Human Pharmaceuticals in the Aquatic Environment: A Challenge to Green Chemistry

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Sushil Khetan was born in India in 1943. He received his B.Sc. and M.Sc. degrees from St. John's College, Agra, in 1961 and 1963. At the Indian Institute of Technology, Kanpur, he joined the research group of Professor M. V. George and earned a Ph.D. degree in 1968 in physical and synthetic organic chemistry. He worked with Dr. Leonard Spialter for his postdoctoral research in organosilicone chemistry at the Aerospace Research Laboratories, Wright-Patterson Air Force Base, Dayton, OH. In 1971, he returned to India and joined the pesticide industry as research manager. Later, he became head of a United Nations funded national program on development of environmentally friendly pesticide formulations. He coauthored a book on Pesticide Formulation (1998) published by the United Nations and was the sole author of a book on Microbial Pest Control (2001) published by Marcel Dekker, New York. In 2001, he joined the Green Chemistry group led by Professor Terry Collins at Carnegie Mellon University, where he has been actively working on the applications of TAML activators of peroxide for decontamination of chemical and biological warfare agents, degradation of thiophosphate pesticides, nitroorganics, and explosives, and removal of micropollutants and biological contaminants from water supply.

Terry Collins was born in New Zealand in 1952. He received his B.Sc. (1973), M.Sc. (1975), and Ph.D. (1978) degrees from the University of Auckland where his research advisor was Warren R. Roper, FRS. He conducted postdoctoral studies with James P. Collman at Stanford University (1978–1980). He is the Thomas Lord Professor of Chemistry at Carnegie Mellon University where he directs the Institute for Green Oxidation Chemistry, a research, education, and development center focused upon developing a holistic approach to sustainability science. He is also an Honorary Professor at the University of Auckland. Professor Collins taught the first university course in green chemistry at Carnegie Mellon, starting in 1992. He writes and lectures widely on how chemists can promote sustainability; he has delivered 400 public lectures all over the world. Professor Collins' research is focused on greening the historically dirty area of oxidation chemistry by designing nontoxic catalysts for activating the natural oxidants, hydrogen peroxide and oxygen. His widely patented, commercializing TAML activators promise to transform industrial peroxide chemistry, allowing it to substitute more effectively for chlorine- and metal-based processes and to enable much more effective processes for destroying in water recalcitrant pollutants and hardy pathogens.

Foreword

Toward the end of the 18th century, Robert Burns while ploughing a field accidentally upturned the nest of a field mouse to the consternation of the hapless creature. Reflecting on the incident, Burns produced "To a Mouse", one of the great English poems written in the Scottish dialect of his time. "To a Mouse" captures in allegory the plight of society with respect to pharmaceuticals released to the environment. The first two stanzas of the eight-stanza poem express pathos over an unintentional assault on a harmless creature by man with one of his overpowering technologies:

> WEE, sleekit, cow'rin, tim'rous beastie,
> O, what a panic's in thy breastie!
> Thou need na start awa sae hasty,
> Wi' bickering brattle!
> I wad be laith to rin an' chase thee,
> Wi' murd'ring pattle!
>
> I'm truly sorry man's dominion,
> Has broken nature's social union,
> An' justifies that ill opinion,
> Which makes thee startle
> At me, thy poor, earth-born companion,
> An' fellow-mortal!
>
> The last two impart the insecurity of Burns, a man who was no stranger to misfortune, in the face of an unknowable future:
>
> But, Mousie, thou art no thy lane,
> In proving foresight may be vain;
> The best-laid schemes o' mice an' men
> Gang aft agley,
> An'lea'e us nought but grief an' pain,
>
> For promis'd joy!
> Still thou art blest, compar'd wi' me
> The present only toucheth thee:
> But, Och! I backward cast my e'e.
> On prospects drear!
> An' forward, tho' I canna see,
> I guess an' fear!

As we will describe in this review, certain pharmaceutical agents are among a growing body of anthropogenic chemicals that, upon release into the environment, are indeed breaking "nature's social union", that symbiosis between man and his fellow creatures upon which the very life of man itself depends. Of the three often-overlapping toxicological endpoints for chemicals, namely, the killing of cells, the mutation of DNA in ways that may lead to cancer, and the disruption of chemical signaling mechanisms controlling cellular development, we understand the least about the third. This last area of toxicity, called "endocrine disruption", was first fathomed by Theo Colborn. Dr. Colborn brought to light new understanding that, at environmentally relevant concentrations, chemicals can interfere with the hormonal command of cellular development and the result can be severe impairment of growing creatures.[1] Impairment by endocrine disrupting chemicals (EDCs) often follows nonmonotonic dose−response profiles. This contrasts with chemicals that kill and chemicals that cause cancer where the risk usually amplifies with increasing exposures and where there is a LONEC or "lowest observed no-effect concentration." However, with the EDCs, the impairing effects are often found at low, but not at higher concentrations. A variety of shapes have been found for nonmonotonic dose−response

curves,[2] and in some cases, the most hazardous concentrations may be the ultralow ones. As with many other classes of environmental pollutants, this makes endocrine disruption the most obvious toxicity issue that we should study with respect to pharmaceuticals in the environment. The research that underpins our increasing insight into the threats of EDCs is catalogued and regularly updated at the website created by J. Peterson Myers.[2] And it is in the solving of the numerous dilemmas that are associated with endocrine disruption effects that we believe green chemistry holds so much promise in the Pharmaceuticals in the Environment (PIE) arena.

