_u \sum U_t \cdot f_i)_{\text{males}} + (S_u \sum U_t \cdot f_i)_{\text{females}}$$

Table B.V. Total per capita daily urinary (U_i) excretion of thyroxine and its conjugated and unconjugated fractions ($F_u \cdot U_i$, $G_u \cdot U_i$, $S_u \cdot U_i$)

Thyroid		U_i					Conjugated fraction of U_i				
		Quantifying U_i			$f_i^{(a)}$	$U_i \cdot f_i$	F_u (%)	G_u (%)	S_u (%)	Reference	
		Reference	I Age (yr)/status	n # of samples							$U_i^{(b)}$ ($\mu\text{g}/\text{cap-d}$)
Thyroxine	Male	(80)	All	61	2.5 ± 0.9	0.5	1.2 ± 0.5	70	30	≈ 0	(21,84,86)
	Female	(80)	All	68	2.1 ± 0.8	0.5	1.1 ± 0.4	70	30	≈ 0	(21,84,86)
Total ($\mu\text{g}/\text{cap-d}$)					$U_i = \sum U_i \cdot f_i$ (n=127) ^(c)	2.3 ± 0.6	$F_u \cdot U_i^{(d),(e)} = 1.7$	$G_u \cdot U_i^{(d),(e)} = 0.7$	$S_u \cdot U_i^{(d),(e)} \approx 0$		

(a) Based on demographic data for the simulation base year 2005 (33); (b) Total urinary loads calculated from reported free fraction (80) and sample weighted correction for level of conjugation from (21,84,86); (c) Total per capita daily load of thyroxine excreted in human urine ($\mu\text{g}/\text{cap-d}$); (d) Total per capita daily load of thyroxine excreted in human urine in free form ($\mu\text{g}/\text{cap-d}$); (e) Total per capita daily load of thyroxine excreted in human urine conjugated to glucuronide ($\mu\text{g}/\text{cap-d}$); (f) Total per capita daily load of a thyroxine excreted in human urine conjugated to sulphate ions ($\mu\text{g}/\text{cap-d}$).

$$* F_u U_i = (F_u \sum U_i \cdot f_i)_{\text{males}} + (F_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\dagger G_u U_i = (G_u \sum U_i \cdot f_i)_{\text{males}} + (G_u \sum U_i \cdot f_i)_{\text{females}}$$

$$\S S_u U_i = (S_u \sum U_i \cdot f_i)_{\text{males}} + (S_u \sum U_i \cdot f_i)_{\text{females}}$$

Table B.VI. Summary of the modeled influent loads, effluent loads and surface water concentrations of endogenous androgens and their *in vivo* metabolites in U. S. waterways

Androgens and their <i>in vivo</i> metabolites	CAS	Parameters for H_{T-inf}			Influent			Effluent		Surface Water	
		$U_i^{(a)}$	$F_i^{(b)}$	N	$H_{T-inf}^{(d)}$	$H_{T-inf}^{(e)}$	$H_{T-inf} - H_{T-inf}^{(e)}$	$Eff^{(f)}$	$H_{T-eff}^{(f)}$	$C_{T-sw-high}^{(g)}$	$C_{T-sw-typical}^{(h)}$
		(μg/cap·d)			(kg/d)			(kg/d)		(ng/L)	
5α-Androstane-3-one	846-46-8	2329	303	763	238	525	0.29	613	781	6.0	
Androst-4-ene-3,6,17-trione	2243-06-3	1332	173	436	363	73	0.12	428	545	4.2	
Androstane-3,17-dione, (5β)	1229-12-5	1577	205	517	124	393	0.29	405	515	4.0	
11β-Hydroxyandrostosterone	57-61-4	710	92	233	0	233	0.46	125	159	1.2	
Dehydroepiandrosterone	53-43-0	395	51	129	106	23	0.35	121	154	1.2	
5βAndrostane-3-11-17-trione	1429-06-7	351	46	115	64	51	0.46	92	117	0.9	
Androstane-3,11,17-trione, (5α)	1482-70-8	303	39	99	39	60	0.46	72	92	0.7	
Androstetriol	4150-30-5	393	51	129	0	129	0.48	67	85	0.7	
11-Ketoetiocholanolone	739-27-5	365	47	120	0	120	0.46	65	82	0.6	
11βHydroxyetiocholanolone	739-26-4	336	44	110	0	110	0.46	59	75	0.6	
11-ketoandrosterone	1231-82-9	333	43	109	0	109	0.46	59	75	0.6	
5βAndrostane-3α17βdiol	1851-23-6	396	51	130	43	87	0.88	53	68	0.5	
Androsterone	53-41-8	1463	190	479	30	450	0.99 ^(g)	34	43	0.3	
Etiocholanolone	53-42-9	1492	194	489	22	467	0.99 ^(g)	26	34	0.3	
5α-Androstane-3α17βdiol	1852-53-5	100	13	33	14	19	0.89	16	20	0.2	
Androstenedione	63-05-8	19	2	6	4	2	0.12	6	7.5	0.1	
Epitestosterone	481-30-1	17	2	5	1	5	0.38	4	4.7	0.0	
Testosterone	58-22-0	28	4	96 ^(e)	37	2	35	0.99 ^(h)	3	3.4	0.0
Dihydrotestosterone	521-18-6	19	2	6	0	6	0.70	2	2.4	0.0	

