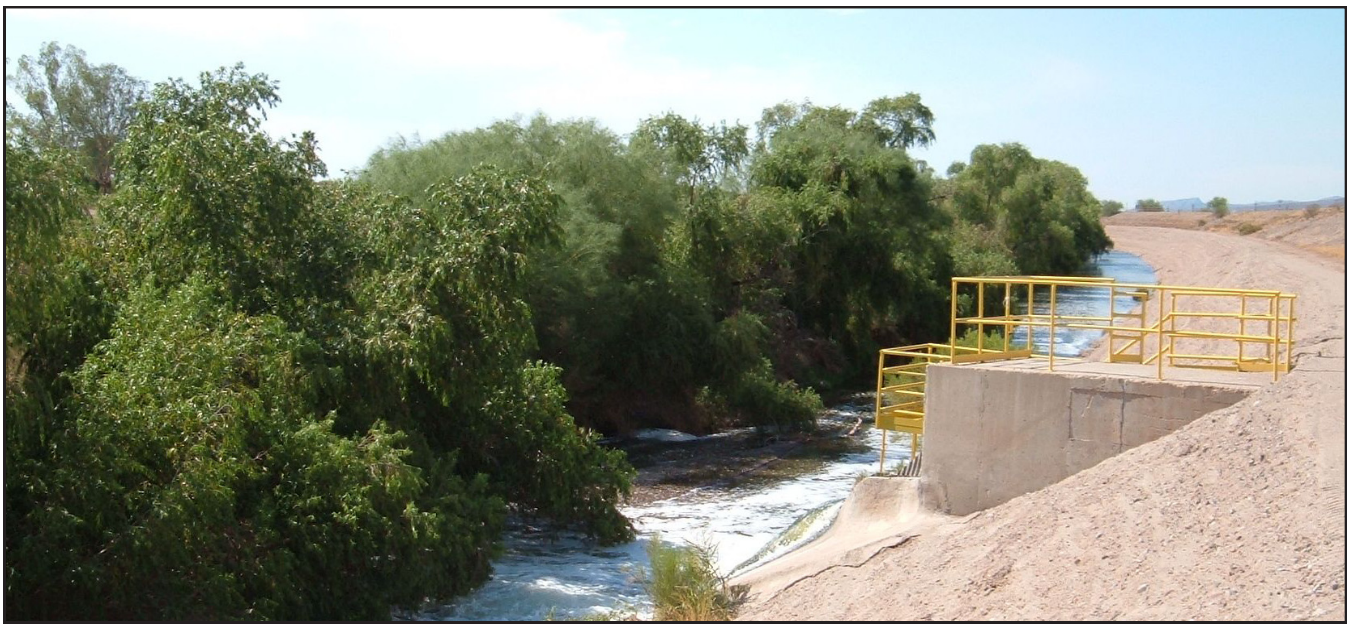
Wastewater Effluent:

Biological Impacts of Exposure and Treatment Processes to Reduce Risk



A literature review by
David Quandrud, PhD
Catherine R. Propper, PhD

Funded by The Nature Conservancy through a grant
from the Nina Mason Pulliam Charitable Trust

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Treated effluent flowing fro Pima County's Roger Road Wastewater Reclamation Facility into the Santa Cruz River. ©Julia Fonseca/Pima County Regional Flood Control District

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Summary

Trace organic compounds of anthropogenic origin are released into the environment through several mechanisms. The discharge of municipal wastewater, either untreated or treated, into the environment is an important source of these contaminants. Most trace organics in wastewater originate from industrial processes and consumer use, including those classified as pharmaceuticals and personal care products (PPCPs). Individually, many of these compounds have been found to disrupt the physiological function of many organisms. These chemicals are commonly called endocrine disrupting compounds (EDCs) because many have been found to impact normal endocrine function in wildlife. It has been recognized that concentrations of EDCs can be reduced, though not eliminated, through conventional wastewater treatment.

There is a significant literature on EDCs in wastewater, their impact on wildlife, and improving removal efficiences through engineered and natural wastewater treatment. We reviewed this literature and summarize it below. We concentrated on summarizing information pertaining to biological impacts of exposure to municipal wastewater effluent, and reducing exposure by manipulating wastewater treatement processes, including conventional, natural, and advanced treatment processes. We also provide a comparison of costs.

We found that there are effects of wastewater exposure on survivorship, health, and reproduction of exposed organisms. The outcomes of exposure range from overt toxicity and increased mortality to impacts on development of reproductive organs and behavior. There are differences among species in endpoint outcomes following exposure to wastewater effluent. Further, understanding which compounds are inducing these effects and how these complex mixes influence physiological function and community dynamics is challenging. Future research should concentrate on understanding complex mixture effects on wildlife not just at the individual level, but also at a population scale and on overall ecosystem function.

Conventional wastewater treatment provides for partial but not complete removal of most EDCs and other trace organics. Natural treatment systems (e.g. recharge basins, artificial wetlands) attenuate many, but not all, EDCs and can be useful as a component in multi-barrier engineered treatment systems. Trace organics removal during advanced wastewater treatment is a very active area of research. Membrane treatment (e.g. reverse osmosis), advanced oxidation processes, and activated carbon have all been shown to be effective in removing or reducing concentrations of EDCs and other trace organics from wastewater. Drawbacks of advanced treatment include capital and operational costs, with energy costs being an important consideration, particularly for membrane treatment. Manipulation of parameters such as increasing the solids retention time (sludge age) during conventional treatment may prove to be nearly as effective as advanced treatment processes for EDC removal and at less cost than advanced treatment. Future research should concentrate on optimizing conventional wastewater treatment processes for EDC removal. In addition, it will be important to couple testing of treatment technologies for EDC removal efficiency with whole animal biological assays to determine if the technology sufficiently eliminates the biological activity of the EDCs in the released wastewater effluent.

List of Acronyms

BFR	Brominated Flame Retardant
BDE	Brominated Diphenyl Ether
BOD	Biochemical oxygen demand
BPA	Bisphenol A
DOM	Dissolved organic matter
EDC	Endocrine disrupting chemical
E1	Estrone
E2	17-β-estradiol
E3	Estriol
EE2	17-α-ethinyl estradiol
HRT	Hydraulic Residence Time
K_{ow}	Octanol-water partitioning coefficient
MBR	Membrane bioreactor
mg/L	milligram per liter
MW	Molecular weight
NAS	National Academy of Science
NOM	Natural organic matter
ng/L	nanogram per liter
NP	Nonylphenol
NP1EO	Nonylphenol monoethoxylate
NP2EO	Nonylphenol diethoxylate
OWC	Organic Wastewater Contaminant
PBDE	Polybrominated Diphenyl Ether
PFC	Perfluorochemical
PFOS	Perfluorooctane sulfonate
PFOA	Perfluorooctanoate
pH	Negative log of the hydronium ion concentration
PPCP	Pharmaceutical and Personal Care Product
ppb	Parts per billion (= micrograms per liter)
ppt	Parts per trillion (= nanograms per liter)
SRT	Solids Retention Time
TOrC	Trace Organic Chemical
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WWE	Wastewater Effluent
WWTP	Wastewater Treatment Plant

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Introduction

Discovery of Potential Endocrine Dysfunction in Wildlife Suggests Chemical Disruption

In the early 1990s, several investigators began to note reproductive abnormalities in wildlife populations around the world; the results of many of these studies were summarized in 1993 by Theo Colborn and her colleagues (Colborn et al. 1993). Soon thereafter investigators began to find gonadal abnormalities such as reversed sex ratios and intersex gonads in fish living downstream from wastewater treatment plants (WWTPs; see review below). These results, along with the increasing collective literature about the impact of low level exposure to individual anthropogenic compounds on animal health (reviewed by Guillette. 2006; Propper 2005), suggest that wastewater treatment does not completely remove many of these chemicals during processing.

Chemicals in Wastewater

In 2002, the United States Geological Survey published its first report investigating the extent of chemical contamination in U.S. streams near wastewater effluent (WWE) outlets (Kolpin et al. 2002). The findings documented the scope of different pharmaceuticals, personal care products, pesticides, steroids and industrial compounds found in many of the U.S. waterways. Prior to and since this report, studies from around the world have found that aquatic species downstream from wastewater facilities were showing reproductive abnormalities (see below). These field studies along with a plethora of laboratory investigations demonstrated that many of the individual compounds found in wastewater impact biological systems. Further investigations evaluated the biological impacts of WWE exposure to organisms living downstream from these facilities, and several studies have also begun to evaluate whether these compounds bioaccumulate. Below we review the outcome of many of the studies demonstrating biological effects of exposure to WWE, and then we summarize the different types of wastewater treatment mechanisms currently available and review their capacity to remove these chemicals.

Sources of Emerging Contaminants to Rivers and the Environment

Recent research has widely documented the presence in the environment of a wide array of chemical compounds that are commonly used in commerce including: prescription and non-prescription pharmaceuticals and personal care products (PPCPs), flame retardants, antimicrobials, detergents, pesticides, and natural and synthetic hormones. The scientific community has not reached consensus on an appropriate term for these compounds, which have been referred to as: emerging contaminants (ECs), contaminants of emerging concern (CECs), trace organic compounds (TOrCs), and microconstituents of potential concern.

A subset of emerging contaminants are known or suspected endocrine disrupting compounds (EDCs) that have the ability to alter the normal functioning of the endocrine system, which is responsible for growth and development in vertebrates. EDCs are naturally occurring and synthetic organic compounds that alter the normal function of endocrine systems. To date (2010), many of the known EDCs refer to compounds that modulate estrogen receptors, resulting in abnormal sexual characteristics such as intersex, atypical male:female sex ratios, and other potentially deleterious reproductive effects observed in fish exposed to these compounds (Vajda et al. 2008). The EDCs best known to produce these specifically estrogenic effects are the naturally occurring steroidal estrogens, including 17-β-Estradiol, synthetic estrogens, such as ethinyl estradiol, used in birth control, and synthetic organic compounds that have been shown to interact with estrogen receptors, including the alyklphenol ethoxylates, bisphenol A, and a number of phthalate plasticizers. A list of the trace organic compounds typically providing the most important contribution to estrogenic activity in municipal wastewater and sludges/biosolids is provided in Table 1. The first five compounds are natural and synthetic steroidal hormones. The next seven compounds are alkylphenols (derived from breakdown of the nonionic surfactants in laundry detergents), and the last four are other prominent trace organic compounds in wastewater. The estrogenic potency of each compound, relative to the synthetic estrogen ethinyl estradiol (EE2) is given in the right-hand column. EE2

Table 1. Trace organic compounds typically contributing to estrogenic activity in municipal wastewater and sludge/biosolids.

Compound Name	*Abbr.*	*Log K_{ow}*	*Mol. Wt. [g/mol]*	*Potency, relative to EE_2 [mol_{EE2}/mol]*
17-ethinylestradiol	EE2	4.15	296.39	1.000000
17-estradiol	E2	3.67	272.37	0.840000
17-estradiol	E2	3.94	272.37	0.840000
Estrone	E1	3.43	270.35	0.319000
Estriol	E3	2.81	288.37	0.002000
4-n-Octylphenol	4nOP	5.50	206.33	0.000360
4-tert-Octylphenol	4tOP	5.28	206.33	0.000360
4-Octylphenol monoethoxylates	OP1EO		250.36	0.000010
4-Octylphenol diethoxylates	OP2EO		294.42	0.000010
4-Nonylphenol	NP	5.92	220.34	0.000010
4-Nonylphenol monoethoxylates	NP1EO	4.17	264.39	0.000001
4-Nonylphenol diethoxylates	NP2EO	4.21	290.43	0.000001
Diethylstilbestrol	DES	5.07	268.34	0.924000
Bisphenol A	BPA	3.64	228.28	0.000563
Benzophenone	benzoph	3.15	182.22	0.000168
Diethylhexyl phthalate	DEHP	8.39	390.56	0.000021

is the most potent estrogenic compound and is commonly used as a standard (positive control) in bioassay tests for estrogenic activity. The estrogenic potency factors, relative to EE2, are based on bioassay tests of the pure compounds. The log K_{ow} value is the octanol-water partitioning coefficient and is a measure of compound hydrophobicity. As a general statement, the higher a compound's K_{ow} value, the lower its solubility in water. Log Kow values greater than 3.0 are considered to be moderately hydrophobic, meaning they will tend to transfer to some degree from the liquid phase to a solid (organic) phase (i.e. sludge) during, for example, wastewater treatment processing. Although these are the best studied of the EDCs, it is vitally important to recognize that these and other compounds may have actions outside of estrogen receptor binding and may impact non-steroidal hormonal function (Guillette et al. 2006; Propper, 2005).

Wastewater contains natural steroidal hormones, including: 17α estradiol (E2α), 17β estradiol (E2), estrone (E1), estriol (E3), testosterone, and progesterones that are excreted by humans. Steroid hormones excreted in feces and urine are either in their free active forms or are in their water-soluble conjugated (either sulfonated or glucoronated) forms (Hanselman et al. 2003; Lee et al. 2007). Conjugation involves the attachment of a sulfate or glucuronide functional group onto the phenoxy or hydroxyl group of each epimer. The conjugated forms are biologically inactive; however, studies at municipal wastewater treatment plants have shown that estrogen conjugates in municipal wastewater are readily hydrolyzed during aerobic treatment processes, converting to their biologically active free hormone forms. The sulfate conjugates are more resistant to breakdown than glucuronide forms and have been detected in municipal effluents (Huang and Sedlak 2001; Gentili et al. 2002; D'Ascenzo et al. 2003; Isobe et al. 2003). The estrogen conjugates are more soluble, more polar, and are expected to be more readily transported through soil (Hanselman et al. 2003). Information is currently lacking in the literature on the distribution, transport, and fate of conjugated hormones in soil and water environments.

Although a substantive causal connection between endocrine disrupting compounds in water and human health has not been established, there is little doubt that physiological changes occur among fish exposed to estrogens (e.g., estrone, 17β-estradiol) in waters impacted by wastewater effluent. Effects include vitellogenin production and elevated incidence of intersex characteristics (Harries et al. 1997; Jobling et al. 1998; Kidd et al. 2006) among continuously exposed males. Although estrogenic hormones have received great attention, other classes of hormones (androgens, progesterones), veterinary drugs (e.g. antibiotics), PPCPs and industrial compounds have all been shown individually to impact physiological function in many animal model systems and wildlife (Kloas et al. 2009, and the effects of exposure to mixtures of contaminants has not been fully investigated and remain a prominent knowledge gap. The first section of this document reviews the available literature on the biological impact of exposure to wastewater effluent and the capacity for bioaccumulation of some of the complex EDC-like compounds.

The fate and behavior of most EDCs once they are released to the environment is poorly understood. The expanding literature on this subject shows that many of these compounds are not fully degraded during wastewater treatment (Drewes et al. 2005). In a USGS survey of 139 streams across the US, it was reported that 80% of the sampled streams contained one or more trace organic contaminant including steroids, nonprescription and prescription drugs, antibiotics, hormones, and personal care products (Kolpin et al. 2002). As many as 38 of the organic wastewater contaminants selected for measurement in that study were observed in a single sample, highlighting the possibility of health effects attributable to simultaneous exposure to mixtures of numerous trace contaminants. The potential sources of these contaminants found in streams are reviewed below.

1. Municipal Wastewater

Wastewater treatment plants (WWTPs) have been identified as a primary source of emerging contaminants to water resources as a function of the waste streams they collect. Connections are being made between WWTP discharges and endocrine disruption in aquatic organisms. This was most recently documented in Colorado where a strong correlation between sexual disruption in fish and environmentally relevant concentrations of emerging contaminants associated with a WWTP effluent discharge was shown (for example, Vajda et al. 2008). There are several other studies from around the world that also find disruption of aquatic organisms downstream from WWTPs and these are reviewed below. Thus WWTPs can be a critical control point for the mitigation of emerging contaminants in the environment.

2. Municipal Sludge/biosolids

In general, it is not well understood whether removal of emerging contaminants from the aqueous phase is the result of chemical or biological transformation, or simply removal by physical means (e.g. sorption to solids). The hydrophobic property of known estrogenic compounds in wastewater suggests that they will be strongly associated with sludges derived from wastewater treatment. For instance, alkylphenol polyethoxylates, a class of surfactants known to be estrogenic, are reported to degrade during the biological activated sludge process to produce metabolites (e.g. alkylphenols, alkylphenol monoethoxylates and alkylphenol diethoxylates) that are more persistent and biologically disruptive than the parent compound. Ahel et al. (1994) reported that while alkylphenol surfactants can be efficiently removed or altered during aerobic treatment, their metabolites have a high octanol-water partitioning co-efficient (log $K_{ow} > 4.5$) which indicates a preference for sorption to the organically rich waste sludge. However the metabolites are not degraded during anaerobic sludge digestion and tend to accumulate in biosolids. Nevertheless, relatively few studies have addressed the fate EDCs during wastewater treatment and, for chemicals that separate with the sludge, survival during solids handling and treatment processes.

Similar to effluents, biosolids are a potential source of these compounds to the environment in their frequent and growing use in landscaping, land reclamation and agriculture and further to surface and ground water via runoff. To date, most research on the occurrence, fate and transport of emerging contaminants in WWTPs has focused on the liquid phase of the wastewater treatment train. This is in part due to the above-referenced link between effluent discharges and endocrine disruption in aquatic organisms. It is also due to the difficulty associated with analyzing solids samples. However, growing interest in the extent the solid phase acts as a sink for emerging contaminants, many of which are hydrophobic, is necessitating analytical method development and research into this area.

Biosolids are the largest by-product resulting from wastewater treatment processes. Federal and state regulatory agencies have generally encouraged the practice of biosolids disposal via addition to soil (USEPA 1981, 1991). Nationwide trends in sludge/biosolids disposal reflect increased reliance on the use of biosolids as soil amendments. In year 2001, 68% of the 8,650 publicly owned treatment works that generated sewage sludge in the United States disposed of biosolids via land application or distribution to the public for use as a soil amendment (National Research Council, 2002). This amounts to $3.4 \cdot 10^6$ dry tons of biosolids each year, or 44% of the sewage sludge now produced (USEPA 1999).