As discussed throughout the review, we now know that certain pharmaceuticals can persist in the environment and, either via the food chain or via drinking water, make their way back to us and, of greatest concern, back to pregnant mothers and children. And we also know that some of these agents are beginning to be associated with adverse developmental effects in aquatic organisms at environmentally relevant concentrations, concentrations we usually consider to be infinitesimal and harmless. Furthermore, we understand that we know almost nothing about the impacts of human exposure to low-dose mixtures of pharmaceuticals or of low-dose pharmaceuticals mixed with other low-dose synthetic pollutants but that the little we do know gives reason for serious concern.

In such circumstances, Burn's foreboding about the future provides an appropriate stance of society toward pharmaceuticals. It is now simple common sense to "guess an' fear" that some of the "best-laid schemes" of the pharmaceutical industry might go "agley". And it is entirely appropriate to question whether we can continue to rejoice in the improvement that an environmentally persistent pharmaceutical agent might bring to the lives of fully developed adults when *we cannot be certain that it is not impairing developing humans because we have not performed appropriate studies to establish this to any reasonable degree of certainty*.

One goal of this review is to bring out that the chemical enterprise needs to work to reduce society's uncertainties with respect to the safety of persistent or pseudopersistent pharmaceutical agents and to curb and ameliorate the impacts of their release to the environment. A further goal is to begin to examine an entirely new set of questions concerning how green chemists might contribute to reducing hazards associated with pharmaceutical pollutants in water.

1. Introduction

Pollution from pharmaceuticals in surface and groundwaters is becoming recognized as an environmental concern in many countries leading to the area of study labeled "PIE" for "Pharmaceuticals in the Environment". The pharmaceutical industry is attaining more effective active pharmaceutical ingredients (APIs) by designing for increased potency, bioavailability and degradation resistance. APIs show a wide range of persistence in aquatic environments, and some are highly persistent. In such cases, the pharmacologically valuable properties of degradation resistance and bioavailability return as hazards because they translate into unwelcomed exposures of humans and the environment to bioactive anthropogenic compounds. In addition, at current environmental concentrations, some APIs are beginning to be associated with adverse developmental effects in aquatic organisms. Questions naturally arise about negative impacts on human health. The most important of these relate to the potential for developmental impairment by trace quantities of pharmaceuticals in drinking water, that is, the subject area of endocrine disruption.

Sentinel research on frogs is illuminating that mixtures of micropollutants may present environmental hazards that are far greater than those for the individual compounds.[3] This highlights an added reason for attempting to understand the possible health and environmental hazards associated with trace pharmaceuticals. It is obviously also important to carry out research into the possible health effects that might arise from the synergistic action of mixtures of pharmaceuticals and micropollutants from other sources.

It is likely that concerns over API-induced developmental disruption should be highest for cities that recycle sewage water or take their drinking water or fish from rivers, lakes, or other water bodies that border highly populated areas where we know persistent APIs are present. Smaller communities with less than state-of-the-art drinking water treatment plants (DWTPs) may also be at higher comparative risk.

In this review, we discuss the background of PIE problems and uncertainties. Our focus is on the APIs themselves rather than other components of formulated pharmaceuticals. We concentrate on pharmaceuticals used in human medicine, some of which are also applied in veterinary medicine, but not on veterinary medicines per se. Occasionally, we include information about personal care products because pharmaceuticals and personal care products (PPCPs) are often grouped together in the literature where it makes no sense for this review to separate them. Veterinary pharmaceuticals have been reviewed by Montforts et al. (1999)[4] and Boxall et al. (2003).[5] This review is limited to what we currently know to be environmentally important APIs, organized according to the different drug categories. We describe major pharmaceutical uses in the United States for the relevant drug classes, because this is tied directly to PIE issues. In section 3, we offer considerable background information into the drugs themselves in the hope that this will be helpful for readers who are not pharmaceutical chemists. As part of this, we decribe what the drugs are used for, because this provides a good mechanism for introducing the chemical structures and emphasizes to the reader the great benefits. But a subtext of the review is that these benefits sit on one pan of a scale. The known negatives, of which PIE problems are one class, sit on the other pan. Most of us would presently consider that the positives decidedly outweigh the negatives. But the negatives pan is becoming increasingly heavy as our understanding advances concerning the unforeseen downsides based upon feedback from nature where human impairment is one of the possible outcomes. At various stages, we point out that certain drugs have had to be withdrawn from the market as startling hazards to human health have come to light. We do this to emphasize that from time to time, the full impacts of drugs are not understood at the time of commercialization. PIE problems are like this. Metabolic conversions of APIs may produce bioactive derivatives. The transformations themselves offer clues into how APIs degrade under oxidative and hydrolytic stress. Therefore we consider that knowledge of the metabolism of environmentally relevant APIs is important for understanding this review, and we have incorporated this. We examine the reasons for the current trend to design metabolic stability into APIs, and we consider the relationship of this to environmental

persistence and its associated consequences. The occurrence of APIs in the environment has been widely reported and reviewed already. Thus, we have not covered this area in detail. But we do highlight what is known about APIs making their way into drinking water. We assess the current status of PIE knowledge derived from ecotoxicological studies on aquatic organisms.

