2011

Active Pharmaceutical Ingredients and Aquatic Organisms

Christian G. Daughton
U.S. Environmental Protection Agency

Bryan W. Brooks
Baylor University, bryan_brooks@baylor.edu

Active Pharmaceutical Ingredients and Aquatic Organisms

Christian G. Daughton
U.S. Environmental Protection Agency
Office of Research and Development
Environmental Sciences Division
National Exposure Research Laboratory
Las Vegas, NV

Bryan W. Brooks
Department of Environmental Science
Center for Reservoir and Aquatic Systems Research
Baylor University
Waco, TX

Published in *Environmental Contaminants in Biota: Interpreting Tissue Concentrations, 2nd edition,* ed. W. Nelson Beyer & James P. Meador (Boca Raton: CRC, 2011).

8 Active Pharmaceutical Ingredients and Aquatic Organisms

Christian G. Daughton
Bryan W. Brooks

CONTENTS

8.1 INTRODUCTION

The presence of active pharmaceuticals ingredients (APIs) in aquatic systems has led in recent years to a burgeoning literature examining environmental occurrence, fate, effects, risk assessment, and treatability of these compounds. The vast preponderance of studies aimed at identifying and quantifying contaminant residues in aquatic tissues have involved the conventional and legacy pollutants. Comparatively few studies have been targeted at APIs, primarily those that are lipophilic. Although APIs have received much attention as "emerging" contaminants of concern, it is important to recognize that traditional approaches to understand and predict exposure and effects of other environmental organic contaminant classes may or may not be appropriate for APIs. For example, traditional approaches for understanding aquatic effects may not be as useful for some APIs (Brooks et al. 2003), but lessons learned from the study of compounds active at the hypothalamic–pituitary–gonadal axis (endocrine disruptors/modulators) may reduce uncertainties associated with environmental assessments of other APIs (Ankley et al. 2007).

Whereas APIs are often considered as a combined class of environmental contaminants, APIs include diverse groups of chemicals with physiochemical properties ranging in pharmacological potencies, environmental fate profiles, and patient usage patterns. Due to the relatively rudimentary state of knowledge for aquatic exposures to these substances, an understanding of critical body residues (CBRs) necessary to elicit pharmacologically and ecologically relevant responses is not available at this time. Because exposure does not necessarily evoke effects or risk, current challenges include understanding the relationship between exposure and effects within an ecological risk assessment framework. It appears particularly critical to understand whether internal pharmacological doses of APIs in target tissues result from exposures at environmentally relevant or realistic concentrations. Such information can inform ecological risk assessments examining the potential effects of APIs based on their specific mechanism/mode of action (MOA).

Tissue residues of contaminants are commonly used in retrospective ecological risk assessments to support an understanding of environmental exposure (Suter et al. 2000). Bioconcentration factors (BCFs) are useful in both retrospective and prospective assessments of traditional contaminants; BCFs are expressed as the ratios of concentrations in tissues (mass/kg) and the respective concentrations in the surrounding aqueous compartment (mass/L), resulting in units of L/kg. APIs are conceptually no different from these conventional contaminants because tissue residues can provide important information in exposure analysis, particularly when used as indicators of exposure in the field. Whole-body and tissue concentrations are essentially proxies for gauging the actual dose at the site(s) of action, which may or may not be known. When bioconcentration occurs, an obvious advantage of measuring internal concentrations is when the external concentrations are below method detection limits (MDLs).

Compared with many conventional pollutants, APIs are in general comparatively more polar. They therefore tend to not partition to particulates and sediments, but rather remain dissolved in the aqueous phase. For those APIs that have significant tendency to become sediment-bound, little attention has been given to their bioavailability as measured by bioaccumulation. The measure of bioaccumulation of a chemical associated with sediment is the biota-sediment accumulation factor (BSAF). The BSAF is expressed as a chemical's tissue concentration normalized to lipid content relative to its concentration normalized to sediment total organic carbon (Burkhard 2009).

In this chapter we examine relevant information on residues in aquatic organisms, select factors influencing exposure, and available methods to understand relationships between exposure scenarios and effects thresholds. Though APIs are often combined with discussions of personal care products (PCPs) in the literature that has rapidly developed following publication of Daughton and Ternes (1999), we specifically focus on APIs for the purposes of this chapter. A broad literature on PCPs in aquatic organisms continues to develop (e.g., Mottaleb et al. 2009) and was recently summarized by Ramirez (2007).

An insight of particular importance concerns the level of knowledge regarding the linkage between exposure to APIs and adverse effects in aquatic organisms. Regardless of the available published data on exposure of aquatic organisms to APIs (and its size is indeed very limited, especially for real-world scenarios), it rarely intersects a complementary body of data for biological effects. When exposure data do exist, the same conditions, concentrations, and species have rarely been used in toxicity studies; parallel data sets are also usually disconnected temporally. Likewise, a considerable body of API data exists for effects, but exposure conditions (especially the API concentration or route of exposure) are often not environmentally realistic. The ability to routinely connect real-world API occurrence data to documented biological effects is therefore not yet available. Evidence for causality is probably strongest for sex steroids, largely because of their ubiquity and potencies; but even here, evidence can be confusing (e.g., see Vögeli 2008). A major challenge in establishing causality—a lack of correlation between tissue levels and observed effects—could well be the result of delayed effects, such as those only manifesting at later life stages but originating from exposure at earlier developmental stages; this is especially true for organisms with longer life cycles.

8.2 EXPOSURE

8.2.1 Background

The published literature on APIs as environmental contaminants is dominated with data on the analysis, occurrence, and fate of these chemicals in the environment, together with evaluation of waste and water treatment technologies. Surprisingly, comparatively little has been published regarding the aquatic toxicology of APIs, especially data relevant to exposure. Little information is available, for example, on the occurrence of APIs in aquatic organisms. This in itself is surprising given that predictive models for bioconcentration in fish are not yet up to the task, and empirical data are needed at least to validate computational approaches. The complexities and limitations of modeling bioconcentration of conventional pollutants (especially the legacy pollutants) in fish are discussed in detail by a number of authors (e.g., Geyer et al. 2000, Gobas and Morrison 2000, Van der Oost et al. 2003, Nichols et al. 2007).

The bulk of the studies on drug residues in aquatic tissues relate to what is known from aquaculture, where exposure is restricted to a very limited number of drugs (almost all being veterinary drugs) and at concentrations orders of magnitude higher than might occur in the ambient environment. Of the thousands of published studies that have been compiled regarding the many aspects of APIs as environmental pollutants (U.S. EPA 2009), roughly only 50 or so are directly relevant to aquatic exposure and tissue levels of APIs, and the majority of these studies have been published since Brooks et al. (2005) reported fish tissue residues of SSRIs (selective serotonin reuptake inhibitors) from an effluent-dominated stream.

APIs have long been assumed to show little propensity to bioconcentrate, no less biomagnify. This has been based largely on their greater water solubility compared with conventional pollutants such as pesticides and many industrial pollutants, especially the persistent organic pollutants (POPs). But APIs are known to sometimes undergo active transport, so this assumption may not be valid. Little is known for assisting the assessment of the bioaccumulation potential in fish (Cowan-Ellsberry et al. 2008); even less is known regarding the bioconcentration of APIs by fish or other aquatic organisms. Bioaccumulation is deemed possible when the BCF (expressed as L/kg) exceeds a range of 500–5000, depending on the standard being applied (Cowan-Ellsberry et al. 2008).

Even when definitive data have been obtained regarding bioconcentration, whether this can be extrapolated among species is unknown. Owen et al. (2007) emphasize the diversity in biology among the 28,000 species of fish. Tissue levels are governed largely by the pharmacokinetics of the API. These authors also point out that almost nothing is known regarding the pharmacokinetics of APIs in aquatic organisms—the xenoestrogen 17α-ethinyl estradiol (EE2) being one exception. Also, while it might be useful, extrapolations between mammals and fish can be challenging and potentially misleading because of key differences in physiology. Owen et al. (2007) stress that a primary route of uptake in fish is via the gills, from where blood is delivered to various organs before reaching the liver, in contrast to oral exposure in mammals, which leads to first-pass metabolism in the liver. Yet a further complication is the wide disparity that can exist among reported K_{ow} data for a particular API; the K_{ow} is the octanol–water partition coefficient—the ratio of a chemical's equilibrium concentration in octanol versus water at a defined temperature. These values can range over several orders of magnitude, pointing to the need for empirical data with which to validate and develop better predictive models. As discussed later, site-specific pH can influence the ionization state of many APIs, reducing the utility of using K_{ow} to predict exposure in retrospective evaluations; methodologies for estimating the BCFs for organic electrolytes have been assessed by Fu et al. (2009).

Exposure is a term commonly used by toxicologists, modelers, and others involved with environmental science. Exposure is a key component of the risk assessment paradigm that can provide insights for ways to reduce biological effects as well as better understand or predict their potential for occurring. Exposure translates the potential of hazard into the reality of risk (see Figure 8.1). But defining what is actually meant by exposure poses significant challenges. It does not necessarily represent a discrete physical or temporal point in the complex series of events that determine the outcomes from physical contact of an organism with a chemical or other stressor. Rather, the processes involved with exposure are spread across a complex spatiotemporal continuum that links a stressor's source or origin with the eventual effects that might occur within biological systems.

Although exposure includes understanding the duration, frequency, and magnitude to which organisms interact with biologically available contaminants, exposure magnitude is available for a limited number of APIs, and exposure duration and frequency is largely unknown for all APIs. Exposure is usually shown in conceptual diagrams as a standalone part of the hazard-risk continuum, an example being shown in Figure 8.1. External factors may influence the bioavailability, absorption, and uptake of an API, while physiochemical properties of an API influences pharmacological bioavailability, and internal factors (e.g., metabolism) will influence the duration of internal dosimetry. Chemical exposure is often visualized as the physicochemical interaction of a biological receptor with the chemical stressor, as when a ligand binds with a receptor. In practice, however, separating exposure from effects can be arbitrary and at times confusing. This is especially true when discussing biomarkers of exposure and biomarkers of effects.

A biomarker can be defined as pathway- or receptor-specific observations that are chemical-induced responses at the biochemical, physiological, or morphological level of an organism (Committee on Biological Markers of the National Research Council 1987). In ecotoxicology, biomarkers represent critical measures to support an understanding of exposure and potential effects to environmental contaminants. Under prospective and retrospective ecological risk assessment frameworks, it is useful to classify the various sublethal responses organisms may exhibit following

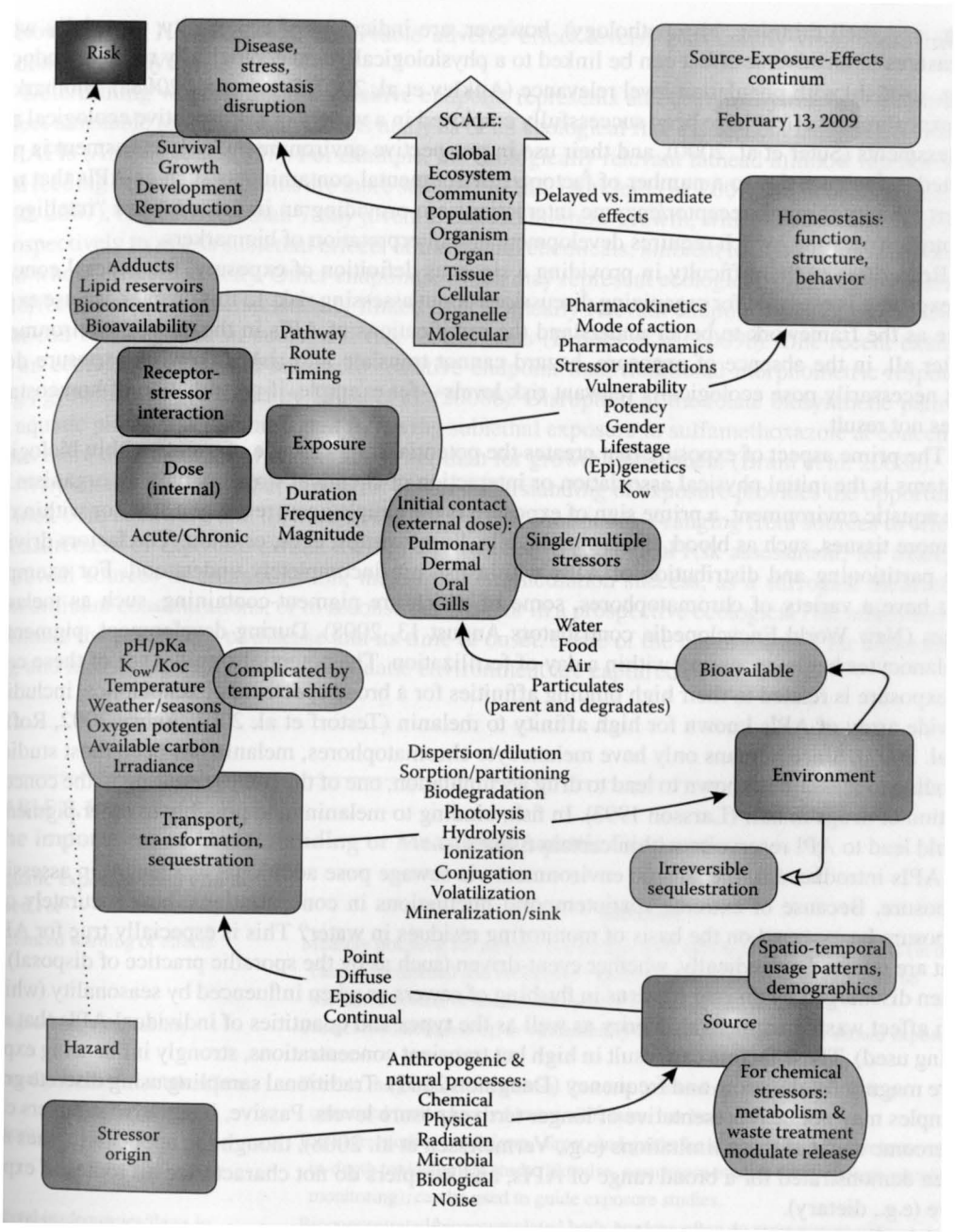

FIGURE 8.1 Source-to-effects continuum generalized for chemical stressors (but all principles also apply to APIs). The rounded rectangular boxes represent major points or processes in time or space along the source-to-effects continuum. The ovalized rectangular boxes and arrow labels represent variables that affect the processes along the continuum. The SCALE arrow represents the level of biological organization that the stressor impacts.

exposure to contaminants as either biomarkers of exposure or biomarkers of effect (Huggett et al. 1992). Biomarkers of exposure differ from biomarkers of effect in that these measures inform whether an organism has been exposed to contaminants (e.g., gene expression) but do not necessarily allow for determination of whether the organism has been adversely impaired. Biomarkers of effect

(e.g., egg shell thinning, histopathology), however, are indicators of ecotoxicity, especially when measures of an adverse effect can be linked to a physiologically and ecologically relevant endpoint (e.g., growth) with population-level relevance (Ankley et al. 2007, Brain et al. 2008a). Biomarkers of exposure and effect have been successfully employed in a variety of retrospective ecological risk assessments (Suter et al. 2000), and their use in prospective environmental risk assessment is projected to increase due to a number of factors. Environmental contaminants such as APIs that may exert toxicity through receptor/enzyme interactions are providing an impetus toward "intelligent" ecotoxicity testing, which requires development and interpretation of biomarkers.

Regardless of the difficulty in providing a rigorous definition of exposure, the general concept of exposure is essential for organizing discussions about assessing risk. In this chapter, we use exposure as the framework to better understand the ramifications of APIs in the aquatic environment. After all, in the absence of exposure, hazard cannot translate into risk. Likewise, exposure does not necessarily pose ecologically relevant risk levels—for example, if perturbation of homeostasis does not result.

The prime aspect of exposure that creates the potential for a cascade of events within biological systems is the initial physical association or interaction of chemical stressors with an organism. In the aquatic environment, a prime sign of exposure is the simple occurrence of a stressor within one or more tissues, such as blood, lipid, muscle, bile, liver, ovaries, eggs, or brain. The factors driving the partitioning and distribution of APIs within fish are incompletely understood. For example, fish have a variety of chromatophores, some of which are pigment-containing, such as melanocytes (New World Encyclopedia contributors August 13, 2008). During development, pigmented melanocytes begin appearing within a day of fertilization. The potential significance of these cells to exposure is related to their high binding affinities for a broad spectrum of xenobiotics, including a wide array of APIs known for high affinity to melanin (Testorf et al. 2001, Aubry 2002, Roffey et al. 2007). Since humans only have melanocyte chromatophores, melanin has been most studied. Binding to melanin is known to lead to drug accumulation, one of the routes resulting in the concentration of drugs in hair (Larsson 1993). In fish, binding to melanin (and possibly to other pigments) could lead to API reservoirs within certain tissues.

APIs introduced to the aquatic environment via sewage pose additional challenges in assessing exposure. Because of extreme spatiotemporal fluctuations in concentrations, how accurately can exposure be assessed on the basis of monitoring residues in water? This is especially true for APIs that are released episodically, whether event-driven (such as by the sporadic practice of disposal) or when discharged by diurnal patterns in flushing of sewers or when influenced by seasonality (which can affect waste treatment efficiency as well as the types and quantities of individual APIs that are being used). These factors can result in high but transient concentrations, strongly influencing exposure magnitude, duration, and frequency (Daughton 2007). Traditional sampling using discrete grab samples may not be representative of longer term exposure levels. Passive, integrative samplers can overcome some of these limitations (e.g., Vermeirssen et al. 2008), though the applicability has not been demonstrated for a broad range of APIs, and samplers do not characterize all routes of exposure (e.g., dietary).

A body of published work points to a spectrum of *potential* toxicological consequences following controlled exposure of certain aquatic organisms to a limited number of APIs, but these studies ignore whether real-world exposures actually occur and whether they occur at the requisite realistic concentrations (e.g., Gaworecki and Klaine 2008). Often, effects using traditional endpoints (e.g., survival, growth, and reproduction) in traditional test species (e.g., *Ceriodaphnia dubia*) are found only at levels orders of magnitude higher than known in the environment (e.g., Henry and Black 2008). Such observations are generally supported by several recent papers that reviewed information on aquatic toxicity of APIs (Crane et al. 2006, Fent et al. 2006, Farré et al. 2008). Rather than repeating such efforts here, it is instead important to keep in mind that the effects level is a function of the sensitivity of the targeted endpoint in the test species selected for study; other endpoints—especially those still not recognized—may have lower effective concentrations

(below current NOAELs—no observable adverse effect levels), particularly from standardized ecotoxicity assays.

Determining whether a more sensitive endpoint represents an ecologically relevant measure of effect amenable to inclusion in effects analysis of an ecological risk assessment must be considered for APIs (Ankley et al. 2007). For example, the ecologically relevant fathead minnow reproduction and feeding behavior endpoints are more sensitive to EE2 (Ankley et al. 2001) and fluoxetine (Stanley et al. 2007), respectively, than 7-day juvenile fathead minnow growth, which is routinely employed prospectively to assess sublethal effects of individual chemicals, ambient toxicity in surface waters, and whole-effluent toxicity. Other endpoints, which may represent ecologically relevant biomarkers of effect, should be mechanistically linked to ecologically relevant endpoints (Ankley et al. 2007) that can be integrated in ecological risk assessments (Brooks et al. 2009b). One recent example of an ecologically relevant and more sensitive endpoint than traditional morphometric responses (e.g., growth) was presented by Brain et al. (2008b). Disruption of the folate biosynthetic pathway in aquatic plants was demonstrated following sublethal exposure to sulfamethoxazole at concentrations more than an order of magnitude lower than for growth impairment (Brain et al. 2008b).

Why should we care about exposure? Better understanding of exposure provides the opportunity to look both backward and forward in the hazard-risk continuum—ranging from sources to effects. Measurement of exposure can be used in a retrospective ecological risk assessment, for example, to reveal sources of contamination, measure site-remediation success, as a surrogate measure of contaminant concentrations, or to reconstruct dose, or in a prospective ecological risk assessment to estimate the possibility of disease and its time of onset. Some of the major reasons for understanding and measuring exposure in the aquatic environment are captured in Table 8.1.

TABLE 8.1
The Importance of Understanding or Measuring Aquatic Exposure

Aquatic Exposure Data Can Be Used For	Rationale
Advanced warning of effects	Measure potential for adverse population-level effects or individual effects (in the case of threatened and endangered species), should the level of exposure be sustained or rise, or should the onset of effects be delayed.
Prognosis or vulnerability	Predict the likelihood of, or vulnerability for, future disease onset should exposure occur.
Reveal potential for subtle effects	Provide insights on the potential for cumulative subtle effects that might go unnoticed until a level of irreversible harm has been reached.
Prioritization of APIs	Exposure studies can inform, direct, and guide the selection of APIs for more in-depth toxicological study; likewise, occurrence studies (such as from water monitoring), can be used to guide exposure studies.
Reveal inadequacies/flaws in models	Bioconcentrated/bioaccumulated body burdens often do not comport with predictive models (e.g., Maunder et al. 2007).
Reveal potential for food chain disturbances	When bioconcentration and bioaccumulation resist predictive modeling, analysis of tissue levels are therefore required.
Sentinel for terrestrial organism exposure	Being more susceptible to exposure than terrestrial organisms and for development of subtle effects, aquatic exposure can serve as a sentinel model for terrestrial exposures (such as might occur from the recycling of treated sewage).
Potential for human exposure	Can provide information on levels of potential stressors that humans might consume via the food chain (e.g., contaminated fish in the diet).
Corroborate representativeness or need for toxicity testing	Establish whether concentrations/doses used in toxicity testing actually occur in the wild.

continued

TABLE 8.1 (continued)
The Importance of Understanding or Measuring Aquatic Exposure

Aquatic Exposure Data Can Be Used For	Rationale
Stressor sources/origins/ distributions	Tissue concentrations can help to reveal the locations, distributions, frequencies, or durations of stressor presence in the ambient environment.
Pollution prevention/source control	Guide actions to prevent or control the source or origin of the stressors, thereby reducing the potential for exposure.
Gauge success/progress of cleanup or remediation efforts, or of new waste treatment technologies	Tissue levels of chemical stressors used to measure ambient concentrations after cleanup actions or remediation, or after implementing new control measures at sewage treatment facilities.
Surrogate measure of pollutant concentrations	Tissue levels of chemical stressors used as surrogate measures of ambient concentrations of APIs in water.
Inadequacy in water monitoring for predicting exposure	Extreme spatiotemporal fluctuations in water concentrations of APIs obscure or complicate evaluation of actual exposure. This is especially true for APIs that are: (i) released episodically (event-driven, such as by the sporadic practice of disposal or events causing large changes in usage rates or in the types of medication being used), (ii) discharged by diurnal patterns in flushing, (iii) influenced by seasonality. Tissue levels may serve to integrate exposure over time.
Magnify levels of stressors not ordinarily toxic	Uptake and bioconcentration of chemical stressors at concentrations below no-effect levels in water can eventually achieve body-burden levels that exceed effects levels.
Magnify levels of stressors not ordinarily detectable	When stressors can be bioconcentrated, exposed organisms serve as sentinels for sources of pollution or ambient levels that might otherwise be too small to directly detect.
Exposure times extended in absence of stressor in ambient environment	Biomagnified residues extend the time during which exposure can occur, even after the API is no longer present in the external environment.
Dose reconstruction	Levels of exposure can be used to reconstruct the original dose.
Correlate ambient, external concentrations with exposure	If actual exposure can be calibrated to external exposure levels, then simple chemical monitoring in water could possibly be used instead of more resource-intensive tissue monitoring for predicting exposure.
Deconvolute contributions to biological effects originating from natural (ambient) background	Natural incidence can play a role in certain biological conditions that also result from exposure to stressors. Natural background can be confused with effect. Understanding exposure helps to distinguish the two causes (e.g., Grim et al. 2007).
Detecting newly emerging stressors	Identifying previously unknown chemicals concentrated in tissues can reveal newly emerging pollutants before any linkage with effects might be suspected; this is particularly germane to new drug entities recently introduced to commerce.

Remarkably, given the voluminous literature on APIs in the aquatic environment, little data exist relevant to the occurrence of APIs as residues in the tissues of aquatic organisms. These limited data come primarily from controlled studies using APIs postulated or known to occur in the environment. These studies involve exposure experiments under controlled conditions (including fish caged in the wild) and especially from studies of veterinary drug residues in aquacultured fish. Another source of data exists in the form of calculated BCFs from predictive models. One of the ramifications of this is that except for the expected residues of certain veterinary APIs used in aquaculture, it is essentially unknown what levels of the numerous APIs occurring in the ambient environment might lead to human exposure via consumption of fish or other aquatic life. No human exposure studies have been done on API residues in wild fish. It is known, however, that tissue residues of certain APIs in aquacultured fish can resist degradation during cooking and can migrate from one

tissue to another during cooking, as shown for the antibiotics oxolinic acid (OA) and flumequine (Steffenak et al. 1994).

8.2.2 Sources/Origins Leading to Exposure

Thousands of distinct chemicals are formulated into tens of thousands of commercial products used worldwide in the practice of human and veterinary medicine or for personal care. Those of most interest in the aquatic environment include the APIs that: (i) are most frequently used or used in the greatest quantities (e.g., NSAIDs: nonsteroidal anti-inflammatory drugs) or which are commonly disposed by flushing into sewers, (ii) are the most potent (e.g., synthetic hormones such as EE2) or that have clear potential for environmental effects (such as antibiotics, serotonin regulators, or oncolytics), (iii) are excreted largely unchanged, (iv) share like-modes or mechanisms of action and which could therefore act by concentration addition (e.g., SSRI antidepressants), and (v) can bioconcentrate.

The occurrence and disposition of APIs in the environment is best viewed in terms of their origins or sources, which comprise the locations from which they enter the environment and the pathways by which they enter (and are initially distributed within) the environment. These are many and varied, and even a simplified network flowchart can seem complex (see Figure 1 in Daughton 2008). The major sources that have the potential for impacting the aquatic environment are summarized in Table 8.2; an overview of the many sources and origins of APIs in the environment is

TABLE 8.2
Sources/Origins of APIs Significant to Aquatic Exposure

API Source/Origin	Description/Explanation of API Source
Human and veterinary medications	Human medications (over-the-counter; prescription only or legend drugs) include thousands of distinct APIs comprising numerous categories used in diagnostics, prophylaxis (including vaccines), cosmetics, lifestyle, and therapy. Veterinary medications (including those used in aquaculture) primarily comprise anabolic steroids, parasiticides, and antibiotics, though other medicines are also used for companion animals (e.g., fluoxetine).
Dual-use	Some APIs also have uses outside the practice of medicine. Some have dual use as pesticides (e.g., lindane, pyrethrins, avermectins, azole fungicides, and warfarin); another example is malachite green, a chemical used illegally in aquaculture but where aquatic exposure has been documented as a result of other uses (Schuetze et al. 2008). Some veterinary medicines have even been evaluated for nonaquaculture pesticidal use directly in the aquatic environment; for example, the sedative medetomidine has been proven effective as an antifouling agent against barnacles (Hilvarsson et al. 2007).
Chemical categories	Lower and higher MW synthetics; biologics (derived from organisms; e.g., antibodies, vaccines, and interleukins); natural products; nanomedicines; halogenated APIs are common (especially those containing fluorine).
DBPs and transformation products	Little is known regarding the halogenated disinfection by-products (DBPs) that might be created from any number of APIs (e.g., Flores and Hill 2008); many metabolites or environmental transformation products can be bioactive.
Manufacturing	Release in effluent from pretreated and untreated API manufacturing; perhaps a minor, localized source in the United States, but possibly of greater significance in less developed countries (e.g., Larsson et al. 2007; Fick et al. 2009).
Excretion	Pharmacokinetics for humans differ radically among APIs, resulting in wide spans in excretion efficiency of unmetabolized parent APIs or in excretion of biologically active metabolites; conjugates can act as reservoirs of parent APIs once hydrolyzed during sewage treatment or in the environment. Excretion apportioned between the feces and urine varies among APIs. Maternal-fetal transport of APIs in fish is not understood.

continued

TABLE 8.2 (continued)
Sources/Origins of APIs Significant to Aquatic Exposure

API Source/Origin	Description/Explanation of API Source
Bathing	Bathing and swimming release APIs directly to sewers or surface waters by two mechanisms, both involving dissolution of APIs: (i) from medications applied directly to the skin, and (ii) after excretion to the skin via sweat (Daughton and Ruhoy 2009).
Sewerage	Only the contributions from distributed sewer systems can directly impact the aquatic environment; septic and advanced onsite systems generally do not serve as direct sources, but can be important in extreme conditions (e.g., improperly installed leach fields, direct discharge from advanced onsite systems). Straightpiping of untreated sewage continues to be practiced in some locales. Wet-weather events can cause discharge of untreated sewage.
Treated sewage effluent	The removal efficiency of APIs from the influents to WWTPs varies greatly—from 0 to essentially 100%, as a function of the properties of the individual API and the type of treatment being used. Removal includes "destruction" (alteration of chemical structure) as well as physical sequestration, such as by filtration or sorption to sludge (and subsequent creation of "biosolids").
Biosolids, manure, pet excrement	One disposal option for biosolids (treated sewage sludges), which can contain sorbed or occluded APIs, is amendment of agricultural soils; wet-weather runoff holds the potential for transporting these APIs to surface water via runoff; manure from farm animals and excrement from medicated pets can also serve as a source (via wet-weather runoff).
Runoff	Primarily relevant to veterinary drugs from CAFOs (e.g., wet-weather flooding of retention ponds) or leaching of manure or feces from domestic animals and pets; can also involve human medications, which are experiencing growing usage with pets.
Wet-weather overflows and straightpiping	Flows that exceed the capacity of sewage treatment plants and rural areas where sewers still discharge directly to surface waters represent worst-case sources (e.g., combined sewer overflows), as the concentrations of APIs in these waste streams would not be reduced by treatment.
Locations where unused medications accumulate	Leftover drugs tend to accumulate (and eventually require disposal from) a wide expanse of locations, extending far beyond the domestic medicine cabinet (Ruhoy and Daughton 2008).
Disposal to sewerage	Unwanted medications are often disposed by flushing down sewer drains; this can involve leftover, unused medications and also the residuals contained in used containers or delivery devices (Ruhoy and Daughton 2008).
Disposal to domestic trash	Instead of disposal to sewerage, leftover medications are also disposed to domestic trash (this is currently the preferred method recommended by the U.S. federal government, when alternative collection options are not available) (U.S. FDA 2010; ONDCP 2009 [updated October]). The leachate collected from trash disposed in engineered landfills is sometimes returned to sewage treatment facilities, possibly serving only as a minor and indirect route to the aquatic environment.
Commercial use	Agriculture (antibiotics) and aquaculture (medicated feeds) serve as sources in localized environments; these sources can involve significant quantities of APIs, especially uneaten medicated feeds (Rigos et al. 2004).
Vaporization/ aerosolization	The vapor pressure (volatility) for the vast preponderance of APIs is insufficient to serve as a source (or route of loss) for the aquatic environment; vaporization might, however, serve as a source for certain PCPs, such as synthetic musks (Aschmann et al. 2001; Peck and Hornbuckle 2004, 2006) and cyclic methylsiloxanes (Xu and Chandra 1999); indeed, Galaxolide has been detected in the blubber from Antarctic fur seal (*Arctocephalus gazella*) from the South Shetland Islands (Corsolini 2009). APIs can also get entrained on particulates that can then become airborne (Hamscher et al. 2003; Cecinato and Balducci 2007).