Hydrophobic contaminants tend to accumulate in biosolids produced via wastewater treatment. Biosolids disposed of as soil amendments can contain part-per-million to part-per-thousand concentrations (dry weight) of hydrophobic contaminants such as flame retarding chemicals and nonylphenol. The discharge of treated wastewater and the land application of biosolids allow the potential for these chemicals to re-enter the biosphere and potable water resources. Persistent hydrophilic compounds are sometimes subject to only minor alteration during wastewater treatment and are, therefore, discharged to receiving waters or reach agricultural fields through irrigation water.

3. Concentrated Animal Feeding Operations

In the United States, production of animals and animal products for human consumption is conducted primarily via concentrated animal feeding operations (CAFOs). It has been estimated that 453 million Mg of manure are produced annually by livestock and poultry animals in the United States (USEPA 2003), representing about 13-fold more solid waste than human sanitary waste (USEPA 1998). In the Western United States alone, the numbers of cattle on feed during January 2009 were 358,000 in Arizona, 490,000 in California, 1,020,000 in Colorado, and 2,800,000 in Texas (USDA Agriculture Statistic Services). Manure and urine from livestock operations are collected through wash down of facilities and the runoff is typically stored onsite in lagoons. Most manure and lagoon effluents are land applied as a fertilizer at agronomic rates based on nitrogen or phosphorous application requirements.

The reuse of manure and wastewater from CAFOs provides significant benefits to soil productivity for plant production. However, potential environmental and human-health impacts associated with the presence of veterinary pharmaceuticals and farm-animal hormones in the applied materials is a concern. Studies have shown that significant levels of veterinary pharmaceuticals and farm-animal hormones may be present in manure and wastewater produced from CAFOs (Hanselman et al. 2003; Khanal et al. 2006; Lee et al. 2007; Burkholder et al. 2007; Bradford et al. 2008). CAFOs are thought to be a substantial contributor of hormones to the environment since onsite waste lagoons are typically anaerobic and provide little if any treatment opportunity, as opposed to municipal wastewater treatment plants that have been shown to degrade hormones substantially during aerobic treatment processes (e.g. Teske et al. 2007). Khanal et al. (2006) estimated that greater than 90% of the total estrogen loading to the environment is due to land application of livestock manure.

4. New Concerns

The trace organic contaminant perfluoronated octosulfonate (PFOS) was detected for the first time in 2009 by the Tucson Water Department in their Microconstituent Sentinel Program, in all four groundwater production wells tested, at concentrations ranging from 3.9 to 65 ng/L. All four wells are located in the northwest part of the Tucson metropolitan area, near the Santa Cruz River. Treated wastewater that is discharged to the Santa Cruz River infiltrates the riverbed in northwest Pima County. There is an Environmental Protection Agency (EPA) health-based advisory guideline of 200 ng/L for PFOS. PFOS was added to the Safe Drinking Water Act Contaminant Candidate List 3 (CCL3) in 2009. The CCL3 represents a chemical "watch list" consisting of chemicals that have been marked for potential regulation via promulgation of maximum contaminant limits. Such regulation will be exceptionally expensive and should be based on a much more comprehensive data set than currently exists.

PFOS and a related compound, perfluorooctanoic acid (PFOA), are perfluorinated anthropogenic chemicals that are very persistent, suspected human carcinogens with half lives in the human body of 4–10 years. The detection of PFOS and other trace organic contaminants (eg. carbamazepine, sulfamethoxazole) in Tucson ground water was brought to the attention of the Tucson City Council and has garnered local attention in the media (Tucson Weekly: October 1, October

15, 2009). PFOS measurements were also described in the *City of Tucson and Pima County Water Quality White Paper*, published September 17, 2009, for the Water and Wastewater Infrastructure, Supply and Planning Study.

PFOS and PFOA are indefinitely persistent in the environment. They are known toxicants and carcinogens in animals. In people, they have been detected in the blood of general populations in the low parts per billion range. In highly exposed groups, some studies have associated PFOA exposure with birth defects, increased cancer rates, and changes to lipid levels, the immune system and liver. Food, drinking water, outdoor air, indoor air, dust, and food packaging are all implicated as sources of PFOS/PFOA to people (Renner, 2007) and contaminated food and drinking water are suspected to be the largest contributors (Trudel et al. 2008). When water is a source, blood levels have been found to be approximately 100 times higher than drinking water levels (Post et al. 2009; Johnson 2009).

The origin of PFOS contamination to groundwater in the Tucson Basin is unknown but it is suspected that effluent recharge along the Santa Cruz River, downstream from the Roger Road and Ina Road wastewater treatment facilities, may be an important source. Recent work by Japanese researchers has indicated that PFOS contamination in groundwater at the Tokyo metropolitan area is due to infiltration of wastewater effluent and surface stormwater runoff, with effluent being a somewhat more important source (Murakami et al. 2009).

Biological impacts and bioaccumulation

We reviewed the literature for studies of the biological impacts following exposure to WWE. We have limited the review to studies involving municipal wastewater facilities, and do not include studies investigating the impacts of exposure to industrial output or to paper and pulp mills. These studies included investigations comparing organisms upstream and downstream from wastewater facilities and those that use laboratory-based studies where exposure occurs under controlled conditions. Overall, 101 peer-reviewed, published were found that examined effects across 54 species (See Table 2 for distribution of studies across major organismal clades). Several studies are summarized in Table 3, and most of these studies to date are further evaluated in detail below.

Table 2. Organisms organized by clade for evaluation of impacts of exposure to WWE.

Group of organisms	# of Studies Evaluated	# of Species Evaluated
Plants		
Algae	3	1
Invertebrates		
Daphnia	5	2
Amphipods	2	2
Snail	1	1
Freshwater Mussels	5	3
Vertebrates		
Fish	75	36
Amphibians	5	6
Reptiles	1	1
Birds	1	1
Mammals	3	1
Total	101	54

Biological impacts

Tissue Culture Systems

Several studies have tried to evaluate the impact of wastewater exposure using in vitro culture systems. These tissues include liver cells (hepatocytes), gonadal fibroblasts, white and red blood cells, and sperm. In general, the source of these tissues has been from several fish species. Many studies have also used genetically engineered yeast cells to evaluate general estrogenicity. Some of these studies are discussed in the following section on treatment removal efficiency.

Several studies have evaluated overt toxicity and general estrogenicity using tissue culture systems. When toxicity tests were conducted using wastewater from a Canadian source of municipal and industrial effluents on Rainbow trout liver and fibroblast cells, cellular toxicity became evident at WWE concentrations between 10%–50% of full concentration (Gagne & Blaise 1998). When raw untreated effluent from Croatia was tested also on rainbow trout liver cells, both toxicity and vitellogenin induction (a marker for estrogenic activity) were found (Grung et al. 2007) at concentrations as low as 10% WWE equivalents. Wastewater effluent from two municipal facilities and water from the Rhine River in Germany were also tested using a trout liver cell vitellogenin assay. Both effluents induced expression of this estrogen-dependent marker (Pawlowski et al. 2003), while the Rhine River water did not. Notably, the river water did contain estrogens, but at lower levels than the effluent waters, suggesting that the water from the Rhine River was diluted below the point of biological acitivity. The vitellogenin response of liver cells to wastewater exposure is not limited to trout. Three-spined sticklebacks (*Gasterosteus aculeatus*) hepatocytes exposed to extracts of municipal WWE in Finland also demonstrate expression of vitellogenin at a concentration of 80% effluent (Bjorkblom et al. 2008). Notably, all of these studies demonstrate estrogenicity at concentrations below full strength WWE.

Another study looking at potential impacts of WWE exposure on reproductive tissues investigated goldfish sperm activation (a critical component of fertility following spawning in fish; Schoenfuss et al. 2009). The results suggest that wastewater exposure may impact sperm motility, but were complicated by similar findings when sperm were exposed to water from a presumable reference site. This study illustrates some of the very complicated issues associated with biological testing of wastewater. First, it may be difficult to distinguish impacts related to osmolarity from those caused by chemical contaminants. Second, as water systems around the world become more contaminated through point source and non-point source pollution, finding associated control or reference sites becomes difficult.

It is important to understand that non-reproductive impacts of WWE exposure may also exist and lead to not just overt toxicity, but to reduced capacity to maintain homeostasis. One in vitro study investigated the impact of WWE exposure on sea bass (*Dicentrarchus labrax*) red blood cells (Boge & Roche 2004). The study found that municipal WWE from France induced changes in cell volume, energy utilization and ultimately led to hemolysis (breakdown of red blood cells). These effects could not be explained by differences in osmotic pressure gradients. This study demonstrates that there are multiple tissue specific endpoints that can be used to evaluate not only toxicity, but also other physiologically important parameters.

Tissue cultures allow for the possibility of evaluating the impact of exposure on immune function. One study compared exposure of mouse spleenocytes to secondary and tertiary treated WWE (Kontana et al. 2008). Although other measures of toxicity in this study demonstrated a reduction in toxicity following tertiary treatment, the mouse spleenocytes maintained a high induction of cytokine production, a measure of immune system induction, even after tertiary treatment. This study further demonstrates the importance of using more than one biomarker to evaluate the impact of WWE exposure.

Plants

Plants have been underutilized as assay measurement tools for evaluating the impact of WWE exposure. A test of toxicity in algae has been developed as a general model for toxicity of individual compounds and is occasionally applied to WWE. For example, one study in Germany using whole water toxicity found low to moderate toxicity with an algae test for WWE from several different treatment plants (Gartiser et al. 2009). Another study found that ammonia in WWE in Australia might be responsible for the decline in brown seaweed populations (Adams et al. 2008). However, it is notable that while algae can be used as a measure of overt toxicity, there are almost no studies on the physiological or growth impact of WWE exposure to most naturally occurring plant species downstream from any treatment facility. As wastewater is reclaimed for agricultural practices and riparian reclamation, much more research needs to be applied to understanding the impact of exposure to these contaminants in WWE.

Invertebrates

Invertebrates have been used as strong potential monitors of environmental quality; and published studies have identified overt toxicity to these animals. Many of these studies are based on the Whole Effluent Toxicity (WET) test developed by the EPA in 1989. For example, using Daphnia, (*Ceriodaphnia dubia*) Hemming et al. (2002) determined that wastewater effluent led to reduced survivorship and reduced fecundity. Similar findings in this species and the closely related *Daphnia magna* have been found in other studies (Keller 1993; Kontana et al. 2008; Ra et al. 2007a; Ra et al. 2007b; Ra et al. 2008).

Mollusks, such as freshwater mussels, are also used as a model system to evaluate the biological impact of WWE exposure in invertebrates. Several different biological endpoints have been tested. One of the standard toxicological tests determines the concentration it takes for exposure to a compound or mix of compounds to induce 50% mortality in exposed organisms over a given time course. This value is termed the lethal concentration for 50% or LC50. For example, the mussel, *Anodonta imbecilis*, exposed to WWE shows a LC50 over the course of 7 days of exposure for WWE diluted to 16% of the full concentration (Keller 1993). In a field study investigating the immune and reproductive systems of the fresh water mussel *Elliptio complanata* upstream and downstream from two treatment facilities in Canada, Bouchard et al. (2009) found greater overall mortality of animals exposed downstream from both facilities, and either suppression or induction of immune responses depending on the site. In the freshwater snail, *Potamopyrgus antipodarum*, exposure to 100% WWE induced a decrease in growth rate, but had no impact on overall mortality; however, embryo production was greatly reduced after 42 days of exposure (Jobling et al. 2003). The mussel, Anodonta cygnea, has been used to determine that exposure to WWE induces increases in expression of specific proteins that can be used as biomarkers for oxidative stress and detoxification processes (Ciccotelli et al. 1998). This study is important because it indicates that WWE exposure causes important gene level changes normally associated with overt toxicity. Similarly, in *E. complanata*, exposure to WWE induces increases in mitochondrial electron transport (Gagne et al. 2006) and changes in neuronal signaling systems (Gagne et al. 2007) suggesting that the exposed animals undergo increases in respiration rates that might lead to more oxidative stress and changes in nervous system function. These studies in daphnia and gastropods suggest that some effluent exerts overt toxicity in these invertebrate species while others may have impacts on overall reproductive output.

However, some invertebrate species may show more subtle effects from endocrine disruption associated WWE exposure. In the amphipod species *Gammarus pulex*, WWE below two treatment facilities led to abnormalities in oocyte structure even though there was no impact on male gonads (Gross et al. 2001). Also, downstream from one treatment facility, there were changes in the size dimorphism that exists naturally in this species between males and females. Similar outcomes with regards to oocyte development downstream from a sewage treatment facility were found in the closely related *G. fossarum* (Schirling et al. 2005). Although little is known about the physiological processes controlling reproduction in these amphipod species, these results demonstrate that as is found in vertebrates (see below), reproduction can be influenced following exposure to WWE in invertebrate species.

Vertebrates: Fish

Many fish species have been used as either model laboratory animals or field available markers for impacts of exposure to wastewater effluent. Of all the model organisms evaluated for the impacts of WWE exposure, fish are the most commonly used (Table 1) probably because fish are readily abundant, there are several biological markers available in fish for understanding shifts in reproductive function, and fish have similar endocrine function to other vertebrate groups. Such similarity is important as the results found in this group may be generalizable to other vertebrates. Therefore, these animals make good models for other animals that may be exposed to complex mixes.

It is important to realize that comparative evaluation of the impact of WWE exposure across studies is complicated by differences in experimental design and treatment. Studies vary in the length of time of exposure, the concentration of wastewater effluent used, and the species used in the evaluation. Furthermore, there are several endpoints that have been used in making a determination of effect. Below we review the impacts of exposure on survivorship, reproductive measures such as vitellogenin concentration, intersex and other gonadal abnormalities, hormonal concentrations and growth.

Survivorship following exposure to WWE is variable across studies, and depends on both the species tested and the wastewater effluent used for exposure. For example, fathead minnows exhibited 100% mortality within 24 hours after exposure to diluted WWE from a Florida treatment facility (Keller 1993). However, the same species exposed to WWE in a constructed wetland for three weeks, even at 100% WWE, showed no mortality (Hemming et al. 2002). Rainbow trout exposed to 15% effluent for 32 weeks showed only 5% mortality over controls (Hoger et al. 2006), and in another study even 100% WWE exposure had no impact over controls on survivorship (Jobling et al. 2003). However, exposure of sand gobies to as low as 0.3% sewage effluent over 7 months induced an increase in mortality (Robinson et al. 2003). Exposure of Chinook salmon eggs to 100% WWE induced 100% mortality shortly after hatching (Fernandez et al. 2007). For the marine marbled spinefoot, the LC50 levels following 96 hr exposure times to domestic WWE were 29% of full concentration (Wahbi & El-Greisy 2007). These studies suggest that the impact of exposure to fish to WWE is dependent on the very specific nature of each facility's output, the length of time of exposure, the stage of animal development during exposure, and the species of fish investigated.

Several studies in fish measure outcomes associated with overt toxicity besides mortality. One relatively rapid measure of overall health in fish is a measure termed *condition factor*. This measure represents a ratio of fish weight to length. Several studies have found lower condition factors in fish associated with exposure to WWE (Hemming et al. 2001) Also, decreases in condition factor are sometimes found in fish downstream from wastewater treatment facilities compared to upstream (Iwanowicz et al. 2009). However, sometimes fish show no difference or a higher condition factor at the downstream sites (Lavado et al. 2004; Porter & Janz 2003; Yeom et al. 2007). These results suggest that condition factor may not be the most sensitive measure of overt toxicity and again is subject to the variables described for mortality.

Tests of specific enzyme expression or activity may provide more reliable measures of toxicity. For example, ethoxyresorufin-O-deethylase (EROD) is a marker of oxidative stress associated with exposure to toxic compounds. Exposure to WWE induces EROD activity in several fish species (Grung et al. 2007; Jeffries et al. 2008; Kosmala et al. 1998; Ma et al. 2005; McArdle et al. 2000). In the field, studies comparing EROD activity upstream and downstream of wastewater treatment facilities have found elevations at the downstream sites (Jeffries et al. 2008; Kosmala et al. 1998; Yeom et al. 2007). These studies demonstrate consistent impacts from exposure on enzymes associated with toxicant exposure.

The impact of WWE exposure on reproductive measures in fish demonstrates the potential for changes in reproductive success that might impact fish population levels. Several different measures have been used to evaluate whether WWE contains chemicals that can act like or inhibit estrogen based functions. Some of the outcomes described below can be directly attributed to these estrogen mimicking actions, and some may be acting through other less well defined endocrine mechanisms.

One marker of estrogen exposure includes an increase in liver gene expression or plasma levels of the protein vitellogenin (VTG), an egg yolk protein made in the liver and normally only expressed in females undergoing oocyte development in the ovaries. The expression of this protein is strongly under the influence of naturally produced estrogens in females. Therefore, finding expression of VTG in juveniles, non-reproductive females, or males suggests that there are exogenous, or outside, sources of estrogens. Notably, many WWEs release estrogenic compounds into the environment (see treatment section). Determination of VTG expression in male or juvenile fish is a good measure of current estrogenicity in water systems, as the fish are expressing VTG as a result of current exposure levels to these compounds.