Pharmaceutical industry design trends essentially guarantee that in the years to come, persistent API-derived pollutants (the APIs themselves and their partial degradation and bioactivated byproducts) will increase in their concentrations and diversity in aquatic systems unless something is done to prevent this from happening. It is our belief that green chemistry will be able to strengthen nonregulatory approaches for ameliorating PIE problems, especially by providing more effective methodologies for decomposing APIs in water. But we also believe that the current regulatory paradigm must be strengthened to better protect society from persistent and pseudopersistent environmentally mobile synthetic compounds, including pharmaceuticals.

In exploring the green chemistry context, starting in section 4, we begin by reviewing photolytic and oxidative processes for degrading and detoxifying different groups of pharmaceuticals in aqueous media. The executers of this research almost invariably did not define it as green chemistry. But because green chemistry, as defined by Paul Anastas, is "the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances", safe processes that allow us to remove hazardous contaminants from our water supplies certainly qualify. In this area of oxidative degradation, the state of knowledge allows us to provide a more detailed analysis of reasonably impressive technologies, but we conclude that the development of much more effective degradation technologies is going to be critical for dealing with PIE problems. In the discussion of what is already available, emphasis is placed upon important aspects of degradation technologies including the degree of pharmaceutical degradation, the identity and characteristics of the degradation intermediates and byproducts, and the possible degradation pathways. Ozonation and ozone-based advanced oxidation processes, such as ozone/hydrogen peroxide, ozone/ultraviolet irradiation, and ozone/hydrogen peroxide/ultraviolet irradiation, already underpin significant technologies for the treatment of wastewaters. There appears to be considerable potential for their expanded use in degrading pharmaceuticals. And finally, the prospective uses of the new catalytic Fe-TAML/peroxide-based processes are briefly discussed as these are starting to be known to apply to the facile degradation of pharmaceuticals in water, including recalcitrant ones. These technologies have been developed to provide a platform technology for water purification in our Institute for Green Oxidation Chemistry at Carnegie Mellon University.

Clean water is so basic to human life that water droplets, bubbling brooks, and waterfalls are enduring symbols of the life force. Obtaining an adequate supply of clean water has likely always been a challenge for much of humanity. But despite the scientific and technological advancements of the modern era and, ironically, sometimes because of them, clean water is becoming an increasingly scarce and coveted resource. Water security is now a critical environmental issue that touches the life of every human being.

The rapid expansion of chemical industry since William Henry Perkin discovered and commercialized in 1856−1857 the first synthetic dye, mauveine, has resulted in the potential for release to the environment of approximately 80 000 xenobiotic compounds[6] that are not natural components of the organisms exposed to them. These alien compositions have worked their way into our lives, usually as uninvited guests and too often with deleterious effects, from a variety of technology sources, including pesticides, personal care products, cleaning materials, building materials, food and drinks, printed goods, houseware, furniture, electronic goods, transportation, sports, laboratory work, education, and pharmaceuticals, in fact, essentially every sector of our chemically based economy. Importantly, even in highly developed countries, we are only a few generations or less into widespread public exposures for many individual xenobiotics, including pharmaceuticals as described herein. New nanotech materials, biologics, genetic therapies, and genetically modified foods are more recent newcomers to this anthropogenic "chemical soup" we swim in. The addition to water of a cocktail of trace quantities of pharmaceuticals, compounds that are designed to exhibit potent physiological activity, is arguably an important emerging water issue. PIE problems are really just beginning to be understood. As detailed below, some of the APIs had estimated environmental persistences of many years, as is often found with xenobiotics.[7]

1.1. Pharmaceuticals: A Perspective

The pharmaceuticals industry is a critical component of the chemical enterprise. Pharmaceuticals are a large and diverse group of both human and veterinary medicinal compounds, as well as nutraceuticals (i.e., bioactive food supplements), which have long been used in significant quantities throughout the world, small molecule drugs of the classical type produced primarily via organic synthesis, and biotech drugs. There are about 4500, including experimental drugs in development, which comprise about 70% of the total. The number of U.S. Food and Drug Administration (FDA) approved small molecule drugs at the end of 2005 was 1090.[8] Drugs, both prescribed and nonprescribed, are becoming an increasingly complex component of health care. These are defined as substances responsible for physiological or pharmacological action and used in the diagnosis, cure, mitigation, treatment, or prevention of disease or nonfood articles intended to affect the structure or any function of the body of man or other animals.[9] They can cure some diseases (e.g., antibiotics), control symptoms (e.g., analgesics or pain relievers and asthma drugs), replace or supplement needed chemicals (e.g., insulin and vitamins), and control the body's self-regulating systems (e.g., high blood pressure and thyroid drugs). Drugs can serve as complements to medical procedures (e.g., anticoagulants during heart valve replacement surgery), deterrents to disease and disability (e.g., lipid-lowering drugs that lessen the risk of coronary artery disease), and new treatments where previously there were none (e.g., drugs for HIV). Thousands of tons of drugs are used by people yearly to treat illnesses, to prevent unwanted pregnancy, or to face the stresses of modern life.[9]