Note: An overview of sources and origins of APIs in the environment is also provided by Daughton (2007, 2008).

provided by Daughton (2007, 2008). Sources and origins are important to understand not just for exposure. A comprehensive inventory of sources and their individual significance relative to each other with respect to contributions to environmental loadings can facilitate the prioritization of actions needed to reduce, minimize, or eliminate the potential for exposure via source reduction or pollution prevention. A complex array of processes acts upon APIs released into sewage to diminish their ultimate concentrations in the aquatic environment. One example situation using the fluoroquinolones is provided by Golet et al. (2002).

8.2.3 Exposure Variables

A vast and complex array of variables and their interactions (involving both the stressor and the target organism) dictate how and to what degree exposure occurs. These include the routes and processes by which exposure occurs. Few of these variables are unique to aquatic exposure involving APIs, and all can play roles, either in concert or in sequence. Not all of the variables, however, have been examined with respect to APIs. Many are relevant to controlled exposure studies and therefore only have hypothetical applicability to exposure in the wild. Table 8.3 summarizes some of the many aspects relevant to aquatic exposure and provides examples from the literature. Some of these factors are incorporated in the conceptualized diagram of the "4Ts": toxicant, totality, tolerance, and trajectory (Daughton 2005). It is also important to recognize that just because a chemical stressor might not be detectable in any tissue does not mean that exposure has not occurred. It could be that the analytical methodology cannot detect the stressor at a sufficiently low level (the MDL is too high). After all, some concentration levels known to have effects are extraordinarily low. For example, effects from EE2 have been documented at the sub-ppt level in surrounding water (i.e., 0.05 ng/L) (Larsen et al. 2008); diclofenac is purported to have pro-inflammatory effects at concentrations as low as 10^{-14} M (~3 pg/L) (Schirmer and Schirmer 2008). Moreover, the kinetics involved with the processes governing exposure might be sufficiently fast that uptake by an organism results in immediate formation of irreversible products of exposure, such as adducts, or in metabolic transformation to bioactive products. These products might merely be biomarkers of exposure or they might alternatively be biomarkers of effect. Regardless, in these situations monitoring for tissue residues of the parent API may well yield negative data, and therefore exposure can be overlooked or underestimated.

The internal dose is a function of the external concentration, chemical state of the API (e.g., ionization), uptake by and distribution within the organism, and bioavailability. Since the amount of stressor that actually interacts with receptors of toxicological consequence cannot be easily determined, surrogate measures are usually used to estimate the actual biologically effective dose. An added difficulty arises in translating "concentrations" associated with sediments and particulates to freely available portions. Studies of uptake from sediments are not common, one example being Higgins et al. (2009), who report on the uptake by an oligochaete of triclocarban.

8.2.4 Some General Perspectives and Background Regarding Aquatic Tissue Levels of APIs

Surprisingly few studies have been published that examine the occurrence of APIs not just in fish but in any aquatic animal or plant. Moreover, most of these data have been obtained for veterinary APIs (primarily antibiotics and estrogenic/androgenic steroids), which are used in aquaculture at levels far exceeding those found in the ambient environment. Most aquatic biomonitoring studies designed to emulate exposure under ambient environmental conditions are performed under controlled conditions, often with exposure concentrations still exceeding those that would be found in the ambient environment. Controlled exposure studies often use exposure concentrations that are one or more orders of magnitude higher than those that exist in the ambient environment, usually to

TABLE 8.3
Variables Involved with Aquatic Exposure

Variable Affecting Exposure	Example
Exposure under controlled conditions	Baths (immersion in static, replacement, or flow-through systems, sometimes using whole effluent from sewage treatment or water collected from native environments), oral (bolus), injection (IP), feeding, caged in wild, whole-effluent toxicity testing.
Route of exposure (free in wild)	Gill (brachial) transport, dermal absorption, oral/gut, olfactory (e.g., transport of nanoparticles via olfactory neurons), lateral-line sensory organ (e.g., Chiu et al. 2008).
Type of organism	Teleost and cartilaginous fish, macroinvertebrates (invertebrates such as insects, crustaceans, mollusks, and worms), periphyton (e.g., Aufwuchs), plants, amphibians, and reptiles, waterfowl, and mammals (e.g., otters).
Environmental location	River, wetland, lake, reservoir, estuary, marine; benthic, pelagic, interfacial monomolecular monolayer; proximity to effluent from treated effluent discharge or raw sewage.
Stressor physical status	Dissolved in water column, particulate-bound (including incorporation into feed), sediment-bound; uptake from water can differ dramatically from dietary uptake.
Stressor properties	Water solubility, K_{ow}, pKa, log D, log K_{lipw}, MW, molecular size or cross section, molecular conformation and steric factors, environmental/metabolic half-life, chirality, vapor pressure.
Ambient environment	pH, temperature (or season), salinity, total dissolved solids, natural organic matter (Galvez et al. 2008), dissolved oxygen or hypoxia, solar irradiance (photolysis), nutrient levels and turbidity (Gordon et al. 2006), geographic locale, and dilution (e.g., effluent-dominated streams yield maximum concentrations). Season can affect performance of sewage treatment (e.g., via temperature) and irradiance (e.g., for photolysis or growth of autotrophs). Season and locale can also affect the types of APIs present in sewage because different medications are used in different seasons and in different locales.
Engineering controls	End-of-pipe treatment can reduce concentrations of some but not all APIs; it can also lead to the formation of biologically active products and API-based DBPs; efficiency is a function of the types of engineered treatments used by manufacturers and municipalities (e.g., Snyder et al. 2007). Types and efficiencies of treatment can vary considerably from country to country (e.g., see Larsson et al. 2007).
Fate	The interactions of the stressor with the ambient environment dictate its fate; key processes are microbial degradation, photolysis, and sequestration via sorption to sediments. But even short environmental half-lives do not preclude a continual presence of those stressors that are continually introduced, such as via treated sewage; this has been termed "pseudopersistence" (Daughton 2002).
Organism status	Food supply, nutrient levels, health, prior exposure history, growth state, lipid content, injuries, disease, age, gender, condition index/factor, body length, over-expression/inhibition of efflux pumps, species/strain. For example, nutrient concentrations and stoichiometry influence lower trophic-level responses to triclosan (Fulton et al. 2009).
Organism behavior and niche	Behavior involving feeding (free-feeding, sessile, filter-feeding), niche, trophic level, swimming/migratory behavior, and attraction/avoidance, and how these influence proximity to source (e.g., sewage outfalls) and aggregation; aquatic, marine, estuarine.
Timing of exposure	Windows of vulnerability, developmental life stage/reproductive status (e.g., embryo, egg, larval, hatchling, fry, juvenile, adult, spawning) (Van Aerle et al. 2002). Simultaneous vs. sequential exposure when multiple stressors are involved.
Magnitude of exposure	Aqueous concentration is a major determinant of actual internal exposure dose for aquatic organisms. For APIs, concentration is partly dictated by the degree of dilution in waters receiving treated or raw sewage effluent (with effluent-dominated streams and streams receiving raw sewage representing worst cases); other determinants include population served, sewage flow rate, and treatment technology. High stressor concentrations in controlled laboratory studies are often not relevant to real-world exposure. Newer exposure studies, however, are exploring lower and lower concentrations; need to ensure that studies are relevant to the real world (Hinton et al. 2005). BCFs are often higher at lower concentrations. Lack of exposure or effects at higher concentrations cannot be used to rule out the importance of lower concentrations because of multiphasic and nonmonotonic dose-response.

TABLE 8.3 (continued)
Variables Involved with Aquatic Exposure

Variable Affecting Exposure	Example
Duration/variability/ frequency of exposure	Constant, pulsed, discrete, episodic, acute/chronic, life cycle, and multigenerational.
Multiple stressors	Simultaneous versus sequential exposure to multiple stressors. Multistressor interaction effects (e.g., additive, potentiated, antagonistic, and synergistic) may follow concentration addition or independent action models; competition for—or facilitation of—transport across membranes.
Exposure history	Prior or simultaneous exposure to other stressors—both chemical and physical (e.g., temperature, salinity, stress from prey, etc.).
Pharmacokinetics	ADME: absorption, distribution, metabolism (e.g., phase I and II), excretion/elimination—all affecting half-life and disposition within organism (including depuration); reactivity within organism (e.g., formation of adducts vs. bioconcentration within lipid).
Pharmacological bioavailability	Residues that are bound versus those that are free (e.g., particulate-bound APIs may not transport across gills); sequestration and/or bioconcentration of body residues, e.g., within lipids, binding with melanins (Larsson 1993; Testorf et al. 2001; Aubry 2002; Roffey et al. 2007) and as other adducts; disposition in blood/plasma (primarily bound to proteins), muscle, bile, liver, brain, gonads, eggs, skin, bone, etc.; these factors also have direct relevance to subsequent human exposure to APIs via the food chain (function of edible tissue distribution).
Aquatic bioavailability	pH influence on ionization state and lipophilicity of APIs. Influence of sorption to suspended particulate matter. Concentration enrichment within sediments. For example, higher K_D values were observed for ciprofloxacin with fine particulate organic matter (FPOM) than coarse particulate organic matter, resulting in a higher magnitude of exposure to benthic macroinvertebrates consuming FPOM (e.g., collector-gatherers) (Belden et al. 2007).
Transformation products	Metabolites and environmental transformation products (e.g., degradates from photolysis or halogenation during disinfection) may prove more important that the parent API.
Resiliency/ vulnerability	Biochemical, physiological, and behavioral repertoire of an organism that determines its ability to avoid exposure or reduce its magnitude or duration; ability to maintain internal homeostasis and adapt to stressors (Clubbs and Brooks 2007).
Biological modulators of exposure	Various biological processes can enhance, facilitate, or reduce exposure. Those that are evolutionarily conserved play key roles. Among the most important considerations include variations of API metabolism among aquatic organisms. Exposure defenses include p-GP efflux pumps, which can also be induced/repressed by other stressors, especially APIs (see: Tan 2007); cellular stress response (e.g., induction of heat shock proteins); facilitation might occur via active transport (e.g., for APIs of low K_{ow} or high MW).

Note: Some of these factors are discussed in detail by Geyer et al. (2000) and van der Oost (2003).

maximize the chances of detecting and quantifying any amount that might accumulate. Most of the aquatic studies involving human APIs have been published only in the last few years.

Some studies examine the presence of APIs (primarily estrogens) indirectly, by way of activity assays. For example, Houtman et al. (2004) examined fish bile for estrogenic activity; but fractionation of the sample is required for assigning activity to a particular API. It is also important to note that in many studies, especially those examining bile, the APIs can be present as metabolically reversible conjugates, which may or may not have been cleaved prior to analysis; so the API is not really present in its parent form, although it can be reabsorbed once the conjugate is excreted into the intestine. The interpretation of tissue levels of many APIs, especially the steroids, is greatly complicated by the relative portions that are free versus conjugated.

The analysis of bile for free and especially conjugated APIs has been well-established for over 30 years. In a summary of data acquired in the 1970s for over a dozen APIs, concentration in the bile of the dogfish shark was already known (Guarino and Lech 1986). Concentrations in the bile versus plasma were known to range up to factors of hundreds (e.g., warfarin and diethylstilbestrol) or thousands (e.g., methotrexate). The utility of bile in exposure monitoring is discussed by Adolfsson-Erici (2005) and Pettersson (2006).

Almost no tissue-monitoring study has examined the optical isomer ratios of racemic APIs; indeed, even the aquatic toxicity of chiral APIs has been little studied, with Stanley et al. (2006) publishing one of the first studies (with propranolol). Stanley et al. (2007) further examined enantiomer-specific sublethal effects of the antidepressant fluoxetine on traditional endpoints (survival and growth) and the ecologically relevant behavioral response of feeding behavior. Both studies by Stanley et al. (2006, 2007) indicated that the more pharmacologically active enantiomer was more toxic to fish when sublethal rather than lethal responses were examined. Clearly enantiomer-specific bioaccumulation and effects of chiral compounds require additional attention and may necessitate *a priori* considerations in ecological risk assessments (Stanley and Brooks 2009).

Although the significance of bioconcentration is largely one of establishing potential internal dose, it is important to note that internal exposure is not necessary for an effect to occur. Certain effects can occur when the target organ is external. This is the case, for example, with exposure of the lateral-line sensory organ (e.g., Chiu et al. 2008) and for olfactory and taste exposure. Theoretically, antibiotics could alter the natural community structure of microorganisms that reside on the external surfaces of any aquatic organism.

An important perspective regarding the range of APIs and metabolites that have been and will continue to be detected in aquatic tissues is that of "self-biasing detectability." Those APIs with the highest probability of being detected are those that (i) can be taken up from water or food, (ii) are present in the highest concentrations, (iii) have the lowest MDLs, and (iv) have available appropriate analytical reference standards. Regarding the second point, those present in the highest concentrations tend to be those with the higher required doses, and which therefore have lower potency (the human acceptable daily intakes [ADIs] for residues of veterinary APIs in food therefore tend to also be higher). The third point is rarely pointed out. MDLs among APIs in a particular tissue can vary by more than 2 orders of magnitude. This means that APIs commonly present in tissues but having high MDLs might not be detected. As an example, Ramirez (2007) targeted 25 APIs/metabolites in fish in effluent-dominated streams and rivers. Of the seven that were detected, three had the lowest MDLs. Of the seven APIs with the highest MDLs, only one (gemfibrozil) was detected. Tissue concentrations below 0.1–1 μg/kg are rarely reported because this usually falls below the current method detection capability, primarily because of matrix interferences. But APIs could nonetheless be present at these levels and will therefore be overlooked or self-censored. In the case of API metabolites, very few deuterated standard compounds are commercially available, which presumably has precluded their analyses in tissues compared with parent drugs.

8.2.5 Predictive Modeling

Empirical data for the uptake and bioconcentration of APIs by aquatic organisms is very limited. Such data are primarily focused on the legacy POPs. The empirical data that do exist are fraught with quality issues and uncertainty; extrapolations across chemical classes or aquatic species are notoriously unreliable. Acquiring empirical data is met with a number of hurdles, not the least of which are cost and animal welfare concerns. Data from real-world field conditions are even more limited than data obtained under controlled laboratory conditions, and these data are poorly covered in the available electronic databases. The bioaccumulation databases for fish have been summarized by Weisbrod et al. (2007); *in vitro* methods for measuring bioavailability in fish have been reviewed by Weisbrod et al. (2009).

A comprehensive review of the literature on uptake and bioconcentration of organic chemicals by aquatic organisms revealed thousands of BCFs and bioaccumulation factors (BAFs) for 842 organic

chemicals in 219 aquatic species (Arnot and Gobas 2006). But nearly half of the BCFs had major sources of uncertainty, and predicted values that usually underestimated empirical values, which were sparse. BAFs under ambient field conditions were generally higher than BCFs obtained under controlled conditions. None of these data, however, included APIs.

Ankley et al. (2005) point out that routine aquatic bioconcentration testing is not common, as conventional APIs tend to be water soluble, with K_{ow} values below 3. Even then, higher molecular weights (MWs) or a propensity for facile transformation (e.g., via hydrolysis) often preclude bioconcentration. On the other hand, given the fact that APIs often rely on active transport for uptake (e.g., Van Bambeke et al. 2000), even low K_{ow} values sometimes may not preclude bioconcentration. A significant aspect of APIs to note is that they are designed to minimize accumulation in the body during intended use, so any build-up that might occur in aquatic tissue could prove toxicologically significant. Another factor that sets APIs apart from conventional POPs is their metabolism—sometimes yielding products that themselves can bioconcentrate. This creates the need to calculate "pseudo" BCFs—the ratio of tissue concentration of a metabolite and the aqueous concentration of the parent API (such as for norfluoxetine).

Van der Oost et al. (2003) also stress that predicting bioaccumulation in fish using simple models (e.g., relying on K_{ow}) is "virtually impossible" and extremely prone to error even with sophisticated models. The dynamics imposed on APIs by pharmacokinetics in particular make prediction extremely difficult to model.

In the absence of empirical data, computed BCFs (e.g., using Quantitative Structure Activity Relationship—QSAR—calculations) are often relied upon—at least to try and inform which APIs might be of concern with regard to bioconcentration. But this approach has major unknowns with respect to pharmaceuticals (Walker et al. 2004a, 2004b). Other approaches for prioritizing which APIs might be of highest exposure concern include those that rely on informatics (e.g., Gunnarsson et al. 2008, Kostich and Lazorchak 2008) or water/sediment monitoring (e.g., Lissemore et al. 2006).

Cunningham et al. (2009) reported on calculated BCFs in fish for 43 APIs. They range up to highs of 353 L/kg (atovaquone), 190 (dutasteride), 64 (beclomethasone), and 51 (nabumetone), but nearly all of the remainder were less than 4 L/kg. In a major analysis of the factors involved with bioconcentration, Geyer et al. (2000) provide calculated K_{ow} values or BCFs for a number of natural and synthetic estrogens and estrogenic chemicals, androgenic steroids, and nonsteroidal antiandrogenic chemicals. Quite a number of computed BCFs are mentioned by Grung et al. (2007), and a number of computed BCFs for lipid-regulators are provided by Hernando et al. (2007). Other predictive modeling approaches are explored in Section 8.4.

The many variables and pitfalls in determining BCFs and their use for predicting BAFs are discussed by Parkerton et al. (2008). These issues, which surround the soundness or quality of BCF/BAF data, were not evaluated in the summary of published data reported in this chapter.

8.2.6 Overview of Fish Tissue and Other Residue Data for APIs

The study of the exposure of fish to APIs is dominated by endocrine-disrupting compounds [EDCs; predominately by the natural and synthetic estrogenic sex steroids—primarily 17β-estradiol (E2) and EE2; comparatively less focus is directed at androgens] and by antibiotics and biocides (such as triclosan). Comparatively few data exist for other drug classes. Most studies regarding EDCs obviously concern reproductive and other direct endocrine effects. Little exists on exposure to APIs having the potential to lead to subtle, difficult-to-detect effects such as alteration of behavior or perturbations of the immune system (e.g., Hoeger et al. 2005, Salo et al. 2007).

Exposure studies regarding endocrine disruption have been dominated by the numerous studies demonstrating that fish are impacted by exposures to treated and untreated sewage. The first of these, which linked exposure to sewage with estrogenicity, was published in 1994 (Purdom et al. 1994). Most evidence for exposure is inferential. Few studies have established the possibility of exposure to either individual or combinations of specific API EDCs.

Excluding the data collected on fish uptake of antibiotics as a direct or indirect result of usage in aquaculture, actual empirical data are rare for fish-tissue residues of APIs resulting from exposures in the environment or exposures under controlled conditions emulating ambient concentrations. Determination of empirical BCFs is rarer yet. These data come from two major categories: (1) fish collected from (or caged in) wild, native environments and (2) fish exposed under controlled conditions in the laboratory. Collectively, these data exist only for about 30 different APIs, some of which were the subject for just a single study (Ramirez et al. 2007).

The limited data on residue levels from exposure in the natural environment are extremely limited, primarily comprising: SSRIs (especially paroxetine, fluoxetine, sertraline, and some principal metabolites such as norfluoxetine and norsertraline), NSAIDs (diclofenac, naproxen, ketoprofen, and ibuprofen), steroids (estrone [E1], E2, and EE2), and diphenhydramine, diltiazem, gemfibrozil, and carbamazepine (CBZ). Also available are data for biocides (triclosan, methyl triclosan, and triclocarban) and malachite green and its leuco metabolite.

Tissue residue levels resulting from exposures under controlled conditions are a bit more common, including many of the same ones as detected under environmental monitoring, but also including: β-blockers (propranolol and atenolol), fungicides (the triazoles: bromuconazole, cyproconazole, metconazole, myclobutanil, penconazole, propiconazole, tebuconazole, tetraconazole, and triadimefon), the macrocyclic lactone avermectin B1, steroids (hydroxyestrone, estriol [E3], 17β-dihydroequilenin, and testosterone), and mono- and di-brominated derivatives of EE2.

These data are discussed in more detail in the succeeding sections. While on the one hand it is interesting that most of these APIs have BCFs greater than unity (considering their generally low K_{ow} values), few have BCFs above 1000 and therefore do not have anywhere near the accumulation potential of the POPs. The tissue levels of low-K_{ow} APIs point to uptake mechanisms beyond passive diffusion. The greatest concentrations tend to be in the bile and liver. Actual bioconcentration of diclofenac seems to be higher than for most APIs. The computed BCF for fluvastatin is among one of the highest for any API. But the highest measured BCFs are for the biocide triclosan, which was found to range upward of 10,000 in the intestines of zebra fish (Orvos et al. 2002). The most empirical data exist for steroids and antibiotics. While residues in edible tissues have clear ramifications for human exposure, residues in organs such as the brain (a focus for monitoring targeted at SSRIs) have implications regarding immediate biological effects. All residues have implications with respect to trophic biomagnification, which has not been studied with respect to APIs.

8.2.6.1 SSRIs/SNRIs (Selective Serotonin and Serotonin-Norepinephrine Reuptake Inhibitors)

Fluoxetine and sertraline and their principal metabolites (norfluoxetine and norsertraline) were detected in all fish tissues (brain, liver, and muscle) from three fish species collected from Pecan Creek TX, an effluent-dominated stream: *Lepomis macrochirus* (bluegill), *Ictalurus punctatus* (channel catfish), and *Pomoxis nigromaculatus* (black crappie) (Brooks et al. 2005). All four analytes were detected in all tissues at levels exceeding 0.1 ng/g; no residues were detected in fish from a reference site not receiving effluent discharges. Compared with average tissue levels of fluoxetine and sertraline, the average levels for norfluoxetine and norsertraline were higher in brain, liver, and muscle. The highest concentrations for each API were detected in the brain, generally followed by the liver: norsertraline (15.6 and 12.94 ng/g), norfluoxetine (8.86 and 10.27 ng/g), sertraline (4.27 and 3.59 ng/g), and fluoxetine (1.58 and 1.34 ng/g); the lowest concentrations were in muscle: norfluoxetine (1.07 ng/g), norsertraline (0.69 ng/g), sertraline (0.34 ng/g), and fluoxetine (0.11 ng/g). These levels are roughly 0–4 orders of magnitude higher than reported in wastewater and effluent-dominated streams, reflecting bioconcentration as well as the possibility of active transport. Although hypothetical human dietary exposure from these fish would yield daily intakes roughly 6 orders of magnitude below therapeutic doses, note that ADIs exist only for veterinary drugs.

Schultz et al. (2010) recently reported extensive data from a monitoring study involving the largest suite of APIs yet targeted from any single therapeutic class (SSRIs/SNRIs); they also collected concomitant data from three matrices (water, bed sediment, and brain tissue of white sucker, *Catostomus commersoni*) from two effluent-impacted streams in Iowa and Colorado. They targeted 10 antidepressants (including two metabolic/transformation products): bupropion, citalopram, duloxetine, fluoxetine (and norfluoxetine), fluvoxamine, paroxetine, sertraline (and norsertraline), and venlafaxine, an SNRI. All but two (duloxetine and fluvoxamine) were found in brain tissue. Of particular interest was the distinct lack of correlation between the types and quantities of these APIs measured in the stream waters versus those in the brain tissues. Venlafaxine was found in most of the stream samples at concentrations consistently higher than the next two most prevalent (bupropion and citalopram), sometimes at levels over an order of magnitude higher. The levels of venlafaxine in the streams sometimes exceeded 0.5 µg/L. In contrast, the primary analytes in brain tissue were norsertraline and sertraline, followed by norfluoxetine and fluoxetine. Indeed little venlafaxine, bupropion, or citalopram were found in brain tissue but were prevalent in sediments (although their relative levels were generally the inverse of those in brain tissue). These data point to the possible involvement of selective uptake of these chemicals into brain tissue. The maximum and the range of mean concentrations (ng/g) in brain tissue were: norsertraline (28.9; 0.01–3), sertraline (4.24; 0.005–1.8), norfluoxetine (3.57; 0.07–0.9), and fluoxetine (1.65; 0.02–0.6). The maximum level reported for norsertraline (28.9 ng/g) is the highest yet reported for an API in brain tissue.

In a more recent study, Brooks et al. (in preparation) observed sertraline, norsertraline, fluoxetine, and norfluoxetine at low ng/g levels in periphyton and three taxa of benthic macroinvertebrates (*Corbicula fluminea*, *Argia* sp., hydropsychidae) collected from Pecan Creek TX, indicating that dietary exposure to fish from these SSRIs and potentially other APIs deserve further study. As noted elsewhere in this document, extraction and analysis approaches to account for matrix differences among aquatic organisms require further development. For example, a recent study advanced extraction techniques for identification of APIs in mollusks (Cueva-Mestanza et al. 2008).

Fish from a Lake Ontario harbor receiving sewage effluent were analyzed for SSRIs by Chu and Metcalfe (2007). Seven fish were collected from three species [three brownbullhead (*Ameiurus nebulosus*), three gizzard shad (*Dorosoma cepedianum*), and one white perch (*Morone americana*)] and analyzed for paroxetine, fluoxetine, and norfluoxetine. Concentrations on the basis of whole wet weight ranged up to 1 µg/kg: paroxetine (0.48–0.58 µg/kg; 3 of 7 samples), fluoxetine (0.14–1.02 µg/kg; 6 of 7 samples), and norfluoxetine (0.15–1.08 µg/kg; 4 of 7 samples); neither fluoxetine nor norfluoxetine was detected in white perch. This occurrence of paroxetine is the first reported in the literature.

In a 2-year study in the Caloosahatchee River, Florida, water samples and the plasma of juvenile bull sharks (*Carcharhinus leucas*) were analyzed for four SSRIs and a metabolite (citalopram, fluoxetine/norfluoxetine, paroxetine, and sertraline) and an SNRI (venlafaxine) (Gelsleichter 2009). Only citalopram, sertraline, and venlafaxine were detected in wastewater or river-water samples. All analytes except for fluoxetine, however, could be detected at very low quantifiable levels in at least one plasma sample from at least one of the 2 years. Sertraline was the only analyte detected in all samples in 2006, while only venlafaxine and citalopram were detectable in 2007.

The bioconcentration of fluoxetine at an exposure concentration of 10 µg/L by Japanese medaka (*Oryzias latipes*) was evaluated under controlled conditions, at three pH values below the pK_a (Nakamura et al. 2008). The empirical BCFs were 8.8, 30, and 260 for the body and 330, 580, and 3100 for the liver, at respective pH values of 7, 8, and 9; the BCF for fluoxetine summed over the body and liver was 11 at pH 7.2. The BCFs increased with increasing pH since fluoxetine is a weak base and uptake of the nonionized molecule is facilitated by diffusion. Not unexpectedly, the N-demethylated metabolite norfluoxetine was similarly recovered. BCFs predicted from liposome/water equilibration did not increase as much with increasing pH as did BCFs predicted by octanol/water partitioning (with control of ionic strength). Bioconcentration of an API such as fluoxetine, while made complicated by pH influencing ionization, differs dramatically from the

bioconcentration of a conventional, long-lived POP. It is further complicated when the API (such as fluoxetine) can be metabolized to another chemical species (in this case, norfluoxetine) that is also subject to bioconcentration. Since S-norfluoxetine binds to the serotonin reuptake transporter with affinity similar to that of R-fluoxetine and S-fluoxetine (Wong et al. 1993), the combined sum of the parent compound and active metabolite should be considered in determining bioconcentration and effects in ecological risk assessment.