Several studies have directly exposed fish to WWE and measured liver expression of the VTG gene or plasma levels of VTG. Again, the methods vary widely in both the species evaluated and the timing of the exposure. Nevertheless, fish exposed to WWE from many different facilities have demonstrated increases in VTG levels (Aerni et al. 2003; Barber et al. 2007; Bjorkblom et al. 2009; Diniz et al. 2005; Hoger et al. 2006; Jobling et al. 2003; Liney et al. 2005; Liney et al. 2006; Ma et al. 2005; McArdle et al. 2000; Rickwood et al. 2008; Robinson et al. 2003; Rodgers-Gray et al. 2001; Rodgers-Gray et al. 2000; Thorpe et al. 2009). In natural settings, fish in rivers downstream from wastewater facilities also demonstrated increases in VTG levels (Folmar et al. 2001; Harries et al. 1997; Iwanowicz et al. 2009; Lavado et al. 2004; Porter & Janz 2003; Sellin et al. 2009; Tarrant et al. 2008; Vajda et al. 2008). Even flatfish down current from WWE outfalls into oceans have demonstrated elevated VTG levels (Rempel M.A. et al. 2006). Generally, the VTG expression parallels other measures of estrogenicity in the effluent waters. These results along with studies that show that WWE diluted to 15% of full strength can induce a VTG impact suggest that some wastewater facilities treatment processes do not eliminate estrogenic impacts. However, there are studies in which no increases in VTG were seen by either exposing fish to WWE directly or by comparing fish downstream from the facilities to those upstream (Angus et al. 2002; de Montgolfier et al. 2008; Giesy et al. 2003; Harries et al. 1997; Hemming et al. 2001; Iwanowicz et al. 2009; McArdle et al. 2000; Nichols et al. 1999; Robinson et al. 2003; Snyder et al. 2004). As little information is available for the treatment removal efficiencies of these facilities, further evaluation of the treatment facilities that were used in these studies may be very useful in informing better treatment plant development for estrogen removal. Last, these results again demonstrate that there are effluent, species, and sometimes sex differences in sensitivity to WWE exposure in VTG responsiveness.

Another biomarker of estrogenicity in WWE is the presence of either female skewed sex ratios and or intersex (presence of oocytes in testes) in fish populations downstream of wastewater facilities. Use of intersex as a biomarker in aquatic vertebrates represents a measure of potential long-term impacts of exposure, as intersex most likely develops during testicular development at a larval period. The first studies demonstrating widespread endocrine disruption in fish populations found that these anomalies were widespread throughout the United Kingdom (see Jobling et al. 1998). Since then many studies have also found intersex and/or female-biased sex ratios either following developmental exposure to wastewater in the laboratory or downstream from treatment facilities in the wild (Afonso et al. 2002; Barnhoorn et al. 2004; Jobling et al. 2002a; Vajda et al. 2008; van et al. 2001; Woodling et al. 2006). One study predicted the concentration of several estrogens and found a significant correlation between these concentrations and the presence of intersex individuals in wild populations of roaches (fish of the genus Rutilus; Jobling et al. 2006). The correlations found for intersex were stronger than those seen for plasma VTG concentrations. However, another study found elevated VTG concentrations in WWE exposed wild brown trout in Ireland, but found no incidence of intersex (Tarrant et al. 2008). To illustrate the complexity of deciding which outcome measure to utilize, another investigation of roach exposed only during development found that exposure to WWE did not induce intersex, but instead 100% of males had permanent feminization of the reproductive ducts (oviducts in males) (Rodgers-Gray et al. 2001). One study also found no indication of gonadal disruption in chinook salmon during developmental exposure (Fernandez et al. 2007), and several studies have noted little change in gonadal morphology with exposure, but in these cases only adult goldfish (Giesy et al. 2003), fathead minnows (Nichols et al. 1999), and common carp (Snyder et al. 2004) were exposed making the assessment of long-term developmental exposure to compounds on gonadal function impossible to determine. These studies demonstrate that developmental exposure to WWE has a strong potential to feminize male reproductive tissues and that evaluating only exposed adults may lead to false assumptions about the biological impacts of exposure.

Other biomarkers for WWE exposure have also been evaluated with mixed findings. For example, estrogen receptor expression has been measured as an indication of exposure to exogenous estrogens. Furthermore, concentrations of plasma hormones such as estradiol and androgens have been measured in some species to determine whether natural hormone levels are impacted by WWE exposure. Exposure to Tronto River water, which receives inputs from several municipal and industrial sources of WWE in Italy, did not induce estrogen receptor gene expression from liver tissue in goldfish (Palermo et al. 2008). However, caged fathead minnows placed downstream of a wastewater treatment facility in Nebraska had elevated levels of estrogen receptor expression compared to animals at other sites including both reference sites that do not receive WWE and sites downstream from other wastewater treatment facilities (Sellin et al. 2009). In fathead minnows, changes in liver gene expression for two enzymes involved in synthesis of reproductive steroids were increased upon exposure to WWE (Kolok et al. 2007). Changes in expression of several other genes in the gonad and livers of fathead minnows following WWE exposure were compared to expression of the same genes after exposure to known estrogens (Filby et al. 2007b). Expression of several genes was sensitive to both estrogen and WWE exposure, but not always in ways that could be predicted by just measuring the estrogenic potency of the WWE. Similarly, expression of several genes in the testis of male brown trout is impacted by WWE exposure (de Montgolfier et al. 2008). These results demonstrate that although WWE has the potential to be estrogenic, other aspects of endocrine and non-endocrine physiology may also be impacted in ways that do not necessarily allow for clear predictions of morphological outcomes.

Measures of the natural hormones estradiol, testosterone and/or 11-keto-testosterone following WWE exposure in fish also have demonstrated mixed results. For example, plasma estradiol levels were not different in flatfish collected near ocean WWE outfall sites compared to levels from fish collected near a reference site (Rempel M.A. et al. 2006). Similar results were found for estradiol and testosterone levels in fathead minnows exposed to WWE (Nichols et al. 1999). In a study of common carp (Snyder et al. 2004), no differences among sites in Lake Mead, Nevada, were found to be associated with distance from WWE outflow in any measure of plasma steroids. In cases where fish are sampled in the wild, results that demonstrate no difference in outcome measures comparing hormone levels among sites can be difficult to interpret. For example, in the above study, the results with the flatfish or the carp could indicate that the WWE at the outfall site did not impact hormone levels, the fish were not particularly sensitive to exposure, or that they are very sensitive to exposure, and the reference sites may also be contaminated. Careful laboratory studies that include control water samples known to have no chemical contamination can help resolve these alternative hypotheses.

There are a few studies that demonstrate differences in hormone levels of fish upstream versus downstream of WWE outlets. A study of walleye collected downstream from a wastewater facility found that males and females had elevated estradiol levels, and males had suppressed testosterone levels (Folmar et al. 2001) compared to upstream fish. Another study with longnose dace also showed decreased testicular secretion of testosterone downstream from a municipal wastewater facility although 11-keto testosterone levels were unaffected (Jeffries et al. 2008). In rainbow trout, both sexes had elevated estradiol levels following exposure, but testosterone levels did not change (Hoger et al. 2006); however, in males, 11-keto testosterone exhibited lower levels following exposure in this study. In largemouth bass from Florida, males downstream from a WWE outlet exhibit higher estradiol levels and lower 11-keto-testosterone levels than do males from an upstream reference site (Sepulveda et al. 2002). In a study of roach in the United Kingdom (Jobling et al. 2002a), females downstream from two wastewater sites had lower levels of plasma estradiol than did females from reference sites. Only females and intersex fish were found in the WWE sites in this study, and the intersex (presumed genetic males) had higher levels of estradiol and testosterone than did males from control sites. These data suggest that WWE from different facilities impact endogenous hormones levels in unpredictable ways. Furthermore, the effect of exposure on plasma hormone levels may be compromised by the animal's breeding cycle and age. Therefore, caution needs to be taken when interpreting any lack of difference among sites. The mixed results from these studies also shows that the generalized use of steroid hormone concentrations as biomarkers for contaminant exposure may not be possible.

A few other measures of potential reproductive endocrine disruption have also been evaluated in fish. For example, gonadosomatic index (GSI - gonad weight/body mass), sperm production, spermatogenesis, and expression of secondary sex characteristics are all measures used to evaluate impacts from WWE exposure. For example, male fathead minnows exposed to WWE show increased GSI and increases in expression of secondary sex characteristics compared to animals exposed to groundwater (Barber et al. 2007). Also, largemouth bass captured downstream from a wastewater facility exhibit higher GSI than do animals from an upstream reference site (Sepulveda et al. 2002). However, as with the measures of endocrine disruption already described, other studies show different results. Again, in fathead minnow exposed at the outlet of a wastewater treatment facility that flows into constructed wetlands, GSI is reduced (Hemming et al. 2001). Male crucian carp exposed to 100% WWE show a decrease in GSI and a complete inhibition of sperm production (Diniz et al. 2005). Inhibition of sperm production is also seen in intersex roach found downstream from wastewater treatment facilities (Jobling et al. 2002b) which leads to a large reduction in fertility through decreases in milt production, sperm motility, and the ability of the sperm to fertilize eggs (Jobling et al. 2002c). Male mosquitofish use an elongated analfin, termed the gonopodium, to deliver sperm to the female's cloaca. One study found that gonopodium length is shorter in males downstream from a wastewater treatment plant compared to upstream and reference sites (Batty & Lim 1999). Goldfish exposed to WWE however, show no decrease in sperm production (Schoenfuss et al. 2002), but do show a decrease in sperm motility (Schoenfuss et al. 2009). Together, these studies suggest that exposure to WWE often reduces fish fertility.

It will become important to monitor impacts of WWE exposure on endpoints that are not directly related to the reproductive tract in order to get an overall understanding on how survivorship and fitness are impacted. For example, Liney et al. (2006) found that roach exposed to WWE from early life stages through 300 days animals exhibited changes in kidney morphology, immune function, and DNA damage in gills and blood. These impacts were found at WWE concentrations diluted below those levels that induce estrogen-like effects such as VTG induction and intersex. Fathead minnows exposed for 21 days to WWE exhibited DNA damage, and changes in metabolic and immune function (Filby et al. 2007a). Palermo et al. (2008) found that fish treated with river water contaminated with several sources of industrial and municipal WWE had impairment of their neuroendocrine stress system. Other aspects of both general and endocrine physiology may be affected by WWE exposure, yet few studies have evaluated such outcomes. Furthermore, there is still little understanding on how both the direct impacts on the reproductive system and indirect effects on other aspects of physiological function may influence overall fitness or reproductive health independently of the overt impacts on the reproductive tract.

Except for measures of survivorship, all of the above markers for impacts of exposure to WWE in fish do not provide information regarding whether exposure has negative impacts on fish reproductive capacity. Few studies provide direct measures of changes in fertility or fecundity. The first, mentioned above, demonstrated that intersex roach living downstream from wastewater facilities have a decrease in fertility (Jobling et al. 2002c). Recently, one study using fathead minnows demonstrated that although there were no overt measures of toxicity upon exposure to WWE from three different facilities, females showed a significant decrease in egg production when exposed to water from two of the facilities (Thorpe et al. 2009). Clearly, if we are to understand the long-term potential impact of exposure to the complex mix of compounds found in

WWE, more studies evaluating the impact of exposure to reproductive output are necessary.

Of particular note are a series of studies conducted by the U. S. Geological Survey evaluating the reproductive health of fish in a series of U.S. watersheds (Blazer et al. 2007; Hinck et al. 2006; Hinck et al. 2007; Hinck et al. 2008; Hinck et al. 2009; Iwanowicz et al. 2009; Schmitt et al. 2005). These studies are summarized in Hinck (2009). Although several fish species showed no evidence of intersex (largescale sucker, longnose sucker, white sucker, spotted bass, northern pike, flathead catfish, burbot, striped bass, white bass and brown trout; note: some of the sample sizes for these species was small), three species—small and largemouth bass and channel catfish—had significant incidences of testicular oocytes and one intersex fish was found in the common carp. Intersex fish were found in 31% of the sites sampled from across the country. Forty-four percent of sites that contained bass had fish with an intersex condition. The range in percent of male individuals exhibiting intersex across all sites ranged from 0–91%. Collectively, these studies demonstrate that intersex fish are occurring across the country and that there are species differences in sensitivity to the development of intersex. Intersex was found in both sites with known contamination and at sites with no known source for estrogenic contaminants. When conducting broad scale studies, more effort should be directed at monitoring water chemistry at collection sites, to determine whether there is a direct correlation between contamination and disruption of gonadal development.

Vertebrates: Amphibians

Amphibians are another group of aquatic vertebrates. However, as they are less diverse and abundant than fish, there have been fewer studies of the impact of wastewater exposure on their health. Using a standard test for toxicity in South African clawed frog larvae, Ciccotelli et al. (1998) exposed tadpoles to either 50% or 100% tertiary treated WWE and evaluated induction of enzyme markers expressed under conditions of toxicant exposure. Several such enzymes were induced in this species following exposure, demonstrating that the WWE did contain compounds that led to physiological responses suggesting toxicity.

One study has investigated the impact of spray irrigation using chlorinated WWE on amphibian populations in nearby ponds (Laposata & Dunson 2000). Compared to nearby ponds that did not receive input from WWE, the number of egg clutches found and both hatching and larval survivorship were decreased in wood frogs, Jefferson salamanders and spotted salamanders. It is unclear whether these changes were a direct result of the WWE impact on the eggs or whether other ecological changes in the ponds such as increases in duckweed cover were responsible for these decreases in reproductive output.

As with fish, there is evidence that exposure during key developmental periods can impact amphibians. Bogi et al. (2003) found that exposure to 50% WWE during development induces an increase in post-metamorphic females VTG gene expression in South African clawed frogs. However, there was little if any significant impact on sex ratios or changes in incidence of intersex in either the clawed frog or in the common frog. A study of leopard frogs (Sowers et al. 2009) found that exposure to 50 or 100% WWE increased survivorship of animals reaching metamorphosis; however, the amount of time to metamorphosis was longer in these treatment groups, suggesting disruption of thyroid hormone systems (the process of metamorphosis is dependent on appropriate secretion of thyroid hormone). Animals in these treatment groups also displayed an increase incidence of intersex compared to control animals. Much more work is necessary to understand whether contaminants in water systems are impacting amphibian survivorship and reproduction, but given that amphibian populations are in decline throughout the world, gaining knowledge about potential impact is critical.

Vertebrates: Reptiles

The impact of municipal WWE on reptiles is virtually unstudied. However, one of the first broadly recognized studies of the impact of chemical pollution on reproductive tract development came from alligators exposed to a chemical spill from a pesticide plant in Florida (Guillette, Jr. et al. 1994; Guillette, Jr. et al. 1995; Guillette, Jr. et al. 1996). These animals expressed ovarian and testicular abnormalities and a 75% decrease in penis size in males. While this spill was dramatic in scope, the results demonstrate that amniotic vertebrates are not protected from the impact of exposure to environmental contaminants. Another study investigating the impact of water contamination on reptiles shows that painted turtles in ponds near cattle pastures found higher VTG levels in females, but not males, compared to control ponds (Irwin et al. 2001). Notably, males in this species did not exhibit a great deal of sensitivity in VTG response to estradiol exposure in the lab. No studies have looked at impacts on reptiles downstream from municipal treatment facilities.

Vertebrates: Birds

There is only one study on the impact of WWE on birds. This work compared reproductive outcomes in tree swallows living near wastewater lagoons to a reference site (Dods et al. 2005). They found lower clutch sizes, decreased fledgling success, and higher liver weights in the birds near the lagoons. However, there was no difference in diet or parental provisioning, suggesting that the differences were not due to changes in parental care.

Vertebrates: Mammals

As with birds and reptiles, there are only a limited number of studies in rats. One study treated laboratory rats with up to 500 times concentrated WWE from a Denver, CO facility for nearly 2 years. The found no impact on survival, growth, organ weight, or organ pathologies (Condie et al. 1994). Another study using 100% WWE from a facility in India investigated the reproductive impact of exposure in male rats (Kumar et al. 2008). This study found WWE-induced changes in weight of sex accessory tissues, and differences in expression of several enzymes involved in the production of steroid hormones. Hormone levels were also affected with both pituitary hormones and testosterone levels affected in a manner that suggested disrupted negative feedback. Last, several measures of overt toxic effects in the livers and kidneys of exposed animals were found. Another study from India also showed severe kidney and liver toxicity with WWE exposure (Tabrez & Ahmad 2009). If we are to better understand the impact of complex chemical mixes on human populations, more studies of WWE impacts on model mammalian species must be initiated.

Impacts at Higher Levels of Organization: Populations and Communities

All of the above studies have examined the impacts of WWE at the molecular, cellular, tissue and individual levels of organization, but in order to determine whether exposure to wastewater contaminants represents true risk to the environment, studies at higher organizational levels need to be conducted. Furthermore, because WWE also contains chemicals and microbial populations that might not directly impact physiology, but might affect other parts of the ecosystem, the overall effects of exposure on population structure might be complicated by changes in the overall biota of the receiving waters.

Only a few studies have evaluated fish population dynamics up and downstream from WWE output. Jeffries et al (2008) examined longnose dace's populations up versus downstream from WWE output in Canada. They found that the number of fish caught per unit effort and the condition factor of the fish was higher immediately downstream from the wastewater facility. Also, there was no dramatic change in age structure of the population between the immediate upstream and downstream sites although there was a shift away from older animals in sites farther downstream that receive effluent from more industrial processes and non-point pollution sources such as agriculture. In a study of pale chub populations in the Republic of Korea, Yeom et al. (Yeom et al. 2007) found that both the abundance and the age structure of the population was dramatically changed. There were many fewer fish downstream compared to upstream from the facilities and there was no young fish found in a site receiving water from both industrial and municipal wastewater facilities. As with studies of impacts on individuals, the impacts of exposure on populations will be affected by the species studied and the effluent evaluated.

A couple of studies have examined fish community effects and found there to be impacts downstream from WWE input. Using an index of biotic integrity for fish communities, Yeom et al. (2007) found that sites downstream from WWE input had degraded fish communities compared to upstream sites. Another study of several streams in South Korea found a lower diversity index and a lower biotic integrity index for fish communities downstream from wastewater facilities compared to upstream sites (Ra et al. 2007b). Obviously, more population and community level studies are necessary in order to understand the overall impact of WWE on a large scale level.

Recently, a few studies have tried to evaluate the impact of WWE on macroinvertebrate communities. These studies have developed a mechanism to compare a biological integrity index to a hazard index calculated from measuring the concentrations of several pollutants in streams exposed to WWE and deriving an index based on known individual compound toxicities in three different species (algal, daphnia and fish;

Table 3. Summary of impact of WWE from several animal studies.