1.2. Population Growth, Inverting Age Structure, and Healthcare Spending in the U.S.

With advances in medical technology and growing healthcare spending, the consumption and uses of pharmaceuticals

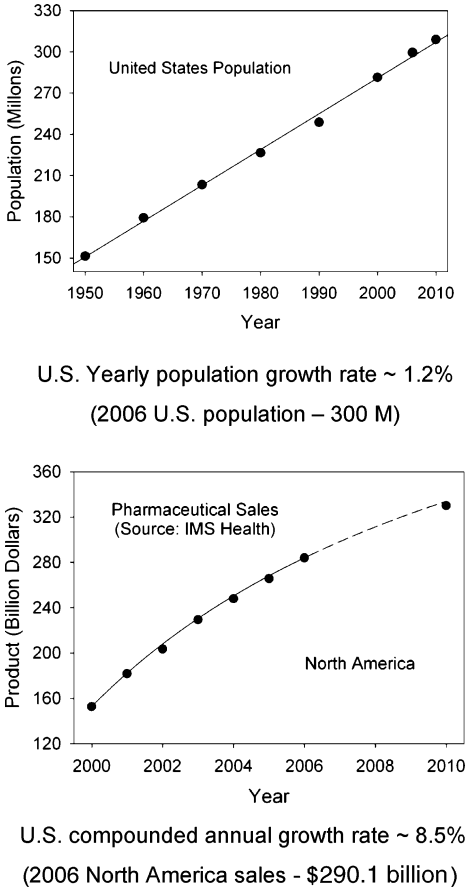


Figure 1. Pharmaceuticals sales in North America have been rising nearly seven times faster than the population growth rate.

has been rising consistently. The use of drugs for some conditions is increasing dramatically (e.g., cholesterol-lowering statins and antidepressants). For other conditions such as asthma, some classes of drugs appear to be replacing older drugs.[9] In 1999−2000, 44% of Americans took at least one prescription drug, and 17% took at least three. Those statistics were up from an average use of 39% and 12% between 1988 and 1994.[9] The annual value of pharmaceutical sales in the United States went up over 5 years from 2000 to 2004 by 62%, from $152.8 to $248 billion.[10] North America continues to be the dominant market for pharmaceuticals representing almost half of global sales.[11] The annual value of US sales is projected to reach $330 billion by the year 2010, showing an annual growth rate of nearly 8.5%. From 1950 to 2006, the total resident population of the United States increased from 151 million to 300 million, representing an average annual growth rate of 1.2% (Figure 1).[12]

Some of the drivers of this growth are the expanding population and the inverting age structure in the general population, the rise of new target age groups, the discovery of new uses for existing drugs, and the increased per capita consumption partly due to expiration of patents with resulting availability of less expensive generics.[13] Older people tend to take more drugs than younger people because they are more likely to have chronic disorders.[14] For example, the prevalences of diabetes, hypertension, and heart disease increase with age.

1.3. Entry of Pharmaceuticals into the Aquatic Environment

Pharmaceuticals are generally absorbed by humans or animals after intake and are then attacked by metabolic degradation processes. However, significant fractions of the original substances often are excreted in unmetabolized form or as active metabolites via urine or feces to be emitted into raw sewage, which may or may not be treated.[15,16] Some pharmaceutical pollutants escape degradation in waste treatment plants and enter the environment.[15−19] In addition to metabolic excretion, disposal by flushing of unused or expired medication and drug-containing waste from manufacturing facilities can also contribute to environmental contamination.[16] Flushing unused medicines down the toilet appears to be of minor importance, while patient excretion following therapy is widely considered to be the primary pathway to the environment.[20] Even posthumously, the drugs administered in the closing phases of our lives likely leach into cemeteries and groundwater.[13]

In a 2002 nationwide study of "emerging pollutants" in waters, the U.S. Geological Survey (USGS) tested pharmaceuticals and personal care products (PPCPs) in several U.S. locations in 139 rivers in 30 states and detected a wide range of biologically active compounds in nearly 80% of these, even in remote areas.[21] According to Lynn Roberts of Johns Hopkins University, compounds that qualify as "emerging pollutants" are those that are "entering into or being generated in the environment in appreciable amounts", that have "a modicum of persistence," and "exhibit deleterious effects on organisms".[22] We consider this to be an appropriate definition. Certain APIs meet the emergent pollutant criteria and are candidates for environmental monitoring.[22] North American waterways were found to contain traces of APIs from a wide spectrum of therapeutic classes, such as, contraceptives, painkillers, antibiotics, anticancer drugs, blood-pressure medications, antidepressants, and drugs used to treat epilepsy.[21] Antibiotics and hormones were found with greatest frequency.[20,21] Trace amounts of drugs have also been found in the tap water of some communities at concentrations ranging between 20 parts per billion (ppb) and less than one part per trillion (ppt).[23,24] While these concentrations are small, drugs are designed to have a physiological effect in small quantities. The USGS study has established a baseline for PPCPs as environmental contaminants in the United States.[21] It indicates that PPCP ecosystem contamination has the potential to impact the health and well being of flora and fauna as well as humans. This unanticipated source of pollution, which is expected to grow, is pitting the need for clean drinking water against our use of modern medicine. Thus, two cornerstones of the revolutions of public health could end up becoming mutually incompatible, unless solutions to water contamination by persistent APIs are developed.[25]