Using caged fathead minnows in the outfall from sewage treatment plants, Metcalfe et al. (2010) detected venlafaxine, citalopram, sertraline, and a demethylated metabolite from each (as well as norfluoxetine) at levels roughly ranging from 1 to 4 ng/g (whole-body wet weight). Only sertraline, however, was detected in more than one of the three sites examined.

Research on the pharmacokinetics of APIs in fish is very sparse. Schultz et al. (2001) published perhaps the first study—on 17α-ethinylestradiol. Paterson and Metcalfe (2008) published an initial examination of the uptake and elimination of fluoxetine. At the outset of exposure to 0.55 µg/L in water over 7 days, accumulation by medaka was noted within the first 5 h; norfluoxetine was also noted at this time at about 40% of the fluoxetine level. A peak tissue concentration of 49 µg/kg was recorded after 3 days for fluoxetine. After 6 days, norfluoxetine exceeded fluoxetine: 64 µg/kg versus 40 µg/kg. This yielded a BCF of 74 for fluoxetine and a pseudo-BCF of 117 for norfluoxetine.

These studies (Brooks et al. 2005, Chu and Metcalfe 2007, Nakamura et al. 2008, Paterson and Metcalfe 2008, Schultz et al. 2010) collectively show that an API metabolite (such as norfluoxetine) can accumulate to equal or greater tissue concentrations than its parent (e.g., fluoxetine), which is not surprising since norfluoxetine is more nonpolar than fluoxetine. This is important when the metabolite (such as norfluoxetine) is bioactive. Metabolic conversion of the parent API means that its measured BCF will be lower than its actual BCF. Presence of an API in tissue might serve as an indicator of higher exposure to a metabolite of similar or higher biological activity. Whether these residues are bioavailable, however, is a key question, as shown by Zhou et al. (2008) who reported that the BCFs for "free" (unbound and directly bioavailable) fluoxetine were less than unity, while those for total fluoxetine were in the range reported by these previous studies. It is also not always clear whether API metabolites in tissues result from endogenous metabolism or from uptake of preexisting metabolites from water.

Clearly, the relationship between coexposure to SSRIs/SNRIs, tissue residues, and potential biological effects requires further study. This was highlighted by the recent work of Painter et al. (2009), which identified environmentally relevant concentrations of a mixture of SSRIs/SNRIs to adversely affect fathead minnow predator escape behavior.

8.2.6.2 NSAIDs (Nonsteroidal Anti-inflammatory Drugs)

Given the widespread usage of NSAIDs and the published data on their environmental occurrence and ecotoxicology, surprisingly few studies have monitored for any of the NSAIDs in aquatic organisms.

Brown et al. (2007) exposed caged juvenile rainbow trout to sewage effluent at three sites and measured plasma levels for various NSAIDs (and gemfibrozil). This was the first reported measurement of fish plasma levels of diclofenac, naproxen, and ketoprofen (as well as gemfibrozil) after exposure in the field. All except ketoprofen showed a propensity to bioconcentrate in plasma. Plasma concentrations ranged from tens to several thousand ng/mL, with the highest being for gemfibrozil. Of particular significance was the wide range of BCFs for any particular API across the study sites. The wide variance in BCFs did not seem to be a function of API concentration in the water, temperature, pH, or exposure time; the authors concluded that some other chemical characteristic of the effluents governed uptake, possibly the presence of colloids or surfactants. Compared with predicted BCFs, all of the APIs, with the exception of ibuprofen, had BCFs that ranged from unity to considerably lower.

Under static exposure to high nominal concentrations (490–1000 µg/L), plasma levels ranged from: 60 ng/mL (ketoprofen), 3440 (diclofenac), and 3640 (naproxen), to 4680 (ibuprofen); under the

same conditions, the level for gemfibrozil was 21,500. These gave empirical BCFs ranging from: 0.1 (ketoprofen), 4 (naproxen), 7 (diclofenac), to 9 (ibuprofen) [and 63 for gemfibrozil].

During the caged study, the exposure levels at the three sites ranged from 4.5 ng/L (ibuprofen) to 2320 ng/L (diclofenac). Resulting plasma concentrations ranged from undetectable (ketoprofen), 12 ng/mL (diclofenac), 14 (naproxen), and 84 (ibuprofen) (and 210 for gemfibrozil). These gave BCFs ranging from: <11 (ketoprofen), 5 (diclofenac), 56 (naproxen), to 18,667 (ibuprofen) (and 199 gemfibrozil).

With juvenile rainbow trout exposed under continuous flow for 96 h to 920 μg/L ibuprofen, after the first 24 h of exposure, the plasma concentrations of ibuprofen increased, beginning at about 7 μg/mL and ending at about 10.6 μg/mL (Huggett et al. 2004), in rough agreement with the data from Brown et al. (2007).

The bioconcentration of diclofenac by fish was reported for the first time by Schwaiger et al. (2004). Rainbow trout (*Oncorhynchus mykiss*) were exposed for 28 days to concentrations ranging from 1 to 500 μg/L. Concentrations in the liver were about 40-fold greater than in muscle. With exposure to 1 μg/L, tissue residue concentrations were about: 2882 ng/g (liver), 1025 ng/g (kidney), 805 ng/g (gills), and 73 ng/g (muscle), yielding BCFs (L/kg) of 2732 (liver), 971 (kidney), 763 (gills), and 69 (muscle); plasma levels were not reported. Tissue concentrations increased linearly with increasing dose, up to 500 μg/L, which yielded tissue concentrations roughly twice those from the 1-μg/L dose. The lower tissue concentrations with respect to dose are the reason the calculated BCFs decreased with increasing concentrations.

Mehinto (2010) reported bile BCFs in the narrow range 509–657 after 21-day exposures of trout to diclofenac at 0.5, 5.0, and 25 ng/L.

8.2.6.3 Lipid Regulators

Gemfibrozil was shown to bioconcentrate in the plasma of goldfish (*Carassius auratus*) after laboratory exposure to an environmentally relevant concentration of 0.34 μg/L (nominal 1.5), as well as a higher concentration of 852 μg/L (nominal 1500) (Mimeault et al. 2005). After 14 days of exposure, plasma BCFs were 500 and 92, respectively, resulting from respective plasma concentrations of roughly 170 and 78,000 μg/L. Uptake was concluded to occur across the gill membrane but passive diffusion or active transport could not be distinguished.

In the same study with four NSAIDs, gemfibrozil was measured in the plasma from rainbow trout caged in effluent-dominated streams and during a controlled static exposure experiment (Brown et al. 2007). This was the first measurement of a fish plasma level of gemfibrozil after ambient exposure in the field. Of the five APIs, the resulting plasma levels were highest for gemfibrozil. Static exposure to a high level of 510 μg/L gave a plasma level of 21,500 ng/mL, yielding a BCF of 63. In the caged field study, the plasma level reached 210 ng/mL, yielding a BCF of 199.

A compendium of calculated K_{ow} and BCF values for a number of fibrates and statins is provided by Hernando et al. (2007). While the $\log K_{ow}$ values all indicate relatively high lipophilicity (most exceeding 4), computational approaches for estimating BCFs showed relatively low values (3.2) for bezafibrate, gemfibrozil, clofibric acid, fenofibric acid, and pravastatin, and 56 for atorvastatin; values ranged from 120 to 380 for clofibrate, fenofibrate, lovastatin, and mevastatin. Higher computed values were found only for simvastatin (800) and fluvastatin (2000). These values might be useful for targeting these latter two statins for biomonitoring to determine actual empirical BCFs. Note, however, that the low computed BCF for gemfibrozil is not predictive of the empirical BCFs, being 1–2 orders of magnitude lower than those measured by Mimeault et al. (2005) and Brown et al. (2007).

8.2.6.4 β-Blockers

Owen et al. (2007; also unpublished data; Owen et al. 2009) exposed juvenile rainbow trout to relatively high levels of propranolol: 10 mg/L for 10 days. Plasma levels of about 5 μg/mL were reached; concentrations were often 40–80% of the water levels after 40 days.

Winter et al. (2008) exposed fathead minnows to relatively high concentrations (0.1–10 mg/L) of atenolol. Compared with water concentrations, plasma concentrations ranged between 1.8% and 6.2% (for males) and 0% and 12.2% (females). The male fish plasma concentration for atenolol corresponding to the exposure concentration for the LOEC (Lowest observed effect concentration) condition index (3.2 mg/L) was 0.0518 mg/L (51 μg/mL).

Cleuvers (2005) reported calculated BCFs of 4.47 and 0.89 for propranolol and metoprolol, and a value for atenolol too low to calculate. But β-blockers were not detected in fish during a study by Brooks et al. (2005, unpublished data).

8.2.6.5 Fungicides

Juvenile rainbow trout (*O. mykiss*) were exposed to nine triazole fungicides (dual-use pesticides and pharmaceuticals) in feed containing each at 23–35 μg/g (wet weight) (Konwick et al. 2006). These triazoles had log K_{ow}'s ranging from 2.9 to 3.9: bromuconazole, cyproconazole, metconazole, myclobutanil, penconazole, propiconazole, tebuconazole, tetraconazole, and triadimefon. Each compound was taken up quickly, reaching steady state after a day of exposure. They quickly reached concentrations in body lipids ranging from roughly 0.5–1 μg/g lipid, yielding biomagnification factors (on the basis of lipid content of fish vs. lipid content of food) ranging from 0.006 (propiconazole) to 0.012 (triadimefon and tebuconazole).

8.2.6.6 Macrocyclic Lactones

The macro-lactone dual-use parasiticides (used in veterinary medicine) are best known as the avermectins. Examples of these large, polycyclic lactones include: abamectin, albendazole, doramectin, emamectin, eprinomectin, ivermectin, morantel, moxidectin, milbemycin, and selamectin. Residues are well-established as occurring primarily in liver and lipid tissues, followed by kidney and muscle. An overview of this chemical class is provided by Danaher et al. (2006). Escher et al. (2007) point out that uptake kinetics and calculated BCFs are lower than predicted based on hydrophobicity. This is hypothesized to result from membrane exclusion because of the large molecular cross section.

Avermectin B1 (abamectin) was shown to resist uptake by sturgeon (into muscle) (Shen et al. 2005); biomagnification therefore would not occur. After a 22-day exposure to 0.2 and 1 ng/mL in water, concentrations in muscle reached steady state in about 2 weeks, giving muscle concentrations of 7.75 and 38.29 ng/g, respectively, yielding BCFs of 41–42.

Exposure for 28 days of bluegill sunfish (*L. macrochirus*) to an aqueous concentration of 0.099 μg/L avermectin B1a gave tissue concentrations of 6.8, 3.0, and 11 μg/kg, in whole fish, fillet, and viscera, respectively, yielding BCFs of 56, 28, and 84 (Van den Heuvel et al. 1996). As with other studies, it was concluded that abamectin does not strongly bioconcentrate and would therefore not be expected to biomagnify.

8.2.6.7 Steroids

Determining the uptake and bioconcentration of steroids is complicated by the fact that many of them have multiple origins. Several of the estrogens, for example, are endogenous to all fish (and some invertebrates but not plankton). Endogenous production can be further complicated by substances that induce synthesis (e.g., via aromatization) or inhibit excretion (e.g., repression of efflux pumps). But they also have at least two other origins. The first is the subject of this chapter—many have origins from the pharmaceutical preparations in which they are used; even β-estradiol is used in certain hormone preparations. The second origin is from other fish, which excrete a variety of steroids, for example as pheromones (Scott and Ellis 2007). These origins become intermingled with that resulting from endogenous synthesis; for estrogens and androgens, this becomes problematic for modeling female and male fish, respectively.

The steroids are also intimately involved in a metabolic cascade that involves interconversion, such as via aromatization, and conjugation. Uptake from surrounding media continually adds to the pool involved with natural metabolic processes. These factors greatly complicate the modeling

of uptake and bioconcentration. Given the dynamic state of uptake, interconversion, and excretion, steady-state concentrations are probably rarely reached in laboratory studies; life-cycle studies are rare. This leads to very wide ranges in both predicted and measured tissue concentrations and BCFs—measured both under controlled laboratory conditions and in the wild. An overview of the environmental occurrence and consequences of exposure of fish to natural and synthetic estrogenic chemicals (of which only a small portion are APIs) is provided by Tyler et al. (2008); further discussion on bioaccumulation of E2 and EE2 is provided by Langston et al. (2005). A method using gas chromatography/mass spectrometry was developed for simultaneously quantifying 12 endogenous steroids in plasma and bile, using flounders (*Platichthys flesus*) as the test species (Budzinski et al. 2006); the steroid analytes spanned the estrogen/androgen metabolic cascades from pregnenolone and progesterone to E2 and 11-ketotestosterone.

One major but very limited source of data on uptake and tissue levels of estrogenic and androgenic steroids is from the aquaculture literature. Steroids are used to induce sex reversal in farmed populations. These data are not covered here. The data of Stewart et al. (2001) serve as one example.

Rainbow trout (*O. mykiss*) and roach (*Rutilus rutilus*) exposed to treated sewage effluent in controlled continuous-flow tanks concentrated E1, E2, and EE2 in the bile—at levels beyond endogenous production (Gibson et al. 2005a, 2005b). Most was present as glucuronide conjugates. Bioconcentration was roughly 4000–6000 for EE2 and 10,000–13,000 for E2 and E1 combined. The conjugated equine estrogen (CEE) metabolite, 17β-dihydroequilenin (17β-Eqn), was also detected; while this is perhaps the first report of 17β-Eqn in an aquatic organism (or in any environmental sample), its specific source was unknown (e.g., whether an endogenous metabolic product vs. an ingredient from a conjugated equine hormone preparation).

This work on CEEs (Gibson et al. 2005a) was extended further in a comprehensive examination of treated and untreated sewage and fish exposed under controlled conditions (Tyler et al. 2009). Treated sewage from wastewater treatment plants (WWTPs) in the United Kingdom were examined for six CEEs: equilin (Eq) and equilenin (Eqn), and four metabolites, 17β-dihydroequilin (17β-Eq), 17α-dihydroequilin (17α-Eq), 17β-dihydroequilenin (17β-Eqn), and 17α-dihydroequilenin (17α-Eqn). The bile from two species of fish (rainbow trout and the common carp, *Cyprinus carpio*) exposed to treated sewage effluent was also analyzed. Among these six CEEs, only two (Eqn and its metabolite 17β-Eqn) were detected in wastewaters. Eqn concentrations ranged from 1.32 to 2.59 ng/L (influent) and 0.32–1.32 ng/L (effluent), and 17β-Eqn ranged from <0.2 (LOD) to 0.37 ng/L (influent) and 0.07–0.18 ng/L (effluent), concentrations on par with those of E2 and EE2. The authors pointed out that since these two CEEs occurred in sewage influent, their origin from hormone replacement products was more probable than from exogenous metabolic processes. 17β-Eqn (as in the prior study) and now Eqn (for the first time) were the only two CEEs detected in bile. BCFs for trout exposed to 17β-Eqn were calculated to be 1.5×10^6 and 2.2×10^6 for trout exposed to Eqn.

Notably, the study of Tyler et al. (2009) is one of the very few focusing on aquatic exposure to also extend its findings to the potential for effects from exposure to environmentally realistic concentrations. Concentrations of 17β-Eqn as low as 0.6 ng/L elicited a vitellogenic response in trout, as well as all but the lowest exposure concentration of Eqn (4.2 ng/L); the carp were nearly three orders of magnitude less sensitive. A 17β-Eqn concentration of 0.6 ng/L nearly intersects with its concentrations detected in the treated UK wastewaters, providing a rare linkage between real-world exposure levels and the potential for adverse effects.

The difficulty in tying exposure to effects is demonstrated in another unique study, involving fish showing signs of possible exposure to estrogens. Three separate projects involved male bream (*Abramis brama*) with ovotestis and vitellogenin from two different locations in the Netherlands and whitefish (*Coregonus lavaretus*) with malformed gonads from Lake Thun, Switzerland (Vögeli 2008). In the ovotestis case, while levels of E1, E2, and EE2 in adipose tissue did not differ from controls, levels of E1 and EE2 in bile showed bioaccumulation in the ovotestis fish; E2 did not differ from the control. In contrast, with the case of elevated vitellogenin, levels of E1, E2,

and EE2 were higher in the bile of the controls. With the group with malformed gonads, only E1 and E2 were present (above the MDLs) in the bile of all fish but the levels were higher in the fish with normal gonads.

In male Rainbow trout exposed to EE2 at relatively high nominal water levels of 125 ng/L, EE2 was shown to be rapidly absorbed (Skillman et al. 2006). EE2 was detected in plasma upon the first sampling time of 15 min and reached a steady-state range of 60–90 ng/mL within 16 h, yielding a BCF of up to 720. Levels in the liver corresponded with those in the plasma. In the bile, levels of free EE2 were also similar to those of the plasma and liver. In the bile, however, conjugated EE2 continued to increase, until 99% of the total EE2 in the bile comprised conjugated glucuronides. The authors conclude that EE2 in plasma, reaching equilibrium levels several hundred-fold higher than in water, represents a viable means for measuring current environmental levels; bile levels, in contrast, were more representative of cumulative exposure. The study also followed the parallel time course synthesis of vitellogenin and gene expression.

A model developed by Lai et al. (2002b) predicted relative bioconcentration of steroids, ranging from fish at the highest trophic level (1.8 for E3) to fish at the lowest trophic level (332 for EE2). In another study (Lai et al. 2002a) examined the uptake of natural (E1, E2, hydroxyestrone, and E3) and synthetic (EE2 and estradiol valerate) estrogens by the freshwater alga, *Chlorella vulgaris*. Under static conditions, all the estrogens were taken up, but E3, hydroxyestrone, E2, and EE2 could not be detected, because of metabolism. No equilibria could be reached, except for E1, for which a BCF of about 27 was calculated. On the basis of K_{ow}, EE2 would be expected to accumulate more, but did not—possibly because of active transport of the endogenous estrogens or active efflux of EE2.

Juvenile rainbow trout (*O. mykiss*), under controlled conditions, were exposed to sewage under continuous flow before and after treatment by sand filtration (Pettersson et al. 2006). After 28 days, bile was sampled. When exposed to untreated water, levels of E1 were two orders of magnitude higher than in controls (4.0 μg/g vs. 0.04 μg/g). Bile levels were also higher compared with controls for EE2 (0.25 μg/g vs. 0.10 μg/g) and E2 (0.17 μg/g vs. 0.04 μg/g). When exposed to treated water (posts and filtration), the bile concentrations for E1 (0.17 μg/g) and E2 (0.04 μg/g) were reduced considerably. The concentration for EE2, however, was slightly higher (0.38 μg/g).

In a subsequent study, Pettersson et al. (2007) examined the bile of perch (*Perca fluviatilis* L.) from the coastal waters of the Swedish Baltic Sea impacted by sewage for E1, E2, and EE2. Studies of fish from the wild are uncommon. EE2 was never detected, in contrast with E1 and E2, which were almost always present. These levels did not differ significantly from samples obtained from reference sites. These findings, however, corroborated lack of signs of endocrine effects, possibly because of efficient sewage treatment practices.

In another study using fish in the native ambient environment, Vermeirssen et al. (2005) used caged brown trout downstream of sewage effluents at five sampling sites. They measured E2, E1, and EE2 but did not report them separately—only as estradiol equivalents. Houtman et al. (2004) also indirectly measured EE2 via estrogen assay (in the bile of male bream, *A. brama*).

In a study of juvenile bull sharks (*C. leucas*) in the Caloosahatchee River, Florida, EE2 was detected in plasma at levels only up to slightly above the MDL (Gelsleichter 2009). EE2 was detected only during the second of 2 years of sampling (2006–2007), being detected in 7 of the 12 sharks sampled; EE2 was not detected in the Myakka River, a control river that did not receive treated wastewater. Levels of EE2 in the river ranged only up to 0.23 ng/L. Of the seven shark plasma samples with detectable residues, the two that could be quantified ranged up to 3.79 ng/mL plasma.

Perhaps the first bioconcentration study of EE2 in fish was reported by Larsson et al. (1999). Caged juvenile rainbow trout (*O. mykiss*) were exposed to an effluent-dominated stream, and E1, E2, and EE2 were measured in bile. The respective concentrations (conjugated and unconjugated combined) for E1, E2, and EE2 in the bile after 2 and 4 weeks were (approximately): 0.6 and 2.5 μg/g; ND and 1.0 μg/g; and 0.3 and 1.1 μg/g. These bile concentrations of roughly 1 ppm were about 4–6 orders of magnitude higher than the water levels. A separate static study using juvenile rainbow

trout exposed for 46 h to 5 μg/L of either E1, E2, or EE2 produced respective bile concentrations of >400, ~200, and ~350 μg/g.

Perhaps the first full life-cycle bioconcentration study of EE2 in fish was reported by Lange et al. (2001). A life-cycle exposure study, using the fathead minnow (*Pimephales promelas*) was done using newly fertilized embryos (24-h old) under continuous flow for 305 days at five concentrations of EE2: 0.2, 1.0, 4.0, 16, and 64 ng/L (Lange et al. 2001); note that the two highest concentrations were toxic. No EE2 could be detected in tissue (<0.38 ng/g) after exposure at 0.2 and 1.0 ng/L test concentrations 192 days posthatch. At 16 ng/L (239 days posthatch) and 64 ng/L (153 days posthatch), the EE2 tissue levels were 7.3 and 31 ng/g, yielding BCFs of 610 and 660, respectively. The authors concluded that the BCF (L/kg) was likely less than 500 (and probably less than 2400) for healthy fish. A more recent study by Caldwell et al. (2008) provided an HC5 value (hazardous concentration predicted to negatively affect 5% of the population) of 0.343 ng/L for a species sensitivity distribution (SSD) of EE2, highlighting the high potency of this API.

The bioconcentration of steroids is yet further complicated by the possibility that uptake is being augmented by facilitated transport to yield tissue levels far beyond what would be predicted with existing models assuming passive brachial uptake.

A study using the three-spined stickleback (*Gasterosteus aculeatus*) used 6-day static exposure concentrations of 1 μg/L (nominal) of either E2, testosterone (T), or E2 and T combined (Maunder et al. 2007). Plasma levels climbed rapidly within the first 6 h to within the range of 20–90 ng/mL. These bioconcentrated levels were 50-fold (E2) and 200-fold (T) greater than the measured exposure concentrations. The authors postulated that the faster and greater uptake than predicted of E2 and T might be due to the presence of a plasma sex hormone-binding globulin (SHBG). Scott et al. (2005) also postulated that SHBG is responsible for enhanced uptake of many of the steroids. This hypothesis is set forth in more detail by Miguel-Queralt and Hammond (2008).

Miguel-Queralt and Hammond (2008) report that natural and synthetic estrogens and androgens are actively taken up by fish via the gills by way of binding to SHBG in the brachial filaments. This uptake mechanism is extremely fast, with up to 70% of T or EE2 being removed from water in 90 min. A broad range of steroids have a high affinity for fish SHBG, whose ligand specificity varies widely across species. Trace amounts (e.g., 50 pmol) of ligand can be taken up from water within minutes. After uptake, residues are rapidly distributed throughout the body; EE2 was reported to then accumulate in the brain, ovaries/eggs, and muscle. The authors point out that SHBG also has a high affinity for at least two of the more common progestin APIs—levonorgestrel and 19-norethindrone. Since these APIs may be frequently present in sewage-derived waters, sometimes at relatively high concentrations, this points to the possibility of progestins occurring in fish. Progestins, however, have only rarely been targeted in environmental monitoring. Sediments in Puget Sound were analyzed for the synthetic progestogen 19-norethindrone. Levels ranged from 419 to 890 ng/g, but the analysis was done with GC-FID rather than GC-MS (Kimball 2008). 19-Norethindrone was the most frequently detected and abundant (26–224 ng/L) of all the synthetic estrogens/progesterones in sewage influent samples (Fernandez et al. 2007). López de Alda et al. (2002) reported 19-norethindrone as frequently occurring in sediments but at low ng/g-levels. Viglino et al. (2008) reported levonorgestrel and 19-norethindrone concentrations in sewage effluent ranging between 30 and 53 ng/L, respectively.

Others postulate that steroid residues in food may contribute more to bioaccumulation by fish than do the residues at significantly lower concentrations in water. Takahashi et al. (2003) report E2 concentrations ranging from 0.0001 to 0.0076 μg/L in water, compared with 0.09–2.26 μg/kg-wet in the periphytons and less than 0.01–0.22 μg/kg-wet in the benthos. Bioaccumulation factors of E2 were estimated at 64–1200 for the periphyton and 100–160 for the benthos.

It is important to note that even though the BCFs for EE2 do not indicate a propensity for bioaccumulation, the extremely low no-effect levels for this steroid have led a number of investigators to recommend more detailed examinations (e.g., Lyssimachou and Arukwe 2007).

Direct uptake from water of E1 by *Daphnia magna* gave a BCF of 228 (Gomes et al. 2004); biomagnification via feeding on *C. vulgaris* was not as efficient.

In a controlled study using artificial sediment and radiolabeled EE2, a benthic freshwater oligochaete (*Lumbriculus variegatus*) was exposed over 35 days to a nominal concentration of 300 ng/g wet-weight sediment (556 ng/g dry weight) (Liebig et al. 2005). Continuous linear uptake never reached steady state. The BSAF was 75 after the 35 days. A calculated steady state (after 360 days of exposure) would yield a BSAF of 190—higher than predicted by K_{ow}. A study with two invertebrates—a midge (*Chironomus tentans*) and amphipod (*Hyalella azteca*)—followed 21-day EE2 exposures using spiked water and water with sediments (Dussault et al. 2009). The exposure concentrations, however, ranged up to 3.1 ppm, orders of magnitude higher than those found in the ambient environment. At one of the lower, but still high, water-only exposures (20 μg/L), the BCFs were 31 for *C. tentans* and 142 for *H. azteca*; BSAFs were 0.8 and 1.5, respectively.

8.2.6.8 Antibiotics: Informing Environmental Exposure with Data from Use of Veterinary Aquaculture Drugs

APIs are used in aquaculture at levels many orders of magnitude higher than their occurrence in the ambient environment. Exposure data in aquaculture settings are obtained usually to assess if therapeutic or prophylactic doses are reached and to assess subsequent depuration of residues to ensure consumer safety. For this reason, the exposure concentrations are orders of magnitude higher than ambient levels, and the antibiotics studied tend to be restricted to those used in veterinary practice (although use of unapproved, illegal drugs also occurs). An overview of antibiotics used in aquaculture is provided by Sapkota et al. (2008). In a Canadian Total Diet Study focused on residues of 39 different veterinary drugs, levels tended to be in the range of low nanograms per gram (Tittlemier et al. 2007).

Even though exposures emulating those during aquaculture occur at higher ambient levels of APIs, they might be useful as worst-case scenarios to inform the potential for bioconcentration under ambient conditions. As one example, trout raised in aquaculture receiving medicated feed with roughly 0.6% oxytetracycline (OTC), which yielded a maximum water concentration of about 0.8 ppm, reached a maximum muscle-tissue concentration of 1.8 ppm (Bebak-Williams et al. 2002). This maximum level rapidly dissipated once the aqueous concentration dissipated. This shows that at high exposure concentrations, the muscle-tissue level shows very little bioconcentration. The literature on veterinary drug exposure is comparatively large, just two examples being Hou et al. (2003) and Chu et al. (2008), who examined the uptake into muscle and depuration of sulfamethazine and nitrofurans.

The study of aquatic exposure to APIs actually began several decades ago. The study of antibiotics used in aquaculture led to the need for examining aquatic tissue levels to assess therapeutic dose levels while assuring levels were sufficiently low for human consumption via the food supply. But even then, the potential for environmental impacts was also a consideration; the early work of Coats et al. (1976) using model ecosystems is an example.

Early studies on the environmental fate and possible biomagnification of veterinary drugs, particularly parasiticides, antibiotics, and other biocides, began in the 1960s and 1970s. Many of these studies were comprehensive and generated considerable data, as they used traditional radiolabeled materials to try and reach closure around mass balances. For example, 3-day uptake in fish of four veterinary drugs was studied in aquatic model ecosystems, using radiolabeled anthelmintic phenothiazine, the coccidiostat clopidol, the bacteriostat sulfamethazine, and the growth promoter diethylstilbestrol (Coats et al. 1976).

Another route of exposure as a result of aquaculture, however, occurs because 70–80% of the APIs used in medicated feed are released to the ambient environment as a result of excretion or escape by way of feed that is not consumed (Pouliquen et al. 2009). Native fish in the vicinity can then be unintentionally exposed—to levels exceeding ambient background concentrations. Usage of antibiotics in aquaculture, however, is episodic and occurs for very limited number of days, but concentrations in sediments immediately below can exceed the ppm-level (Pouliquen et al. 2009). Samuelson et al. (1992) reported that levels of several antibiotics in aquatic organisms nearby aquaculture exceeded levels considered safe for human consumption; also see Cabello (2006).