Tissue/species	*Outcome measured*	*Outcome*	*Citation*
Daphnia, (*Ceriodaphnia dubia*)	Whole effluent toxicity (WET) test	Reduced survivorship and reduced fecundity	Hemming et al. 2002
Anodonta imbecilis	7-days of exposure	High LC50 at 16% concentrated WWE	Keller 1993
Elliptio complanata	Mortality; immune responses	Greater overall mortality; suppression or induction of immune responses depending on source	Bouchard et al. 2009
Potamopyrgus antipodarum	Mortality, growth, fertility	Decrease in growth rate; no impact on overall mortality; embryo production was reduced	Jobling et al. 2003
A. cygnea	Gene expression	Important gene level changes normally associated with overt toxicity	Ciccotelli et al. 1998
E. complanata	Respiratory; nervous system	Induces increases in mitochondrial electron transport, changes in neuronal signaling systems	Gagne et al. 2006; Gagne et al. 2007
Gammarus pulex	Gonadal changes	Abnormalities in oocyte structure, no impact on male gonads	Gross et al. 2001
G. fossarum	Gonadal changes	Oocyte abnormalities	Schirling et al. 2005
Rainbow trout	Mortality; hormone levels	0-5% mortality over controls; elevated estradiol levels; testosterone levels did not differ	Hoger et al. 2006; Jobling et al. 2003
Sand gobies	Mortality	Increase in mortality	Robinson et al. 2003
Chinook salmon eggs	Mortality	Induced 100% mortality shortly after hatching	Fernandez et al. 2007
Marine marbled spinefoot	Mortality; gonadal changes	LC50 levels occurred at 29% diluted WWE; ovarian abnormalities	Wahbi and El-Greisy 2007
Demersal flatfish	VTG levels; gonadal changes	Elevated VTG levels; sperm damage; masculinization	Rempel M.A. et al. 2006
Roaches	Gondal and reproductive tract changes	In some populations there was a correlation between water estrogen concentrations and the presence of intersex individuals in wild populations; in other populations there was no intersex, but 100% of males had permanent feminization of the reproductive ducts	Jobling et al. 2006; Rodgers-Gray et al. 2001; Jobling et al. 2002
Goldfish (liver tissue)	Estrogen receptor expression changes; sperm production	No induction of estrogen receptor gene expression or impact on sperm production	Palermo et al. 2008; Schoenfuss et al. 2002

Table 3, continued. Summary of impact of WWE from several animal studies.

Tissue/species	*Outcome measured*	*Outcome*	*Citation*
Fathead minnows	Estrogen receptor expression changes; gonadal changes	Elevated levels of estrogen receptor expression; increased GSI (males) and increased expression of secondary sex characteristics; DNA damage, and changes in metabolic and immune function	Sellin et al. 2009; Kolok et al. 2007; Barber et al. 2007; Filby et al. 2007
Brook trout	Expression of VTG and several genes in the testis; spermatogenesis	All genes had changes in expression over controls; spermatogenesis was accelerated	de Montgolfier et al. 2008
Walleye	Steroid hormone levels	Males and females had elevated estradiol levels; Males had suppressed testosterone levels	Folmar et al. 2001
Longnose dace	Steroid hormone levels	Decreased testicular secretion of testosterone; 11-keto testosterone levels were unaffected	Jeffries et al. 2008
Largemouth bass	Steroid Hormone Levels	Males exhibit higher estradiol levels and lower 11-keto-testosterone levels than do males from an upstream reference site	Sepulveda et al. 2002
Common carp	Steroid hormone levels; EROD levels	No differences in any measure.	Snyder et al. 2004
Male crucian carp	Gonadal abnormalities	Decrease in GSI and a complete inhibition of sperm production	Diniz et al. 2005
African-clawed frog larvae	Induction of enzymes used to evaluate toxicity; VTG expression	Evaluated induction of enzyme markers expressed under conditions of toxicant exposure; induced VTG expression	Bogi et al. 2003; Ciccotelli et al. 1998
Leopard frogs	Metamorphic timing; gonadal abnormalities	Increased survivorship of animals reaching metamorphosis, amount of time to metamorphosis was longer, suggesting disruption of thyroid hormone system, increase incidence of intersex compared to control animals	Sowers et al. 2009
Painted turtles	VTG levels	Higher VTG levels in females, but not males	Irwin et al. 2001
Tree swallows	Reproductive parameters; liver weight	Lower clutch sizes; decreased fledgling success; higher liver weights in the birds near the WWE lagoons. No difference in diet or parental provisioning	Dods et al. 2005
Laboratory rats	Several outcomes for overall health	No impact on survival, growth, organ weight, or organ pathologies	Condie et al. 1994

Ginebreda et al. 2009; Gros et al. 2010). The results of this index suggested that complex mixes at concentrations found in river water may not represent a significant hazard (Gros et al. 2010). However, when the WWE hazard index is calculated based on known concentrations of several compounds and compared to the downstream diversity index, significant strong negative correlations were found (Ginebreda et al. 2009) indicating that there is an overall decrease in macroinvertebrate community structure with increasing pollution from WWE.

One study used complex network analysis mechanisms to evaluate inter-taxa community structure effects associated with different levels of pollution along a stream (Kim et al. 2008). They found that there were sets of microbial communities that were correlated to the level of stream pollution, and the macroinvertabrate community structure followed, to some degree, the microbial community changes. The complexity of these interacting networks makes it very difficult to determine the causative links between pollution and shifts in inter-taxa community structure; however, future studies investigating any one taxa must take into account that the impacts of exposure to chemicals in wastewater also probably interacts with changes in other taxa within the communities. Studies combining mathematical modeling and real life biological outcome measures at the community level are an area of research that needs further development to assess how WWE contamination may remodel community structure.

General Conclusions Regarding Biological Impacts

It is critical to recognize that the reproductive disruption in wildlife populations exposed to the complex mix of chemicals in WWE may not be due solely to the actions of specific compounds on limited physiological endpoints. A recent study modeling the impacts of WWE on reproductive disruption of roach in the United Kingdom demonstrates that these chemical mixes have varied and complicated effects on endocrine function that lead to differential influences on reproductive physiology (Jobling et al. 2009). Also, aside from those studies mentioned above, there is very little information available regarding the impact on non-steroidogenic physiological processes such as stress responses, thyroid physiology, and sugar metabolism. Furthermore, each WWE mix is different from others, which combined with the complexity of endocrine physiology and species differences in sensitivity, makes straight forward predictions about outcomes immensely difficult. The EPA has generated a series of assays to test for reproductive disruption and thyroid hormone disruption through its Endocrine Disruptor Screening Program (http://www.epa.gov/endo/index.htm). One possibility is that these assays be adapted to testing which forms of wastewater treatment reduce the possibility of disruption. Furthermore, the development of tests that go beyond the individual animal and test the impacts on population and community structures can be devised and potentially standardized using both mesocosm designs and natural field populations.

Bioaccumulation

The above section demonstrates that exposure to chemicals in WWE induces changes in organismal physiology with different species having variable levels of sensitivity. The obvious conclusion from these studies is that these chemicals can accumulate in body tissues where they then interact with cellular mechanisms to induce changes in how an organism functions.

The bioaccumulation of environmental contaminants in any given organism does not end with that individual. Other organisms that rely on organisms lower on the food web receive the prey item's contaminant "history." The animal higher on the food chain will not only accumulate the contaminants in the water or sediment of its environment, but also those in its food resources. This process is known as biomagnification. As with the tests for biological effects, tests for bioaccumulation of chemicals found in WWE are most common using fish, but a few other species have also been investigated. Notably, some compounds may be present in the environment, but may not bioaccumulate in the tissues of exposed organisms. However, the presence of such compounds in tissues strongly suggests bioaccumulation. Many of these studies are reviewed below and the results are summarized in Table 4.

Few studies have evaluated bioaccumulation of a wide range of environmental contaminants directly relating to WWE exposure; however, one of the most extensive evaluations of bioaccumulation of compounds derived from WWE is a USGS study from a wetlands receiving WWE from a Phoenix, Arizona treatment facility (Barber et al. 2006). Two fish species, mosquitofish and *Tilapia* were evaluated for uptake of several compounds and trace elements found in the effluent from the facility. The mosquitofish showed consistently higher levels of bioaccumulation of several pesticides and PCBs (Table 3) than did Tilapia, and the accumulation reflected the concentrations of the compounds found in water where the fish were collected. Also, the Tilapia showed greater bioaccumulation in the liver than in the muscle fillets for all compounds except p,p'-DDE and trans-nonachlor. Another study of largemouth bass from a Florida stream system that evaluated bioaccumulation from a reference site compared to sites receiving industrial or municipal effluent, determined that many compounds, including DDTs, cycldeine pesticides, PCBs and heavy metals, were higher in fish immediately downstream from these sites than in fish from the reference site (Sepulveda et al. 2002). The comparison between sites demonstrates that if these compounds are available in the environment, they will bioaccumulate in the fish tissues. These results are important because they suggest that commercial and sports fish like *Tilapia* and bass, used for human consumption, bioaccumulate contaminants in their environment and therefore become a source for human exposure.

Other studies have evaluated bioaccumulation of specific classes of compounds found in WWE or in the general environment. The most commonly evaluated compounds include heavy metals, and there have been many studies over the last several decades in a variety of species (some reviews include: Dallinger et al. 1987; Goodyear & McNeill 1999; Kouba et al. 2010). Although there is some evidence that these compounds impact endocrine function, little is understood about the endocrine disrupting capacity of these compounds outside of overt toxicity and neurotoxicity, and they will not be further reviewed here.

Polybrominated diphenyl ethers (PBDEs) are chemicals manufactured as flame retardants and are widespread aquatic environmental pollutants. Evaluation of small mouth bass upstream versus downstream from WWE facilities demonstrates a 607% increase in the concentration of PBDE's in muscle tissue between a site near the beginning of a river in Maine and downstream from a wastewater facility. White suckers exhibit a 19% increase in PBDE's in whole fish homogenates when comparing sites more immediately upstream to the downstream sites (Anderson & MacRae 2006). In the marine environment off of the coast of the United States, ppt-ppb levels of PBDEs are found in organisms ranging from mussels to whales (please see Table 1, Yogui & Sericano 2009 for details). Although this study did not investigate compounds from WWE directly, the results demonstrate how ubiquitous PBDEs are in our environment.

Another group of environmental chemicals are those derived from the antimicrobial triclosan. In a study evaluating bioaccumulation of triclosan, triclocarban, and methyl triclosan in algae and snails downstream from a Texas wastewater treatment facility, Coogan and La Point (2008) found ppb levels of all three compounds in both organisms. The bioaccumulation factor was about 3 fold. In an evaluation of fish from several lakes in Switzerland, Balmer et al. (2004) found that whitefish and roach in remote lakes that receive no WWE had no measurable concentrations of methyl triclosan, while fish in lakes receiving WWE had measureable methyl triclosan in their tissues. Furthermore, the levels of triclosan were correlated with the levels in the lakes, and the bioconcentration factor was more than 10,000 fold (Balmer et al. 2004). Fish from around the United States also have detectable levels of triclosan in their tissues (Ramirez et al. 2009). This compound is also bioaccumulating in bottlenose dolphins sampled from the southeast coast of the U.S. with up to 31% of the animals sampled having detectable levels of triclosan in the ppt range (Fair et al. 2008). These studies demonstrate that antimicrobial compounds do not get fully removed through wastewater processing and do bioaccumulate in the tissues of organisms living downstream from WWE outflow.

Sunscreens like benzophenone-3 (BP-3), 4-methylbenzylidene camphor (4-MBC), ethylhexyl methoxy cinnamate (EHMC) and octocrylene (OC) were measured in several fish species across Swiss lakes. Fish from reference lakes that receive little human impact did not have any of these compounds in their tissues; however, fish from lakes receiving WWE and recreational use did exhibit bioaccumulation of each of these compounds. The results varied across lakes and species (Balmer et al. 2005; Buser et al. 2005). Similarly, brown trout were evaluated from several rivers in Switzerland for the occurrence of 4-MBC and OC (Buser et al. 2006). The levels found in these fish were considerably higher than those found from remote lake sites. While in these studies it was not possible to separate whether the compounds were derived from WWE or from direct recreational use, nevertheless, these studies determined that the active ingredients of sunscreen do bioaccumulate in exposed aquatic organisms.

Sources of pesticide contamination are multiple and not always solely from WWE exposure; however, WWE does represent a point source for pesticide exposure. Bass and carp from streams throughout the southeast United States bioaccumulate pesticides and pesticide residues such as DDE, DDT, toxaphene, aldrin, dieldrin, endrin, mirex, dacthal, endosulfan, methoxychlor, and other organochlorine residues (Hinck et al. 2008). In a Chinese lake receiving WWE, levels of organochlorine pesticides are also in the ppb range in zooplankton, fish, and turtles (Li et al. 2008). Again, while the source of the pesticides may be multiple, these compounds do bioaccumulate.

Polychlorinated biphenyls (PCBs) have been evaluated in both marine and freshwater fish. The waters of the marine shelf off the coast of San Diego receive effluent from several treatment facilities, but also receive potential contamination from the dumping of sediments dredged from San Diego Bay. Parnell et al. (2008) evaluated PCB loads in the livers of four families of fish living in this coastal marine environment. Several congeners of PCBs were found at part per billion levels (ug/kg), and the types and quantities of PCBs differed among collection sites, species and individual fish liver lipid content. The authors believe the source of PCB contamination is probably not from local WWE exposure as PCBs have not been detected from these facilities in more than 20 years. In a broad scale USGS study to evaluate levels of contaminants in fish throughout the southeast U.S., Hinck et al. (2008) determined levels of PCBs in freshwater largemouth bass and carp and found levels also in the ppb range (see Table 3), Notably, these levels were above those known to have negative health impacts on fish. Similar results were found from bass in a stream system in Florida (Sepulveda et al. 2002). Zooplankton, carp, goldfish, tilapia, catfish and softshelled turtles from a lake in China receiving WWE from a Beijing treatment facility also demonstrate significant levels of PCBs that biomagnify up the food web (Li et al. 2008). These data demonstrate that even though the use of PCBs ended decades ago, there is still widespread bioaccumulation of PCBs in fish tissues.

Some bioaccumulated compounds, like steroids and pharmaceuticals, clearly result from point source pollution like wastewater treatment facilities. Rainbow trout and roach experimentally exposed to wastewater effluent for 10 days have higher bile content of several

Table 4. Compounds evaluated for bioaccumulation.

Compound	*Species*	*Concentration Mean Upstream or in Lakes Not Receiving WWE*	*Concentration Downstream or in Lakes Receiving WWE*	*Bio-concentration factor*	*References*
Polybromiated diphenyl ethers	Small mouth bass	2800 ng/g lipid	17,000 ng/g lipid Mean	Not determined	Anderson & MacRae 2006
Polybromiated diphenyl ethers	White sucker	6300 ng/g lipid	7500 ng/g lipid (Mean)	Not determined	Anderson & MacRae 2006
Methyl Triclosan, triclosan and triclocarban	Algae, snails, roach, white fish brown trout	Not determined	5 ng/g fish-760 ng/g lipid	3-2.6 X 10^5	Balmer et al. 2004; Buser et al. 2006; Coogan & La Point 2008
Benzophenone-3,	Whitefish, roach and perch	Not determined	66-123 ng/g lipid	Not determined	Balmer et al. 2005
4-methylbenzylidene camphor	Whitefish, roach, perch, brown trout	N/A	80 ng/g -1800 ng/g lipid weight depending on species and location	1-2.3 x 10^4	Balmer et al. 2005; Buser et al. 2006
Ethylhexyl methoxy cinnamate	Whitefish, roach and perch	Not determined	Nd-64 ng/g lipid	Not determined	Balmer et al. 2005
Octocrylene	Whitefish, roach and perch, brown trout	Not determined	25-2400 ng/g lipid weight	Not determined	Balmer et al. 2005

Table 4, continued. Compounds evaluated for bioaccumulation.

Compound	*Species*	*Concentration Mean Upstream or in Lakes not receiving WWE*	*Concentration Downstream or in Lakes Receiving WWE*	*Bio- concentration factor*	*Reference(s)*
Chlordanes	Mosquito-fish, Tilapia, largemouth bass	Not determined	nd~12 ug/kg body weight	0-9400	Barber et al. 2006; Sepulveda et al. 2002
p,p'-DDE and other DDTs	Mosquito-fish, Tilapia, largemouth bass, carp	Not determined	Nd-44 ug/kg body weight	43,000-150,000	Barber et al. 2006; Hinck et al. 2008; Sepulveda et al. 2002
Other organochlorine pesticides and residues	Carp, bass	Not determined	Nd-100 ug/kg	Not determined	Hinck et al. 2008
Lindane	Mosquito-fish, Tilapia	Not determined	Nd-6.4 ug/kg body weight	0-530	Barber et al. 2006
Trans-nonachlor	Mosquito-fish, Tilapia	Not determined	Nd-61 ug/kg body weight	1600-9500	Barber et al. 2006
PCB totals	Mosquito-fish, Tilapia, largemouth bass, carp	Not determined	Nd-2400 ug/kg body weight	Not determined	Barber et al. 2006; Hinck et al. 2008
PCB totals	Sanddabs, sole, turbot, croakers, scorpionfish, rockfish	Not determined	0-60 ug/kg lipid	Not determined	Parnell et al. 2008
PCB totals	Zooplankton, carp, goldfish, catfish, tilapia, turtle, largemouth bass	4.7-43.8 ug/l	0.65-158 ug/kg liver or muscle weight	2.0-4.4 (Food web magnification factor)	Li et al. 2008; Sepulveda et al. 2002
4-nonylphenol	Insects, benthos, phytoplankton	Not determined	8-310 ug/kg dry weight	63-990 benthos	Dods et al. 2005; Takahashi et al. 2003
Galaxolide and tonalide	Largemouth bass, carp, bowfin, white sucker and smallmouth buffalo	Not determined	21-2100 ug/kg fillet weight	Not determined	Ramirez et al. 2009
Norluoxetine, sertraline, diphenhydramine, Diltiazem, carbamazepine	Largemouth bass, carp, bowfin, white sucker and smallmouth buffalo	Not determined	0-19 ug/kg tissue	Not determined	Ramirez et al. 2009; Schultz et al. In press.

estrogenic steroids such as estrone, estradiol, ethynyl estradiol (birth control estrogen), and 17-beta-dihydroequilenin (estrogen replacement therapy estrogen) than fish exposed to control tap water or river water from upstream sites (Gibson et al. 2005). Ethynyl estradiol was also found in the testes and ovaries of these animals. In river water receiving WWE in Japan, estradiol levels were also up to ppb levels in benthos animals and plants and phytoplankton in river water receiving WWE (Takahashi et al. 2003). These studies demonstrate that naturally occurring estrogens, their metabolites, and their pharmacological mimics can bioaccumulate in vertebrates and other non-vertebrate organisms.