The fates of human and veterinary drugs after urinary and fecal excretions are quite different. The excreted human pharmaceuticals pass through sewage treatment plants (STP) prior to entering rivers or streams. Veterinary drugs are more likely to directly contaminate soil and groundwater without any sewage treatment. Manure is an important resource for topsoil dressing (Figure 2). After rainfall, surface waters can be polluted with human or veterinary drugs by runoff from fields treated with digested sludge or livestock slurries. The groundwater can also be contaminated.[26]

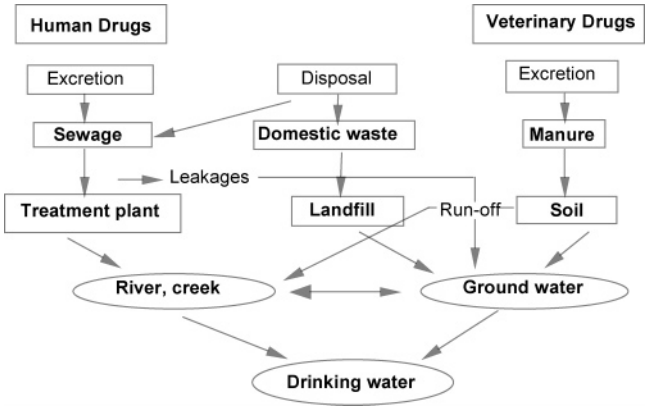


Figure 2. Fate of pharmaceuticals in the environment. Modified from ref 15.

1.4. Sewage Treatment Plant (STP) Effluents: Temporal and Spatial Distributions and the Fate of Pharmaceuticals in Environmental Waters

Many pharmaceutical compounds pass, at least in part, through sewage treatment plants to end up in environmental waters. STPs were often not designed to handle pharmaceuticals. Nevertheless, a recent study has shown that operating an STP with longer solids retention times will remove more pharmaceuticals and other chemicals.[27]

Many studies have determined that the attenuation of pharmaceutical contaminants occurs in a river with time and distance from the source outlet as multiple degradation processes are at work. Seasonal variation in temperature and light intensity are considered to be the factors determining the fate of pharmaceuticals in surface waters.[24,28] Boreal winter climate conditions with low temperatures and low daylight hours may lead to decreased bio- and photodegradation of pharmaceuticals compared with summer. These processes are likely to be even less effective in rivers covered by ice and snow. Several reports have described the temporal and the spatial distributions of pharmaceuticals in surface water systems.[24,28,29]

In the Finnish city of Turku, the effects of seasonal variation in climate conditions have been examined on the degradation of pharmaceuticals that were released from an STP to the River Aura. The seasonal impacts on natural degradation processes were also examined at Turku's drinking water treatment plant (DWTP), which is located downstream from the STP.[28] The occurrence of five pharmaceuticals (ibuprofen, naproxen, ketoprofen, diclofenac, and bezafibrate) in the influent and effluent water of the STP were followed for winter, spring, and summer, showing that the elimination of the pharmaceuticals decreased significantly in wintertime (about 25% reduction compared with spring and summer), leading to 3−5-fold increases in concentrations of pharmaceuticals in the effluent water. Pharmaceuticals were also carried longer distances downstream from the STP when the river was covered by ice and snow. During snowmelting, more rapid transportation of the pharmaceuticals was observed resulting from the increased water flow rate. The DWTP located downstream from the STP produced water that contained about 8 ng/L of ibuprofen and ketoprofen in the winter sample, whereas in spring and summer, the studied pharmaceuticals were not detected. The results showed that cold seasons in boreal areas could severely increase the environmental risk of pharmaceuticals and the risk for contamination of drinking water.[28]

The likelihood that pharmaceuticals will end up in drinking water is also increased when treated sewage water is used to meet drinking water needs in areas of water scarcity.[30] Water reclamation is becoming increasingly important in the arid western states of the U.S.A. For example, Southern California imports nearly all of its potable water from the Colorado River and the Sacramento−San Joaquin River Basin. Sewage treatment plant effluents heavily impact both of these sources and effluent-dominated streams represent worse case scenarios for evaluating and predicting aquatic responses to contaminants such as PPCPs.[30] The raw and treated drinking water from water filtration plants in San Diego showed the occurrence of several polar organic PPCPs, including clofibrate, clofribric acid, ibuprofen, triclosan, phthalate esters, sunscreens, and DEET.[24] The occurrences and concentrations of these compounds were found to be highly seasonally dependent, reaching maximums approaching that of reclaimed nonpotable wastewater when the flow of the San Joaquin River in the summer months was low and the quantity of imported water was high.[24] At the sites where secondary or tertiary treated effluents are used for subsequent groundwater recharge, soil-aquifer treatment showed that removal of antiepileptics, carbamazepine, and primidone did not seem to occur in more than 8 years of subsurface travel time.[31] Although a "toilet-to-tap" program has been proposed as a safe solution to California's water shortage problems, concerns remain about the possibilities for effluent-derived microcontaminants impacting health and safety issues.[32]