Blue mussels (*Mytilus edulis*) were evaluated for their ability to bioconcentrate two veterinary antibiotics: OA and OTC (Le Bris and Pouliquen 2004). Exposure concentrations were intended to emulate unintended exposure by what might be encountered near aquaculture. Exposure concentrations were roughly 0.95 mg/L for OTC and 1.46 mg/L for OA. Uptake was determined for foot, muscle, mantle, viscera, gills, and shell. OTC concentrations were higher in viscera (1.83 mg/kg) than gills (0.37 mg/L), with other parts less than 0.2 mg/kg. OA concentrations were highest in gills (0.79 mg/kg) followed by shell (0.19 mg/kg). BAFs less than 1.0 were expected for these two highly ionized APIs.

Nie et al. (2008) found the bioaccumulation of ciprofloxacin by carp (*Allogynogenetic crucian*) under controlled feeding conditions to vary greatly, depending on several exposure scenarios. Feeding resulted in much higher residues (in visceral and muscle tissues) than via exposure to water. Uptake was fast, with maximum levels being reached within a day. The tissue concentrations (μg/kg) resulting from each type of exposure were: water (muscle: 10; viscera: 42); feeding (muscle: 73; viscera: 645); and dual exposure (muscle: 43; viscera: 368).

8.2.6.9 Carbamazepine (CBZ)

Juvenile rainbow trout were exposed under continuous flow for 96 h to 200 μg/L CBZ (Huggett et al. 2004). After 24 h of exposure, the plasma concentrations of CBZ decreased, beginning at about 2.5 ng/mL and ending at less than 1 ng/mL, showing a low propensity to bioconcentrate. See the results for CBZ published by Ramirez (2007), Ramirez et al. (2007), and Zhou et al. (2008) summarized under the section "Multianalyte Studies." In those studies, CBZ was also shown to poorly bioconcentrate, having a low BCF (<1).

After a 60-day exposure to a high 19-ppm concentration of CBZ, no intracellular accumulation could be detected in the algae *Ankistrodesmus braunii* (Andreozzi et al. 2002). A method developed for determining CBZ in tissues was used to analyze a crustacean (*Thamnocephalus platyurus*) after it fed on algae (*Pseudokirchneriella subcapitata*) that had been previously exposed to CBZ at 250 mg/L (Lajeunesse et al. 2009). The mean CBZ concentration in dried *T. platyurus* was 129 (±57) μg/mg.

8.2.6.10 Triclosan (and Methyl Triclosan) and Triclocarban

With respect to the most heavily used biocides, triclosan [TCS: 5-chloro-2-(2,4-dichlorophenoxy)phenol] has been studied more frequently than triclocarban [TCC: N-(4-chlorophenyl)-N′-(3,4-dichlorophenyl)urea]. In general, the transformation of triclosan to the more lipophilic methyl triclosan (MTCS: 4-chloro-1-(2,4-dichlorophenoxy)-2-methoxybenzene) leads to lower tissue levels of TCS compared with MTCS. Most of the research has been conducted in Europe and Scandinavia, with very recent studies in the United States (e.g., Leiker et al. 2009). Tissue residue levels of MTCS generally exceed those of any API—a result of higher BCFs and higher exposure levels.

MTCS was first identified in fish by Miyazaki et al. (1984). Up to 38 ng/g was detected in the whole bodies of a freshwater fish (*Carassius carassius*) collected from Tama River, Tokyo Bay. Samsøe-Petersen et al. (2003) report on a monitoring study that sampled various aquatic species from 12 locations in Sweden, where concentrations ranged from less than 0.1 to 13 μg/kg (wet weight). Much lower concentrations (in blood plasma) were reported in perhaps the first study from the United States, where Alaee et al. (2003) reported on fish from the Detroit River (Michigan-Ontario) having TCS in the blood plasma of all 13 species surveyed; levels ranged from 0.61 ng/g wet weight (brown bullhead) to 10.4 ng/g (white bass). In contrast, MTCS was detected in the plasma of all 13 species but ranged only from 0.0004 ng/g for common carp to 0.0132 ng/g for largemouth bass; the presence of TCS at 3 orders of magnitude higher concentration than MTCS was ascribed to the higher lipophilicity of MTSC and its possible preferential partitioning to lipid tissue.

Bile was analyzed for TCS in fish subjected to various exposure scenarios involving three WWTPs in Sweden, ranging from caged within effluent-dominated flows, to wild (or directly exposed to sewage under controlled compositions) (Adolfsson-Erici et al. 2002). Concentrations

ranged from 0.24 to 4.4 mg/kg bile (for wild fish) to 34–120 mg/kg (for those exposed directly to treated sewage effluent).

Orvos et al. (2002) assessed TCS bioconcentration in zebrafish (*Danio rerio*) using continuous flow with 3 and 30 μg/L for 5 weeks followed by 2 weeks of depuration. During the 5-week exposure, the BCFs ranged between 2000 and less than 3500 for the 39-μg/L exposure and from 3500 to about 5200 for the 3-μg/L exposure, giving average BCFs during the exposure period of 4157 at 3 μg/L and 2532 at 30 μg/L. BCFs for head/scale and fillet ranged from about 1000 to 2000, whereas they ranged from about 8000 to 11,000 in the intestines for the low and high exposures, respectively. After depuration, the BCFs were 30 and 41 for the high and low exposures, respectively, so half-life residence time within the body was short compared with POPs.

Boehmer et al. (2004) performed a rare 10-year retrospective study (1994–2003) of breams (*A. brama*) from representative German rivers. The study revealed that TCS was rarely present in muscle while MTCS was detected in all specimens collected. In general, when present, TCS muscle-tissue concentrations remained relatively constant but low for any given river—less than 1 ng/g wet weight. TCS concentrations were always lower than MTCS, which had excursions above 30 ng/g wet-weight muscle.

Balmer et al. (2004) reported MTCS in fish (white fish, *Coregonus* sp.; roach, *R. rutilus*) from various lakes in Switzerland receiving treated sewage effluents. Concentrations ranged up to 35 ng/g (wet weight) or 365 ng/g (lipid basis) and fell within narrow ranges for a given lake. In another study of Swiss lakes, Balmer et al. (2005) measured lipid levels of MTCS in fish lipids, where levels (ng/g) ranged from undetectable (perch) and 4–233 (roach), to 4–56 (whitefish). Buser et al. (2006) examined the muscle tissue of brown trout (*Salmo trutta fario*), from seven Swiss rivers that receive treated sewage effluent, for MTCS. All concentrations were higher than those reported by Balmer et al. (2005) for lake fish (white fish, *Coregonus* sp. and roach, *R. rutilus*). Concentrations ranged from 130 to 2100 ng/g, compared with the previous lake fish study of 4–370 ng/g. The concentrations for river fish had considerable variation, possibly due to a more fluctuating input from sewage; river fish had higher concentrations probably because the exposure levels were higher.

In a recent study, a survey of common carp from Las Vegas Bay revealed MTCS (but not TCS) in all 29 male common carp at a mean whole-body concentration of 600 μg/kg wet weight (7400 μg/kg on the basis of lipid, giving a BCF of 1.8×10^5) (Leiker et al. 2009). Three chlorinated analogs (3- and 5-chloromethoxy triclosan and 3,5-dichloromethoxy triclosan) were also present but less often and at lower concentrations, ranging from 0.5 to 13 μg/kg in 21–76% of the samples; the brominated analog (bromomethoxy triclosan) was detected but not quantified.

In contrast with MTCS, reported TCS levels are not as common and almost always lower (with the exception of blood plasma). The average TCS accumulation factor for zebrafish over a 5-week test period was 4157 at 3 mg/L and 2532 at 30 mg/L (Orvos et al. 2002). TCS concentrations were highest in the digestive tract; head and muscle concentrations were similar. Following a 2-week depuration, the average BAF was 41 for 3 mg/L exposure and 32 for 30 mg/L exposure. The BCF was predicted to be roughly 2500.

Houtman et al. (2004) identified TCS at ppm levels in the nonpolar residual fraction of bile from wild fish in the Netherlands. Bile concentrations were about 14 μg/mL for fish from the North Sea Canal and 80 μg/mL for fish from the River Dommel.

In a rare cross-species survey, TCS and MTCS were measured in the blood plasma of 13 species of fish (both benthic and pelagic) from a stretch of the "highly contaminated" Detroit River (Valters et al. 2005). TCS levels ranged from 0.750 to 10.0 ng/g, while MTCS was present at 0.4–13.4 pg/L, 3 orders of magnitude lower. TCS in the estuarine water samples averaged 7.5 ng/L, although the tissue and water sampling were temporally disconnected. Another feature of this study was the parallel analyses for a spectrum of brominated diphenyl ethers.

Algae were shown to bioconcentrate TCS, MTCS, and TCC (Coogan et al. 2007) by roughly 3 orders of magnitude when collected from Pecan Creek, the same effluent-dominated stream in north Texas, U.S., previously studied by Brooks et al. (2005) and Ramirez et al. (2007). This may

be the first report of the bioconcentration of any of these three chemicals in algae; it may also be the first report of the bioconcentration of TCC by any organism. Dissolved concentration ranges (and algal wet-weight bioconcentration ranges and BAFs, L/kg) from four sampling sites for each of the three analytes were: TCS levels of <10–120 ng/L (<10–146 μg/L; BAFs nil-2100); MTCS levels of <5–80 ng/L (<5–89 μg/L; BAFs nil-1500); and TCC levels of <15–190 ng/L (<10–401 μg/L; BAFs nil-2700).

Coogan and La Point (2008) extended these initial algal bioconcentration studies to examine snail (*Helisoma trivolvis*) bioaccumulation of TCS, MTCS, and TCC from the effluent outfall to Pecan Creek. Dissolved concentration ranges (and snail wet-weight bioconcentration level and BAFs) were: TCS level of 112 ng/L (58.7 μg/L; BAF 500); MTCS level of 41 ng/L (49.8 μg/L; 1200); and TCC 191 ng/L (299 μg/L; 1600). Bioaccumulation of antimicrobials has been observed in other macroinvertebrates; adult grass shrimp accumulated MTCS after a 14-day exposure to 100 μg/L TCS (Delorenzo et al. 2008).

More recently, Mottaleb et al. (2009) reported mean (n = 11, ±SD) TCS levels at 21 ng/g (±4) in *L. macrochirus* (bluegill) from Pecan Creek, Texas. Although TCS was not detected in fish (Sonora sucker) collected from a relatively pristine location in the East Fork Gila River, New Mexico, TCS was detected at 12 ng/g in bluegill from Clear Creek, Texas, a regional reference site studied by Brooks et al. (2005) and Ramirez et al. (2007). This site does not receive point-source municipal effluent, but may be influenced by onsite wastewater. In the Mottaleb et al. (2009) study, fish samples examined from Pecan Creek were the same organisms analyzed previously for target APIs by Ramirez et al. (2007).

The most in-depth controlled study of TCC involved its uptake from sediments by the freshwater oligochaete *L. variegatus* (Higgins et al. 2009); depuration was also studied. TCC BSAFs were calculated and determined empirically during a 56-day study. Sediment spiked with TCC maintained a constant (and environmentally relevant) concentration over 56 days (22.4 ± 7.6 μg/g dry weight); the TCC concentration in the surrounding water also maintained constant, at 820 ± 220 ng/L. Uptake by *L. variegatus* was rapid, reaching a maximum of 1310 ± 60 μg/g lipid or 42 ± 2 μg/g wet weight at 5 days, after which levels began to decline. Bioaccumulation comported with predictions from conventional models. Depuration was rapid. After 21 days in clean sediment, the TCC concentration in *L. variegatus* had declined to 9.6 ± 0.3 μg/g lipid (0.31 ± 0.01 μg/g wet weight). The BSAF ([mass of sediment organic carbon]/[mass of tissue lipid organic carbon]) after 56 days was calculated as 1.6 ± 0.6.

In a very rare study of higher-tropic-level aquatic wildlife, triclosan was measured for the first time in a marine mammal—bottlenose dolphins (*Tursiops truncatus*) from two estuarine sites (Charleston, South Carolina, and Indian River Lagoon, Florida) (Fair et al. 2009). Both sites are influenced by discharged treated wastewaters. Blood-plasma levels of TCS for one site ranged from 0.12 to 0.27 ng/g (with 4 of the 13 having levels exceeding the MDL of 0.033 ng/g), and for the other site ranged from 0.085 to 0.106 (with 3 of 13 having detectable levels). These are possibly the highest plasma levels yet reported for any aquatic organism. TCS levels in the respective waters for the two sites averaged 7.5 ng/L, with a maximum of 13.7 ng/L.

8.2.6.11 Miscellaneous APIs

In perhaps its first reported occurrence in fish from the wild, diazepam was quantified in liver samples from 10 hornyhead turbot (*Pleuronichthys verticalis*) collected near MWTP ocean discharges in southern California (Kwon et al. 2009). The levels in five females ranged from 23 to 45 ng/g (wet weight) and in five males from 58 to 110 ng/g (wet weight); EE2, CBZ, simvastatin, and oxybenzone were also targeted but not detected.

Malachite green is a multiuse chemical. Although it has useful properties in aquaculture, its use in food is prohibited worldwide (see Sudova et al. 2007); nonetheless, it still experiences clandestine use in aquaculture and can be used legally for ornamental fish. Because it is a chromophore, it also has a variety of other commercial uses unrelated to veterinary medicine—particularly as a dye.

Malachite green bioconcentrates readily in the lipid of aquatic organisms, primarily as its metabolite leuco malachite green, which occurs at a ratio of 5–7:1. It persists in tissues, being found in the highest concentration in the liver. Schuetze et al. (2008) documented the occurrence of malachite green in the European eel (*Anguilla anguilla* L.) from lakes, rivers, and a canal in Berlin, Germany. Total concentrations of malachite green and the leuco form ranged up to 0.765 μg/kg (wet weight) in 25 of the 45 eels collected. Exposure was concluded to result from treated sewage. Although some of the bioconcentrated residue may have come from the use of malachite green for illegal and legal treatment of fish, an unknown but possibly large portion undoubtedly resulted from other commercial uses, such as dyed textiles.

8.2.6.12 API Disinfection By-Products (DBPs) and Metabolites

Chlorination of either drinking water or wastewater containing steroids is known to produce mono- and di-chlorinated products of varying estrogenic activity. Little has been published on DBPs from APIs. In the presence of bromide, which often occurs in surface waters and wastewaters, multiply-brominated analogs can be formed (Lu and Korshin 2008). In particular, Lu and Korshin (2008) demonstrated the formation of stable dibromo-EE2. Buth et al. (2007) identified a number of products from the reaction of cimetidine with chlorine; Dodd and Huang (2004) identified products from sulfamethoxazole; and DellaGreca et al. (2009) identified various chlorinated and nonchlorinated products from atenolol. Similarly, Nakamura et al. (2007) identified a number of chlorinated estrones. The bioaccumulative potential for these reaction products is unknown. Similar issues surround the complex array of potential metabolites and other transformation products from parent APIs; many examples are reviewed by Farré et al. (2008) and by Kosjek and Heath (2008). Little work has been published on the possible metabolites from aquatic organisms. The recent work of Mehinto et al. (2010) revealed some possible metabolites from diclofenac in fish.

Despite an increasing number of studies on API DBPs and other transformation products, there are very few studies regarding their uptake by aquatic organisms. In one of the only such studies, roach (*R. rutilus*) were exposed for 5 days in an aquarium filled with drinking water and spiked with EE2 at a nominal concentration of 30 ng/L; the measurable concentration in the test situation, however, was below the limit of detection (0.6 ng/L) (Flores and Hill 2008). EE2 was found to be rapidly brominated (yielding mono- and di-brominated EE2). Di-brominated EE2 (but no detectable mono-brominated EE2) accumulated in the ovaries and liver to levels 18- to 67-fold greater than the parent EE2. Concentrations (ng/g wet weight) of EE2 and dibromo-EE2 detected were: liver (EE2: 2.7 and dibromo-EE2: 92.3) and ovaries (EE2: 0.2 and dibromo-EE2: 2.3), yielding a BCF for the ovaries of 130 and for the liver of 7894.

8.2.7 Uptake by Aquatic Plants and Aerial Invertebrates

The uptake of APIs by plants and algae, which compose an important part of the aquatic food chain, might prove a significant part of dietary exposure. Indeed, uptake of APIs by certain plants is so efficient that they have been evaluated for *in-situ* phytoremediation of contaminated waters and sediments (e.g., Forni et al. 2002). Plant uptake has been particularly germane to aquaculture sites.

A discrete body of work has been published on the uptake of APIs by aquatic plants. The aquatic bryophyte *Fontinalis antipyretica* is known to bioconcentrate metals, pesticides, and PAHs and has therefore been used *in situ* as a bioindicator for integrative monitoring. A study of the uptake of three antibiotics widely used in aquaculture (OA, flumequine, and OTC) showed BCFs ranging from 75 (flumequine) to 450 (OTC) (Delepee et al. 2004). These antibiotics had mean tissue residence times of 18 and 59 days. The study was conducted at relatively high concentrations of 100 and 1000 ppb. BCFs were higher at the lower concentrations and were an inverse function of K_{ow}—increasing according to ionization instead of lipophilicity.

In a study of transpiration stream concentration factors (TSCFs) versus polarity, Dettenmaier et al. (2009) showed that polar but nonionizable, highly water soluble organic compounds can be

easily taken up by plant roots and translocated to shoot tissue. Studies on uptake of APIs by plants (primarily bryophyte) have generally revealed rather high levels, and sometimes the source was not necessarily related to aquaculture, as upstream samples have at times shown similar levels. Pouliquen et al. (2009) examined bryophytes as biomonitors downstream of aquaculture and sewage. They reported maximum tissue concentrations (ng/g) for OA (47), flumequine (~600), OTC (1200), and florfenicol (513).

Migliore et al. (2000) exposed an aquatic weed (*Lythrum salicaria* L.) to flumequine. After 35 days, the dry-weight tissue concentrations were in the ppm range: 13.3, 8.7, 0.7, 0.3, and 0.2 μg/g at flumequine aqueous concentrations of 5000, 1000, 500, 100, and 50 μg/L, respectively. Exposure of an aquatic fern (*Azolla filiculoides* Lam.) to sulfadimethoxine for 5 weeks at concentrations of 50, 150, 300, and 450 mg/L resulted in uptake at the mg/g dry-weight level (1000 ppm) (Forni et al. 2002). Typha was shown to rapidly absorb clofibric acid at 20 μg/L, removing more than 50% within 48 h (Dordio et al. 2008).

Redshaw et al. (2008) recently used a *Brassicaceae* (cauliflower) model to examine fluoxetine uptake by plants. Following a 12-week exposure to 280 μg/L fluoxetine in growth media, fluoxetine concentrations were higher in the stems (0.49 μg/g wet weight) than in leaf tissues (0.26 μg/g wet weight) of *Brassicaceae*. This study did not examine steady-state tissue levels of fluoxetine; this is important because fluoxetine is photolabile and should have degraded over the 12-week study period (Redshaw et al. 2008). However, presence of low μg/g-levels of fluoxetine suggest bioconcentration, which did not correlate with lipid content in leaf and stem tissues of *Brassicaceae*. Although cauliflowers are terrestrial plants and this study was specifically interested in estimating potential fluoxetine uptake in terrestrial plants exposed to biosolid-amended soils, it suggests that fluoxetine accumulation by nonrooted aquatic macrophytes such as *Lemna* sp. should be considered (Redshaw et al. 2008).

Coexposure to APIs will often occur with varying nutrient ratios and stoichiometries. Nutrient enrichment was previously demonstrated to influence the magnitude of triclosan toxicity to *L. gibba*, for both traditional morphometric endpoints (Fulton et al. 2009) and nontraditional responses, such as internal C:N:P and nitrate uptake kinetics (Fulton et al. 2010). Because nutrient stoichiometry can also influence internal lipid metabolism and concentrations in plants and algae, site-specific nutrient enrichment differences may result in differential bioconcentration of APIs (Fulton et al. 2010).

The potential for trophic transfer of APIs out of the aquatic realm was recently shown by Park et al. (2009). EE2 was determined in aerial invertebrates (primarily Diptera) whose larval stages develop in STP percolating filter beds. EE2 concentrations in insects captured near STPs were significantly higher than in those over 2 km away. The median EE2 tissue concentration was 42 ng/g (with the 75th percentile 140 ng/g) from insects near the STPs, compared with a median level of less than 3 ng/g (and 9 ng/g 75th percentile level) detected in the insects more distant from the STPs. Further transfer to insectivorous bats and birds was postulated. Rough calculations estimated that daily exposure to EE2 for bats feeding on insects near the STPs could range from 9 to 159 ng/g.

8.2.8 Multianalyte Studies

Studies that target multiple APIs to gauge ambient exposure are indeed rare. The recent study of Schultz et al. (2010) targeting 10 antidepressants (including two metabolic/transformation products) was discussed earlier. The first and most comprehensive multianalyte study to date on fish tissue was by Ramirez et al. (2007). From a target list of 23 APIs and 2 metabolites, only four were reported as being detected. Fish (*Lepomis* sp.) were sampled in Texas from an effluent-dominated stream ($n = 11$) and from another creek ($n = 20$) that served as reference. The four APIs were detected in muscle from all samples in the study site. The range (and mean; ng/g wet weight) were: diphenhydramine [0.66–1.32 (0.96)]; diltiazem [0.11–0.27 (0.21)]; CBZ [0.83–1.44 (1.16)]; and norfluoxetine [3.49–5.14 (4.37)]. This is the first report of diphenhydramine, diltiazem, and CBZ in wild fish.

With impetus provided by the Brooks et al. (2005) study, the U.S. EPA initiated the National Pilot Project of pharmaceuticals and PCPs (PPCPs) in Fish Tissue (U.S. EPA 2008a), which represents the first national-scale reconnaissance study of PPCPs in fish tissue (Ramirez 2007, Ramirez et al. 2009). Sample collection and processing procedures followed approaches previously used during the U.S. EPA's National Study of Chemical Residues in Lake Fish Tissue. Analytical methods for PPCPs in the National Pilot Project employed approaches previously developed by Ramirez et al. (2007) and Mottaleb et al. (2009). Because effluent-dominated and effluent-dependent ecosystems represent worst-case scenarios for API exposure (Brooks et al. 2006), five effluent-dominated river systems were selected for study: Phoenix, AZ; Orlando, FL; Chicago, IL; West Chester, PA; and Dallas, TX. The Gila River, NM was selected as a reference site for this study. Ramirez et al. (2009) targeted 25 APIs/metabolites in the fillets and livers from wild-caught fish: acetaminophen, atenolol, caffeine, *CBZ**, cimetidine, clofibric acid, codeine, *diltiazem**, 1,7-dimethylxanthine, *diphenhydramine**, erythromycin, fluoxetine*, gemfibrozil*, ibuprofen, lincomycin, metoprolol, miconazole, norfluoxetine*, propranolol, sertraline*, sulfamethoxazole, thiabendazole, trimethoprim, tylosin, *warfarin*. The four shown in italics had MDLs below 1 ng/g. The seven with asterisks were detected in multiple fish from multiple locations. Of these seven that were detected, three had the lowest MDLs (CBZ, diltiazem, and diphenhydramine) in both fillet (less than 1 ng/g) and liver (less than 2 ng/g), while only two (fluoxetine and gemfibrozil) were among those with the highest MDLs (greater than 6 ng/g in fillet and 12 ng/g in liver). Of the seven APIs with the highest MDLs, only one (gemfibrozil) was detected.

At only one of the five sites, receiving effluent from a sewage treatment facility using tertiary treatment, none of the target APIs was detected in the fillet from any fish; also no API was detected in any fish from a nonimpacted reference site. All of the seven APIs detected among the 25 targeted APIs were detected in fish from only one site, which received effluent from secondary treatment. Mean concentrations in fillet for all the detected APIs were generally less than 3 ng/g and ranged from 0.04 to 11 ng/g. The majority of the mean concentrations in liver for all the detected APIs were generally greater than 6 ng/g and ranged from 0.03 to a high of 380 ng/g (sertraline). Except for one site where fluoxetine was found in fillet but not in liver (and where the liver also contained substantially more norfluoxetine), the API concentrations in livers were always larger—by several fold or by over one order of magnitude. Of significance, API concentrations did not correlate with lipid content—a finding shared with other published studies.

Another study targeted five APIs during the course of ground truthing a new *in vivo* tissue sampling method using implanted solid-phase microextraction (SPME) fibers (Zhou et al. 2008). The targeted APIs were the ones previously reported by Ramirez et al. (2007): diltiazem, diphenhydramine, CBZ, and norfluoxetine. Under controlled exposure conditions, rainbow trout (*O. mykiss*) gave BCFs for CBZ in muscle after 7- and 14-day exposures of 0.44 and 0.22, respectively. Significantly, free and total tissue levels after 14 days were lower than those after 7 days. The authors postulated that CBZ metabolism was upregulated during the exposure time. The bioconcentration of fluoxetine differed markedly. While the respective free concentrations in muscle after 7 and 14 days of exposure were only 0.30 and 0.65 times those in the aqueous media, the BCFs for total fluoxetine in muscle were 62 and 84, respectively. The same approach was used for determining muscle levels of free API in wild fish captured from streams that received treated sewage. These are the only reports of "free" APIs in aquatic tissues. In the wild fish, only diltiazem and diphenhydramine were detected. Free diltiazem muscle concentrations were 2.04 and 5.69 pg/g in the white sucker (*C. commersoni*) and Johnny darter (*Etheostoma nigrum*), respectively. Free diphenhydramine concentrations were 32.0 and 81.6 pg/g for white sucker and Johnny darter, respectively. These concentrations are several orders of magnitude lower than the conventional "total" levels reported by all previous investigations.

A more recent study (Fick et al. 2010), which was built on previous efforts by Brown et al. (2007), exposed rainbow trout to final treated effluent for 14 d at three different sites in Sweden (Umeå, Stockholm, and Gothenburg). Of the 25 API analytes targeted, 16 were detected in fish plasma (sampled from at least one study location) at levels exceeding one-thousandth of their

respective human plasma levels associated with therapeutic dose (C_{max}). One of these 16 APIs of particular significance was the synthetic progestin levonorgestrel—at plasma levels of 8.5 and 12 ng/mL, a level 4-fold greater than human plasma C_{max}. This plasma level of levonorgestrel represented an empirical BCF of 12,000, which was 200-fold higher than the predicted BCF. Such approaches for relating internal-dose API exposures to potential effect thresholds are explored further below.

Finally, the study of Kwon et al. (2009), as discussed earlier, targeted five APIs: EE2, diazepam, CBZ, simvastatin, and oxybenzone. Only the first two, however, were detected in liver samples from hornyhead turbot (*P. verticalis*) from southern California.

8.2.9 Summary of Published Data

Much of the data for APIs/metabolites and related DBPs in this chapter on aquatic tissue-levels and BCFs/BAFs compiled from the published literature is summarized in Table 8.4. Included in the table is an indication of historical precedence—whether the data were the first to be reported; most of the data are "firsts," revealing that the depth of the published data in terms of repeated measurements is very shallow.