Several other compounds used for a multitude of industrial and manufacturing purposes are found in WWE including alkylphenols and bisphenol A. For example, insects taken from a lagoon receiving WWE had significantly higher levels of 4-nonylphenol in their tissues than did those taken from a reference site (Table 3; Dods et al. 2005). Rainbow trout exposed to WWE accumulated nonylphenol and nonylphenol ethoxylates in their bile, however, the concentrations were not measured directly, but were evaluated based on the estrogenicity equivalence via the yeast estrogen screen (Gibson et al. 2005). The levels were determined to be about 20 ng/ml estradiol equivalents. Interestingly, roach exposed to WWE in the same study showed less bioaccumulation of not only nonylphenols, but also other estrogenic compounds again demonstrating that there are strong species differences in the potential for bioaccumulation. Takahashi et al. (2003) found ppb levels of nonylphenols and bisphenol A (BPA) in benthos plants and animals and in phytoplankton collected from a river downstream from WWE outlets. These compounds are found in many WWEs throughout the world, and have been shown to have impacts on reproductive function at ppb dosage levels in many organisms (Scares et al. 2008; Vandenberg et al. 2009).

Recently, the USGS undertook a large-scale study to evaluate bioaccumulation of pharmaceuticals and personal care products in fish from five sites throughout the United States that receive WWE (Ramirez et al. 2009). Each site evaluated different fish species because of lack of similar fish communities across areas. One reference area in the Gila Wilderness in New Mexico was included, and notably, no PPCPs were detected from animals at this site. However, the compounds galaxolide and tonalide, commonly used fragrances in many personal care products, were detected in fish tissues at all other sites in the ppb-ppm range. Five pharmaceuticals, norfluoxetine, sertraline, diphenhydramine, diltiazem and carbamazepine, were found in samples from some of the sites in up to the low ppb range (Ramirez et al. 2009; Schultz et al. in press). As the source of these compounds is almost always through WWE, they make excellent markers demonstrating the wastewater treatment facilities are a point source of pollution for chemicals that bioaccumulate.

Treatment processes available to reduce chemical contamination

Removal of EDCs during Wastewater Treatment

Removal of EDCs during wastewater treatment can occur by four mechanisms: adsorption onto solids, aerobic and anaerobic biodegradation, chemical (abiotic) degradation, and volatilization. In general, adsorption and biodegradation are the most important EDC removal mechanisms. A comprehensive description of EDC behavior during wastewater treatment is given in Birkett and Lester (2003).

Adsorption onto Solids

The octanol-water partitioning coefficients (K_{ow} values) of estrogenic EDCs (Table 1) suggest they should tend to transfer to some extent from the liquid phase to the solid phase (sludge) during wastewater treatment processing. Several studies have examined the removal of EDCs to sludge/biosolids during wastewater treatment. For example, Holbrook et al. (2002) and Takigami et al. (2002) both showed that estrogenic hormones were transferred to sludge and that biosolids contained greater estrogenic activity than the secondary effluents.

Aerobic and Anaerobic Biodegradation

Biodegradation of estrogenic hormones during conventional wastewater treatment has been demonstrated. In a study at six wastewater treatment facilities, Baronti et al. (2000) reported that effluent concentrations of estrone (E1) were higher than influent concentrations for four of the six sites, likely due to the aerobic biodegradation of estradiol (E2) to estrone during the treatment process. The biodegradation of alkylphenolic surfactants during wastewater treatment can also lead to the formation of more estrogenic alkylphenols including nonyl- and octyl-phenols.

Chemical (Abiotic) Degradation

Abiotic degradation of hormones in the environment has been demonstrated. Gray and Sedlak (2005) reported a half-life of 3.5 days for E2 when photolytic decay is the only removal mechanism. Field investigations have shown lower levels of removal in wetlands. Mansell et al. (2004) reported E2 and testosterone were removed by 10% over a 24 hour period.

Volatilization

Volatilization is not expected to be an important removal process during wastewater treatment for EDCs/PPCPs due to their relatively low vapor pressures.

Arizona-based Research

A series of studies have been conducted to evaluate removal efficiencies of estrogenic activity at municipal wastewater treatment facilities in Arizona, including an effort by Northern Arizona University/ University of Arizona during 2005-2007 funded by The Arizona Water Institute. Results (Table 5) indicate that influent-to-effluent reductions in total estrogenic activity (3rd column in the table) are highly variable and probably treatment plant- or process-dependent. Up to ninety-nine percent removals were observed for the Avra Valley oxidation ditch, the Randolph Park membrane bioreactor and the Rio de Flag nitrifying/denitrifying wastewater treatment plants. On the other hand, the Roger Road Wastewater Treatment Plant, which provides secondary treatment in a biotower (trickling filter), removed only one-third of the measured influent estrogenic activity. Clearly, the type and efficiency of biochemical treatment provided are major determinants of estrogen transformation efficiency. The fourth column in the table represents the across-the-plant removal of estrogenic activity after accounting for the activity present in sludge/biosolids leaving the facility. An emphasis of current research is understanding the partitioning of EDCs to the solid phase during municipal wastewater treatment, including sludge digestion/stabilization processes, and after land application of biosolids.

To facilitate understanding of biological wastewater treatment impacts on removal of estrogenic contaminants, the authors (Quanrud and Propper) are currently performing a bench-scale simulation of activated sludge processes in research funded by the Arizona Water Institute. Hypotheses regarding the impact of temperature and sludge age on the removal rate of selected EDCs are being tested. The project is also evaluating the value of advanced oxidation processes (AOPs) as a tertiary treatment process to destroy estrogenic compounds in wastewater treatment plant (WWTP) effluent. Dependent variables for these experiments are through-process removal of specific estrogenic compounds (estradiol and ethinyl estradiol) and the removal of total estrogenic activity. In addition, further development and validation of an immuno-based lateral flow assay is underway to rapidly and inexpensively evaluate estrogenic compounds in aqueous media. A final project report is expected in late 2010.

Conventional Wastewater Treatment

During the last ten years or so, there have been quite a few efforts to evaluate conventional wastewater treatment processes for trace organic compound (TOrC) removal efficiency (Table 6). Categories of TOrCs in wastewater include pharmaceuticals and personal care products (PPCPs), pesticides, industrial chemicals, and flame retardants. Some of these compounds are known to be endocrine disrupting compounds (EDCs), meaning they can elicit endocrine response in exposed animals and/or humans. EDC fate during wastewater treatment processes has been a focus of intense research in recent years. Consequently, there is now a body of literature on which to base certain general expectations regarding the fates of EDCs and other trace organics during conventional wastewater treatment processes.

While wastewater treatment plants (WWTPs) represent a significant and ongoing point source of EDCs, they also present opportunities for applying effective, centralized removal strategies. Treatment facilities that utilize activated sludge (AS) and perform nitrification are strong candidates for removing EDCs. Nitrifying bacteria can be classified as either ammonia-oxidizing or nitrite-oxidizing. There is support for the notion that the ammonia-oxidizers are primarily responsible for EDC destruction in nitrifying AS systems. Specific evidence suggests that the enzyme ammonia monooxygenase (AMO) is responsible for EDC transformations. Activated sludge systems are managed for nitrification by manipulating the solids retention time (SRT).

The natural estrogens E2 and particularly estrone (E1) are responsible for most of the estrogenic activity in conventionally treated wastewater (Tan et al. 2007). Estrogenic activity represents the sum effect due to the mixture of estrogenic EDCs present. Representative removals (influent to effluent) of E2 during conven-

Table 5. Removals of estrogenic activity at wastewater treatment facilities in Arizona. Influent and effluent values were obtained using the YES bioassay.

Facility	*Treatment Process*	*Estrogenic activity*	
		Percent Removal (influent to effluent)	*Percent Removal (overall)*
Avra Valley	Oxidation ditch with no digestion	99.8, 98[1]	97[1]
Sundog	Oxidation ditch with anaerobic digester	89[2]	9[2]
Roger Road	Trickling filter with anaerobic digester	33, 31[1]	26[1]
Ina Road	Activated sludge with 100% oxygen and anaerobic digester.	88, 71[1], 76[2]	65[1], 54[2]
Randolph Park	Denitrification/ Nitrification / Membrane bioreactor and no digester	>99, 98[1], 28[2]	97[1], 28[2]
Wildcat	Trickling filter with enhanced solids contact	96, 85[1]	54[1]
91st Ave.	Activated sludge with nitrification/denitrification	0[2]	99[2]
Rio de Flag	Nitrification/denitrification with no digester.	>99.6, 99[1], 73[2]	71[1], -36[2]

[1] Teske et al. 2006

[2] Propper et al. 2007

tional wastewater treatment vary from 70 to 99% (Servos et al. 2005; Baronti et al. 2000; Nasu et al. 2001; Tan et al. 2007). Influent-to-effluent losses of E1, however, are highly variable, ranging from net increases to near complete removal. High concentrations of E1 in plant effluents have been attributed to hydrolysis of conjugated natural estrogens, oxidation of E2 to E1 and relatively slow E1 oxidation kinetics during wastewater treatment (Ternes et al. 1999).

Researchers have noted that some TOrCs are not effectively removed by conventional wastewater treatment processes (Johnson and Sumpter, 2001; Heberer, 2002a,b; Castiglioni et al. 2006) and thus are present in receiving waters (Kolpin et al. 2002). Examples of persistent TOrCs include carbamazepine, methoxazole, and iopromide.

With respect to endocrine disrupting compounds, the results of several broad surveys have produced a weak consensus on the ability of conventional wastewater treatment processes to remove estrogenic activity and/or specific chemicals that contribute to total estrogenic activity in wastewater and wastewater effluent. A comprehensive review of these studies was performed by Teske and Arnold (2008). Summaries of estrogenic compound/activity removal efficiencies during activated sludge and trickling filter wastewater treatment processes are given in Tables 7 and 8, respectively. Most researchers agree that there is a direct relationship between the removal efficiency of natural estrogens and both sludge age and hydraulic retention time (HRT) in activated sludge aeration basins (secondary treatment). Nitrifying plants are generally more successful at removing estrogenic activity, and there is mild debate over whether or not nitrifying bacteria are directly responsible for the biochemical transformations of interest. Success in removing trace organics with longer sludge

Table 6. Compilation of recent peer-reviewed studies examining fate of EDCs during conventional wastewater treatment processes.

Wastewater treatment process(s)	*Trace organic compound parameter(s)*	*Reference*
Activated sludge, trickling filter	55 PPCPs	Kasprzyk-Hordern et al. 2009
23 conventional WWTPs	DBP precursors	Krasner et al. 2009
13 onsite treatment systems	13 PPCPs	Matamoros et al. 2009
Activated sludge	31 PhACs	Radjenovic et al. 2009
chlorination	Estrogenic/antiestrogenic activities	Wu et al. 2009
Activated sludge	E2	Zeng et al. 2009
Activated sludge	Retinoic acid receptor agonists	Zhen et al. 2009
Primary sedimentation	3 phthalate plasticizers	Barnabe et al., 2008
Primary or secondary treatment	9 antibiotics	Gulkowska et al., 2008
Secondary treatment	Estrogenic activity; vitellogenin assay	Martinovic et al., 2007
Biological treatment	Iopromide	Schultz et al. 2008
6 biological treatment plants	5 EDCs	Stasinakis et al. 2008
Activated sludge	Ketoprofen, naproxen, carbamazepine, clofibric acid	Wang et al. 2008
Sand filtration	13 PPCPs	Matamoros et al. 2007
Sand filtration	24 PhACs	Nakada et al. 2007
Activated sludge	28 antibiotics	Watkinson et al. 2007
Activated sludge	8 antibiotics	Xu et al. 2007
Activated sludge		Servos et al. 2005
Onsite wastewater treatment	Surfactant metabolites, steroids, stimulants, disinfectants, antimicrobial agents, several PhACs	Conn et al. 2006

age could also be due to greater metabolic diversity in activated sludge under those conditions, for example. Several studies have shown that removals of the natural estrogens 17β-estradiol and estriol are generally greater than the removal of estrone during conventional wastewater treatment. Estrone removal varies greatly from plant to plant and may account for a significant fraction of overall estrogenic activity in effluents from facilities that do not perform well in this regard.

Advanced Wastewater Treatment

Recognition that conventional wastewater treatment processes are insufficient to completely remove all TOrCs from effluent has led to a substantial amount of research activity examining the efficacy of advanced wastewater treatment processes. Advanced processes may be employed either before or after conventional biological wastewater treatment operations, with the exception of membrane bioreactors which provide secondary biological treatment concurrent with advanced membrane filtration.

Advanced treatment strategies for removal of trace organics from wastewater can be divided into two general categories: *physical destruction* of compounds and *physical removal* of compounds. The main types of physical destruction methods are *ozonation* and *advanced oxidation processes* (AOPs). Examples of physical removal processes include membrane treatment and activated carbon.

In the following section, the different types of advanced wastewater treatment processes are described, along with discussion of the pros and cons of each process.

Physical Destruction Techniques

We have identified 34 peer-reviewed research articles published during 2008-2009 that have examined PPCP and EDC removal efficiencies during advanced wastewater treatment processes that *physically destroy* trace organics (Table 9). The articles in Table 9 are sorted by type of advanced treatment process: ozone, advanced oxidation, Fenton reaction, and other.

Ozone

Ozonation of drinking water and wastewater for disinfection purposes has been practiced for many years. Application of ozone for micro pollutant removal in wastewater has been examined more recently. Ozonation treatment of wastewater differs from drinking water treatment in that the amount of dissolved organic matter is higher in wastewater than in drinking water.

Table 7. Summary of reported removal efficiencies for estrogenic activity and specific estrogenic compounds during activated sludge wastewater treatment.

Compound/Measurement Parameter	*Percent Reduction (influent to effluent)*	*Reference*
Estrogenic activity	74	Svenson et al. 2003
Estrogenic activity	-50 to 100	Servos et al. 2005
Estrogenic activity	88	Propper et al. 2007
Estrogenic activity	96	Propper et al. 2007
E1	64	D'Ascenzo et al. 2003
E1	61	D'Ascenzo et al. 2003
E1	-55 to 98	Servos et al. 2005
E1	61	Baronti et al. 2000
E1	74	Johnson et al. 2000
E1	83	Ternes et al. 1999
E1	0	Ternes et al. 1999
E2	85	D'Ascenzo et al. 2003
E2	40-99	Servos et al. 2005
E2	87	Baronti et al. 2000
E2	88	Johnson et al. 2000
E2	99.9	Ternes et al. 1999
E2	64	Ternes et al. 1999
EE2	85	Baronti et al. 2000
EE2	78	Ternes et al. 1999
NP	-623 to 95	Sole et al. 2000
NP	9 to 94	Ahel et al. 1994

Thus, the amount of ozone needed to destroy target trace organics is higher for wastewater due to the ozone demand exerted by non-target dissolved effluent organic matter in wastewater.

Ozone can promote partial breakdown of compounds that are then amenable to biodegradation during conventional treatment. Use of ozone as a pretreatment can enhance removal of trace compounds during subsequent biological conventional treatment. Treatment with ozone prior to biological treatment can also reduce the amount of trace organics accumulation in activated sludge. This is an important consideration when sludge/biosolids are used for land application on agricultural land.

Drawbacks of using ozone include production of oxidation byproducts (organic and inorganic) and incomplete mineralization of target chemical compounds. Ozone typically provides a partial transformation of target organic species to other compounds that may exhibit toxicity. Thus, it is important to assess toxicity of ozone transformation byproducts as well as the parent target compounds. Bromate, a suspected carcinogen, is produced by a reaction between ozone and bromide ion. The amount of bromate production depends upon bromide ion concentration and ozone dosage. Research has shown however, that the partial oxidation can be sufficient to destroy the biological activity of compounds, including estrogenic and antimicrobial compounds (Huber et al. 2004; Dodd et al. 2009). Ozone also decomposes into hydroxyl radicals that can nonselectively oxidize trace organics. Ozonation of secondary effluent has been shown to be successful for removing a wide variety of PPCPs, including diclofenac, carbamazepine, estradiol, estrone, and EE2 (Huber et al. 2005; Nakada et al. 2007)

Advanced Oxidation Processes

AOPs are generally defined as oxidation processes that generate hydroxyl radicals (·OH). Hydroxyl radicals are highly potent, extremely reactive chemical oxidants. They are short lived but have very strong oxidizing power and are able to oxidize and mineralize almost any organic molecule, yielding CO_2 and inorganic ions. Most types of AOPs make use of a combination of either oxidants and irradiation (ozone/hydrogen peroxide/UV) or a catalyst and irradiation (ferrous iron/hydrogen peroxide; UV/titanium dioxide). A common drawback of all AOPs is the high demand for electrical energy to operate ozonators, UV lamps, etc., often making such treatment strategies economically disadvantageous. Thus, at the present time, treatment systems utilizing AOPs are still relatively scarce and are typically only utilized by larger municipalities.

Wert et al. (2009) compared ozone and ozone/H_2O_2 for removal of 31 organic micropollutants. Those compounds with relatively fast reaction rate constants with ozone were >95% removed (carbamazepine, diclofenac, naproxen, sulfamethoxazole, and triclosan). Those compounds with relatively less fast reaction rate constants with ozone were removed at proportionally slower rates (atrazine, iopromide, ibuprofen, diazepam). The amount and character of effluent-derived organic matter in the wastewater impacted the micropollutant removal efficiencies.