In a study conducted in Sweden, screening for antibiotics in the raw sewage water and final effluent in five STPs revealed the presence of trimethoprim, fluoroquinolones (norfloxacin, ofloxacin, and ciprofloxacin), a sulfonamide (sulfamethoxazole), and a tetracycline (doxycycline).[33] With sewage treatment, the concentrations of the fluoroquinolones were decreased by 86−87% in the effluent. Golet et al. (2003) have reported similar results.[34] The average STP removal efficiency of sulfamethoxazole was found to be ca. 42%. A removal efficiency of 60% was reported for a Spanish STP.[35] Antibiotics in Canadian STP effluents show similar patterns of occurrence.[36] Removal of doxycycline was found to be strongly influenced by the treatment process and other variables at individual STPs, with high amounts occurring in sludge in some cases.[33]

Estrogenic activity has been observed in STP effluent contaminated aquatic systems. This has been attributed mainly to the presence of steroidal estrogens.[29] In a recent study, the Eysines STP effluent near Bordeaux in France was analyzed for the steroidal compounds estrone, 17β-estradiol, estriol, 17α-ethynylestradiol, mestranol, progesterone, norethindrone, and D-norgestrel.[29] Only estrone and, on one occasion, estradiol and its metabolite estriol were detected in the effluent. These are compounds from the natural steroid hormone family, emphasizing that natural contributions to the endocrine disrupting potential of sewage effluents, in this case the estrogenicity, are a key part of the total story. The receiving Valle D'Eysines River was examined to provide both summer and winter temporal and spatial distributions. In summer, the decay rates were high such that 50% of the initial amount of estrone, the most persistent of the family, was degraded 1.7 km downstream from the effluent discharge. In winter, estrone levels had not significantly decreased 10 km downstream from the effluent discharge.[29]

1.5. Pharmaceutical Detection in Drinking Water

Growing populations and increasing intensification of land and water use for industry and agriculture have increased the need to reclaim wastewater for reuse, including supplementing the drinking water supply. Concurrently this has increased the risk of water resource contamination. The contaminants can leach from the contaminated watercourses into the groundwater aquifers and appear at trace-level concentrations in drinking water. Because of their polar structure, several pharmaceuticals are not significantly adsorbed in the subsoil, thus reaching the groundwaters, which constitute a major source of drinking water. The conventional process (C−F−S) for drinking water treatment plants consists of the following sequence of treatments: coagulation, the addition of coagulant salts and polymers to destabilize colloidal particles; flocculation, the agitation of coagulated water to promote the aggregation of suspended materials; and sedimentation, the stilling of flocculated water to promote settling of suspended solids and floccules. The C−F−S water is then usually chlorinated. For the same reason of polarity, C−F−S treatment plants are also not able to completely remove many pharmaceuticals.

Several studies have identified APIs in finished drinking water.[37−43] A number of reservoirs tapped for drinking water were monitored along the Lergue River (Hérault watershed) in Southern France, where were found pharmaceuticals and other wastewater-related dominant contaminants such as paracetamol, diclofenac, and carbamazepine.[44] Clofibric acid and diazepam were detected in treated drinking water in Milan, Italy.[18] Heberer and colleagues[39−43] have reported the presence of clofibric acid, propylphenazone, and diclofenac in the drinking water of Berlin in the concentration range of several hundreds of nanograms per liter. Berlin tap water samples were found to contain clofibric acid in concentrations up to 165 ng/L. A correlation was found between clofibric acid concentrations in tap water samples with the proportion of groundwater recharge used in drinking water production.[45]

Frick detected three widely used nonprescription drugs, caffeine, cotinine, and acetaminophenone, in samples of potable water collected near Atlanta, Georgia.[46] Stackelberg and colleagues identified 17 organic contaminants including carbamazepine (0.258 μg/L) in the finished water of a drinking water treatment plant using the C−F−S process.[23] Loraine and colleagues identified and quantified ibuprofen (0.93 μg/L) and ibuprofen methyl ester (4.95 μg/L) in finished water, but DEET, clofibrate, and clofibric acid, which were present in the raw water, were not found.[24]

Tauber detected carbamazepine and gemfibrozil in point-of-use drinking waters in ten cities in Canada that were examined for a 44-drug subset consisting of commonly used neutral and acidic pharmaceuticals; sulfonamide, quinolone, tetracycline, and macrolide antibiotics; and various transformation products.[47] Similarly, the National Water Research Institute (NWRI) and Health Canada surveyed 20 southern Ontario drinking water facilities for nine acidic pharmaceuticals. They detected trace amounts of these drugs (not specifically named) in the drinking water of four Canadian communities, including Montreal and Hamilton where the water sources from lakes, rivers, and groundwater were contaminated with wastewater effluents.[48]

Ye and colleagues detected several antibiotics from the finished water samples collected from five drinking water treatment plants (WTP) in North Carolina that use conventional treatment processes.[45] Before treatment, occurrences of fluoroquinolones were the most frequent in the source waters, followed by sulfonamides, lincomycin, tetracyclines, and macrolides, all in low nanogram per liter concentrations. In the finished water, some of these were present in much lower levels, indicating their partial removal. However, the incomplete removal of antibiotics, such as ciprofloxacin, is of concern due to their unknown health effects if they do persist in finished waters even at nanogram per liter levels. Townsand reported the building up of fluoxetine (Prozac) in rivers and groundwater used for drinking water supplies quoting the British Environment Agency.[49] It was believed that the Prozac found its way into the water table from treated sewage.