TABLE 8.4
Summary of Bioconcentration Data for APIs in Aquatic Tissues

APIs Studied in Wild Specimens (Controlled *In Situ* Studies Indicated by Asterisk*)	Maximum Concentration in Wild Specimens (μg/kg) (Controlled Studies Indicated by Asterisk*)	Historical Precedence in Literature	Notes	Reference
		Antidepressants		
Bupropion	0.013–0.07 brain	Probably 1st report	Detected in samples from 5 of 8 streams; upper range = 0.348 ng/g. Water = 20–50 ng/L.	Schultz et al. (2010)
Citalopram	0.57 plasma	Possibly 1st report	Sharks.	Gelsleichter (2009)
	0.01–0.07 brain	Probably 1st report	Detected in samples from 4 of 8 streams; upper range = 0.212 ng/g. Water = 4.5–70 ng/L.	Schultz et al. (2010)
Fluoxetine	1.58 brain 1.34 liver	Probably 1st reports	Lowest concentrations in muscle. Empirical BCFs up to 260 for body and 3100 for liver (Nakamura et al. 2008); but BCF for "free" fluoxetine less than unity (Zhou et al. 2008). Controlled exposure to 0.55 g/L gave peak concentration of 49 μg/kg tissue (Paterson and Metcalfe 2008).	Brooks et al. (2005) [also Chu and Metcalfe (2007)]
	0.02–0.6 brain		Detected in samples from 6 of 8 streams; upper range = 1.6 ng/g. Water = 1–9 ng/L.	Schultz et al. (2010)

continued

TABLE 8.4 (continued)
Summary of Bioconcentration Data for APIs in Aquatic Tissues

APIs Studied in Wild Specimens (Controlled *In Situ* Studies Indicated by Asterisk*)	Maximum Concentration in Wild Specimens (µg/kg) (Controlled Studies Indicated by Asterisk*)	Historical Precedence in Literature	Notes	Reference
Norfluoxetine	10.27 liver 8.86 brain	Probably 1st reports	Lowest concentrations in muscle. Controlled exposure to 0.55 ng/L of fluoxetine gave peak concentration of 64 µg/kg tissue (Paterson and Metcalfe 2008).	Brooks et al. (2005) [also Chu and Metcalfe (2007)]
	0.07–0.9 brain		Detected in samples from 5 of 8 streams; upper range = 3.6 ng/g. Water = 0.9–4 ng/L.	Schultz et al. (2010)
Paroxetine	0.58 whole body	Probably 1st report		Chu and Metcalfe (2007)
	0.005–1.8 brain		Detected in samples from 6 of 8 streams; upper range = 4.2 ng/g. Water = 2–4 ng/L.	Schultz et al. (2010)
Sertraline	4.27 brain 3.59 liver	Probably 1st reports	Lowest concentrations in muscle.	Brooks et al. (2005)
	1.1–1.2 plasma		Fish exposed to treated sewage effluent; 2 of 3 sites in Sweden; BCF > 138–240 (predicted = 959).	Fick et al. (2010)
Norsertraline	15.6 brain 12.94 liver	Probably 1st reports	Lowest concentrations in muscle.	Brooks et al. (2005)
	0.01–3 brain		Detected in samples from 7 of 8 streams; upper range = 28.9 ng/g. Water = 1.1–6 ng/L.	Schultz et al. (2010)
	0.01–0.02 brain		Detected in samples from 3 of 8 streams; upper range = 0.113 ng/g. Water = 0.8–4 ng/L.	Schultz et al. (2010)
Venlafaxine	0.32 plasma	Possibly 1st report	Sharks.	Gelsleichter (2009)
	0.02–0.1 brain		Detected in samples from 2 of 8 streams; upper range = 1.12 ng/g. Water = 102–220 ng/L.	Schultz et al. (2010)
		NSAIDs		
Diclofenac	12 plasma	Probably 1st report	BCF = 5. First report of bioconcentration (under controlled conditions) gave mg/kg concentrations in liver and kidney, with BCFs of nearly 3000 [Schwaiger et al. (2004)].	Brown et al. (2007)
	2.2–20 plasma		Fish exposed to treated sewage effluent; 3 of 3 sites in Sweden; BCF = 2.5–29 (predicted = 93).	Fick et al. (2010)
	328 bile	Probably 1st report in bile	21-day exposure of trout to 0.5 ng/mL; BCF = 657.	Mehinto et al. (2010)

TABLE 8.4 (continued)
Summary of Bioconcentration Data for APIs in Aquatic Tissues

APIs Studied in Wild Specimens (Controlled *In Situ* Studies Indicated by Asterisk*)	Maximum Concentration in Wild Specimens (μg/kg) (Controlled Studies Indicated by Asterisk*)	Historical Precedence in Literature	Notes	Reference
Ibuprofen	84 plasma	Possibly 1st report	BCF = 18,667.	Brown et al. (2007)
	5.5–102 plasma		Fish exposed to treated sewage effluent; 3 of 3 sites in Sweden; BCF = 21–58 (predicted = 77).	Fick et al. (2010)
Ketoprofen	Undetected	Probably 1st attempted analysis	Did not bioconcentrate.	Brown et al. (2007)
	15–107 plasma	Probably 1st report	Fish exposed to treated sewage effluent; 3 of 3 sites in Sweden; BCF = 3.5–48 (predicted = 20).	Fick et al. (2010)
Naproxen	14 plasma	Probably 1st report	BCF = 56.	Brown et al. (2007)
	33–46 plasma		Fish exposed to treated sewage effluent; 3 of 3 sites in Sweden; BCF = 22–28 (predicted = 24).	Fick et al. (2010)
Lipid Regulators	Calculated BCFs for a number of fibrates and statins are provided by Hernando et al. (2007); values ranged 120–380 for clofibrate, fenofibrate, lovastatin, and mevastatin, but higher for simvastatin (800) and fluvastatin (2000).			
Gemfibrozil	210 plasma	Probably 1st report	BCF = 199. Plasma concentration of 170 μg/L yielded a BCF of 500 after controlled exposure to 0.34 μg/L (Mimeault et al. 2005).	Brown et al. (2007)
***β*-Blockers**			Not detected in studies of Brooks et al. (2005) and Brooks (unpublished data).	
Atenolol*	51 plasma*	Probably 1st report (for controlled exposure)	Controlled exposure to 3.2 mg/L. Calculated BCF diminishingly low (Cleuvers 2005).	Winter et al. (2008)
Propranolol*	5 plasma*	Probably 1st report (for controlled exposure)	Controlled exposure to 10 mg/L for 10 days.	Owen et al. (2007; also unpublished data)
		Fungicides		
Nine triazoles*	500–1000 body lipid*	Probably 1st report (for controlled exposure)	Exposed via feed at concentrations of 23–35 mg/kg w/w.	Konwick et al. (2006)

continued

TABLE 8.4 (continued)
Summary of Bioconcentration Data for APIs in Aquatic Tissues

APIs Studied in Wild Specimens (Controlled *In Situ* Studies Indicated by Asterisk*)	Maximum Concentration in Wild Specimens (µg/kg) (Controlled Studies Indicated by Asterisk*)	Historical Precedence in Literature	Notes	Reference
Macrocyclic Lactones (avermectins)			Residues well-established as occurring in liver and lipids.	See overview: Danaher et al. (2006).
Abamectin*	38.29 muscle*		Controlled 22-d exposure to 1 µg/L; BCF = 42.	Shen et al. (2005)
Avermectin B1a*	6.8, 3.0, and 11* in whole fish, fillet, and viscera	One of earliest reports for controlled exposure	Controlled 28-d exposure to 1 µg/L; BCFs of 56, 28, and 84.	Van den Heuvel et al. (1996)
Steroids			Considerable data exist on uptake of endogenous estrogens under controlled *in situ* conditions (not summarized here).	See overview: Tyler et al. (2008).
EE2	1100 bile	Perhaps 1st bioconcentration study	4-week exposure in effluent-dominated stream; controlled 46-h exposure to 5 µg/L gave 350,000 µg/L in bile; bioconcentration is in the range of 4–6 orders of magnitude.	Larsson et al. (1999)
	3.79 plasma		Juvenile sharks in wild.	Gelsleichter (2009)
EE2*	31 tissue*	Perhaps the first full life-cycle bioconcentration study of EE2	Lifecycle/posthatch exposure to 64 ng/L. BCFs probably less than 500–2400. No detectable residues after exposure to 1 ng/L.	Lange et al. (2001)
	32–40 (E2eq) bile*		Also detected in testes and ovaries.	Gibson et al. (2005a)
EE2 dibrominated*	92.3 liver* 2.3 ovaries*	Perhaps 1st study (controlled) targeted at API DBPs	Roach exposed for 5 days to drinking water with measurable EE2 of 0.6 ng/L. Accumulated concentrations 18–67 greater than those measured for EE2. BCFs 7894 (liver) and 130 (ovaries).	Flores and Hill (2008)
Levonorgestrel	8.5–12 plasma	Probably 1st report	Fish exposed to treated sewage effluent; 2 of 3 sites in Sweden; BCF = 12,000 (predicted = 46).	Fick et al. (2010)
Testosterone*	80 plasma*		6-day exposure to 1 µg/L; plasma levels dropped quickly upon cessation of exposure.	Maunder et al. (2007)

TABLE 8.4 (continued)
Summary of Bioconcentration Data for APIs in Aquatic Tissues

APIs Studied in Wild Specimens (Controlled *In Situ* Studies Indicated by Asterisk*)	Maximum Concentration in Wild Specimens (μg/kg) (Controlled Studies Indicated by Asterisk*)	Historical Precedence in Literature	Notes	Reference
Equilenin*		Probably 1st report (for exposure to treated sewage)	BCF = 2.2×10^6	Tyler et al. (2009)
17β-Dihydroequilenin*	30–40 (E2eq) bile*	Probably 1st report (for exposure to treated sewage)	BCF = 1.5×10^6	Gibson et al. (2005a, 2005b)
Antibiotics	Considerable data exist for tissue levels resulting from the high exposures used in aquaculture. Some data exists from controlled studies that simulate the indirect exposure that might occur for organisms in the vicinity of an aquaculture operation. See text for discussion.			
Miscellaneous				
Carbamazepine (CBZ)	2.5 plasma*	Perhaps 1st report (for controlled exposure)	24-h exposure to 200 μg/L; BCF < 1.	Huggett et al. (2004)
	0.3–1.0 plasma		Fish exposed to treated sewage effluent; 3 of 3 sites in Sweden; BCF = 0.8–4.2 (predicted = 6).	Fick et al. (2010)
	0.83–1.44 muscle	1st report in wild fish	11 specimens from effluent-dominated stream.	Ramirez et al. (2007)
	129,000* crustacean	Trophic-level transfer	Concentration on basis of dried weight; fed algae that had been exposed to CBZ.	Lajeunesse et al. (2009)
	None detected in algae		Exposed to 19 ppm.	Andreozzi et al. (2002)
Cilazapril	0.1–0.7 plasma	Possibly 1st report	Fish exposed to treated sewage effluent; 2 of 3 sites in Sweden; BCF > 100–700 (predicted = 6).	Fick et al. (2010)
Diazepam	23–110 liver	Possibly 1st report	CBZ and simvastatin also targeted but not detected.	Kwon et al. (2009)
Diltiazem	0.11–0.27 muscle	1st report in wild fish	11 specimens from effluent-dominated stream.	Ramirez et al. (2007)
	0.002–0.0056 muscle	1st report of "free" concentrations in wild		Zhou et al. (2008)
	0.9 plasma		Fish exposed to treated sewage effluent; 1 of 3 sites in Sweden; BCF = 24–139 (predicted = 14).	Fick et al. (2010)

continued

TABLE 8.4 (continued)
Summary of Bioconcentration Data for APIs in Aquatic Tissues

APIs Studied in Wild Specimens (Controlled *In Situ* Studies Indicated by Asterisk*)	Maximum Concentration in Wild Specimens (µg/kg) (Controlled Studies Indicated by Asterisk*)	Historical Precedence in Literature	Notes	Reference
Diphenhydramine	0.66–1.32 muscle	1st report in wild fish	11 specimens from effluent-dominated stream.	Ramirez et al. (2007)
	0.032–0.082 muscle	1st report of "free" concentrations in wild		Zhou et al. (2008)
Haloperidol	1.2 plasma	Possibly 1st report	Fish exposed to treated sewage effluent; 1 of 3 sites in Sweden; BCF = 3.2 (predicted = 153).	Fick et al. (2010)
Meclozine (meclizine)	0.1–0.7 plasma	Possibly 1st report	Fish exposed to treated sewage effluent; 2 of 3 sites in Sweden; BCF > 200–1400 (predicted = 2521).	Fick et al. (2010)
Memantine	2.3 plasma	Probably 1st report	Fish exposed to treated sewage effluent; 1 of 3 sites in Sweden; BCF < 50–164 (predicted = 36).	Fick et al. (2010)
Orphenadrine	0.9 plasma	Probably 1st report	Fish exposed to treated sewage effluent; 1 of 3 sites in Sweden; BCF < 63–100 (predicted = 61).	Fick et al. (2010)
Oxazepam	0.2–0.7 plasma	Probably 1st report	Fish exposed to treated sewage effluent; 3 of 3 sites in Sweden; BCF = 0.7–3.6 (predicted = 7).	Fick et al. (2010)
Risperidone	0.3–2.4 plasma	Probably 1st report	Fish exposed to treated sewage effluent; 3 of 3 sites in Sweden; BCF > 60–480 (predicted = 47).	Fick et al. (2010)
Tramadol	1.1–1.9 plasma	Probably 1st report	Fish exposed to treated sewage effluent; 3 of 3 sites in Sweden; BCF = 2.3–3.3 (predicted = 20).	Fick et al. (2010)
Verapamil	0.7 plasma	Probably 1st report	Fish exposed to treated sewage effluent; 1 of 3 sites in Sweden; BCF < 33–175 (predicted = 40).	Fick et al. (2010)
Triclosan, Methyl triclosan, Triclocarban	TCS: 5-chloro-2-(2,4-dichlorophenoxy)phenol MTCS: 4-chloro-1-(2,4-dichlorophenoxy)-2-methoxybenzene TCC: N-(4-chlorophenyl)-N′-(3,4-dichlorophenyl)urea			
TCS	0.61–10.4 plasma	1st report in U.S.	Detected in all 13 species surveyed.	Alaee et al. (2003)

TABLE 8.4 (continued)
Summary of Bioconcentration Data for APIs in Aquatic Tissues

APIs Studied in Wild Specimens (Controlled *In Situ* Studies Indicated by Asterisk*)	Maximum Concentration in Wild Specimens (μg/kg) (Controlled Studies Indicated by Asterisk*)	Historical Precedence in Literature	Notes	Reference
	0.75–10.0 plasma		13 species from Detroit River. MCTS levels 0.0004–0.013 μg/kg, 3 orders of magnitude lower.	Valters et al. (2005)
	85–270 plasma	1st report in marine mammal	Detected in 7 of 26 dolphins. Possibly highest plasma level reported for any aquatic organism.	Fair et al. (2009)
	240 to 4400 bile	Possibly 1st report (in bile of wild fish)		Adolfsson-Erici et al. (2002)
	14,000–80,000 bile		The Netherlands	Houtman et al. (2004)
	35 whole body; 365 lipid		Swiss lakes	Balmer et al. (2004)
	21 whole body			Mottaleb et al. (2009)
	146 algae whole body	1st report in snail	Algal BAF: <2100	Coogan et al. (2007)
	58.7 snail whole body		Snail BAF: 500	Coogan and La Point (2008)
TCS—halogenated analogs	0.5–13 whole body	1st report of halogenated products	3- and 5-chloromethoxy TCS; 3,5-dichloromethoxy TCS; unidentified bromomethoxy TCS; detected in 21–76% of 29 carp from Las Vegas Bay.	Leiker et al. (2009)
MTCS	38 whole bodies	1st report		Miyazaki et al. (1984)
	0.1–13 whole bodies		Monitoring study of multiple species at 12 locations in Sweden.	Samsøe-Petersen et al. (2003)
	600 whole body; 7000 lipid		Detected in all 29 carp from Las Vegas Bay. TCS not detected. BCF = 1.8×10^5.	Leiker et al. (2009)
	0.0004–0.0132 plasma	1st report in U.S.	Detected in all 13 species surveyed.	Alaee et al. (2003)
	30 muscle		10-year retrospective study of breams. MTCS was always detected, but TCS was rarely present.	Boehmer et al. (2004)
	130–2100 muscle		Seven Swiss rivers	Buser et al. (2006)

continued

TABLE 8.4 (continued)
Summary of Bioconcentration Data for APIs in Aquatic Tissues

APIs Studied in Wild Specimens (Controlled *In Situ* Studies Indicated by Asterisk*)	Maximum Concentration in Wild Specimens (μg/kg) (Controlled Studies Indicated by Asterisk*)	Historical Precedence in Literature	Notes	Reference
	4–233 lipid		Swiss lakes	Balmer et al. (2005)
	89 algae whole body 49.8 snail whole body	1st report in snail	Algal BAF: <1500 Snail BAF: 1200	Coogan et al. (2007) Coogan and La Point (2008)
TCC	401 algae whole body 299 snail whole body 42,000 worm whole body*	1st report in any organism 1st report of BSAF	Algal BAF: <2700 Snail BAF: 1600 Worm BSAF: ca 2	Coogan et al. (2007) Coogan and La Point (2008) Higgins et al. (2009)

Considering the data for all APIs (excluding triclosan and its derivatives) on aquatic tissue levels or bioconcentration, the following can be noted. Only a handful of studies predate 2003. The concentrations for the majority of APIs range from 1 to 100 μg/kg regardless of tissue type. Those APIs showing higher concentrations include gemfibrozil and triazole fungicides. Most data are for controlled *in situ* exposures rather than for organisms sampled in the wild. Data for tissue levels in wild samples exist for roughly 21 APIs and metabolites. Data for controlled studies exist for about 9 APIs; the study of Fick et al. (2010) exposed fish to treated sewage effluent and quantified an additional 10 unique APIs. Data for tissue levels exist for roughly 40 human APIs/metabolites (excluding antibiotics) but many are from single studies. Steroids are commonly quantified as total (conjugates combined with free). Of the existing calculated empirical BCFs, except those for steroids, nearly all are lower than several thousand, most being lower than 100. More data exist for estrogens (especially endogenous estrogens) and triclosan (including MTCS and other derivatives) than for any other class of APIs; surprisingly, despite its high usage (similar to triclosan), very little data exists for triclocarban. MTCS, unlike TCS, does not concentrate in plasma. Tissue concentrations for both TCS and MTCS can exceed tens of thousands μg/kg, with BCFs up to the range of several million.

8.3 FACTORS INFLUENCING EXPOSURE

8.3.1 General Considerations

It is important to keep in mind the difficulty in comparing BCFs between APIs (or even for a given API) or between species of fish and other aquatic organisms. The wide range of variables in Table 8.3 can add tremendous variability to these values. But moreover, BCFs are reported on different bases, not just whole body; these include different tissues or on a wet-weight basis or on the basis of lipid content. They can also use empirical data generated by static (steady-state equilibration and nonsteady-state) or kinetic uptake measurements, as well as nominal exposure levels that span one or more orders of magnitude (sometimes exceeding the solubility, and other times the uptake rate is

the limiting factor, resulting in lower BCFs at higher exposures). These factors make it difficult to distill existing data into succinct generalizations.

Various models have been developed in attempting to link aquatic tissue residues with biological effects. As an example, attempting to establish a more realistic measurable linkage of exposure with effects, the CBR concept holds that the whole-body concentrations across species does not vary wildly among chemical stressors sharing the same MOA for a given biological endpoint. The CBR is supposedly relatively consistent for a given endpoint, whether acute or chronic. Its appeal derives from the assumption that levels of chemical stressors internal to an organism more directly dictate receptor interaction than doses calculated from surrounding ambient concentrations. By the nature of its definition, CBR should be relatively independent of the stressor's ambient concentration in the immediate aqueous environment. The CBR concept supposedly accounts for a measure that is more closely associated with the level of stressor that would actually interact with the receptor. However, in a critical examination of CBR by Barron et al. (2002), published data were not found to support the CBR concept among members from groups of chemicals sharing the same MOA; variability in correlation with effects was found to be as great as other measures such as ambient concentration. Many variables may be at work here. For example, it is not known whether bioaccumulated residues are readily bioavailable, or if rather, only the free residues are (e.g., Zhou et al. 2008).

These issues, together with the many terms used in aquatic exposure (e.g., bioconcentration, bioaccumulation, biomagnification, bioavailability, and biomarkers) and exposure's role in assessing aquatic health, are discussed in the comprehensive work of Geyer et al. (2000), Gobas and Morrison (2000), and van der Oost et al. (2003). What measure of stressor level experienced by an organism serves as the best surrogate for true dose remains elusive. Below we examine several important variables that may be critical for ecological risk assessments of APIs.

8.3.2 Select Site-Specific Factors Influencing Exposure

8.3.2.1 Hydrology

Effluent-dominated ecosystems may be defined as receiving systems in which more than 50% of the in-stream flow results from effluent discharges. Effluent-dependent conditions result seasonally when the in-stream flow of these receiving systems is entirely dependent on effluent discharges. In more arid or semiarid regions experiencing rapid urbanization, effluent-dominated or dependent conditions are common (Brooks et al. 2006). Examples of effluent-dominated large river systems include the Trinity River in Texas and the South Platt River in Colorado (Brooks et al. 2006). Prospective environmental assessments of APIs often include a default in-stream dilution factor of 10 when predicting expected environmental concentrations (Brooks et al. 2003), which are not representative or protective of effluent-dominated or dependent ecosystems.

In an attempt to estimate effluent-dominated conditions in the United States, Brooks et al. (2006) examined information from the U.S. EPA on receiving system critical dilution limits included in the National Pollutant Discharge Elimination System (NPDES) program (U.S. EPA 1991). Under annual mean flow, it was estimated that less than 20% of discharges entered receiving systems with less than 10-fold dilution, but this value increased three fold to approximately 60% of in-stream dilution occurring at less than 10 fold during low flow conditions (e.g., 7Q10, the seven consecutive-day lowest flow with a 10-year recurrence interval) (Brooks et al. 2006).

The NPDES data summarized above was quite dated. New discharges or increased treatment demands on existing dischargers frequently result from increasing population growth. Thus, Brooks et al. (2006) examined a representative sample of NPDES permits (582) in U.S. EPA Region 6, which comprises the states of Arkansas, Louisiana, Oklahoma, New Mexico, Texas, and a number of Tribes. The minimum (or critical) dilution limit for a wastewater stream is the smallest degree of dilution that can avoid reasonable potential to exceed water quality criteria. Of the permits examined during the late 1990s and early 2000s by U.S. EPA Region 6 staff, 58% included critical

dilution limits of >50%, suggesting effluent-dominated or dependent conditions under low flows. Critical dilution limits of 100%, indicating effluent-dependent conditions, were observed in 37% of permits evaluated (Brooks et al. 2006).

As noted earlier, in-stream hydrology is an important consideration because effluent-dominated conditions present worst-case locations for API exposures in developed countries. Daughton (2002) proposed the term "pseudopersistent" to describe the unique exposure scenarios to APIs in these ecosystems. Although APIs are designed to be stable enough to ensure parent stability through the manufacturing-distribution-prescription-treatment continuum, APIs are generally considered to have lower environmental persistence than conventional priority pollutants. However, human APIs (and ingredients from PCPs) may be unique compared with conventional contaminants because they can be continuously introduced via effluent to a receiving system (Daughton 2002). Under these conditions the half-lives of the compounds may exceed in-stream hydrologic retention times, increasing the effective exposure duration experienced by organisms residing in the receiving system (Ankley et al. 2007). Of course, increased effective exposure duration could also apply to other effluent contaminants in these scenarios. Unfortunately very little information is available for in-stream magnitude, frequency, and duration of exposure to APIs originated from any of numerous sources, so the influence of hydrology on "pseudopersistence" of APIs requires more study.

8.3.2.2 Wastewater Treatment Technologies

Though effluent-dominated or effluent-dependent conditions described earlier deserve particular attention for API exposures in developed countries, treatment capabilities of WWTPs discharging to these systems are likely to be relatively high because effluent dilution limits are generally more stringent to meet effluent quality goals (e.g., water quality criteria, whole effluent toxicity). An understanding of treatment capabilities for APIs has grown in recent years, though an understanding of site-specific loading of APIs will be influenced by a number of factors. The most comprehensive study to date was commissioned by American Water Works Association Research Foundation (Snyder et al. 2007). During this study, various treatment technologies were evaluated singularly and in combination for their efficiencies in removing select APIs, PCPs, and endocrine-disrupting compounds.

Snyder et al. (2007) concluded that conventional processes for coagulation, flocculation, sedimentation, and ultraviolet radiation (for disinfection) were largely ineffective for many of the target analytes examined, including a number of APIs. More advanced treatment technologies such as reverse osmosis, activated carbon, advanced oxidation processes, and nanofiltration were considered relatively highly effective for target analytes, though API structural properties influenced treatability among tested technologies (Snyder et al. 2007). This study highlighted the importance of understanding ecological risk from specific APIs prior to making risk-based management decisions (U.S. EPA 1999), because risk mitigation technologies such as advanced treatment processes for APIs may be cost-prohibitive for municipal dischargers.

In developing countries, however, advanced WWTP technologies might not be employed, regulatory guidelines not be developed, or enforcement of regulations may not be as prevalent as in the developed world. A recent study by Larsson et al. (2007) examined select APIs in effluent from a WWTP in Patancheru, India. This WWTP was reported to primarily receive influent wastewater from approximately 90 pharmaceutical manufacturers. Although isotope dilution was not employed and extraction efficiencies were not reported in this screening study, high levels of several APIs were reported in grab samples collected on two consecutive days, ranging from 90 (ranitidine) to 31,000 (ciprofloxacin) μg/L. Further, 21 of 59 target pharmaceuticals were reported earlier to be 1 μg/L (Larsson et al. 2007). Levels of most of these APIs represent the highest concentrations reported in the peer-reviewed literature, highlighting the importance of understanding site-specific ecological exposure and risks in less developed countries. A follow-up study (Fick et al. 2009) revealed concentrations of APIs surface- and well-water levels that may be the highest yet reported in the ambient environment—above the ppm (mg/L) level. Lakes receiving treated wastewater effluent

contained ppm levels of three fluoroquinolone antibiotics (ciprofloxacin, norfloxacin, and enoxacin) and cetirizine, an antihistamine. These studies show that in special cases, aquatic exposure levels have the potential to reach concentrations that exceed human plasma levels achieved during therapeutic treatment.

8.3.3 Site-Specific pH and API pKa

Many APIs are weak acids or weak bases. Because these compounds are ionizable, their pKa and the pH of the medium influence the proportion of the molecules present in a nonionized form. The nonionized/ionized ratio of an API in a matrix (e.g., body compartment) is an important consideration in pharmacology and toxicology—influencing absorption and disposition profiles of APIs following dosage in target organisms (Klaassen and Watkins 2003). As noted earlier, additional uptake mechanisms are possible for APIs, but the nonionized form of a drug is more nonpolar and thus considered to passively cross membranes more readily than the ionized form of an API (Kah and Brown 2008). Such observations for APIs are included in physiological-based pharmacokinetic models, which are discussed in greater detail later.

For conventional contaminants, such as pentachlorophenol and ammonia, the more nonionized form is believed to be more bioavailable and toxic to aquatic life. Subsequently, the U.S. EPA developed National Ambient Water Quality Criteria for ammonia (U.S. EPA 1985) and pentachlorophenol (U.S. EPA 1986) that incorporate adjustment factors for site-specific differences in pH. Similarly, the nonionized forms of APIs are likely more bioavailable and potentially more toxic to aquatic life residing in receiving systems (Kah and Brown 2008). An example is provided in Figure 8.2 for the SSRIs fluoxetine (Figure 8.2a) and sertraline (Figure 8.2b), which were reported in three fish species in a receiving system with in-stream pH commonly >8.0 (Brooks et al. 2005).

For chemicals that can ionize, distribution into lipid is a function of the pH. For these dissociative systems, a "distribution" coefficient "D" (as opposed to partition coefficient) is calculated; D can be viewed as an "apparent" partition coefficient—one that depends on pH and the degree of ionization. Both fluoxetine (pKa = 10.05 ± 0.10) and sertraline (pKa = 9.47 ± 0.40) are weak bases with log D values and associated BCFs that are predicted to increase over environmentally relevant pH ranges (Figure 8.2); however, the liposome-water distribution coefficient (log D_{lipw}) may be more useful than log D for predicting accumulation of ionizable compounds (Escher et al. 2000).

As presented previously, Nakamura et al. (2008) observed fluoxetine toxicity for, and BCFs in, Japanese medaka to increase with increasing pH in laboratory studies. Valenti et al. (2009) reported similar toxicity-pH observations with juvenile fathead minnows exposed to sertraline. Further, Valenti et al. (2009) performed a time-to-death fathead minnow study with 500 µg/L of sertraline, and estimated associated LT50 values of >48, 31.9, and 4.9 h at pH treatment levels of 6.5, 7.5, and 8.5, respectively. Such observations support the findings of Nakamura et al. (2008) because if more nonionized sertraline exists at higher pH treatment levels, then sertraline should be more bioavailable and more readily absorbed by juvenile fathead minnows, resulting in the observed more rapid onset of mortality at increasingly higher pHs.

Nakamura et al. (2008) further used pH and the BCF values calculated in their study to predict aqueous fluoxetine levels in Pecan Creek, TX that would result in reported levels of accumulation of fluoxetine in fish (Brooks et al. 2005). Interestingly Nakamura et al. (2008) imputed that the fluoxetine concentrations in Pecan Creek, TX should be ~11 ng/L, which is representative of fluoxetine levels routinely observed in Pecan Creek over the past few years (Brooks unpublished data). However, these estimates do not account for other routes of exposure such as diet. Brooks et al. (in preparation) have quantitated levels of sertraline, norsertraline, fluoxetine, and norfluoxetine in periphyton and benthic macroinvertebrates from Pecan Creek, suggesting that future studies should understand the relative contribution of bioconcentration to bioaccumulation of these and other APIs in aquatic life.

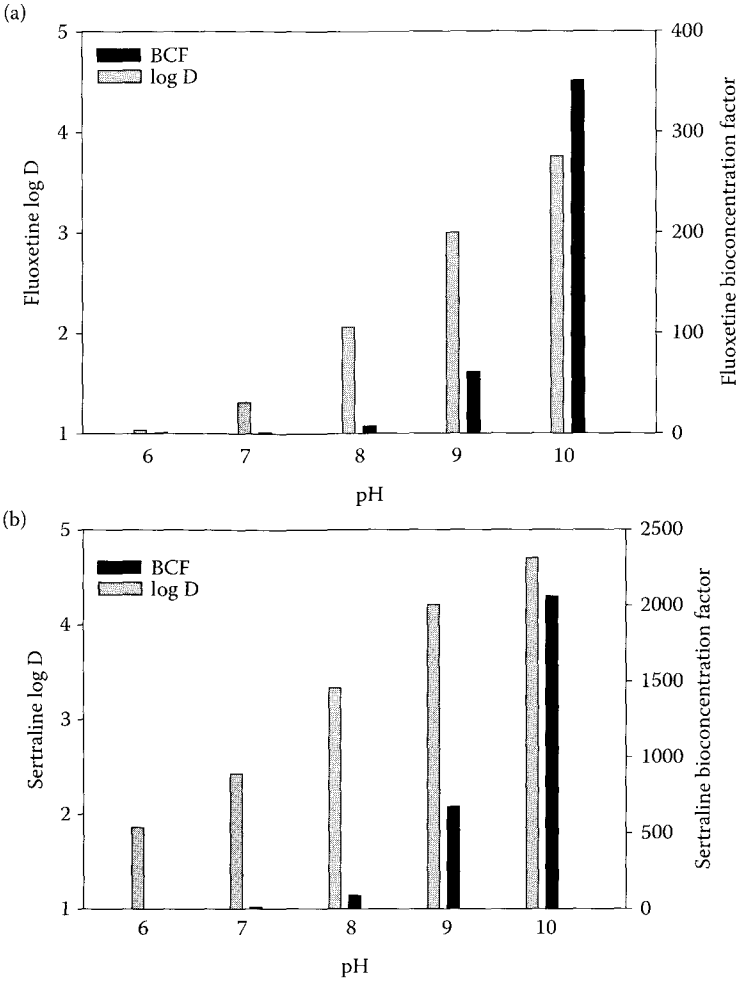

FIGURE 8.2 Bioconcentration factors and log D values for the selective serotonin reuptake inhibitors fluoxetine (a) and sertraline (b) across environmentally relevant pH ranges. Values calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14.