There is currently increasing interest in pursuing AOP methods that can utilize solar radiation as an energy source. These AOP methods include heterogeneous photocatalysis using titanium dioxide (TiO_2) and homogeneous photocatalysis by photo-Fenton reaction. Heterogeneous photocatalysis is based on the use of wide band-gap semiconductors typically plated with titanium dioxide (TiO_2). Hydroxyl radicals are

Table 8. Summary of reported removal efficiencies for estrogenic activity and specific estrogenic compounds during trickling filter wastewater treatment.

Compound/measurement parameter	*Percent Reduction (influent to effluent)*	*Reference*
YES	54	Svenson et al. 2003
YES	33	University of Arizona unpublished
E2	92	Ternes et al. 1999a
E1	67	Ternes et al. 1999a
EE2	64	Ternes et al. 1999a

Table 9. Compilation of recent peer-reviewed studies examining fate of EDCs during advanced wastewater treatment (physical destruction processes).

Advanced wastewater treatment process(s)	*Trace Organic Compound Parameter(s)*	*Reference*
Ozone	220 TOrCs	Hollender et al. 2009
Ozone	31 TOrCs: EDCs, PPCPs	Wert et al. 2009
Ozone	Ames test, Microtox test	Petala et al. 2008
Ozone, activated carbon	4 nitroimidazoles	Sanchez-Polo et al. 2008
Ozone	E1, E2, E3, nonylphenol, bisphenol A	Zhang et al. 2008a
Ozone (modeling)	62 TOrCs	Lei and Snyder 2007
Ozone	24 PhACs	Nakada et al. 2007
Ozone/H_2O_2	TOX, bromated, total aldehydes, total carboxylic acids	Wert et al. 2007
Ozone, chlorine	E2, EE2, BPA	Alum et al. 2004
UV/TiO_2	32 PhACs and EDCs	Benotti et al. 2009
Electrochemical oxidation, Ozonation, Fenton		Canizares et al. 2009
Ozone, Fenton, UV/H_2O_2	Dichlorodiethyl ether (DCDE)	Christensen et al. in press
Ozone, UV/H_2O_2	Dilantin, DEET, meprobamate, iopromide	Dickenson et al. 2009
UV/H_2O_2	Benzene, toluene, 1,2-xylene, dichloromethane, trichloromethane	Ji et al. 2009
TiO_2	Amoxicillin, Carbamazepine, diclofenac	Rizzo et al. in press
UV/TiO_2	16 PhACs in RO retentate	Westerhoff et al. 2009
UV/H_2O_2	Ibuprofen, Diphenhydramine, Phenazone, Phenytoin	Yuan et al. 2009
Activated carbon, electrochemical, UV/TiO_2, sonolysis	DOC	Dialynas et al. 2008
Microwaves/H_2O_2	Anaerobically digested sludge	Eskicioglu et al. 2008
Ozone/H_2O_2	33 PhACs	Rosal et al. 2008
Fe-TAML/peroxide catalysis	E1, E2, E3, androsterone, testosterone	Shappell et al. 2008
MnO_2	E1, E2, E3, EE2	Xu et al. 2008
UV, UV/H_2O_2	Ketoprofen, naproxen, carbamazepine, ciprofloxacin, clofibric acid, iohexol	Pereira et al. 2007
Solar photocatalysis		Radjenovic et al. 2009
UV	EE2, diclofenac, sulfamethoxazole, ioromide	Canonica et al. 2008
Solar photolysis	NDMA	Plumlee and Reinhard 2007
Fenton-biological	8 PhACs	Badawy et al. in press
Solar photo-Fenton TiO_2	Acetaminophen, antipyrine, atrazina, caffeine, diclofenac, isoproturaon, progesterone, sulfamethoxazole, triclosan	Klamerth et al. 2009
Solar photo-Fenton	Nalidixic acid	Sirtori et al. 2009
Solar photo-Fenton	Nalidixic acid	Sirtori et al. 2009
Fenton	1,4-dioxane	Son et al. 2009
Photo-Fenton	E2	Zhao et al. 2008
ultrasound	ibuprofen	Mendez-Arriaga et al. 2008
Molecular imprinted polymer	E1, E2, E3, bisphenol A	Zhongbo and Hu 2008

generated on the plated surface when it is illuminated with UV energy. The photo-Fenton homogeneous photocatalytic reaction promotes oxidation of trace organic compounds using iron (FE^{2+}) salts and hydrogen peroxide (H_2O_2) that are added directly to wastewater. The photo-Fenton reaction is accelerated in the presence of UV light, which regenerates the ferrous ions, increasing the formation of hydroxyl radicals. The photo-Fenton process is gaining increasing attention due to its simplicity and the possibility of using sunlight to drive the reaction, thus lowering the operating cost significantly. Several studies have reported on using photo-Fenton successfully to destroy pharmaceuticals such as antibiotics, hormones, analgesics, anti-inflammatory drugs, and others (Trovo et al. 2008; Bautitz et al. 2007). A drawback of the conventional Fenton process is the catalytic reaction is most efficient at low pH (2.5–3), necessitating readjustment of pH prior to discharge of the treated water.

Several research studies have reported success in destroying many types of trace organics using Photo-Fenton and TiO2 treatments but their main drawback is their relatively high operating cost. There is currently research underway to examine mild solar TiO_2 treatment and Photo-Fenton as tertiary treatments (Klamerth et al. 2009).

Most AOP treatment methods will lead to accumulation of oxidation-refractory organics. A new electrochemical technology AOP that does not produce oxidation-refractory organics but does generate large amounts of hydroxyl radicals is conductive-diamond electrochemical oxidation (CDEO). Recently, a comparison of treatment results and operation costs of CDEO versus ozonation and Fenton oxidation was performed by Canizares et al. (2009). They found that all three AOP methods could achieve complete removal (mineralization) of trace organic pollutants. An economic analysis showed that the operating cost of Fenton oxidation was lower than either CDEO or ozonation and capital costs were higher for ozonation than for CDEO and Fenton oxidation, regardless of type of organic micropollutant treated.

Use of AOPs as a pretreatment stage before conventional biological treatment has attracted attention in recent years (Mantzavinos and Psilakis 2004; Tabrizi and Mehrvar 2004). Studies have shown that breakdown products of pharmaceuticals can be biodegraded during conventional biological wastewater treatment (Tunay et al. 2004; Varatharajan and Kanmani 2007).

Perhaps the main obstacle to use of advanced oxidation processes in wastewater treatment is process cost. Cost cutting proposals include use of renewable energy sources (e.g. solar) for irradiation source for running the AOP. Costs are discussed further below.

Physical Removal Techniques

Membrane Processes

Membranes used for water/wastewater treatment are classified by decreasing pore size: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO). The water that passes through a membrane is called permeate and the water/brine mixture retained by the membrane is called retentate. MF and UF are considered low pressure membranes and NF and RO are considered high pressure membranes. NF and RO are both pressure driven membrane processes, where an applied pressure forces water through the pores and contaminants are retained due to size and charge interactions. NF is a newer process and is defined as lying between relatively porous ultrafiltration and RO. NF is distinguished from RO in that it only retains multivalent ions, which makes it a more economical alternative where the removal of monovalent salts is not required. MF/UF are typically used as a pretreatment to NF or RO in order to reduce the amount of clogging on the tight NF or RO membranes.

As a general statement, membrane systems used in advanced wastewater treatment systems can be divided into two groups: 1) *large-scale centralized systems* typically using dual membrane processes in series, e.g. microfiltration (MF) and reverse osmosis (RO) and 2) *small-scale systems* employing membrane bioreactors (MBR) that combine membrane separation processes with biological treatment.

A summary listing of recent peer-reviewed research on membrane processes for removing trace organics from wastewater is provided in Table 10. Most studies conclude that NF and RO are capable of complete to near complete removal of EDCs and other trace organics from wastewater. Snyder et al. (2007) found that RO and NF were capable of removing most of the 36 EDCs and PhACs to below detection levels (<25 ng/L) in pilot-scale and full-scale membrane treatment systems. In another recent study, Kim et al. (2007) reported greater than 95% removals of 25 EDCs and PPCPs during reverse osmosis and nanofiltration.

One drawback of membranes is fouling. All membranes become fouled, or clogged, over time. Membranes are constructed of polyamide or cellulose acetate; much current research on membranes is focused on testing of new material formulations designed to reduce the amount of fouling. Membrane fouling has the potential to affect rejection mechanisms of organic solutes as a result of modified electrostatic, steric, and hydrophobic/hydrophilic solute-membrane interactions (Yangali-Quintanilla et al. 2009). The ability of membranes to remove trace organic contaminants such as hormones may decrease with development of fouling on the membrane surface.

Other drawbacks of membrane technology are the production/disposal of brine and the energy costs of pushing water through the membranes. Brine is a particularly challenging issue for Arizona because watersheds do not flow to the ocean and there is currently no infrastructure in place for brine disposal. Costs are discussed further in Section 5 of this report.

Activated Carbon

Granular or powdered activated carbon is very efficient in removing trace hydrophobic organics from water and has been used for a long time in drinking water treatment; activated carbon has been used for removing conventional micropollutants such as pesticides. Current research is looking at the use of activated carbon for removing PPCPs and EDCs. Studies indicate that removal efficiencies using activated carbon vary with physicochemical properties of the contaminants.

Activated carbon adsorption efficiency for trace organics is reduced with increasing amounts of natural organic matter in water due to competition for sorption sites. Eventually, all sorption sites become filled, leading to breakthrough of contaminants. A drawback of activated carbon is that it has a finite capacity to adsorb trace organics and thus has to be replaced or regenerated on a routine basis to maintain removal efficiency.

It is generally recognized that advanced treatments such as advanced oxidation processes (AOPs) and high pressure membrane separation (e.g. reverse osmosis) can produce effluents that are free of estrogenic activity and other trace organics. There is some concern, how-

Table 10. Compilation of recent peer-reviewed studies examining fate of EDCs during advanced wastewater treatment (physical removal processes).

Membrane process	*Trace Organic Compound Parameter(s)*	*Reference*
Activated carbon, AOPs	BPA, DBP, BBP, DEHP	Asakura and Matsuto, 2009
Granular activated carbon	Naproxen, Carbamazepine, Nonylphenol	Yu et al. 2009a
Granular activated carbon	Naproxen, Carbamazepine, Nonylphenol	Yu et al. 2009b
nanofiltration	Acetaminophen, carbamazepine, estrone, gemfibrozil, oxybenzone	Comerton et al. 2009
nanofiltration	9 pharmaceuticals, 5 EDCs	Yangali-Quintanilla et al. 2009
nanofiltration	15 perfluorochemicals	Steinle-Darling and Reinhard 2008
nanofiltration, ultrafiltration	E2, fluoranthene	Yoon et al. 2004
nanofiltration, reverse osmosis	12 PhACs	Radjenovic et al. 2008
nanofiltration, reverse osmosis	Trace neutral compounds	Kim et al. 2007
microfiltration, reverse osmosis	28 antiobiotics	Watkinson et al. 2007
reverse osmosis	NDMA	Plumlee et al. 2008
Membrane bioreactor	31 PhACs	Radjenovic et al. 2009b
Membrane bioreactor	12 polar micropollutants	Weiss and Reemtsma 2008

ever, that the economic costs of such processes cannot be justified for removal of trace organics alone in most situations (Jones et al. 2007). The incentive for advanced treatment processes increases when wastewater is to be reclaimed for indirect potable reuse or when effluent discharge leads to near-term incidental potable reuse. See below for discussion of economic analysis and possible alternatives to advanced treatment for removal of trace organics from wastewater prior to environmental discharge.

Natural Treatment Systems

Natural treatment systems for wastewater can be classified as managed or unmanaged. Managed systems include rapid infiltration (soil aquifer treatment) and constructed wetlands (either surface or subsurface flow). Unmanaged systems include surface transport in rivers and percolation occurring along riverbeds that receive municipal wastewater effluent. Recharge of wastewater effluent to aquifers in Arizona occurs during managed rapid infiltration operations in Phoenix and Tucson and also during unmanaged infiltration in effluent-dependent reaches of the Salt and Santa Cruz Rivers. In Phoenix, the 91st Avenue Wastewater Treatment Plant discharges nitrified/denitrified effluent to the Salt River. Discharges of secondary effluent to the Santa Cruz River originate from the Nogales International Wastewater Treatment Plant to the Upper Santa Cruz River; and from the Roger Road and Ina Road wastewater treatment facilities in the City of Tucson.

Peer-reviewed research studies examining trace organic compound fate in natural treatment systems are compiled in Table 11. The studies are listed by the type of treatment process examined: soil aquifer treatment (rapid infiltration), constructed wetlands, and river transport/percolation.

The fate of trace organics through rapid infiltration processes (e.g. soil aquifer treatment, SAT) has received considerable attention. Almost all of the research conducted to date at recharge facilities along rivers in the State of Arizona has been in the Phoenix and Tucson municipal regions. In the Phoenix area, existing recharge operations along the Salt River include the City of Mesa's Northwest Water Reclamation Plant (NWWRP) and the City of Phoenix's Tres Rios Wetlands. In Tucson, the Sweetwater Recharge Facilities, located along the Santa Cruz River, has been in operation since 1990 and has been the subject of research studies conducted by the University of Arizona, Arizona State University, University of Colorado, Colorado School of Mines, Stanford University, and the University of California at Berkeley.

The Sweetwater Recharge Facilities (SRF) (Figure 1) is an effluent Underground Storage and Recovery Project which annually provides approximately 6,500 acre-feet of the City's reclaimed water supply. The facility consists of a constructed wetland and constructed recharge basins. The SRF serves as a recharge and recovery facility, a nationally recognized research platform for SAT, and a public recreation and education site. The recharge component of the SRF consists of eight excavated recharge basins which occupy approximately 28 acres. These surface-spreading basins recharge secondary effluent obtained from the Pima County Roger Road Wastewater Treatment Plant, secondary quality effluent from the constructed wetlands, and excess reclaimed water produced during low demand periods.

Effluent from Pima County's Roger Road Wastewater Treatment Plant (RRWTP) above the 6,500 AF per year that is infiltrated and recovered at the Sweetwater Recharge Facilities is discharged to the Santa Cruz River. Chlorinated secondary effluent from Pima County's Ina Road Water Pollution Control Facility (IRWPCF) (activated sludge process) is also discharged into the Santa Cruz, about 8 km downstream from the RRWTP outfall. Together, the plants provide about 50,000 AFY ($6.17 \cdot 10^8$ m^3) to the river, most of which recharges the Tucson aquifer.

Infiltration processes that contribute to soil-aquifer treatment have a significant beneficial effect on water quality. Infiltration through ~100 feet of unconsolidated sediment, from the Sweetwater Recharge Facilities infiltration basins to monitoring wells at the water table produces significant reductions in dissolved organics.

Estrogens and estrogenic activity have been found to decrease rapidly with depth during percolation of secondary effluent at the Sweetwater Recharge Facilities (Mansell and Drewes 2004; Conroy et al. 2005, 2007; Zhang et al. 2008) and at the NWWTP in Mesa (Drewes et al. 2003; Mansell et al. 2004). Estrogenic activity decreased by about 85%, from 2.6 ng/L (EE2 equivalents) to 0.41 ng/L after percolation through the top 4.6 m of unconsolidated sediment at the Sweetwater Recharge Facilities, and remained essentially constant from there to the water table at 37 m below land surface (BLS) (Zhang et al. 2008). Most of the

Table 11. Peer reviewed research studies examining fate of emerging trace organics during natural treatment processes.

Natural treatment process	*Trace organic compound Parameter(s)*	*Reference*
Soil aquifer treatment	Dissolved organic matter	Xue et al. 2009
Soil aquifer treatment	PBDEs, nonylphenol, estrogenic activity	Zhang et al. 2008
Soil aquifer treatment	E2, E3, testosterone, antiepileptics, analgesics, lipid regulators, xray contrast agents	Amy and Drewes 2007
Soil aquifer treatment	E1, E2, E3,	Lim et al. 2007
Soil aquifer treatment	EDTA, NTA, alkylphenol ethoxylate	Yoo et al. 2006
Soil aquifer treatment	Carbamazepine, phenazone	Massmann et al. 2006
Soil aquifer treatment	Estrogenic activity; anti-estrogenic activity	Conroy et al. 2007
Soil aquifer treatment	Estrogenic activity; anti-estrogenic activity	Conroy et al. 2005
Soil aquifer treatment	E2, E3, testosterone	Mansell and Drewes 2004
Soil aquifer treatment	Alkylphenol polyethoxylates metabolites	Montgomery-Brown et al. 2003
Soil aquifer treatment	THMFP	Quanrud et al. 2003
Wetlands, agricultural drains, river transport	Trihalomethanes	Engelage and Stringfellow 2009
Constructed wetlands	12 pharmaceuticals and personal care products	Matamoros et al. 2008
Constructed wetlands	13 pharmaceuticals and personal care products	Matamoros et al. 2007
Constructed wetlands	THMFP	Quanrud et al. 2004
River transport	5 estrogens; 9 androgens; 9 progestogens; 6 glucocorticoids	Chang et al. 2009
River transport	EDTA, gemfibrozil, ibuprofen, metoprolol, naproxen	Fono et al. 2006
River transport	105 organic wastewater contaminants	Kolpin et al. 2004
River transport	Estrogenic activity	Quanrud et al. 2004b
Indirect and direct artificial recharge	10 pharmaceuticals; 19 pesticides; 6 industrial chemicals	Diaz-Cruz and Barcelo 2008

observed reduction in aqueous-phase estrogenic activity occurred in the first meter of percolation below the surface of the basin. Mansell and Drewes (2004) monitored the estrogen hormones E2 and E3 during infiltration of secondary effluent at the Sweetwater Recharge Facilities. The concentration of E2 decreased from 7.2 to 1.8 ng/L during percolation through the first 4.6 m of sediment and fell below the method detection limit (0.4 ng/L) before water reached the unconfined aquifer at 37 m BLS. The concentration of estriol (E3) was 21.3 ng/L in the spreading basin and fell below the method detection limit (0.6 ng/L) at a depth of 4.6 m. Estrone (E1) was not measured. These results reinforce the idea that major contributors to total estrogenic activity in conventionally treated wastewater are rapidly attenuated during infiltration through unconsolidated sediments. However, it is important to realize that the degree of attenuation will depend on sediment hydraulic characteristics. For example, fractured or highly porous sediment in which preferential pathways are established between the surface and the aquifer might produce limited removal of estrogenic activity.