1.6. Possible Health Effects of Chronic Exposure to Pharmaceuticals

Although pharmaceutical concentrations characteristically measured in water can only lead to human exposures that are much lower than those producing targeted pharmacological effects, whether there are any human developmental disruption outcomes of long-term exposures to low doses of individual drugs or combinations thereof, as well as combinations of drugs with other common micropollutants, have yet to be determined. Some drugs (e.g., antiepileptics) are persistent.[13,50] Others are "pseudopersistent"; while they degrade in the environment at reasonable rates, they are continuously being replaced by ongoing widespread use.[13] Some drug compounds dissolve readily in water, but about 30% have high fat solubility, which enables them to engage in the reverse-entropy process of bioaccumulation by entering cells and moving up food chains becoming more concentrated in the process.[51]

Multiple unanswered questions are associated with the health and environmental impacts of chronic exposures to low levels of multiple bioactive substances. Other recent studies have shown a combination of multiple chemicals to have an additive effect.[52,53] These studies used the yeast estrogen screen (YES), which detects the ability of chemicals to bind to the alpha human estrogen receptor and implicates binders as chemicals that can affect reproductive endpoints. The investigators tested mixtures of eight such chemicals, each at a concentration below the level of observable effects. In combination, their effects were additive and produced a detectable effect.[52]

2. Pharmaceuticals of Environmental Concern: Their Pharmacodynamics and Pharmacokinetics (Metabolism)

In our reading to understand what is known about the impact of PIE on human health and the environment, we had to learn a great deal about the drugs themselves as neither of us has worked in the pharmaceutical industry. Sections 2 and 3 summarize background material that was helpful to us and are intended to assist the nonspecialist reader with background knowledge that is key to the PIE area. Pharmacodynamics describes the therapeutic effects of drugs, their side effects, the site and mechanisms of drug action and the relationship between drug concentration and effect. Summarily stated, pharmacodynamics is the study of what a drug does to the body. Pharmacokinetics involves what the body does to a drug, including the processes of absorption,

Table 1. Selected Pharmaceutical Groups and Their Environmental Risk Indicators[54]

drug	examples	risk indicator	refs
painkillers	nonsteroidal anti-inflammatory drugs (NSAIDS, e.g., ibuprofen); other analgesics (e.g., acetaminophen)	very high prescription and OTC volumes; detected in the environment	66
antibiotics	penicillins, sulfamethoxazole	high volumes; detected in the environment; concerns over toxicity and anti-bacterial resistance	206, 210, 364
beta blockers	propranolol, metoprolol	high volume; detected in the environment	17
antiepileptics	carbamazepine, phenobarbital	high volumes; long-term prescriptions; persistent	285
lipid regulators	statins (e.g., atorvastatin, clofibrate)	long-term prescriptions; commonly detected	76, 365
antidepressants	fluoxetine, risperidone	subject of toxicity testing	366
hormones	contraceptive pill, 17α-ethinylestradiol	most extensively studied treatments; toxicological properties; hormone replacement; widely detected	186, 367, 368, 369
antihistamines	loratadine, cetirizine	commonly held nonprescription medicine	

distribution, metabolism, and elimination and how long these processes will take.[14]

Pharmaceuticals are a large and varied class of compounds with diverse properties and applications. To facilitate their study, they are often grouped according to their therapeutic action. Eight therapeutic groups have been identified in our research as being particularly significant for PIE, namely, nonsteroidal anti-inflammatory drugs, antibiotics, beta-blockers, antiepileptics, blood lipid-lowering agents, antidepressants, hormones, and antihistamines. More categories may become important as PIE research unfolds. Various criteria have been used in the selection of the therapeutic classes and specific drugs, including the volume of prescription, the toxicity, and the evidence for presence in the environment. The environmental occurrence of a pharmaceutical is a function of many variables including, the quantity manufactured, the dosage amount and frequency, the environmental persistence, the compound's metabolism, and the effectiveness of STPs in degrading it.[15,16] Table 1 summarizes risk indicators according to Bound and Voulvoulis presented with examples of pharmaceuticals within the different groups.[54]

2.1. Metabolic Transformations

APIs, which are mostly lipophilic organic molecules, undergo two principal metabolic biotransformation phases in mammals.[55] Phase I transformations proceed by oxidative, reductive, and hydrolytic pathways, leading to the introduction of a functional group, such as −OH, −SH, $(>C)_2O$ (epoxide), $-NH_2$, or −COOH, with a usually modest increase in hydrophilicity. Common API phase I oxidative enzymes include the cytochrome P450 family of hemoproteins, the flavoproteins, the flavin monooxygenases (FMOs), the monoamine oxidases (MAOs), the heme-containing peroxidases such as myeloperoxidase (MPO), and cyclooxygenases, such as the prostaglandin synthases, COX-1 and COX-2.[55]