8.3.4 Advancement in Tissue Sampling and Surrogate Monitoring

Site-specific exposure may be estimated *in situ* using surrogate measures of API bioavailability. Passive sampling devices have long been used for obtaining estimates of aquatic uptake; see overviews in Greenwood et al. (2007). A variety of devices have been developed for field-deployment to emulate the uptake of xenobiotics by fish via diffusion into lipid. These include semipermeable membrane devices (SPMD) (Barber et al. 2006) and the polar organic chemical integrative sampler (POCIS) (Vermeirssen et al. 2005). Bayen et al. (2009) discuss the variables in the use of passive sampling devices for predicting uptake of hydrophobic chemicals, which would be applicable to only a portion of APIs. Key characteristics for the device and organism are surface-to-volume/weight ratios.

Nonlethal sampling via biopsy has been used for other pollutants but not yet for APIs. One example is the acquisition of tissue samples from fins (Rolfhus et al. 2008).

Conventional sampling devices and tissue sampling approaches suffer from cost for the devices and expenses associated with sample preparation, including organic solvents (and disposal) and

analyst time. More significantly, with respect to exposure studies, tissue extraction usually only measures total levels of the analyte as opposed to free, unbound residues that are more readily bioavailable. A recently developed approach uses *in-vivo* sampling by way of implanted fibers coated with the sorbent poly(dimethylsiloxane) in a SPME format (Zhou et al. 2008); another approach for establishing chemical activity in tissues is the silicone membrane equilibrator developed by Mayer et al. (2009). SPME avoids many of these limitations and serves to collect only free residues. However, an understanding of the utility of various SPMDs, SPMEs, and POCIS technologies across API classes ranging in physiochemical properties under varied environmentally relevant pH ranges is not available at this time.

8.4 MODELS FOR PREDICTING EXPOSURE AND POTENTIAL EFFECTS OF APIs

8.4.1 Background and Prioritization

Although several recent book chapters reviewed approaches for predicting human API (Versteeg et al. 2005) and veterinary API (Metcalfe et al. 2008) concentrations in aquatic systems, limited approaches are available for predicting exposure within an organism and linking exposure to potential ecological effects. Prospective assessments often include trigger values for further testing based on predicted environmental concentrations (e.g., 1 μg/L for human APIs in the United States). These predicted concentrations are often driven by production volumes and associated patient uses, and do not consider API potency. Ankley et al. (2007) reviewed assumptions associated with API trigger values based on usage, noting that a trigger value of 1 μg/L equates to a production volume in the United States of 44,000 kg/year, but that this approach is not appropriate for highly potent APIs such as EE2. Despite its very low production volume, it is highly potent (C_{max} is less than 100 pg/mL, where C_{max} is the maximum plasma level reached during therapeutic dosing) and lipophilic (log P ~ 4).

Because APIs represent compounds with a wide range of potencies and physiochemical properties (log P or D, pKa), screening approaches that examine similar properties for the large expanse of thousands of APIs may be useful for prioritizing substances for further bioaccumulation or ecotoxicity studies. Although risk-based prioritization approaches have been developed for veterinary APIs (Boxall et al. 2003, Capleton et al. 2006, Kools et al. 2008) and pesticide transformation products (Sinclair et al. 2006), few approaches have been published for prioritizing human APIs (see Gunnarsson et al. 2008, Kostich and Lazorchak 2008). A powerful tool for such studies may be derived from probabilistic hazard/risk assessment. Chemical toxicity distributions (CTDs) represent robust probabilistic approaches for predicting a specific toxicological response in a model organism (e.g., fathead minnow reproduction) associated with the universe of chemicals that share a common MOA. CTDs are derived by plotting toxicity property data (e.g., NOAELs for fathead minnow reproduction) for a number of chemicals against a probability scale. This represents an approach conceptually similar to SSDs, which plot a distribution of toxicity benchmarks for various species exposed to a common chemical. Much like SSDs, which allow an assessor to estimate the concentration below which a certain percentage of aquatic species would respond to a chemical (e.g., an HC5 or 5th centile value), a CTD allows for predictions of the concentration below which a specific percentage of chemicals with a common MOA (or theoretically any other common data property) will still elicit a specific response (e.g., below the NOAEL for fathead minnow reproduction). For example, CTDs were previously demonstrated to predict toxicity of carcinogens (Munro 1990), antibiotics (Brain et al. 2006), estrogen agonists (Dobbins et al. 2008), and the antimicrobial parabens (Dobbins et al. 2009). This approach is particularly useful for environmental contaminants such as APIs that have limited environmental exposure information (Brain et al. 2006, Dobbins et al. 2009). CTDs were further demonstrated to exhibit diagnostic capabilities to predict differences in sensitivities among common *in vitro* and *in vivo* models of estrogen agonist activity (Dobbins et al. 2008). CTDs are conceptually similar to but provide a more quantitative approach than Threshold

of Toxicological Concern methodologies (Gross et al. 2010) previously used in human health risk assessment (Brooks et al. 2009a).

We explored the utility of using probabilistic therapeutic distributions (PTDs), which are identical to CTDs with the exception being that therapeutic plasma data (C_{max}) are examined, to represent the full spectrum of API potencies. Figure 8.3 presents a PTD of C_{max} values for 275 human APIs, and Table 8.5

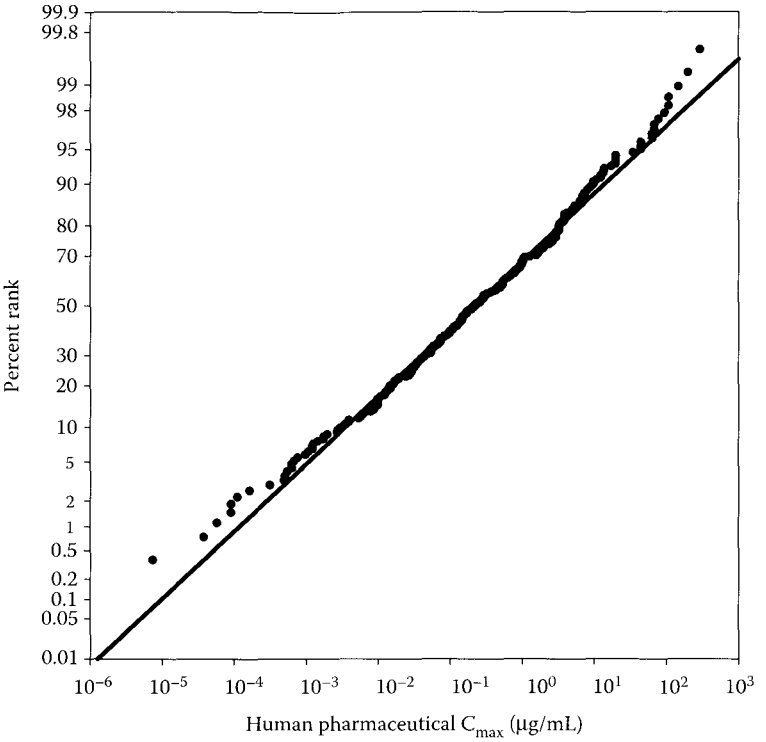

FIGURE 8.3 Probabilistic therapeutic distribution (PTD) of human plasma C_{max} values for 275 Active Pharmaceutical Ingredients (APIs; $r^2 = 0.99$). For APIs with multiple C_{max} values, distribution values are C_{max} concentrations associated with the most common dosage; also see Table 8.5.

TABLE 8.5
Probabilistic Therapeutic Distribution Centiles and Predicted C_{max} Values Derived from C_{max} Plasma Concentrations for 275 Human Pharmaceuticals; also See Figure 8.3

Centile Value	C_{max} (μg/mL)
1%	0.0001169
5%	0.00107
10%	0.00347
25%	0.0248
50%	0.221
75%	1.97
90%	14.15
95%	45.97
99%	419.32

identifies concentrations associated with specific centiles (e.g., 5th, 95th centile) of the distribution. Using this PTD approach ≤ 25% of human APIs are predicted to have C_{max} values less than 0.0248 or greater than 1.97 μg/mL (Table 8.5). Chemical classes with relatively high potencies include endocrine active substances (n = 12; range: 0.0000922–0.0595 μg/mL), whereas NSAIDs (n = 11; range: 0.705–110 μg/mL) have relatively lower potencies.

Although the PTD approach presented here only examined C_{max} values to compare relative potencies among a wide range of APIs, PTDs could be developed for other API property data useful for predicting accumulation (e.g., BCF). For example, maximum log D (or log K_{lipw}) values for weak acids and weak bases could be examined over an environmentally relevant pH range (e.g., pH 6–9). Maximum log D PTDs could be developed for weak acids and weak bases (e.g., pH 9 used for a weak base with a pKa > 9) to predict the proportion of APIs that may be expected to have log D values greater than some screening threshold (e.g., ~3) at environmentally relevant conditions. Such approaches could provide useful rankings of relative therapeutic property data and for predicting potential property data of new medicines within API classes (Brooks et al. 2009a). For example, Berninger and Brooks (2010) provide a more extensive examination of the utility of PPDs. Specifically PPDs to prioritize pharmaceutical classes for further study, based on a statistically significant relationship between a mammalian margin of safety corollary and fish acute-to-chronic ratios, when fish chronic responses were plausibly linked to therapeutic MOA (Berninger and Brooks 2010).

8.4.2 Physiological-Based Pharmaco(Toxico)Kinetic (PBPK) Models

Pharmacokinetics often utilizes one- and two-compartment models to examine potential systemic effects following exposure. These relatively simple approaches model the distribution of a contaminant (or therapeutic) in a whole body or plasma compartment (one compartment). In a two-compartment model, disposition in a whole body or plasma compartment is coupled with a second compartment, which represents movement to storage depots (e.g., fat) or metabolism. Although these models are useful for deriving parameters such as clearance rates, multicompartment PBPK models are also useful tools for predicting uptake and disposition of environmental contaminants. These more advanced models can: incorporate physiological processes to predict distribution of a compound among various tissues; extrapolate among organisms, exposure routes and ages; and estimate internal dose (Andersen and Dennison 2002, Barton et al. 2007). Subsequently, PBPK models are routinely used in human health risk assessments (U.S. EPA 2006) and increasingly developed for ecotoxicological applications in fish models (see other chapters in this book).

Whereas a number of investigators have examined the utility of physiological models for predicting environmental contaminant uptake and distribution in fish (Erickson et al. 2006a, 2006b, 2008), Erickson et al. (2006a), recently developed a model in trout for describing uptake and elimination of ionizable organic chemicals (chlorophenols) at fish gills. Erickson et al. (2006b) further applied this model to several weak acids with pKa values ranging from 4.74 to 8.62 and log K_{ow} values ranging from 2.75 to 5.12. This model was found to predict uptake of ionizable chemicals based on physiochemical properties under the exposure conditions evaluated with trout (Erickson et al. 2006b). Similar approaches with APIs would be useful for predicting uptake. Although multicompartment PBPK models have not been developed for ionizable APIs and fish, such efforts could be critical to estimate internal dose of APIs to target tissues under environmentally realistic API exposures and pH gradients. This area deserves additional study to characterize API exposure in various tissues of aquatic organisms where therapeutic targets are present.

8.4.3 The Huggett Model

In addition to exhibiting wide ranges in potency, lipophilicity of APIs demonstrates marked variability along a polar-nonpolar continuum. Accounting for such differences in lipophilicity and

potency, Huggett et al. (2003) proposed the following model to prioritize human APIs for additional chronic testing (Equation 8.1):

$$F_{SS}PC = EC \times (P_{Blood:Water}) \tag{8.1}$$

where $F_{SS}PC$ is predicted fish steady-state plasma concentration, EC is the aqueous exposure concentration, and $P_{Blood:Water}$ is the predicted partition coefficient in blood from aqueous exposure medium. Fish were selected for model development because more information is available for these organisms, and fish appear to contain relatively high evolutionary conservation of API targets (Huggett et al. 2003, Gunnarsson et al. 2008). The Huggett Model (Huggett "mammalian-fish leverage model") simply proposes that the higher an API's predicted plasma concentration in fish (FSSPC) compared with that of a mammal (e.g., human therapeutic plasma concentration [HTPC] or a C_{max} value) the higher the likelihood of chronic adverse effects (Huggett et al. 2003). As the effect ratio (ER) (Equation 8.2) inflates, the likelihood of an API causing chronic effects drops. As the ER drops, and especially when it becomes less than 1, adverse effects become more probable.

$$ER = H_TPC/F_{SS}PC \tag{8.2}$$

The core calculation of this model ($P_{Blood:Water}$) employed an empirical relationship between log K_{ow} and plasma concentrations in trout and *in vitro* partitioning data, which was developed for hydrophobic compounds by Fitzsimmons et al. (2001; Equation 8.3):

$$\log P_{Blood:Water} = 0.73 \times \log K_{ow} - 0.88 \tag{8.3}$$

Though Huggett et al. (2003) used Equation 8.3 for development of Equation 8.2, another relationship (Equation 8.4) reported by Fitzsimmons et al. (2001) appears even more important for APIs with apparent log P values lower than 2:

$$\log P_{BW} = \log [(10^{0.73 \log K_{ow}} \times 0.16) + 0.84] \tag{8.4}$$

To derive Equations 8.3 and 8.4, Fitzsimmons et al. (2001) coupled *in vivo* log $P_{Blood:Water}$ values for compounds having log K_{ow} values ranging from 3.1 to 8.2 with previously published *in vitro* data for compounds with lower log K_{ow}'s (Bertelsen et al. 1998).

Brooks et al. (2009a) extended this approach for fish exposed to veterinary medicines (Equation 8.5):

$$ER = EIC_{Plasma}/C_{max} \tag{8.5}$$

where EIC_{Plasma} is the concentration in fish plasma resulting from environmental exposure. Similar to the Huggett Model, the EIC_{Plasma} value proposed by Brooks et al. (2008) would include an uptake prediction from aqueous exposure, and C_{max} would be derived from animal efficacy studies (e.g., in livestock). As previously noted, the study by Fick et al. (2010) identified accumulation of select pharmaceuticals approaching or exceeding human therapeutic levels in plasma of caged fish below effluent discharges.

It is important to note, however, that the models of Huggett et al. (2003) and Brooks et al. (2009b) considered neither bioaccumulation through dietary sources nor the metabolism potential once an API is absorbed into the fish. They also did not include log D in model derivation. Despite its limitations, the Huggett Model appears to provide a reasonable screening approach that is amenable to further refinement. Remaining to be determined is whether: (i) a relationship similar to the equation of Fitzsimmons et al. (2001) (Equation 8.3) would be appropriate for ionizable APIs if, for example, log K_{ow} were substituted with log D; (ii) dietary exposure is a concern for specific APIs; (iii) clearance rates could be predicted in fish using mammalian or target organism information and allometric scaling approaches

(if the metabolic pathway for eliminating an API [e.g., CYP450 isoenzyme] is present in a study species); or (iv) API target densities and functional responses would be different between mammals and fish. Similar approaches have not been developed for other organisms (e.g., invertebrates).

Summary

The preponderance of studies published on APIs as contaminants in the aquatic environment have focused on establishing the presence of APIs in the abiotic environment—primarily levels in water and sediment. Comparatively few studies document tissue concentrations. Even fewer studies examine bioaccumulation from sediments. This is a major limitation in being able to establish correlations between biological effects observed in the field with exposure, especially because exposure usually involves multiple chemical (and other) stressors acting in unison or in sequence. This is a critical step in being able to establish cause and effect. Few data are available on tissue levels from free-ranging, migratory fish in locales not directly impacted by sewage effluent. Even fewer controlled exposure studies follow the emergence of any type of biological effect. As one example, a range of effects have been reported for trout chronically exposed to a minimum of 500 ng/L of diclofenac for 21 days (Mehinto et al. 2010).

As part of the EPA's *National Rivers and Streams Assessment (NRSA)* study (U.S. EPA 2008b), plans include analysis of water and fish fillets from 183 urban rivers in the United States for 54 APIs targeted from a range of therapeutic classes, as well as for four synthetic musks and two of their metabolites (Blocksom et al. 2009). The NRSA will attempt to provide the largest dataset yet on the occurrence of multiple APIs in fish tissue. Sample collection began in 2008, and plans are to report on the data by 2011.

A concerted effort is needed to synthesize the data and knowledge that have already been published, especially in the non-English literature not covered in this review (Daughton 2009). This knowledge basically languishes in the published literature, reducing the ability to rationally prioritize and design the most needed research. As such, it is also unable to prevent duplication of effort. While actual empirical data on BCFs or tissue levels of APIs are extremely scarce, a range of modeling techniques can be used to prioritize APIs according to predicted BCF or known tendency for active transport. A limited list of APIs could then be targeted for in-field monitoring to corroborate predictions. Computed BCFs from models are more available than empirical BCFs, but these have never been compiled in any database. Further, BAFs (and BSAFs) for APIs should be further considered to account for site-specific pH influences on ionization and partitioning, dietary exposures, and potential trophic transfer. The literature focusing on veterinary drug use in aquaculture, although limited primarily to antibiotics and steroids, may be of use in extrapolating to the ambient environment.

Predictive models and studies under controlled laboratory conditions directed at APIs that have yet to be identified in aquatic tissue from the wild could be more widely used to better inform the prioritization of APIs for field biomonitoring. For example, the fact that some synthetic progestins (i.e., levonorgestrel and 19-norethindrone) have a high affinity for fish sex hormone-binding globulin, points to the possibility that their enhanced uptake could lead to their being found in tissues. Observations by Fick et al. (2010) for levonorgestrel appear to confirm this perspective. This is critical because synthetic progestins have been identified to adversely affect fish reproduction at low or even sub-part-per-trillion concentrations (Zeilinger et al. 2009).

More study is needed on the bioaccumulation of metabolites, especially those that are bioactive themselves. Likewise, more needs to be known about halogenated DBPs, which might very well have higher BCFs than their parent APIs. Such data are important with regard to trophic chain transfer and accumulation and with respect to human consumption, particularly in effluent-dominated or dependent ecosystems. Given the probably significant role in medicine to be played by nanomaterials (especially in better targeting drug delivery), studies are needed on the uptake of engineered nanoparticles; see overview by Baun et al. (2008).

Exposure studies under controlled laboratory conditions need to use concentrations that have relevance to the environment. Earlier studies (as late as the 1990s) focused on acute toxicity studies, yielding results using concentrations that far exceeded realistic ambient levels. Concentrations that occur in the aquatic environment are often orders of magnitude lower than those used in many controlled studies. Such low concentrations are capable of eliciting sublethal effects that are much more difficult to detect, especially those leading to subtle behavioral effects or changes that are of delayed onset. Further studies relating tissue-specific internal exposures to sublethal responses predicted by API MOAs are warranted.

Perhaps the gold standard for the study of exposure is the "lossless" model using radiolabeled APIs. This permits attempts at achieving mass balance closures for residues distribution over the whole body (e.g., Junker et al. 2006) and in target tissues where therapeutic targets for many APIs are present at highest densities. Even with radiolabeled materials, however, it is rare that mass balances can be fully achieved (Roffey et al. 2007). Unfortunately, radiolabeled APIs are expensive and not widely available, presumably precluding such studies to date.

A major question is whether measurements of whole body or of certain tissues or compartments accurately reflect the internal dose at target organs better than exposure measurements imputed from aqueous concentrations. While assumed to better represent exposure than do concentrations external to the organism, the actual bioavailability of these residues is essentially unknown. Given the exposure continuum that ultimately leads to stressor-receptor interaction, stressor residues residing in tissues such as fat depots, cartilage, bone/scale, gonads, and blood may not yet have reached their ultimate destinations where maximal biological activity can be affected. New approaches using biopsy or *in vivo* equilibrative extraction of target tissues could illuminate this unknown. As an example, SPME has been used to examine *in vivo* the body/tissue concentrations of free stressors (Wen et al. 2006, Zhou et al. 2008). Other new approaches for measuring tissue concentrations include caudal fin biopsy (e.g., Rolfhus et al. 2008).

The difficulty in assessing tissue concentrations is the driving force behind the development of physical model systems that can emulate biological exposure. A number of approaches have evolved, primarily aqueous sampling devices that act as surrogates for whole-body exposure (to estimate concentrations within the body). These devices include SPMD, SPME, and POCIS. However, the utility of such technologies to be predictive of accumulation in organ systems within an organism where API targets are present is not understood. Coupling radiolabeled API studies with development of PBPK fish models presents a useful approach for future efforts, particularly for understanding uptake of ionizable APIs across environmentally relevant pH gradients.

Perhaps the ultimate question is whether the assessment of exposure is truly meaningful if the "totality" of exposure is not considered—that is, all the stressors to which an organism is exposed, both anthropogenic and naturally occurring. This includes the universe of chemical stressors and the numerous other stressors, including those that are physical, electromagnetic, radiological, or biological. Relative risks of APIs for aquatic organisms in the field, compared with other stressors, remains a significant research need. Perhaps the major question with regard to APIs in the aquatic environment is the importance of long-term, low-level, multistressor exposure.

A range of other gaps and limitations with respect to better understanding APIs in aquatic organisms is summarized in Table 8.6.

The United States Environmental Protection Agency through its Office of Research and Development partially funded and collaborated in the research described here. It has been subjected to Agency review and approved for publication.

ACKNOWLEDGMENTS

We thank Jason Berninger for assistance developing Figure 8.3 and Table 8.5. BWB was supported by grants from the U.S. Environmental Protection Agency and WateReuse Foundation during development of this manuscript.

TABLE 8.6
Tissue Levels of APIs in Fish: Limitations, Gaps, and Unique Aspects

Limitation of Data	Example/Explanation	Additional Details
Limited data on tissue concentrations	Of the thousands of publications covering the many aspects of APIs as environmental contaminants, only about 50 published studies have reported API concentrations in fish tissues.	Only a subset of these studies has reported residues in fish exposed in wild, native environments.
Ecotoxicity studies rarely report tissue occurrence of APIs	Ecotoxicity studies of APIs have been conducted to assess effects endpoints. They generally provide little data or insights regarding either the parameters involved with internal exposure or tissue concentrations.	
Linkage between tissue residues and biological effects	Do concentrations in aquatic tissues better represent the potential for toxicity than concentrations free in the surrounding aquatic environment? Do they better reflect the actual dose? Tissue residues in target systems could reflect relevant internal dose compared to whole organism CBR approaches.	Very few studies have examined internal dose (in plasma) following exposure in the field.
Tissue data almost always report total concentrations	The portion of the total tissue-level of an API that is bioavailable is almost always unknown; unknown portions can be sequestered as adducts. Free versus bound residues are rarely distinguished, likely because radiolabeled APIs are scarce.	Total residue levels often involve conjugates, which do not pose a readily bioavailable source.
Limited scope of targeted analytes	Extremely few APIs have been targeted for tissue analysis. Empirical tissue levels in nonaquacultured fish have been published for roughly only 30 different APIs, excluding antibiotics (roughly only 20 in samples from native environments); the vast majority of all possible APIs identified in the aquatic environment (and those not yet identified) have never been targeted for tissue analysis.	The limited published data for tissue residues are focused primarily on antibiotics/biocides and natural and synthetic sex steroids; very few studies have targeted the simultaneous presence of multiple APIs.
Exposure concentration verification in laboratory studies	With aqueous exposure under controlled conditions, the actual dosage needs to be measured; the nominal added dosage is likely to be different than the measured dosage (as a result of sorption to container walls and other solids, degradation, etc).	Inaccurate exposure concentrations can lead to calculated BCFs that are too high or too low by one or more orders of magnitude.
Exposure duration and frequency	Little is known regarding the life-cycle body burden of APIs. Multigenerational studies are extremely rare.	Multiyear monitoring studies are even rarer (e.g., Boehmer et al. 2004).
Self-biasing	APIs with the lowest MDLs have the best chance of being detected. Those with the highest MDLs have the lowest chance of being detected. API MDLs for a particular tissue can vary by more than 2 orders of magnitude. This means that APIs commonly present in tissues but having high MDLs might not be detected.	Tissue concentrations below 0.1–1 μg/kg are rarely reported because this usually is below the method limit of detection—because of matrix interferences. So an unknown number of APIs could be present at these levels.
Tissue and measurement basis	API residues have been reported in a wide range of tissues. They are also normalized on different bases (e.g., wet weight, dry weight, lipid content, etc.).	This makes intercomparisons between studies very difficult.

continued

TABLE 8.6 (continued)
Tissue Levels of APIs in Fish: Limitations, Gaps, and Unique Aspects

Limitation of Data	Example/Explanation	Additional Details
Mass balance	Few studies attempt to achieve mass closure around the total body burden of APIs and their distribution across all tissues.	Rigorous closure studies usually require the use of radiolabeled APIs, but the commercial availability of radiolabeled compounds is extremely limited.
Endogenous contributions	For the endogenous sex steroids, levels metabolically synthesized are augmented by unknown levels contributed by uptake of exogenous residues in water and sorbed to food.	Exogenous contributions, even for endogenous steroids, can have origins from pharmaceuticals.
Veterinary medicine exposure studies	The vast preponderance of bioconcentration studies for fish has been conducted because of the concern for food residues resulting from API usage in aquaculture. Knowledge gained from these studies could possibly be evaluated for relevance to exposure in the ambient environment.	Aquaculture studies comprise two major scenarios: (1) direct incorporation of API in fish being treated with high levels during aquaculture, and (2) indirect incorporation of API in wild fish that become exposed to aquaculture residues leftover from uneaten feed and excreted residues.
Controlled exposure studies versus real-world settings	Tissue data are primarily obtained under controlled exposure studies. Of the very limited studies on tissue concentrations, only about two-thirds have been obtained under ambient conditions with fish in their native environments.	Even in native environments, exposure studies are often controlled by using caged wild fish. More studies are needed using fish captured from waters less dominated by sewage effluents.
Real-world exposure scenarios and exposure concentrations	Tissue data obtained under controlled conditions are often derived from exposure concentrations far higher than those that occur in native environments. Controlled exposure studies often use exposure concentrations that are one or more orders of magnitude higher than those that exist in the ambient environment, to maximize the chances of detecting and quantifying any amounts that have been accumulated.	BCFs usually drop off as exposure concentrations increase, leading to gross underestimates for real-world settings at much lower concentrations.
Inadequate uptake models	Lipid-solubility (e.g., as modeled by octanol–water coefficient) is an inadequate predictor of API uptake. K_{ow} coupled with pK_a (log D, log K_{lipw}) provides a more realistic model.	Other mechanisms may be involved, especially those involving active transport, facilitating the uptake of polar (ionizable) APIs.
K_{ow} data unreliable	Published K_{ow} data are extremely variable, probably a result of ionization and localized charges and because of interaction with other ions.	Models employed for predictions of physicochemical properties inherently vary.
Models for predicting BAFs	Bioaccumulation of APIs cannot currently be modeled.	Too many variables compared with the conventional nonpolar pollutants (e.g., legacy POPs), whose accumulation is dictated primarily by lipid-solubility and metabolism is minimal.
Metabolites	The metabolism of APIs creates the potential for bioconcentration of metabolites, some of which are bioactive themselves.	Little is known regarding pharmacokinetics of APIs in fish or other aquatic organisms.

TABLE 8.6 (continued)
Tissue Levels of APIs in Fish: Limitations, Gaps, and Unique Aspects

Limitation of Data	Example/Explanation	Additional Details
Tissue depots/ reservoirs	It is poorly understood how APIs are stored and accumulate in aquatic organisms.	Mechanisms could include adducts with DNA, pigments such as melanin, and a wide array of endogenous proteins (especially those in plasma).
Tissue levels are not necessary for adverse effects	Important to note that internal exposure is not necessary for an effect when the target organ is external.	This is the case, for example, with exposure of the lateral-line sensory organ.
Significant BCFs are not necessary for adverse effects	APIs are designed to minimize accumulation during therapy in the body. Any build-up in aquatic tissue could be important, regardless of how low the BCF might be.	
Interspecies extrapolations	Variance in uptake and pharmacokinetics of fish species makes usefulness of extrapolations questionable, requiring further investigation.	Comparative metabolism and other potential applications of pharmacological "read-across" are largely unknown.
Surrogates for bioconcentration	Sampling devices based on partitioning of analytes from aqueous media (e.g., semipermeable membranes) have not been calibrated for fidelity to bioconcentration under varying field conditions and do not account for bioaccumulation through dietary routes of exposure.	