(a) From tables I-III in main text and tables A.1-XVI in appendix A; (b) Estimated to be 13% of the respective U_i (from testosterone urine to fecal load ratio for adult males - see text for details); (c) Exogenous testosterone consumption estimated from Canadian and Swedish consumption data (see text for details); (d) From equation (8); (e) Using equation (11); (f) Estimated using STPWIN (47), unless otherwise indicated; (g) From (86,87); (h) From (87); (i) Equation (10); (j) Equation (13) with a DF of 4; (k) Equation (13) with a DF of 130.

Table B.VII. Summary of the modeled influent loads, effluent loads and surface water concentrations of endogenous thyroids and their *in vivo* products in U.S waterways

Thyroids and their <i>in vivo</i> metabolites	CAS	Parameters for H_{T-inf}			Influent			Effluent		Surface Water	
		$U_t^{(a)}$	F_t	N	$H_{T-inf}^{(e)}$	$H_{T-inf}^{(f)}$	$H_{T-inf} - H_{T-inf}^{(f)}$	$Eff^{(g)}$	$H_{T-eff}^{(h)}$	$C_{T-sw-high}^{(i)}$	$C_{T-sw-typical}^{(j)}$
		(μg/cap·d)			(kg/d)			(kg/d)		(ng/L)	
Triiodothyronine	6893-02-3	2.2	9.7		3.4	0.0	3.4	0.14	2.96	3.8	0.03
Thyroxine	51-48-9	2.3	9.2	3.6 ^(d)	3.6	0.0	3.6	0.37	2.30	2.9	0.02
3,3'-Diiodothyronine	70-40-6	1.3	5.5 ^(b)		2.0	0.2	1.9	0.23	1.59	2.0	0.02
Thyronine	1596-67-4	4.1	4.1 ^(c)	3.6 ^(d)	2.4	0.0	2.4	0.75	0.60	0.8	0.01
Reverse triiodothyronine	5817-39-0	0.5	2.1 ^(b)		0.8	0.1	0.7	0.14	0.67	0.9	0.01
3',5'-Diiodo-L-thyronine	4192-14-7	0.4	1.5 ^(b)		0.5	0.1	0.5	0.26	0.42	0.5	0.00

(a) From Tables IV-V in main text and Tables A.XVII in appendix A; (b) Estimated to be 4.2x the respective U_t (see text for details); (c) Estimated to be 1.0x the respective U_t (see text for details); (d) Due to exogenous levothyroxine consumption estimated from Canadian and French consumption data (see text for details); (e) From equation (8); (f) Using equation (11); (g) Estimated using STPWIN (47); (h) Equation (10); (i) Equation (13) with a DF of 4; (j) Equation (13) with a DF of 130.

Table B.VIII. Predicted No–Effect Concentrations (*PNECs*) and Risk Quotients (*RQs*) for endogenous hormones for which eco-toxicological data was available

Hormones	Endpoint	Taxon	Species	Duration (d)	Effect	Concentration ($\mu\text{g/L}$)	Reference	<i>AF</i>	<i>PNEC</i> (ng/L)	<i>RQ</i>	
										high	typical
Testosterone	NOEC	Fish	<i>O. ishawytscha</i>	29	Sex differentiation	1	(92)				
	NOEC	Algae	<i>S. subspicatus</i>	3	Growth	335	(93)	10 ^(a)	100	3.4x10 ⁻²	2.6x10 ⁻⁴
	NOEC	Crustacean	<i>B. calyciflorus</i>	4	Females fertilized	1	(94)				
Androstenedione	NOEC	Fish	<i>G. affinis</i>	24	Masculinization	0.04	(95)	10 ^(b)	4	1.9x10 ⁰	1.44x10 ⁻²
	NOEC	Crustacean	<i>D. magna</i>	21	Abnormalities	2291	(96)				
Dihydrotestosterone	NOEC	Fish	<i>G. aculeatus</i>	18-35	Spiggin induction	2	(97)	100 ^(c)	20	1.2x10 ⁻¹	8.8x10 ⁻⁴
Thyroxine	LOEC	Fish	<i>C. auratus</i>	15d	No. of fins	10	(98)	1000 ^(d)	10	2.9x10 ⁻¹	2.2x10 ⁻³
Triiodothyronine	LOEC	Fish	<i>C. auratus</i>	40d	Tail fin length	10	(98)	1000 ^(d)	10	3.8x10 ⁻¹	2.9x10 ⁻³

(a) *AF*=10 is used, considering 3 chronic endpoints from three different taxa were available;(b) *AF* =10 is used, even though only data from two taxa was available because the chronic endpoint for algae is unlikely to be below the one reported for fish;(c) *AF* = 100 is used, since only one chronic endpoint was available; (d) *AF* =1000 is used conservatively, as the endpoint available was an LOEC.

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1. References cited refer to those in Chapter 22.

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