While research has shown aqueous-phase concentrations of estrogenic activity and estrogen hormones are substantially removed during percolation in soil, there remains the question of whether these trace organics are being biodegraded and/or sorbed onto soil particles. If sorption is occurring, there is a possibility of compound breakthrough if the sorption capacity of the soil is exceeded. Zhang et al. (2008) showed that only a small fraction of the cumulative mass of estrogenic activity in the effluent applied to the basins (3% in basin RB-1 and 7% in basin RB-8) was accounted for in sediment extracts derived from the top 0.85 m of basin sediments. Mass balance calculations indicated that estrogenic activity is biodegraded or incorporated into humic substrates during infiltration. Assuming process kinetics are first-order in sorbed concentrations of total estrogenic activity, then half times for disappearance of estrogenic activity are expected to be on the order of 2–3 months. Under this scenario, estrogenic compounds in effluent are rapidly sorbed to sediment particles on a time scale of hours to days during SAT, then biodegraded / incorporated into humus over months, perhaps during basin drying periods when molecular oxygen is available. Unfortunately, there remains the possibility that estrogenic contaminants are leached from the top meter of sediments via subsequent infiltration. A mass balance study was also performed on brominated flame retardants—polybrominated diphenyl ethers (PBDEs). Zhang et al. (2008) found that PBDEs were retained in near surface sediments with little or no degradation occurring over time.

In contract to the conclusions of Mansell and Drewes (2004), Zhang et al. (2008) concluded that the 30–40 m of unconsolidated sediments through which secondary effluent percolates before encountering the unconfined aquifer at the Sweetwater Recharge Facilities is an imperfect barrier to the transport of hydrophobic trace contaminants, as estrogenic activity and brominated flame retardants (PBDEs) were detectable in the infiltrate at considerable depths. The ability of estrogenic contaminants and PBDEs in municipal effluent to partially survive conventional wastewater treatment and soil–aquifer treatment suggests the need for groundwater quality monitoring during artificial recharge. However, their results also indicate that soil–aquifer treatment can be an important compo-

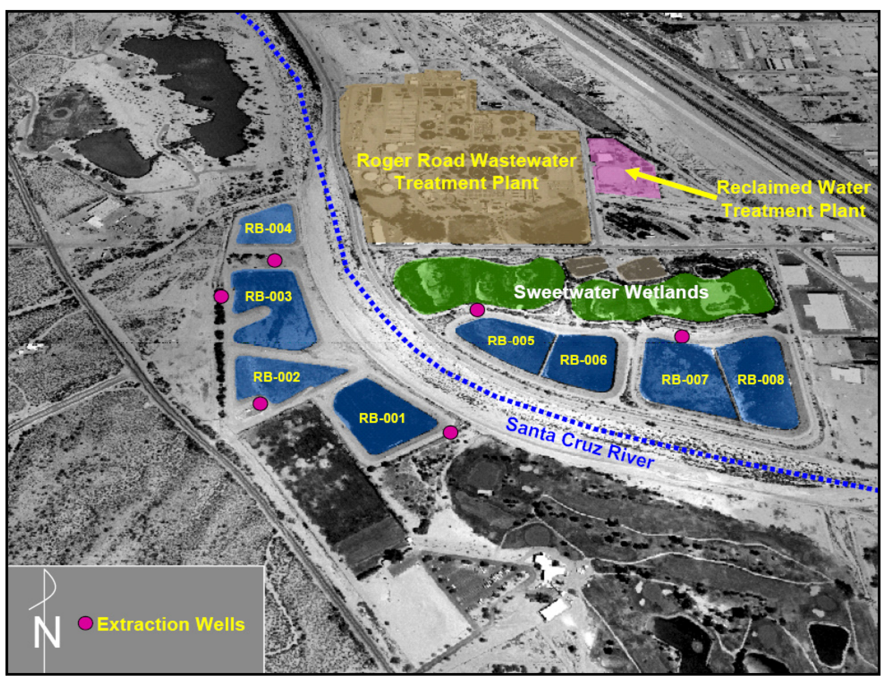

Figure 1. Site map of the City of Tucson's Sweetwater Recharge Facilities (Graphic courtesy of Tucson Water).

nent of a multi-barrier treatment system for restoring wastewater to near-potable quality. On the other hand, PBDEs and perhaps other hydrophobic contaminants that are removed via sorption on infiltration basin sediments are apparently conserved for periods of decades or longer. The accumulation and persistence of PBDEs and potentially other EDCs in shallow sediment layers raises concerns regarding the bioavailability of these compounds and their possible future uptake by plants, and even the eventual transfer of the most volatile compounds (such as BDE-47) to the atmosphere after plant uptake (Gouin and Harner 2003).

The Northwest Water Reclamation Plant (NWWRP) is located in the northwest corner of the City of Mesa and has a treatment capacity of 18-million gallons per day. Treatment at the Mesa Northwest WRP consists of activated sludge treatment including nitrification/denitrification with disinfection and tertiary filtration. The plant receives sewage from the City of Mesa whose drinking water supply consists mainly of surface water from the Salt and Verde Rivers Salt River Project. The nitrifying-denitrifying system provides a consistent effluent for application to the recharge basins with DOC concentrations of 5–7 mg/L and total nitrogen concentrations less than 10 mg-N/L. The effluent from the NWWRP is discharged to two recharge sites and the Salt River, which also recharges the aquifer. The NWWRP has used soil aquifer treatment (SAT) since it began operation in 1990.

A review of other SAT research in Arizona is provided in Amy and Drewes (2007), describing additional field studies performed at the Sweetwater Recharge Facilities in Tucson, AZ and at the Northwest Water Reclamation Plant (NWWRP) in Mesa, AZ. Types of trace compounds examined included pharmaceuticals, EDCs, chlorinated flame retardants, and caffeine (a wastewater indicator compound). The authors conclude that most effluent-derived trace organic compounds are removed to some degree as a function of travel time and presence of oxygen, although a few compounds (carbamazepine, primidone, and x-ray contrast agents such as iopromide) persist even after longer term aquifer treatment/storage.

Although it is too early to generalize with confidence, it seems likely that compound hydrophobicity, as indicated by octanol-water partitioning, is a good correlate for physical removal during SAT, particularly on organic-rich sediments such as those at the surface of effluent infiltration basins. Most of the estrogenic compounds are at least mildly hydrophobic (Table 1), perhaps accounting for the rapid decline of estrogens observed during SAT. In such cases, soil-aquifer treatment remains an effective barrier to biodegradable organics. Many other trace organics are also hydrophobic, but a few are not, and some are even charged at neutral pH, so that their transport through sediments during infiltration is likely. A few compounds, including carbamazepine (an anti-epileptic) and iodinated x-ray contrasting agents (e.g. iopromide), seem capable of transport through sediments and are poorly attenuated even after months to years of underground residence time. In this context, it should be pointed out that a few extremely hydrophobic trace contaminants in wastewater such as polybrominated diphenyl ethers (PBDEs, flame retardants) are present in wastewater effluents at concentrations that are higher than predicted from straightforward partitioning models, which suggest that they should partition with sludges produced via wastewater treatment. It has been suggested that colloidal organics in wastewater effluent tend to stabilize these compounds in the aqueous phase, and that interactions of the same nature may produce an unexpected degree of transport among hydrophobic contaminants during infiltration processes. Second-order effects of this kind have not been studied extensively and they may prove to be significant. To illustrate, the transport of hydrophobic hormones such as 17β-estradiol in blood is facilitated by protein chaperones—molecules that are produced to distribute hydrophobic chemicals that would otherwise experience transport difficulties.

Constructed Wetlands

As of 1998, there were reported to be over 600 constructed wetlands used for treatment of municipal wastewater in North America (Cole 1998). Constructed wetlands provide an attractive method for polishing municipal wastewater, offering such additional benefits as public recreational use and wildlife habitat (Knight 1997). In the Sonoran desert region of Arizona, several constructed wetland projects were developed within the past 15 years and more are planned.

Constructed wetlands provide water quality treatment benefits for wastewater. However, they also can provide a couple of negative water quality impacts. Evapotranspiration by wetland vegetation can increase the concentration of salts and other solutes in the water. Also, wetlands are known to be a source of natural organic matter that could have a negative impact on water quality, depending on the end use of the wetland-treated water. If water is eventually disinfected using a chlorine-based oxidant, natural organic matter (NOM) can serve as a precursor for production of trihalomethanes (THMs) and other carcinogenic disinfection by products (DBPs).

The fate of DBP-precursors during treatment of tertiary effluent at the Tres Rios Demonstration Wetlands (near Phoenix, Arizona) was examined by Rostad et al. (2000). Specific formation potentials of nonpurgable total organic halogen (μg NPTOX per mg DOC) increased by a factor of 1 to 1.7 and specific THMFP increased by a factor of 2 to 7.5 after wetland treatment. Increases were attributed to DBP-reactive natural organic matter added by the wetlands.

Hot summer temperatures in Arizona significantly impact water quality changes during wetland treatment of wastewater effluent. Season-dependent variations in evapotranspiration (ET) and production of autochthonous wetland-derived natural organic matter (NOM) and their effects on concentrations of organics in subsurface wetlands at the Constructed Ecosystems Research Facility (CERF) (Tucson, Arizona) were described by Quanrud et al. (2001). In summary, dissolved organic matter (DOM) reduction was greatest in the cooler winter months. Dissolved solute concentrations increased substantially (up to 100 percent) during hot summer months due to removal of water via ET. Thus, trace organics that are not amenable to biodegradation and/or sorption during wetland treatment (e.g. hydrophilic pharmaceutical compounds such as carbamazepine) could be present at higher concentrations after treatment in constructed wetlands. Colloidal natural organic matter could facilitate the transport of trace organic compounds during wetland treatment, reducing their retention in the wetland.

River Transport

There is limited knowledge at the present time on impacts to Arizona Rivers from existing effluent recharge facilities. Most information has been obtained from reaches of the Santa Cruz River in Tucson and the Salt River in Phoenix/Tempe that receive treated effluent.

Santa Cruz River

Although there has not been continuous surface flow in the Santa Cruz River of Arizona and Mexico since the early 1900s, it still sustains some of the most diverse riparian habitats in the Southwest (in the U.S. and Mexico) and the groundwater it recharges is critical to agriculture and human development along its length. The river's only remaining continuous flowing surface reaches are entirely or largely dependent on treated effluent discharged from the Nogales International Wastewater Treatment Plant (NIWTP) near Nogales, AZ in the upper Santa Cruz River (SCR) and two large wastewater treatment plants in Tucson, AZ in the lower SCR.

Among potential pollutants of concern, wastewater-derived endocrine disrupting compounds (EDCs) and pharmaceuticals and personal care products (PPCPs) have significant potential to introduce adverse biological impacts on resident biota even at vanishingly low concentrations (Drewes et al. 2005). The non-compliance of discharge limits by the NIWTP culminated in a lawsuit by the Sierra Club in 2000. The resulting Consent Decree obligated the NIWTP to upgrade to comply with state and federal standards. The $60M upgrade (an activated sludge treatment system with UV disinfection) was completed in July 2009, with commissioning of the new treatment train in February 2009. Previously, treatment was provided by wastewater lagoons and chlorine disinfection. Because the upgraded plant is designed to greatly improve nutrient and organic carbon removal, it is expected it will also improve removal of a broad suite of trace organic compounds, including EDCs and PPCPs.

The fates of trace organics in effluents that reenter the environment are governed by physical properties of the compounds, by their biodegradability and by their vulnerability to photolysis reactions. The Santa Cruz River provides an excellent opportunity to study attenuation processes in surface waters, where biodegradation, photolysis and sorption to sediments and vegetation are candidate mechanisms. In the Tucson Basin, two large municipal wastewater treatment plants operated by Pima County at Roger Road and at Ina Road in the City of Tucson provide secondary effluent that is the only source of permanent water in the lower Santa Cruz River. Hence, this section of the Santa Cruz River is an effluent dependent stream.

The fate of estrogenic activity along the 25-mile effluent-dependent reach of the lower Santa Cruz River and in a series of monitoring wells located along the same reach of the river was evaluated by Quanrud et al. (2004a). Estrogenic activity in the river was found to decrease with distance traveled downstream from the effluent outfalls. Ten miles below the Roger Road and Ina Road Water Reclamation Facility outfalls the total estrogenic activity in Santa Cruz River water is about 80% lower that measured levels in plant effluents (Quanrud et al. 2004). A 60 percent reduction in estrogenic activity occurred over the order of a few days. However, the contributions of individual estrogenic compounds, and the mechanisms of contaminant attenuation are unknown. The hydrophobic character of major estrogenic contaminants (Table 1) suggests that they will readily partition on organic-rich solids when there is liquid–solid contact. This could occur, e.g., in shallow streams or during the infiltration of wastewater through sediments. Nevertheless, detectable estrogenic activity was present at groundwater monitoring wells at 100–200 ft below land surface along the same reach of the Santa Cruz. Estrogenic activity was highest in groundwater with the largest fractional wastewater contribution, as determined from boron isotope measurements. Results suggested that water quality in shallow monitoring wells at some locations along the Santa Cruz River is affected by the infiltration of reclaimed water from the Roger Road and Ina Road Wastewater Treatment Plants. Groundwater from the monitor wells along the Santa Cruz showed a strong relationship between the fractional (volume) contribution of reclaimed water and estrogenic activity. There was a weaker, yet significant relationship between dissolved organic carbon concentration and estrogenic activity in the same set of samples. In the Santa Cruz River monitor wells with large volume contributions of reclaimed water, estrogenic activities and DOC concentrations were high relative to values in wells dominated by reclaimed water at the Sweetwater Recharge Facilities. These findings suggested that managed infiltration of wastewater effluent can produce water quality benefits that are not realized during the unmanaged, incidental recharge of reclaimed water in a riverbed.

Murray Springs/San Pedro River

We have communicated with Nick Paretti, USGS Arizona Water Science Center, on their ongoing work to identify emerging contaminants in Murray Springs near the San Pedro River. As of December 2009, the USGS has released some preliminary monitoring results but has not yet made any conclusion regarding the source of the trace contaminants detected in Murray Springs or whether these contaminants are subsequently transported into the San Pedro River.

Verde River

Recent unpublished data (Table 12) provided by Dr. Wen-Yee Lee (University of Texas at El Paso) shows the presence of several alkylphenolic compounds, including nonylphenol, 4-tert octylphenol, nonylphenol monoethoxylate (NP1EO), and nonylphenol diethoxylate (NP2EO) in effluent from the Chino Valley wastewater treatment facility and at locations along the Verde River. These compounds derive from the biodegradation of alkylphenol polyethoxylates (APEs) (nonionic surfactants used in laundry detergents) and are mildly estrogenic (Table 1). Presence of alkylphenols in the Verde River suggests that other trace organics typically associated with wastewater effluent may also be present, including estrogenic steroidal hormones. The concentrations of nonylphenol reported by Dr. Lee at the Chino Valley WTP (9.95, 0.24 μg/L) and in the Verde River (ranging from below detection up to 14.3 μg/L) are somewhat less than found in the lower Santa Cruz and Salt Rivers by the USGS in their earlier national reconnaissance study (Kolpin et al. 2002) where nonylphenol concentrations were on the order of 40 to 60 μg/L (ppb).

Table 12. Alkylphenol Concentrations (ug/L, ppb) along the Verde River, Arizona (unpublished data provided by Wen-Yee Lee, University of Texas at El Paso).

Sampling Location	*4-nonylphenol*	*NP1EO*	*NP2E0*	*4-tert octylphenol*
Chino Valley WTP	9.95 / 0.24	9.19 / 0.23	14.26 / 3.79	0.81 / 0.14
Verde Spring	BCL	BCL	0.13 / 0.33	ND
Verde Spring	2.0 / 14.3	ND	2.52 / 6.14	ND
Bear Siding	BCL	BCL	BCL	BCL
Perkinsville	1.9 / 4.54	ND	3.2 / 12.62	No data

BCL: below calibration limit; signal is lower than the lowest calibration standard used in the analysis (4-tertpoctylphenol, 0.01ppb; NP, 0.05 ppb; NPE1, 0.1 ppb; and NPE2, 0.2 ppb)

ND: not detected

What is on the horizion with respect to EDCs and treatment/recharge?

Alternative monitoring approaches for organic micropollutants

There are a few research studies currently underway examining new approaches for monitoring trace organic fate during conventional and advanced wastewater treatment processes. Given that there are literally thousands of different trace organic compounds in wastewater present at ng to µg per L concentrations, a comprehensive chemical monitoring program is cost prohibitive. An alternative approach gaining increased attention by researchers is to identify and monitor an appropriate set of "indicator" compounds or surrogates that are then used to predict the fates of other trace compounds that are more difficult and expensive to monitor. An indicator compound is defined as an individual chemical used to measure the effectiveness of a process for a group or family of compounds in the treatment process of interest. A surrogate is a parameter that serves as a performance measure of a treatment process that relates to removal of specific contaminants of interest.

Dickenson et al. (2009) presents a scheme by which to monitor a select set of indicator compounds during chemical oxidation following conventional wastewater treatment. They developed treatment removal categories for proposed indicator compounds during ozonation (Table 13). The most sensitive compounds to assess treatment performance are those that exhibit intermediate or good removal under normal operating conditions. For example, a treatment system failure is indicated by poor removal of a compound that is normally removed. Dr. Jorg Drewes (Colorado School of Mines) and other researchers are currently performing a follow-up 2-year study, funded by a grant from the WateReuse Foundation, to develop indicator compound lists appropriate for use in membrane treatment processes and during soil-aquifer treatment. A final project report is expected in late 2010.