REFERENCES

Adolfsson-Erici, M. 2005. Fish bile in environmental analysis. Doctoral diss., Department of Applied Environmental Science, Stockholm University, Stockholm, Sweden. http://www.diva-portal.org/su/abstract.xsql?dbid=494 (accessed February 3, 2010).

Adolfsson-Erici, M., M. Pettersson, J. Parkkonen, and J. Sturve. 2002. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. *Chemosphere* 46(9–10):1485–1489.

Alaee, M., I. D'Sa, E. Bennett, and R. Letcher. 2003. Levels of triclosan and methyl triclosan in the plasma of fish from the Detroit river. *Organohalogen Compd.* 62:136–139.

Andersen, M., and J. Dennison. 2002. Toxicokinetics models: where we've been and where we need to go! *Hum. Ecol. Risk Assess.* 8(6):1375–1396.

Andreozzi, R., R. Marotta, G. Pinto, and A. Pollio. 2002. Carbamazepine in water: persistence in the environment, ozonation treatment and preliminary assessment on algal toxicity. *Water Res.* 36(11):2869–2877.

Ankley, G. T., M. C. Black, J. Garric, T. H. Hutchinson, and T. Iguchi. 2005. A framework for assessing the hazard of pharmaceutical materials to aquatic species. In *Human Pharmaceuticals: Assessing the Impacts on Aquatic Ecosystems*, ed. R. T. Williams, pp. 183–237. Pensacola, FL: SETAC Press.

Ankley, G. T., B. W. Brooks, D. B. Huggett, and J. P. Sumpter. 2007. Repeating history: pharmaceuticals in the environment. *Environ. Sci. Technol.* 41(24):8211–8217.

Ankley, G. T., K. M. Jensen, M. D. Kahl, J. J. Korte, and E. A. Makynen. 2001. Description and evaluation of a short-term reproduction test with the fathead minnow (*Pimephales promelas*). *Environ. Toxicol. Chem.* 20(6):1276–1290.

Arnot, J. A., and F. A. Gobas. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environ. Rev.* 14(4):257–297.

Aschmann, S. M., J. Arey, R. Atkinson, and S. L. Simonich. 2001. Atmospheric lifetimes and fates of selected fragrance materials and volatile model compounds. *Environ. Sci. Technol.* 35(18):3595–3600.

Aubry, A.-F. 2002. Applications of affinity chromatography to the study of drug-melanin binding interactions. *J. Chromatogr. B* 768 (1):67–74.

Balmer, M. E., H.-R. Buser, M. D. Müller, and T. Poiger. 2005. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. *Environ. Sci. Technol.* 39(4):953–962.

Balmer, M. E., et al. 2004. Occurrence of methyl triclosan, a transformation product of the bactericide triclosan, in fish and from various lakes in Switzerland. *Environ. Sci. Technol.* 38(2):390–395.

Barber, L. B., S. H. Keefe, R. C. Antweiler, H. E. Taylor, and R. D. Wass. 2006. Accumulation of contaminants in fish from wastewater treatment wetlands. *Environ. Sci. Technol.* 40(2):603–611.

Barron, M. G., J. A. Hansen, and J. Lipton. 2002. Association between contaminant tissue residues and effects in aquatic organisms. *Rev. Environ. Contam. Toxicol.* 173:1–37.

Barton, H., et al. 2007. Characterizing uncertainty and variability in physiologically based pharmacokinetic models: state of the science and needs for research and implementation. *Toxicol. Sci.* 99(2):395–402.

Baun, A., N. Hartmann, K. Grieger, and K. Kusk. 2008. Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. *Ecotoxicology* 17(5):387–395.

Bayen, S., T. L. ter Laak, J. Buffle, and J. L. M. Hermens. 2009. Dynamic exposure of organisms and passive samplers to hydrophobic chemicals. *Environ. Sci. Technol.* 43(7):2206–2215.

Bebak-Williams, J., G. Bullock, and M. C. Carson. 2002. Oxytetracycline residues in a freshwater recirculating system. *Aquaculture* 205(3–4):221–230.

Belden, J. B., J. D. Maul, and M. J. Lydy. 2007. Partitioning and photo degradation of ciprofloxacin in aqueous systems in the presence of organic matter. *Chemosphere* 66(8):1390–1395.

Berninger, J. P., and B. W. Brooks. 2010. Leveraging mammalian pharmaceutical toxicology and pharmacology data to predict chronic fish responses to pharmaceuticals. *Toxicol. Lett.* 193(1):69–78.

Bertelsen, S. L., A. D. Hoffman, C. A. Gallinat, C. M. Elonen, and J. W. Nichols. 1998. Evaluation of log kow and tissue lipid content as predictors of chemical partitioning to fish tissues. *Environ. Toxicol. Chem.* 17(8):1447–1455.

Blocksom, K., Batt, J. Lazorchak, et al. 2009. National rivers and streams assessment: fish tissue contaminants. In *8th Annual Surface Water Monitoring and Standards (SWiMS) Meeting* (http://www.epa.gov/region5/water/wqb/swims.htm). Chicago, IL: US EPA.

Boehmer, W., H. Ruedel, A. Wenzel, and C. Schroeter-Kermani. 2004. Retrospective monitoring of triclosan and methyl-triclosan in fish: results from the German environmental specimen bank. *Organohalogen Compd.* 66:1516–1521.

Boxall, A. B. A., L. A. Fogg, P. Kay, P. A. Blackwell, E. J. Pemberton, and A. Croxford. 2003. Prioritisation of veterinary medicines in the UK environment. *Toxicol. Lett.* 142(3):207–218.

Brain, R. A., M. L. Hanson, K. R. Solomon, and B. W. Brooks. 2008a. Aquatic plants exposed to pharmaceuticals: effects and risks. *Rev. Environ. Contam. Toxicol.* 192:67–115.

Brain, R. A., A. J. Ramirez, B. A. Fulton, C. K. Chambliss, and B. W. Brooks. 2008b. Herbicidal effects of sulfamethoxazole in lemna gibba: using p-aminobenzoic acid as a biomarker of effect. *Environ. Sci. Technol.* 42(23):8965–8970.

Brain, R. A., H. Sanderson, P. K. Sibley, and K. R. Solomon. 2006. Probabilistic ecological hazard assessment: evaluating pharmaceutical effects on aquatic higher plants as an example. *Ecotoxicol. Environ. Saf.* 64(2):128–135.

Brooks, B., et al. [in preparation]. Determination of selective serotonin reuptake inhibitors in macroinvertebrates and periphyton from an effluent-dominated stream.

Brooks, B. W., G. T. Ankley, J. F. Hobson, J. M. Lazorchak, R. D. Meyerhoff, and K. R. Solomon. 2008. Assessing the Aquatic Hazards of Veterinary Medicines. In *Veterinary Medicines in the Environment*, eds. M. Crane, A. Boxall, and K. Barrett, pp. 97–153. Pensacola, FL: SETAC Press.

Brooks, B. W., et al. 2005. Determination of select antidepressants in fish from an effluent-dominated stream. *Environ. Toxicol. Chem.* 24(2):464–469.

Brooks, B. W., et al. 2003. Aquatic ecotoxicology of fluoxetine. *Toxicol. Lett.* 142(3):169–183.

Brooks, B. W., D. Huggett, and A. Boxall. 2009a. Pharmaceuticals and personal care products: research needs for the next decade. *Environ. Toxicol. Chem.* 28(12):2469–2472.

Brooks, B. W., D. B. Huggett, R. A. Brain, and G. Ankley. 2009b. Risk asessment considerations for veterinary medicines in aquatic systems. In *Veterinary Pharmaceuticals in the Environment*, eds. K. Henderson and J. Coats, pp. 205–223. Washington, DC: American Chemical Society.

Brooks, B. W., T. M. Riley, and R. D. Taylor. 2006. Water quality of effluent-dominated ecosystems: ecotoxicological, hydrological, and management considerations. *Hydrobiologia* 556(1):365–379.

Brown, J. N., N. Paxeus, L. Forlin, and D. G. J. Larsson. 2007. Variations in bioconcentration of human pharmaceuticals from sewage effluents into fish blood plasma. *Environ. Toxicol. Pharmacol.* 24(3): 267–274.

Budzinski, H., M. H. Devier, P. Labadie, and A. Togola. 2006. Analysis of hormonal steroids in fish plasma and bile by coupling solid-phase extraction to GC/MS. *Anal. Bioanal. Chem.* 386(5):1429–1439.

Burkhard, L. 2009. Estimation of biota sediment accumulation factor (BSAF) from paired observations of chemical concentrations in biota and sediment EPA/600/R-06/047. Cincinnati, OH: U.S. Environmental Protection Agency, Ecological Risk Assessment Support Center, 35 pp. http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=488260 (accessed February 3, 2010).

Buser, H.-R., M. E. Balmer, P. Schmid, and M. Kohler. 2006. Occurrence of UV filters 4-methylbenzylidene camphor and octocrylene in fish from various Swiss rivers with inputs from wastewater treatment plants. *Environ. Sci. Technol.* 40(5):1427–1431.

Buth, J. M., W. A. Arnold, and K. McNeill. 2007. Unexpected products and reaction mechanisms of the aqueous chlorination of cimetidine. *Environ. Sci. Technol.* 41(17):6228–6233.

Cabello, F. C. 2006. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ. Microbiol.* 8(7):1137–1144.

Caldwell, D. J., et al. 2008. Derivation of an aquatic predicted no-effect concentration for the synthetic hormone, 17a-ethinyl estradiol. *Environ. Sci. Technol.* 42(19):7046–7054.

Capleton, A. C.,et al. 2006. Prioritising veterinary medicines according to their potential indirect human exposure and toxicity profile. *Toxicol. Lett.* 163(3):213–223.

Cecinato, A., and C. Balducci. 2007. Detection of cocaine in the airborne particles of the Italian cities Rome and Taranto. *J. Sep. Sci.* 30(12):1930–1935.

Chiu, L., L. Cunningham, D. Raible, E. Rubel, and H. Ou. 2008. Using the zebrafish lateral line to screen for ototoxicity. *JARO J. Assoc. Res. Otolaryngol.* 9(2):178–190.

Chu, P.-S., M. I. Lopez, A. Abraham, K. R. El Said, and S. M. Plakas. 2008. Residue depletion of nitrofuran drugs and their tissue-bound metabolites in channel catfish (ictalurus punctatus) after oral dosing. *J. Agric. Food Chem.* 56(17):8030–8034.

Chu, S., and C. D. Metcalfe. 2007. Analysis of paroxetine, fluoxetine and norfluoxetine in fish tissues using pressurized liquid extraction, mixed mode solid phase extraction cleanup and liquid chromatography-tandem mass spectrometry. *J. Chromatogr.* 1163(1–2):112–118.

Cleuvers, M. 2005. Initial risk assessment for three β-blockers found in the aquatic environment. *Chemosphere* 59(2):199–205.

Clubbs, R. L., and B. W. Brooks. 2007. *Daphnia magna* responses to a vertebrate estrogen receptor agonist and an antagonist: a multigenerational study. *Ecotoxicol. Environ. Saf.* 67(3):385–398.

Coats, J. R., R. L. Metcalf, P. Y. Lu, D. D. Brown, J. F. Williams, and L. G. Hansen. 1976. Model ecosystem evaluation of the environmental impacts of the veterinary drugs phenothiazine, sulfamethazine, clopidol, and diethylstilbestrol. *Environ. Health Perspect.* 18:167–179.

Committee on Biological Markers of the National Research Council. 1987. Biological markers in environmental health research. *Environ. Health Perspect.* 74:3–9.

Coogan, M. A., R. E. Edziyie, T. W. La Point, and B. J. Venables. 2007. Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in a North Texas wastewater treatment plant receiving stream. *Chemosphere* 67(10):1911–1918.

Coogan, M. A., and T. W. La Point. 2008. Snail bioaccumulation of triclocarban, triclosan, and methyltriclosan in a North Texas, USA, stream affected by wastewater treatment plant runoff. *Environ. Toxicol. Chem.* 27(8):1788–1793.

Corsolini, S. 2009. Industrial contaminants in Antarctic biota. *J. Chromatogr.* 1216(3):598–612.

Cowan-Ellsberry, C. E., et al. 2008. Approach for extrapolating *in vitro* metabolism data to refine bioconcentration factor estimates. *Chemosphere* 70(10):1804–1817.

Crane, M., C. Watts, and T. Boucard. 2006. Chronic aquatic environmental risks from exposure to human pharmaceuticals. *Sci. Total Environ.* 367(1):23–41.

Cueva-Mestanza, R., Z. Sosa-Ferrera, M. E. Torres-Padrón, and J. J. Santana-Rodríguez. 2008. Preconcentration of pharmaceuticals residues in sediment samples using microwave assisted micellar extraction coupled with solid phase extraction and their determination by HPLC-UV. *J. Chromatogr. B* 863(1): 150–157.

Cunningham, V. L., S. P. Binks, and M. J. Olson. 2009. Human health risk assessment from the presence of human pharmaceuticals in the aquatic environment. *Regul. Toxicol. Pharmacol.* 53(1):39–45.

Danaher, M., L. C. Howells, S. R. H. Crooks, V. Cerkvenik-Flajs, and M. O'Keeffe. 2006. Review of methodology for the determination of macrocyclic lactone residues in biological matrices. *J. Chromatogr. B* 844(2):175–203.

Daughton, C. G. 2002. Environmental stewardship and drugs as pollutants. *The Lancet* 360(9339): 1035–1036.

Daughton, C. G. 2005. Emerging chemicals as pollutants in the environment: a 21st century perspective. *Renewable Resources Journal* 23(4):6–23.

Daughton, C. G. 2007. Pharmaceuticals in the environment: sources and their management. In *Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle*, eds. M. Petrovic and D. Barcelo, pp. 1–58. Amsterdam, The Netherlands: Elsevier Science.

Daughton, C. G. 2008. Pharmaceuticals as environmental pollutants: the ramifications for human exposure. In *International Encyclopedia of Public Health*, eds. K. Heggenhougen and S. Quah, pp. 66–102. Oxford, England: Academic Press.

Daughton, C. G. 2009. Chemicals from the practice of healthcare: challenges and unknowns posed by residues in the environment. *Environ. Toxicol. Chem.* 28(12):2490–2494.

Daughton, C. G., and I. S. Ruhoy. 2009. Environmental footprint of pharmaceuticals—the significance of factors beyond direct excretion to sewers. *Environ. Toxicol. Chem.* 28(12):2495–2521.

Daughton, C. G., and T. A. Ternes. 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environ. Health Persp.* 107(Suppl 6):907–938.

Delepee, R., H. Pouliquen, and H. Le Bris. 2004. The bryophyte *Fontinalis antipyretica* Hedw. bioaccumulates oxytetracycline, flumequine and oxolinic acid in the freshwater environment. *Sci. Total Environ.* 322(1–3):243–253.

DellaGreca, M., M. R. Iesce, P. Pistillo, L. Previtera, and F. Temussi. 2009. Unusual products of the aqueous chlorination of atenolol. *Chemosphere* 74(5):730–734.

Delorenzo, M. E., J. M. Keller, C. D. Arthur, M. C. Finnegan, H. E. Harper, V. L. Winder, and D. L. Zdankiewicz. 2008. Toxicity of the antimicrobial compound triclosan and formation of the metabolite methyl-triclosan in estuarine systems. *Environ. Toxicol.* 23(2):224–232.

Dettenmaier, E. M., W. J. Doucette, and B. Bugbee. 2009. Chemical hydrophobicity and uptake by plant roots. *Environ. Sci. Technol.* 43(2):324–329.

Dobbins, L., R. Brain, and B. Brooks. 2008. Comparison of the sensitivities of common *in vitro* and *in vivo* assays of estrogenic activity: application of chemical toxicity distributions. *Environ. Toxicol. Chem.* 27(12):2608–2616.

Dobbins, L., S. Usenko, R. Brain, and B. Brooks. 2009. Probabilistic ecological hazard assessment of parabens using *Daphnia magna* and *Pimephales promelas*. *Environ. Toxicol. Chem.* 28(12):2744–2753.

Dodd, M. C., and C. H. Huang. 2004. Transformation of the antibacterial agent sulfamethoxazole in reactions with chlorine: kinetics mechanisms, and pathways. *Environ. Sci. Technol.* 38(21):5607–5615.

Dordio, A. V., C. Duarte, M. Barreiros, A. J. P. Carvalho, A. P. Pinto, and C. T. da Costa. 2008. Toxicity and removal efficiency of pharmaceutical metabolite clofibric acid by Typha spp.—potential use for phytoremediation? *Bioresour. Technol.* 100(3):1156–1161.

Dussault, È. B., V. K. Balakrishnan, U. Borgmann, K. R. Solomon, and P. K. Sibley. 2009. Bioaccumulation of the synthetic hormone 17α-ethinylestradiol in the benthic invertebrates *Chironomus tentans* and Hyalella azteca. *Ecotoxicol. Environ. Saf.* 72(6):1635–1641.

Erickson, R. J., J. M. McKim, G. J. Lien, A. D. Hoffman, and S. L. Batterman. 2006a. Uptake and elimination of ionizable organic chemicals at fish gills: I. Model formulation, parameterization, and behavior. *Environ. Toxicol. Chem.* 25(6):1512–1521.

Erickson, R. J., J. M. McKim, G. J. Lien, A. D. Hoffman, and S. L. Batterman. 2006b. Uptake and elimination of ionizable organic chemicals at fish gills: II. Observed and predicted effects of pH, alkalinity, and chemical properties. *Environ. Toxicol. Chem.* 25(6):1522–1532.

Erickson, R., J. Nichols, P. Cook, and G. Ankley. 2008. Bioavailability of chemical contaminants in aquatic systems. In *The Toxicology of Fishes*, eds.R. T. Di Giulio and D. E. Hinton, pp. 9–54. Boca Raton, FL: CRC Press.

Escher, B. I., et al. 2007. Membrane-water partitioning, membrane permeability and baseline toxicity of the parasiticides ivermectin, albendazole, and morantel. *Environ. Toxicol. Chem.* 27(4):909–918.

Escher, B., R. Schwarzenbach, and J. Westall. 2000. Evaluation of liposome-water partitioning of organic acids and bases. 2. Comparison of experimental determination methods. *Environ. Sci. Technol.* 34(18):3962–3968.

Fair, P. A.,et al. 2009. Occurrence of triclosan in plasma of wild Atlantic bottlenose dolphins (*Tursiops truncatus*) and in their environment. *Environ. Pollut.* 157(8–9):2248–2254.

Farré, M. L., S. Pérez, L. Kantiani, and D. Barceló. 2008. Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. *TrAC, Trends Anal. Chem.* 27(11):991–1007.

Fent, K., A. A. Weston, and D. Caminada. 2006. Ecotoxicology of human pharmaceuticals. *Aquat. Toxicol.* 76(2):122–159.

Fernandez, M. P., M. G. Ikonomou, and I. Buchanan. 2007. An assessment of estrogenic organic contaminants in Canadian wastewaters. *Sci. Total Environ.* 373(1):250–269.

Fick, J., R. H. Lindberg, J. Parkkonen, B. Arvidsson, M. Tysklind, and D. G. J. Larsson. 2010. In press. Therapeutic levels of Levonorgestral detected in blood plasma of fish: results from screening rainbow trout exposed to treated sewage effluents. *Environ. Sci. Technol.* 44(7): 2661–2666.

Fick, J., H. Söderström, R. H. Lindberg, C. Phan, M. Tysklind, and D. G. J. Larsson. 2009. Contamination of surface, ground, and drinking water from pharmaceutical production. *Environ. Toxicol. Chem.* 28(12):2522–2527.

Fitzsimmons, P. N., J. D. Fernandez, A. D. Hoffman, B. C. Butterworth, and J. W. Nichols. 2001. Branchial elimination of superhydrophobic organic compounds by rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* 55(1–2):23–34.

Flores, A., and E. M. Hill. 2008. Formation of estrogenic brominated ethinylestradiol in drinking water: implications for aquatic toxicity testing. *Chemosphere* 73(7):1115–1120.

Forni, C., A. Cascone, M. Fiori, and L. Migliore. 2002. Sulphadimethoxine and *Azolla filiculoides* Lam.: a model for drug remediation. *Water Res.* 36(13):3398–3403.

Fu, W., A. Franco, and S. Trapp. 2009. Methods for estimating the bioconcentration factor of ionizable organic chemicals. *Environ. Toxicol. Chem.* 28(7):1372–1379.

Fulton, B., R. Brain, S. Usenko, J. Back, R. King, and B. Brooks. 2009. Influence of nitrogen and phosphorus concentrations and ratios on Lemna gibba growth responses to triclosan in laboratory and stream mesocosm experiments. *Environ. Toxicol. Chem.* 28(12):2610–2621.

Fulton, B. A., R. A. Brain, S. Usenko, J. A. Back, and B. W. Brooks. 2010. Exploring Lemna gibba thresholds to nutrient and chemical stressors: differential effects of triclosan on internal stoichiometry and nitrate uptake across a N:P gradient. *Environ. Toxicol. Chem.* In press. 10.1002/etc.289.

Galvez, F., A. Donini, R. C. Playle, D. S. Smith, M. J. O'Donnell, and C. M. Wood. 2008. A matter of potential concern: natural organic matter alters the electrical properties of fish gills. *Environ. Sci. Technol.* 42(24):9385–9390.

Gaworecki, K. M., and S. J. Klaine. 2008. Behavioral and biochemical responses of hybrid striped bass during and after fluoxetine exposure. *Aquat. Toxicol.* 88(4):207–213.

Gelsleichter, J. 2009. Project profile: exposure of freshwater sharks to human pharmaceuticals. Evaluating the risks that pharmaceutical-related pollutants pose to Caloosahatchee River wildlife: observations on the bull shark, *Carcharhinus leucas*. In *Final Report: Charlotte Harbor National Estuary Program*, CAC 2–18-09. Sarasota, FL: Mote Marine Laboratory, 18 pp. http://www.chnep.org/NEP/agendas-2009/CAC/CAC2–18-09_3c_pharmaceuticals.pdf (accessed February 3, 2010).

Geyer, H., G. Rimkus, I. Scheunert, A. Kaune, K.-W. Schramm, A. Kettrup, M. Zeeman. D. Muir, L. Hansen, and D. Mackay. 2000. Bioaccumulation and occurrence of endocrine-disrupting chemicals (EDCs), persistent organic pollutants (POPs), and other organic compounds in fish and other organisms including humans. In *Bioaccumulation—New Aspects and Developments*, ed. B. Beek, pp. 1–166. Heidelberg: Springer Berlin.

Gibson, R., M. D. Smith, C. J. Spary, C. R. Tyler, and E. M. Hill. 2005a. Mixtures of estrogenic contaminants in bile of fish exposed to wastewater treatment works effluents. *Environ. Sci. Technol.* 39(8):2461–2471.

Gibson, R., C. R. Tyler, and E. M. Hill. 2005b. Analytical methodology for the identification of estrogenic contaminants in fish bile. *J. Chromatogr.* 1066(1–2):33–40.

Gobas, F. A. P. C., and H. A. Morrison. 2000. Bioconcentration and biomagnification in the aquatic environment. In *Handbook of Property Estimation Methods for Chemicals*, eds. D. Mackay and R. S. Boethling, pp. 189–231. Boca Raton, FL: CRC Press.

Golet, E. M., A. C. Alder, and W. Giger. 2002. Environmental exposure and risk assessment of fluoroquinolone antibacterial agents in wastewater and river water of the Glatt Valley Watershed, Switzerland. *Environ. Sci. Technol.* 36(17):3645–3651.

Gomes, R. L., H. E. Deacon, K. M. Lai, J. W. Birkett, M. D. Scrimshaw, and J. N. Lester. 2004. An assessment of the bioaccumulation of estrone in *Daphnia magna*. *Environ. Toxicol. Chem.* 23(1):105–108.

Gordon, D. A., et al. 2006. Effects of eutrophication on vitellogenin gene expression in male fathead minnows (*Pimephales promelas*) exposed to 17a-ethynylestradiol in field mesocosms. *Environ. Pollut.* 142(3):559–566.

Greenwood, R., B. Vrana, and G. Mills, eds. 2007. *Passive Sampling Techniques in Environmental Monitoring.* Wilson & Wilson's Comprehensive Analytical Chemistry series ed. D. Barcelo. Amsterdam, The Netherlands: Elsevier.

Grim, K. C., M. Wolfe, W. Hawkins, R. Johnson, and J. Wolf. 2007. Intersex in japanese medaka (oryzias latipes) used as negative controls in toxicologic bioassays: a review of 54 cases from 41 studies. *Environ. Toxicol. Chem.* 26 (8):1636–1643.

Gross, M., et al. 2010. Thresholds of toxicological concern for endocrine active substances in the aquatic environment. *Integr. Environ. Assess. Manage.* 6 (1):2–11.

Grung, M., et al. 2007. Human and veterinary pharmaceuticals, narcotics, and personal care products in the environment: current state of knowledge and monitoring requirements, TA-2325/2007. Oslo, Norway: Swedish Environmental Research Institute, 98 pp. http://www.sft.no/publikasjoner/2325/ta2325.pdf (accessed February 3, 2010).

Guarino, A. M., and J. J. Lech. 1986. Metabolism, disposition, and toxicity of drugs and other xenobiotics in aquatic species. *Vet. Hum. Toxicol.* 28 (Suppl. 1):38–44.

Gunnarsson, L., A. Jauhiainen, E. Kristiansson, O. Nerman, and D. G. J. Larsson. 2008. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. *Environ. Sci. Technol.* 42(15):5807–5813.

Hamscher, G., H. T. Pawelzick, S. Sczesny, H. Nau, and J. Hartung. 2003. Antibiotics in dust originating from a pig-fattening farm: a new source of health hazard for farmers? *Environ. Health Perspect.* 111(13):1590–1594.

Henry, T., and M. Black. 2008. Acute and chronic toxicity of fluoxetine (selective serotonin reuptake inhibitor) in western mosquitofish. *Arch. Environ. Contam. Toxicol.* 54(2):325–330.

Hernando, M., A. Agüera, and A. Fernández-Alba. 2007. LC-MS analysis and environmental risk of lipid regulators. *Anal. Bioanal. Chem.* 387(4):1269–1285.

Higgins, C. P., Z. J. Paesani, T. E. A. Chalew, and R. U. Halden. 2009. Bioaccumulation of triclocarban in *Lumbriculus variegatus*. *Environ. Toxicol. Chem.* 28(12):2580–2586.

Hilvarsson, A., H. P. Halldorsson, and A. Granmo. 2007. Medetomidine as a candidate antifoulant: sublethal effects on juvenile turbot (Psetta maxima L.). *Aquat. Toxicol.* 83(3):238–246.

Hinton, D. E., et al. 2005. Resolving mechanisms of toxicity while pursuing ecotoxicological relevance? *Mar. Pollut. Bull.* 51(8–12):635–648.

Hoeger, B., B. Hitzfeld, B. Kollner, D. R. Dietrich, and M. R. van den Heuvel. 2005. Sex and low-level sampling stress modify the impacts of sewage effluent on the rainbow trout (*Oncorhynchus mykiss*) immune system. *Aquat. Toxicol.* 73(1):79–90.

Hou, X., J. Shen, S. Zhang, H. Jiang, and J. R. Coats. 2003. Bioconcentration and elimination of sulfamethazine and its main metabolite in sturgeon (Acipenser schrenkii). *J. Agric. Food Chem.* 51(26):7725–7729.

Houtman, C. J., A. M. Van Oostveen, A. Brouwer, M. H. Lamoree, and J. Legler. 2004. Identification of estrogenic compounds in fish bile using bioassay-directed fractionation. *Environ. Sci. Technol.* 38(23):6415–6423.

Huggett, D. B., J. C. Cook, J. F. Ericson, and R. T. Williams. 2003. A theoretical model for utilizing mammalian pharmacology and safety data to prioritize potential impacts of human pharmaceuticals to fish. *Hum. Ecol. Risk Assess.* 9(7):1789–1799.

Huggett, D. B., J. F. Ericson, J. C. Cook, and R. T. Williams. 2004. Plasma concentrations of human pharmaceuticals as predictors of pharmacological responses in fish. In *Pharmaceuticals in the Environment—Sources, Fate, Effects and Risks*, ed. K. Kümmerer, pp. 373–386. Berlin, Germany: Springer.

Huggett, R., R. Kimerle, P. Mehrle, and H. Bergman, eds. 1992. *Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress*. Boca Raton, FL: Lewis Publishers.

Junker, T., R. Alexy, T. Knacker, and K. Kummerer. 2006. Biodegradability of 14C-labeled antibiotics in a modified laboratory scale sewage treatment plant at environmentally relevant concentrations. *Environ. Sci. Technol.* 40(1):318–324.

Kah, M., and C. D. Brown. 2008. Log D: Lipophilicity for ionisable compounds. *Chemosphere* 72(10): 1401–1408.

Kimball, B. 2008. Endocrine disrupting chemical (EDC) accumulation in Puget Sound sediments and the implications for native fish populations. Senior Thesis diss., School of Oceanography, University of Washington. https://digital.lib.washington.edu/xmlui/handle/1773/3770 (accessed February 3, 2010).

Klaassen, C., and J. Watkins. 2003. *Casarett & Doull's Essentials of Toxicology*. New York, NY: McGraw-Hill.

Konwick, B. J., A. W. Garrison, J. K. Avants, and A. T. Fisk. 2006. Bioaccumulation and biotransformation of chiral triazole fungicides in rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* 80(4):372–381.

Kools, S. A., A. Boxall, J. F. Moltmann, G. Bryning, J. Koschorreck, and T. Knacker. 2008. A ranking of European veterinary medicines based on environmental risks. *Integr. Environ. Assess. Manage.* 4(4):399–408.

Kosjek, T., and E. Heath. 2008. Applications of mass spectrometry to identifying pharmaceutical-transformation products in water treatment. *TrAC, Trends Anal. Chem.* 27(10):807–820.

Kostich, M. S., and J. M. Lazorchak. 2008. Risks to aquatic organisms posed by human pharmaceutical use. *Sci. Total Environ.* 389(2–3):329–339.

Kwon, J. W., K. L. Armbrust, D. Vidal-Dorsch, and S. M. Bay. 2009. Determination of 17α-ethynylestradiol, carbamazepine, diazepam, simvastatin, and oxybenzone in fish livers. *J. AOAC Int.* 92(1):359–69.

Lai, K. M., M. D. Scrimshaw, and J. N. Lester. 2002a. Biotransformation and bioconcentration of steroid estrogens by *Chlorella vulgaris*. *Appl. Environ. Microbiol.* 68(2):859–64.

Lai, K. M., M. D. Scrimshaw, and J. N. Lester. 2002b. Prediction of the bioaccumulation factors and body burden of natural and synthetic estrogens in aquatic organisms in the river systems. *Sci. Total Environ.* 289(1–3):159–168.

Lajeunesse, A., G. Vernouillet, P. Eullaffroy, C. Gagnon, P. Juneau, and S. Sauvé. 2009. Determination of carbamazepine in aquatic organisms by liquid–liquid extraction and liquid chromatography-tandem mass spectrometry. *J. Environ. Monit.* 11:723–725.

Lange, R., et al. 2001. Effects of the synthetic estrogen 17α-ethinylestradiol on the life-cycle of the fathead minnow (*Pimephales promelas*). *Environ. Toxicol. Chem.* 20(6):1216–1227.

Langston, W. J., G. R. Burt, B. S. Chesman, and C. H. Vane. 2005. Partitioning, bioavailability and effects of oestrogens and xeno-oestrogens in the aquatic environment. *J. Mar. Biol. Assoc. U.K.* 85(1):1–31.

Larsen, M., K. Hansen, P. Henriksen, and E. Baatrup. 2008. Male zebrafish (*Danio rerio*) courtship behaviour resists the feminising effects of 17α-ethinyloestradiol—morphological sexual characteristics do not. *Aquat. Toxicol.* 87(4):234–244.

Larsson, B. S. 1993. Interaction between chemicals and melanin. *Pigment Cell Res.* 6(3):127–133.

Larsson, D. G. J., C. de Pedro, and N. Paxeus. 2007. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. *J. Hazard. Mater.* 148(3):751–755.

Larsson, D. G. J., et al. 1999. Ethinyloestradiol—an undesired fish contraceptive? *Aquat. Toxicol.* 45(2–3):91–97.

Le Bris, H., and H. Pouliquen. 2004. Experimental study on the bioaccumulation of oxytetracycline and oxolinic acid by the blue mussel (*Mytilus edulis*). An evaluation of its ability to bio-monitor antibiotics in the marine environment. *Mar. Pollut. Bull.* 48(5–6):434–440.

Leiker, T. J., S. R. Abney, S. L. Goodbred, and M. R. Rosen. 2009. Identification of methyl triclosan and halogenated analogues in male common carp (Cyprinus carpio) from Las Vegas Bay and semipermeable membrane devices from Las Vegas Wash, Nevada. *Sci. Total Environ.* 407(6):2102–2114.

Liebig, M., P. Egeler, J. Oehlmann, and T. Knacker. 2005. Bioaccumulation of ^{14}C-17a-ethinylestradiol by the aquatic oligochaete *Lumbriculus variegatus* in spiked artificial sediment. *Chemosphere* 59(2):271–280.

Lissemore, L., C. Hao, P. Yang, P. K. Sibley, S. Mabury, and K. R. Solomon. 2006. An exposure assessment for selected pharmaceuticals within a watershed in Southern Ontario. *Chemosphere* 64(5):717–729.

López de Alda, M. J., A. Gil, E. Paz, and D. Barceló. 2002. Occurrence and analysis of estrogens and progestogens in river sediments by liquid chromatography-electrospray-mass spectrometry. *Analyst* 127(10):1299–1304.

Lu, J., and G. V. Korshin. 2008. A spectroscopic study of the bromination of the endocrine disruptor ethynylestradiol. *Chemosphere* 72(3):504–508.

Lyssimachou, A., and A. Arukwe. 2007. Alteration of Brain and Interrenal StAR Protein, P450scc, and Cyp11β mRNA Levels in Atlantic Salmon after Nominal Waterborne Exposure to the Synthetic Pharmaceutical Estrogen Ethynylestradiol. *J. Toxicol. Environ. Health, A* 70(7):606–613.

Maunder, R. J., P. Matthiessen, J. P. Sumpter, and T. G. Pottinger. 2007. Rapid bioconcentration of steroids in the plasma of three-spined stickleback *Gasterosteus aculeatus* exposed to waterborne testosterone and 17ß-oestradiol. *J. Fish Biol.* 70(3):678–690.

Mayer, P., L. Toraäg, N. Glaesner, and J. Å. Jönsson. 2009. Silicone membrane equilibrator: measuring chemical activity of nonpolar chemicals with poly(dimethylsiloxane) microtubes immersed directly in tissue and lipids. *Anal. Chem.* 81(4):1536–1542.

Mehinto, A. C., E. M. Hill, and C. R. Tyler. 2010. Uptake and biological effects of environmentally relevant concentrations of the nonsteroidal anti-inflammatory pharmaceutical diclofenac in rainbow trout (*Oncorhynchus mykiss*). *Environ. Sci. Technol.* 44(6):2176–2182.

Metcalfe, C. D., A. Boxall, K. Fenner, D. Kolpin, M. Servos, E. Silberhorn, and J. Staveley. 2008. Exposure assessment of veterinary medicines in aquatic systems. In *Veterinary Medicines in the Environment*, eds. M. Crane, K. Barrett and A. B. A. Boxall, pp. 57–96. Pensacola, FL: SETAC Press.

Metcalfe, C. D., S. Chu, C. Judt, H. Li, K. D. Oakes, M. R. Servos, and D. M. Andrews. 2010. Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an urban watershed. *Environ. Toxicol. Chem.* 29(1):79–89.

Migliore, L., S. Cozzolino, and M. Fiori. 2000. Phytotoxicity to and uptake of flumequine used in intensive aquaculture on the aquatic weed, Lythrum salicaria L. *Chemosphere* 40(7):741–750.

Miguel-Queralt, S., and G. L. Hammond. 2008. Sex hormone-binding globulin in fish gills is a portal for sex steroids breached by xenobiotics. *Endocrinology* 149(9):4269–4275.

Mimeault, C., A. J. Woodhouse, X. S. Miao, C. D. Metcalfe, T. W. Moon, and V. L. Trudeau. 2005. The human lipid regulator, gemfibrozil bioconcentrates and reduces testosterone in the goldfish, *Carassius auratus*. *Aquat. Toxicol.* 73(1):44–54.

Miyazaki, T., T. Yamagishi, and M. Matsumoto. 1984. Residues of 4-chloro-1-(2,4-dichlorophenoxy)-2-methoxybenzene(triclosan methyl) in aquatic biota. *Bull. Environ. Contam. Toxicol.* 32(2):227–32.

Mottaleb, M. A., S. Usenko, J. G. O'Donnell, A. J. Ramirez, B. W. Brooks, and C. K. Chambliss. 2009. Gas chromatography-mass spectrometry screening methods for select UV filters, synthetic musks, alkylphenols, an antimicrobial agent, and an insect repellent in fish. *J. Chromatogr.* 1216(5):815–823.

Munro, I. 1990. Safety assessment procedures for indirect food additives: an overview. Report of a workshop. *Regul. Toxicol. Pharmacol.* 12(1):2–12.

Nakamura, H., R. Kuruto-Niwa, M. Uchida, and Y. Terao. 2007. Formation of chlorinated estrones via hypochlorous disinfection of wastewater effluent containing estrone. *Chemosphere* 66(8):1441–1448.

Nakamura, Y., H. Yamamoto, J. Sekizawa, T. Kondo, N. Hirai, and N. Tatarazako. 2008. The effects of pH on fluoxetine in Japanese medaka (Oryzias latipes): acute toxicity in fish larvae and bioaccumulation in juvenile fish. *Chemosphere* 70(5):865–873.

New World Encyclopedia contributors. 13 August 2008. Chromatophore. In *New World Encyclopedia*.

Nichols, J. W., P. N. Fitzsimmons, and L. P. Burkhard. 2007. *In vitro-in vivo* extrapolation of quantitative hepatic biotransformation data for fish. II. Modeled effects on chemical bioaccumulation. *Environ. Toxicol. Chem.* 26(6):1304–1319.

Nie, X.-P., J.-F. Chen, X. Wang, X.-Z. Zhou, J.-Y. Lu, and Y.-F. Yang. 2008. Bioaccumulation of Ciprofloxacin in *Allogynogenetic crucian* carp and its toxic effects (in Chinese). *Acta Ecologica Sinica* 28(1):246–252.

ONDCP. 2009 [updated October]. Proper disposal of prescription drugs: Federal Guidelines. Washington, DC: White House Office of National Drug Control Policy, http://www.whitehousedrugpolicy.gov/publications/pdf/prescrip_disposal.pdf (accessed February 3, 2010).

Orvos, D. R., D. J. Versteeg, J. Inauen, M. Capdevielle, A. Rothenstein, and V. Cunningham. 2002. Aquatic toxicity of triclosan. *Environ. Toxicol. Chem.* 21(7):1338–1349.

Owen, S. F., et al. 2007. Comparative physiology, pharmacology and toxicology of ß-blockers: mammals versus fish. *Aquat. Toxicol.* 82(3):145–162.

Owen, S. F., et al. 2009. Uptake of Propranolol, a cardiovascular pharmaceutical, from water into fish plasma and its effects on growth and organ biometry. *Aquat. Toxicol.* 93(4):217–224.

Painter, M. M., et al. 2009. Antidepressants at environmentally relevant concentrations affect predator avoidance behavior of larval fathead minnows (*Pimephales promelas*). *Environ. Toxicol. Chem.* 28(12): 2677–2684.

Park, K. J., C. T. Müller, S. Markman, O. Swinscow-Hall, D. Pascoe, and K. L. Buchanan. 2009. Detection of endocrine disrupting chemicals in aerial invertebrates at sewage treatment works. *Chemosphere* 77(11):1459–1464.

Parkerton, T. F., et al. 2008. Guidance for evaluating *in vivo* fish bioaccumulation data. *Integr. Environ. Assess. Manage.* 4(2):139–155.

Paterson, G., and C. D. Metcalfe. 2008. Uptake and depuration of the anti-depressant fluoxetine by the Japanese medaka (Oryzias latipes). *Chemosphere* 74(1):125–130.

Peck, A. M., and K. C. Hornbuckle. 2004. Synthetic musk fragrances in Lake Michigan. *Environ. Sci. Technol.* 38(2):367–72.

Peck, A. M., and K. C. Hornbuckle. 2006. Synthetic musk fragrances in urban and rural air of Iowa and the Great Lakes. *Atmos. Environ.* 40(32):6101–6111.

Pettersson, M. 2006. Endocrine disrupting compounds in effluent waters: Chemical analysis to evaluate exposure of fish. Doctoral diss., Department of Applied Environmental Science (ITM), Stockholm University, Stockholm. http://www.diva-portal.org/su/theses/abstract.xsql?dbid=1373 (accessed February 3, 2010).

Pettersson, M., M. Adolfsson-Erici, J. Parkkonen, L. Forlin, and L. Asplund. 2006. Fish bile used to detect estrogenic substances in treated sewage water. *Sci. Total Environ.* 366(1):174–186.

Pettersson, M., E. Hahlbeck, I. Katsiadaki, L. Asplund, and B.-E. Bengtsson. 2007. Survey of estrogenic and androgenic disruption in Swedish coastal waters by the analysis of bile fluid from perch and biomarkers in the three-spined stickleback. *Mar. Pollut. Bull.* 54(12):1868–1880.

Pouliquen, H., et al. 2009. Comparison of water, sediment and plants for the monitoring of antibiotics: a case study on a river dedicated to fish farming. *Environ. Toxicol. Chem.* 28(3):496–502.

Purdom, C. E., P. A. Hardiman, V. V. J. Bye, N. C. Eno, C. R. Tyler, and J. P. Sumpter. 1994. Estrogenic effects of effluents from sewage treatment works. *Chem. Ecol.* 8(4):275–285.

Ramirez, A., et al. 2009. Occurrence of pharmaceuticals and personal care products (PPCPs) in fish tissues: results of a national pilot study in the U.S. *Environ. Toxicol. Chem.* 28(12):2587–2597.

Ramirez, A. J. 2007. Determination of Pharmaceuticals and Personal Care Products in Fish Using High Performance Liquid Chromatography-Tandem Mass Spectrometry and Gas Chromatography-Mass Spectrometry. PhD diss., Department of Chemistry and Biochemistry, Baylor University, Waco, TX. https://beardocs.baylor.edu/handle/2104/5119 (accessed February 3, 2010).

Ramirez, A. J., M. A. Mottaleb, B. W. Brooks, and C. K. Chambliss. 2007. Analysis of pharmaceuticals in fish using liquid chromatography-tandem mass spectrometry. *Anal. Chem.* 79(8):3155–3163.

Redshaw, C. H., V. G. Wootton, and S. J. Rowland. 2008. Uptake of the pharmaceutical Fluoxetine Hydrochloride from growth medium by Brassicaceae. *Phytochemistry* 69(13):2510–2516.

Rigos, G., I. Nengas, M. Alexis, and G. M. Troisi. 2004. Potential drug (oxytetracycline and oxolinic acid) pollution from Mediterranean sparid fish farms. *Aquat. Toxicol.* 69(3):281–288.

Roffey, S. J., R. S. Obach, J. I. Gedge, and D. A. Smith. 2007. What is the objective of the mass balance study? A retrospective analysis of data in animal and human excretion studies employing radiolabeled drugs. *Drug Metab. Rev.* 39(1):17–43.

Rolfhus, K. R., M. B. Sandheinrich, J. G. Wiener, S. W. Bailey, K. A. Thoreson, and C. R. Hammerschmidt. 2008. Analysis of fin clips as a nonlethal method for monitoring mercury in fish. *Environ. Sci. Technol.* 42(3):871–877.

Ruhoy, I. S., and C. G. Daughton. 2008. Beyond the medicine cabinet: an analysis of where and why medications accumulate. *Environ. Int.* 34(8):1157–1169.

Salo, H. M., N. Hébert, C. Dautremepuits, P. Cejka, D. G. Cyr, and M. Fournier. 2007. Effects of montreal municipal sewage effluents on immune responses of juvenile female rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* 84(4):406–414.

Samsøe-Petersen, L., M. Winther-Nielsen, T. Madsen, and DHI Water and Environment. 2003. Fate and effects of Triclosan, Environmental Project No. 861 2003: Danish EPA, 47 pp. http://www.miljoestyrelsen.dk/udgiv/publications/2003/87-7972-984-3/pdf/87-7972-985-1.pdf (accessed February 3, 2010).

Samuelsen, O. B., B. T. Lunestad, B. Husevag, T. Holleland, and A. Ervik. 1992. Residues of oxolinic acid in wild fauna following medication in fish farms. *Dis. Aquat. Org.* 12:111–119.

Sapkota, A., et al. 2008. Aquaculture practices and potential human health risks: current knowledge and future priorities. *Environ. Int.* 34(8):1215–1226.

Schirmer, K., and M. Schirmer. 2008. Who is chasing whom? A call for a more integrated approach to reduce the load of micro-pollutants in the environment. *Water Sci. Technol.* 57(1):145–150.

Schuetze, A., T. Heberer, and S. Juergensen. 2008. Occurrence of residues of the veterinary drug malachite green in eels caught downstream from municipal sewage treatment plants. *Chemosphere* 72(11):1664–1670.

Schultz, I. R., G. Orner, J. L. Merdink, and A. Skillman. 2001. Dose-response relationships and pharmacokinetics of vitellogenin in rainbow trout after intravascular administration of 17α-ethynylestradiol. *Aquat. Toxicol.* 51(3):305–318.

Schultz, M. M., et al. 2010. Antidepressant pharmaceuticals in two U.S. effluent-impacted streams: occurrence and fate in water and sediment, and selective uptake in fish neural tissue. *Environ. Sci. Technol.* 44(6):1918–1925.

Schwaiger, J., H. Ferling, U. Mallow, H. Wintermayr, and R. D. Negele. 2004. Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part I: histopathological alterations and bioaccumulation in rainbow trout. *Aquat. Toxicol.* 68(2):141–150.

Scott, A., M. Pinillos, and M. Huertas. 2005. The rate of uptake of sex steroids from water by *Tinca tinca* is influenced by their affinity for sex steroid binding protein in plasma. *J. Fish Biol.* 67(1):182–200.

Scott, A. P., and T. Ellis. 2007. Measurement of fish steroids in water—a review. *Gen. Comp. Endocrinol.* 153 (1–3):392–400.

Shen, J., Q. Zhang, S. Ding, S. Zhang, and J. R. Coats. 2005. Bioconcentration and elimination of avermectin B1 in sturgeon. *Environ. Toxicol. Chem.* 24(2):396–399.

Sinclair, C. J., A. B. A. Boxall, S. A. Parsons, and M. R. Thomas. 2006. Prioritization of pesticide environmental transformation products in drinking water supplies. *Environ. Sci. Technol.* 40(23):7283–7289.

Skillman, A. D., J. J. Nagler, S. E. Hook, J. A. Small, and I. R. Schultz. 2006. Dynamics of 17a-ethynylestradiol exposure in rainbow trout (*Oncorhynchus mykiss*): absorption, tissue distribution, and hepatic gene expression pattern. *Environ. Toxicol. Chem.* 25(11):2997–3005.

Snyder, S. A., E. C. Wert, H. Lei, P. Westerhoff, Y. Yoon, and AWWA Research Foundation. 2007. Removal of EDCs and Pharmaceuticals in Drinking and Reuse Treatment Processes [Project #2758] Denver, CO: Awwa Research Foundation, 331 pp. http://ualweb.library.ualberta.ca/uhtbin/cgisirsi/x/0/0/57/5?user_id=WUAARCHIVE&searchdata1=4198864 (accessed February 3, 2010).

Stanley, J. K., and B. W. Brooks. 2009. Perspectives on ecological risk assessment of chiral compounds. *Integr. Environ. Assess. Manage.* 5(3):364–373.

Stanley, J. K., A. J. Ramirez, C. K. Chambliss, and B. W. Brooks. 2007. Enantiospecific sublethal effects of the antidepressant fluoxetine to a model aquatic vertebrate and invertebrate. *Chemosphere* 69(1):9–16.

Stanley, J. K., A. J. Ramirez, M. Mottaleb, C. K. Chambliss, and B. W. Brooks. 2006. Enantiospecific toxicity of the ß-blocker propranolol to *Daphnia magna* and *Pimephales promelas*. *Environ. Toxicol. Chem.* 25(7):1780–1786.

Steffenak, I., V. Hormazabal, and M. Yndestad. 1994. Effect of cooking on residues of the quinolones oxolinic acid and flumequine in fish. *Acta Vet. Scand.* 35(3):299–301.

Stewart, A. B., A. V. Spicer, E. K. Inskeep, and R. A. Dailey. 2001. Steroid hormone enrichment of Artemia nauplii. *Aquaculture* 202(1–2):177–181.

Sudova, E., J. Machova, Z. Svobodova, and T. Vesely. 2007. Negative effects of malachite green and possibilities of its replacement in the treatment of fish eggs and fish: a review. *Vet. Med. (Praha).* 52(12):527–539.

Suter, G. W., R. A. Efroymson, B. E. Sample, and D. S. Jones. 2000. *Ecological risk assessment for contaminated sites*. Boca Raton, FL: Lewis Publishers.

Takahashi, A., T. Higashitani, Y. Yakou, M. Saitou, H. Tamamoto, and H. Tanaka. 2003. Evaluating bioaccumulation of suspected endocrine disruptors into periphytons and benthos in the Tama River. *Water Sci. Technol.* 47(9):71–76.

Tan, X. 2007. P-glycoprotein and Membrane Permeability as Determinants for Xenobiotic Bioavailability and Bioaccumulation PhD diss., Comparative Biomedical Sciences Shandong Medical University Shandong, China. http://etd.lsu.edu/docs/available/etd-11152007–101053/ (accessed 3 February 2010).

Testorf, M. F., R. Kronstrand, S. P. S. Svensson, I. Lundström, and J. Ahlner. 2001. Characterization of [3H] flunitrazepam binding to melanin. *Anal. Biochem.* 298(2):259–264.

Tittlemier, S. A., et al. 2007. Analysis of veterinary drug residues in fish and shrimp composites collected during the Canadian Total Diet Study, 1993–2004. *Food Addit. Contam.* 24(1):14–20.

Tyler, C., E. Routledge, and R. van Aerle. 2008. Estrogenic effects of treated sewage effluent on fish: steroids and surfactants in English rivers. In *The Toxicology of Fishes*, eds. R. T. DiGiulio and D. E. Hinton, pp. 971–1002. Boca Raton, FL: CRC Press.

Tyler, C. R., et al. 2009. Environmental health impacts of equine estrogens derived from hormone replacement therapy. *Environ. Sci. Technol.* 43(10):3897–3904.

U.S. EPA. 1985. Ambient water quality criteria for ammonia—1984, EPA-440–5-85–001. Washington, DC: Office of Regulations and Standards, Criteria and Standards Division, http://www.epa.gov/waterscience/criteria/library/ambientwqc/ammonia1984.pdf (accessed February 3, 2010).

U.S. EPA. 1986. Ambient water quality criteria for pentachlorophenol-1986, EPA-440–5-86–009. Washington, DC: Office of Regulations and Standards, Criteria and Standards Division.

U.S. EPA. 1991. Technical Support Document for Water Quality-Based Toxics Control, EPA/505/2–90-001, 335 pp. http://www.epa.gov/npdes/pubs/owm0264.pdf (accessed February 3, 2010).

U.S. EPA. 1999. Guidelines for Ecological Risk Assessment, April 1998, Risk Assessment Forum, EPA/630/R-95/002F. Washington, DC, http://www.epa.gov/ncea/raf/pdfs/ecotxtbx.pdf (accessed February 3, 2010).

U.S. EPA. 2006. Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment (Final Report), EPA/600/R-05/043F, http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=157668 (accessed February 3, 2010).

U.S. EPA. 2008a. *EPA Pilot study of pharmaceuticals in fish tissue: Office of Water*, http://www.epa.gov/waterscience/ppcp/files/fish-pilot.pdf (accessed February 3, 2010).

U.S. EPA. 2008b. *National Rivers and Streams Assessment (NRSA)*. US Environmental Protection Agency. Washington, DC. http://www.epa.gov/owow/riverssurvey (accessed February 3, 2010).

U.S. EPA. 2009. *Pharmaceuticals and Personal Care Products (PPCPs): Relevant Literature*. US Environmental Protection Agency. http://www.epa.gov/ppcp/lit.html (accessed March 10, 2009).

U.S. FDA. 2010. *How to Dispose of Unused Medicines, Consumer Health Information: US Food and Drug Administration*, http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm101653.htm (accessed February 3, 2010).

Valenti Jr, T. W., P. Perez Hurtado, C. K. Chambliss, and B. W. Brooks. 2009. Aquatic toxicity of sertraline to *Pimephales promelas* at environmentally relevant surface water pH. *Environ. Toxicol. Chem.* 28(12):2685–2694.

Valters, K., et al. 2005. Polybrominated diphenyl ethers and hydroxylated and methoxylated brominated and chlorinated analogues in the plasma of fish from the Detroit River. *Environ. Sci. Technol.* 39(15):5612–5619.

Van Aerle, R., N. Pounds, T. H. Hutchinson, S. Maddix, and C. R. Tyler. 2002. Window of sensitivity for the estrogenic effects of ethinylestradiol in early life-stages of fathead minnow, *Pimephales promelas*. *Ecotoxicology* 11(6):423–434.

Van Bambeke, F., E. Balzi, and P. M. Tulkens. 2000. Antibiotic efflux pumps. *Biochem. Pharmacol.* 60(4):457–470.

Van den Heuvel, W. J. A., A. D. Forbis, B. A. Halley, C. C. Ku, T. A. Jacob, and P. G. Wislocki. 1996. Bioconcentration and depuration of avermectin B1a in the bluegill sunfish. *Environ. Toxicol. Chem.* 15(12):2263–2266.

Van der Oost, R., J. Beyer, and N. P. E. Vermeulen. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol.* 13(2):57–149.

Vermeirssen, E., R. Eggen, B. Escher, and M. Suter. 2008. Estrogens in Swiss rivers and effluents—sampling matters. *Chimia* 62(5):389–394.

Vermeirssen, E. L. M., O. Körner, R. Schönenberger, M. J.-F. Suter, and P. Burkhardt-Holm. 2005. Characterization of environmental estrogens in river water using a three pronged approach: active and passive water sampling and the analysis of accumulated estrogens in the bile of caged fish. *Environ. Sci. Technol.* 39(21):8191–8198.

Versteeg, D. J., A. C. Alder, V. L. Cunningham, D. W. Kolpin, R. Murray-Smith, and T. Ternes. 2005. Environmental exposure modeling and monitoring of human pharmaceutical concentrations in the environment. In *Human Pharmaceuticals: Assessing the Impacts on Aquatic Ecosystems*, ed. R. T. Willams, pp. 71–110. Pensacola, FL: SETAC Press.

Viglino, L., K. Aboulfadl, M. Prévost, and S. Sauvé. 2008. Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS/MS. *Talanta* 76(5):1088–1096.

Vögeli, A. C. 2008. Endocrine disrupting chemicals—linking internal exposure to effects in wild fish. Doctoral diss., ETH Zurich, Zurich, Switzerland. http://e-collection.ethbib.ethz.ch/eserv/eth:30864/eth-30864-02.pdf (accessed February 3, 2010).

Walker, J., D. Dnaebel, K. Mayo, J. Tunkel, and D. Gray. 2004a. Use of QSARs to promote more cost-effective use of chemical monitoring resources. 1. Screening industrial chemicals and pesticides, direct food additives, indirect food additives and pharmaceuticals for biodegradation, bioconcentration and aquatic toxicity potential. *Wat. Qual. Res. J. Canada* 39(1):35–39.

Walker, J. D., D. Plewak, O. Mekenyan, S. Dimitrov, and N. Dimitrova. 2004b. Use of QSARs to promote more cost-effective use of chemical monitoring resources. 2. Screening chemicals for hydrolysis half-lives, Henry's law constants, ultimate biodegradation potential, modes of toxic action and bioavailability. *Wat. Qual. Res. J. Canada* 39(1):40–49.

Weisbrod, A. V., et al. 2007. Workgroup report: review of fish bioaccumulation databases used to identify persistent, bioaccumulative, toxic substances. *Environ. Health Perspect.* 115(2):255–61.

Weisbrod, A. V., et al. 2009. The state of *in vitro* science for use in bioaccumulation assessments for fish. *Environ. Toxicol. Chem.* 28(1):86–96.

Wen, Y., Y. Wang, and Y.-Q. Feng. 2006. Simultaneous residue monitoring of four tetracycline antibiotics in fish muscle by in-tube solid-phase microextraction coupled with high-performance liquid chromatography. *Talanta* 70(1):153–159.

Winter, M. J., et al. 2008. Defining the chronic impacts of atenolol on embryo-larval development and reproduction in the fathead minnow (*Pimephales promelas*). *Aquat. Toxicol.* 86(3):361–369.

Wong, D. T., F. P. Bymaster, L. R. Reid, D. A. Mayle, J. H. Krushinski, and D. W. Robertson. 1993. Norfluoxetine enantiomers as inhibitors of serotonin uptake in rat brain. *Neuropsychopharmacology* 8(4):337–344.

Xu, S. H., and G. Chandra. 1999. Fate of cyclic methylsiloxanes in soils. 2. Rates of degradation and volatilization. *Environ. Sci. Technol.* 33(22):4034–4039.

Zeilinger, J., T. Steger-Hartmann, E. Maser, S. Goller, R. Vonk, and R. Länge. 2009. Effects of synthetic gestagens on fish reproduction. *Environ. Toxicol. Chem.* 28(12):2663–2670.

Zhou, S. N., K. D. Oakes, M. R. Servos, and J. Pawliszyn. 2008. Application of solid-phase microextraction for *in vivo* laboratory and field sampling of pharmaceuticals in fish. *Environ. Sci. Technol.* 42(16):6073–6079.