Optimization of Conventional Wastewater Treatment Processes

Manipulation / optimization of conventional wastewater treatment facilities to improve trace contaminant removal efficiencies is an active area of research. The most important design parameter for wastewater treatment plants is the solids retention time (SRT), also known as sludge age. The SRT is a measure of the mean residence time of microorganisms in the reactor. Only those organisms that can reproduce in the SRT are retained/enriched in the system. Thus, a higher SRT allows for enrichment of more slowly growing bacteria and leads to a more diverse microbial population with broader physiological capabilities (e.g. carbon removal, nitrification). Sludge age relates the growth rate of microorganisms to effluent concentrations of target compounds. For example, if degradation of a specific EDC is dependent on the SRT, a critical sludge age can be determined. Thus, if a WWTP operates at an SRT below the critical value, it is expected that removal of the specific EDC will not occur or will be incomplete, whereas if the SRT is higher than the critical value, then complete degradation will be expected to occur. It has been shown that wastewater treatment plants utilizing nutrient removal processes (nitrification/denitrification) and/or high sludge ages exhibit better removal rates for EDCs and pharmaceuticals than those facilities using standard operating conditions (Andersen et al. 2003; Ternes et al. 2004). The longer hydraulic residence time and/or solids retention time, along with greater microbial diversity in facilities employing nutrient removal, are thought to be important factors promoting greater removal of trace organic compounds. Clara et al. (2005) determined critical SRT values for several different trace organic contaminants at five full-scale wastewater treatment facilities, including activated sludge systems and a membrane bioreactor. Some of the target compounds (bisphenol A, ibuprofen, bezafibrate, and the natural estrogens) exhibited removal efficiency dependence on SRT and no significant differences were found in treatment efficiency at comparable SRT at the five treatment facilities. In a similar study, removals of clofibric acid and gemfibrozil were not affected by increased sludge age (Jones et al. 2005). Clara et al. (2005) concluded that high removal efficiencies of these target compounds can be achieved at an SRT higher than 10 days. Contradictory results were seen for diclopfenac and the synthetic estrogen 17a-ethinylestradiol; carbamazapine was not affected during treatment.

The authors (Quanrud and Propper), in a project funded by the Arizona Water Institute, are involved in a bench-scale activated sludge bioreactor study evaluating the effect of SRT on removal efficiency of natural estrogens, 17a-ethinylestradiol, and nonylphenol as a function of sludge age. Sludge ages of 4, 8, 12, and 16 days are being evaluated. The study is also examining the role of nitrifying bacteria on biodegradation of estrogenic compounds. A final project report is expected in late 2010.

Table 13. Example of treatment removal bins (categories) for indicator compounds during advanced treatment using ozone (adapted from Dickenson et al., 2009)

Good removal (>90%)	*Intermediate removal 90–50 %*	*Intermediate removal 50–25%*	*Poor removal (<25%)*
Acetaminophen	Iopromide	NDMA	Chloroform
Triclosan	Mush ketone		TCEP
Bisphenol A	Musk xylene		TCPP
Estrone			TDCPP
Nonylphenol			
Oxybenzone			
Sulfamethoxazole			
triclocarban			
Carbamazepine			
Diclofenac			
EDTA			
Naproxen			
DEET			
Ibuprofen			
Primidone			
Tonalide			

Comparison of costs: advanced vs. conventional wastewater treatment

There are a limited number of existing full-scale advanced wastewater reclamation facilities upon which to gather financial cost data. Examples which are considered for evaluation of treatment costs, compared to conventional treatment, include the Scottsdale Water Campus (Arizona), Orange County Groundwater Replenishment System (California), and the Fred Hervey Water Reclamation Plant (Texas).

Scottsdale Water Campus

The City's primary Water Reclamation Plant (WRP) located at the Water Campus provides reclaimed water for irrigation of turf. The WRP process includes Nitrification – DeNitrification followed by tertiary treatment and disinfection which provides Class A+ reclaimed water, as defined by the Arizona Department of Environmental Quality (ADEQ). The City also conducts groundwater recharge at the Water Campus using Class A+ reclaimed water from the WRP further treated through the Advanced Water Treatment (AWT) Plant which is also located at the Water Campus. The AWT consists of microfiltration, reverse osmosis, post treatment stabilization and a series of vadose zone recharge wells. Total cost of the Scottsdale Water Campus was about $200 million. The City of Scottsdale is currently conducting conceptual design efforts for the expansion of the Water Campus Advanced Water Treatment Facility. The expansion will increase capacity of the AWT from 8 to 27 mgd. The 2008 Wastewater Master Plan for the City of Scottsdale recommended increasing the capacity of the AWT to meet increased flow demands generated by growth and inflow and infiltration related to storm events. The City is also considering treatment technology beyond what is currently implemented at the Water Campus to address recently identified compounds of potential concern (CPC) that can impact the quality of groundwater due to recharge.

The advanced wastewater treatment system at the Scottsdale Water Campus facility consists of microfiltration/ultrafiltration followed by reverse osmosis (RO) and UV disinfection, producing water suitable for landscape irrigation and groundwater recharge via direct injection. . Since its implementation, Scottsdale's reuse programme has saved over 94 million m3 (25 billion gallons) of potable water, and the Scottsdale Water Campus is one of the largest municipal facilities in the world that treats raw wastewater to potable quality for aquifer recharge.

Construction of the first two phases of the Scottsdale Water Campus was completed in the late 1990s and included tertiary and advanced water treatment facilities; costs totaled $75 million. Scottsdale estimates its cost to produce potable quality water via this method is less than $1.30 per 1,000 gallons (Grenoble 2009). Costs would be considerably larger for a comparative facility built today.

Orange County Groundwater Replenishment System (California)

The Groundwater Replenishment (GWR) System is the world's largest water purification and reuse project of its kind. The project replaced Water Factory 21, a smaller advance treatment facility that previously provided 14 million gallons per day (mgd) of product water for injection to the local aquifer. The GWR System is operated by the Orange County Water District (OCWD) in Fountain Valley, CA and treats wastewater effluent to produce 70 mgd (265 million liters/day, mLd) of finished product water that is injected into the local groundwater basin to prevent seawater intrusion. The GWR System treatment process includes microfiltration, reverse osmosis, and advanced oxidation processes (UV light combined with hydrogen peroxide). Construction on the GWR system began in 2003 and the facility was completed and went on line in January 2008. Total capital cost was $481 million and the annual operating cost is approximately $30 million, which includes energy costs. With grants and subsidies factored in, the cost to recharge or inject GWR System water when the facility is operating at full design capacity is approximately $600 per acre foot (one AF= 325,900 gallons), equivalent to approximately $1.84 per 1000 gallons, or about 40% higher than the unit water cost at the Scottsdale Water Campus.

Fred Hervey Water Reclamation Plant (Texas)

The Fred Hervey Water Reclamation Plant is located in Northeast El Paso. The Fred Harvey Water Reclamation Plant recovers and treats wastewater, which is then injected into the groundwater. The water eventually travels to one of El Paso's potable water fields to become part of the drinking water supply. In 2004, a total of 577 million gallons of reclaimed water were returned to the Hueco Bolson aquifer.

The Fred Hervey Water Reclamation Plant is designed to receive up to 38,000 cubic meters per day (m^3/day) or 10 million gallons per day (mgd) of influent wastewater from Northeast of El Paso. Wastewater is treated and turned into potable water by two separate treatment processes. The first process (primary) removes particulate matter from the wastewater through screening, degritting, and primary settling in sedimentation tanks. The second process consists of biological treatment, chemical coagulation and a two-stage powdered activated carbon (PAC) treatment, lime treatment, filtration, disinfection and granular activated carbon filtration. The lime treatment process raises the pH to kill viruses and remove hardness, phosphorus and heavy metals. Carbon dioxide is added to the water to lower its pH value afterward. Sand filters are used to reduce turbidity of treated water. Ozone disinfection is used to sterilize the treated water. Finally, water is further filtered by activated carbon to remove any remaining trace organics (Sheng 2005).

Since its startup in 1985, The Fred Hervey Plant has produced approximately 121.6 million cubic meters (m^3) (32.1 billion gallons) of reclaimed wastewater, of which 2/3 (74.7 million m^3) have been injected into the Hueco Bolson aquifer, and 1/3 has been used for other purposes. The annual injection peaked at 7 million m3 (1.86 billion gallons) in 1990; however, the annual injection rate has been reduced thereafter due to increases in demands for other uses of reclaimed water, primarily for cooling purposes at El Paso Electric Company. Injection currently accounts for only approximately 35–50% of total produced reclaimed wastewater. It should be noted that the recharge basin has been in operation since 2001, and accounted for about 40% of annual injection in 2003. It is expected that the infiltration basin recharge method may become the most viable method for recharge, and be expanded due to its low construction and operation costs as well as its easy maintenance (Sheng 2005). The treatment cost at the Fred Hervey Plant was about $1.60 per 1,000 gallons (year 2000 figures).

Singapore NEWater

NEWater is the brand name given to reclaimed water produced by Singapore's Public Utilities Board. Specifically, it is treated wastewater that has been purified using a similar process as employed at the Orange County Groundwater Replenishment System. Treatment includes microfiltration, reverse osmosis, and ultraviolet disinfection, in addition to conventional water treatment processes. The water is considered potable and is consumed by humans, but is mostly used for industry requiring high purity water. Total treatment capacity is currently about 20 MGD. Treatment cost for NEWater is about $3.78 per 1,000 gallons (Wong, 2007).

Economic Critique of Advanced Wastewater Treatment

The United Kingdom is currently conducting a demonstration program, estimated to cost approximately US $80 million, to evaluate advanced treatment technologies to remove EDCs from wastewater. An economic analysis of the efficacy of using the proposed advanced treatment processes for removing trace organic contaminants from wastewater was performed by Jones et al. (2007). This peer-reviewed study performed an economic analysis of the two major options being considered in the UK demonstration program: i) granular active carbon combined with ozone treatment and ii) membrane filtration using reverse osmosis. A summary of their cost calculations (converted into $USD) for three population sizes is shown in Table 14. Jones et al. (2007) concluded that the capital cost of advanced treatment (GAC + ozone) represented approximately 40% of the capital cost of a standard conventional wastewater treatment plant. The capital cost of adding advanced membrane treatment (MF + RO) exceeded the capital cost of the standard plant for the larger population sizes. Operating costs for a membrane treatment process also exceeded the operating costs for conventional wastewater treatment, reflecting that energy costs are a significant component of total operating costs for membrane treatment. Overall, membrane treatment had higher capital and operating costs than GAC + ozone. Jones et al. (2007) concluded that use of advanced treatment methods may be economically and environmentally undesirable due to increased energy consumption, economic costs, and increased CO_2 emissions. However, their economic analysis did not consider the environmental benefit of removing EDCs from the wastewater stream. They concluded that modification/optimization of existing conventional wastewater treatment, including increasing the solids retention time (SRT) (sludge age) and hydraulic retention time, combined with nutrient removal processes (e.g. nitrification/denitrification), may be almost as effective in removing EDCs from wastewater but with much lower financial and environmental costs.

As far as the authors are aware, Jones et al. (2007) is they only study of this type currently available in the literature. However, critical evaluations of energy use and carbon footprints associated with water treatment operations have begun to appear in the literature. For example, Stokes and Horvath (2009) used a life cycle assessment (LCA) decision support tool to evaluate water supply costs of desalination (membrane treatment) versus water importation in California. They found that desalination has an energy and air emission footprint 1.5 to 2.4 times greater than that of imported water. Seawater desalination energy use would result in 800 kg of CO_2 emissions per year for the typical Californian's water needs. As more communities contemplate strategies to reduce effluent concentrations of trace organic contaminants, similar types of analyses comparing advanced wastewater treatment processes, and/or optimized conventional wastewater treatment processes, may begin to appear in the literature over the next few years.

Another option available to communities that are interested in reducing inputs of trace organic compounds to the environment is source control. Fono and McDonald (2008) list four source control strategies that local governments can undertake that do not require wastewater treatment facility upgrades or changes in operational procedures.

1. Pharmaceutical take-back programs;

2. Ecolabeling of household and personal care products to encourage consumers to choose products with non-toxic ingredients;

3. Reduction of over- and unnecessary medication;

4. Phasing out persistent or toxic pharmaceuticals when less toxic alternatives exist.

Information Gaps and Recommendations for Future Research

There is a wealth of literature currently available describing the occurrence of a spectrum of trace organic compounds in the environment and our collective knowledge is increasing everyday due to growing scientific, regulatory, and public interest. As analytical methods continue to improve over time, we are able to find more and more compounds at smaller and smaller concentrations. Current analytical methods can detect organic contaminants at part per trillion levels and in the future may be able to detect at part per quadrillion levels. Given the rapid improvements in our ability to detect trace levels of contaminants, some scientists argue that the critical question is not whether the compounds exist, but rather at what concentrations in the environment are they harmful to human and/or eco-

Table 14. Comparison of total costs of advanced wastewater treatment options for three WWTP sizes (adapted from Jones et al. 2007).

Treatment option	*Population size*	*Capital cost (Standard WWTP) ($ million)*	*Capital cost (Advanced treatment) ($ million)*	*Operating cost Standard treatment ($ million)*	*Operating cost Advanced treatment ($ million)*	*Total cost (per m^3 wastewater treated) ($)*
Activated sludge combined with GAC + ozone	5000	3.25	1.12	0.30	0.03	4.91
	50000	11.1	4.32	0.22	0.22	2.48
	200000	33.1	12.8	0.99	0.86	1.87
Activated sludge combined with MF + RO	5000	3.25	2.08	0.30	0.19	6.22
	50000	11.1	15.3	0.22	1.50	3.85
	200000	33.1	35.5	0.99	5.77	2.64

logical health? There is a definite need to further assess the toxicology of individual trace organic compounds and more importantly mixtures of compounds at environmentally relevant concentrations. These types of studies are inherently complicated and very expensive and are most likely to require federally supported research funding from e.g. the National Institutes of Health or National Science Foundation, or the United States Environmental Protection Agency.

Given the financial costs of advanced wastewater treatment, many communities may be unable or unwilling to add advanced treatment processes to their existing wastewater treatment facilities, especially in the absence of federal regulation of discharge quality requirements for emerging contaminants. Given this reality, optimization of conventional wastewater treatment processes may prove to be a preferred option in many cases. There is a need to further evaluate how manipulation of basic treatment parameters, such as solids retention time and hydraulic residence time, can improve trace contaminant removal efficiency during conventional treatment (e.g. activated sludge) at existing facilities.

Conclusions and recommendations

The results of this overview demonstrate that the issue of EDC release into the environment from WWTPs is extraordinarily complicated. The shear number of chemicals released and the complexity of their interaction with biological systems, individually and at the community level, makes quantifying varying levels of impacts to the environment difficult. In addition, the complexity of interactions during the treatment process and the fact that each compound type might behave differently when treated with different wastewater treatment processes demonstrates the need for not only more organized studies on the different processes, but also for development and testing of innovative engineering techniques for increasing removal efficiencies. Last, it should be recognized that different countries have dramatically different regulations, infrastructure and capital capacity for wastewater management. Finding inexpensive, effective mechanisms to limit wide-scale chemical contamination of water systems around the world is of vital importance.

The findings from the review of the biological literature demonstrate clearly that WWE around the world induces changes in biological systems from cell physiology through ecosystem function. The impacts range from overt toxicity to clear endocrine disruption that is not always easy to evaluate because different species show differences in exposure sensitivity. To complicate the exposure issue further, sensitivity may be limited to specific life stages. Furthermore, the endocrine system is a complex compilation of integrative physiological systems, and although most of the research evaluating the impact of WWE on endocrine function has focused on steroidal mechanisms of disruption, it is clear that many of the anthropogenic compounds in the environment impact non-steroidal hormonal systems (Guillette et al. 2006; Propper, 2005). Last, only a few studies (see above) have begun to address the complex ecosystem consequences of exposure to WWE. These studies are critical if we are to gain a full understanding of the potential long-term biological impacts of how complex mixes of compounds will change our environment.

The fate of EDCs and other emerging organic contaminants during conventional and advanced wastewater treatment processes has received considerable scientific attention in recent years. Studies have shown EDC removal during conventional wastewater treatment is incomplete; EDCs remain present (to some degree) in treated effluent and in biosolids. Removal efficiencies of EDCs during wastewater treatment correlate roughly to overall process efficiency. That is, the better the attenuation of conventional water quality parameters such as BOD during wastewater treatment, the better the removal of trace organics such as EDCs is expected to be. Natural treatment processes including rapid infiltration (soil-aquifer treatment) and in-stream transport can remove EDCs via biodegradation and/or sorption and may be useful as part of a multi-barrier treatment system for EDC removal. Advanced wastewater treatment technologies such as advanced oxidation processes and membrane treatment (e.g. reverse osmosis) are capable of removing EDCs to below detection levels and a few examples of full-scale advanced systems are in use in the U.S. where indirect potable reuse is a primary consideration. However, capital, operational, and energy costs of advanced wastewater treatment are significant and may prove to exceed what most communities are willing to pay, especially given the absence of federal regulation pertaining to maximum contaminant levels for EDCs in effluent discharge.

We have two overriding recommendations:

1. Develop a tiered series of biological assays to better standardize biological testing of WWE for endocrine disrupting capacity. The development of these assays could be modeled after the mechanisms used by the USEPA's Endocrine Disruptor Screening program which evaluates individual compounds for steroidal hormone and thyroid hormone disruption (http://www.epa.gov/endo/). Such a series of carefully designed assays could be coupled with evaluations of chemical removal efficiencies from different treatment processes in order to better design future wastewater treatment facilities.

2. Optimization of conventional wastewater treatment processes (e.g. increasing the solids retention time and/or hydraulic residence time; sequential anaerobic/aerobic sludge digestion) may prove to be a viable alternative for achieving discharge water quality objectives at less cost than advanced treatment technologies. Further study of optimization strategies for improving conven-

tional wastewater treatment and nutrient removal processes (nitrification/denitrification) for EDC removal is warranted. Again, these studies should be tied to biological assays for evaluation low level contaminant activity on biological systems.

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