Phase II conjugations involve the attachment of a generally polar, readily available in vivo molecule to a susceptible functional group to form O- and N-glucuronides, sulfates, and acetate esters, carboximides, and glutathionyl adducts, all with increased hydrophilicity relative to the unconjugated metabolite. Reactive functional groups are often, but not necessarily, a result of phase I reactions. Many pharmaceuticals are conjugated with glucuronic acid or sulfate to make the whole molecules more polar, which are then readily filtered through the kidneys to be eliminated from the body through renal excretion.[55]

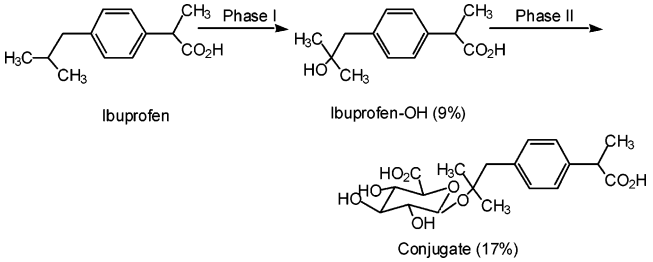


Figure 3. Ibuprofen and metabolites conjugate with glucuronic acid to enhance their polarity to excretion.

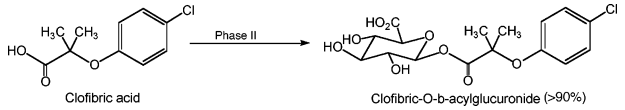


Figure 4. Metabolites of clofibric acid excreted by humans.

Acidic pharmaceuticals, such as the anti-inflammatories ibuprofen and acetylsalicylic acid and the lipid regulators clofibrate metabolite clofibric acid and bezafibrate are negatively charged at neutral pH, because their carboxylic moieties are deprotonated. Ibuprofen, for example, is first hydroxylated and then conjugated (Figure 3). Approximately 15% of ibuprofen is excreted unchanged or as its glucuronide. The remaining percentage is allocated to further metabolites such as hydroxy-ibuprofen, carboxy-ibuprofen, carboxy-hydratropic acid, and their respective conjugates[56] (Figure 3), portions of which are also excreted.

Clofibric acid, the principal and active metabolite of the cholesterol-lowering drug clofibrate, is conjugated to clofibric-*O*-*β*-hydroxyglucuronide and excreted (Figure 4).

Many pharmaceuticals are excreted mainly as metabolites. However, phase II metabolites are likely cleaved during sewage treatment to yield the nonmetabolized pharmaceuticals and hence may increase the relevant environmental concentrations.[26] Examples include the natural hormone 17*β*-estradiol (E_2)[57,58] and 17α-ethinylestradiol (EE_2), the synthetic steroid hormone of the contraceptive pill.[59]

2.2. High Volume Drugs

As a first approximation, the occurrence of a blockbuster drug that generates huge dollar annual sales could give an indication that relatively large quantities are entering the environment. Unlike bulk drugs such as antibiotics and over-the-counter painkillers, blockbuster drugs are often patented new drugs or a family of drugs used to treat a common illness for which there are no effective alternatives. From 1991−2000, there were 32 blockbuster drugs launched by 15 pharmaceutical companies. In 2006, there were more than 101 drugs that had sales exceeding $1 billion per year, 35 of these had sales exceeding $2 billion, 16 surpassed the $3 billion mark, and the top selling drug, Pfizer's Lipitor, became the first drug to have annual sales exceeding $12 billion.[60] Several health conditions have been the target of multiple blockbusters including cholesterol and triglyceride lowering statins (HMG-CoA reductase inhibitors), anti-ulcerants (gastric acid production reducing and heartburn soothing proton pump inhibitors), antidepressants (selective serotonin reuptake inhibitors or SSRIs), and antihistamines (histamine H1 receptor antagonists).

Another indicator is the use of a pharmaceutical to treat major geriatric diseases. The prevalence of many chronic conditions and diseases increases with age, as does the use of medications designed to treat them. Prescription drug use is greater among middle-aged and older adults than among younger individuals for conditions such as, arthritis, diabetes, high blood pressure, and elevated cholesterol. In addition to the cardiovascular diseases, drugs for pain relieving and hypoglucomia are consumed in very large quantities.[9] The general population in the United States is growing older with the +65 population increasing from 35 million in 2000 to an anticipated 54 million in 2020, requiring the expanded use of pharmaceuticals used for treating chronic and acute health conditions in the elderly.[61]

In the following sections of the review, we introduce the individual drugs belonging to high volume use categories and emphasize their therapeutic uses that are so valuable for human health maintenance. Information on the different pharmaceuticals and the related pharmacological information have been obtained from various sources, but we have relied significantly on the Merck Manual of Medical Information[14] and the Internet drug index.[62] We also indicate where drugs have been withdrawn from the market over toxicity concerns, because these incidents emphasize that toxicity surprises happen from time to time when carefully executed clinical trials have been conducted.

2.2.1. Analgesic–Antipyretic Agents

2.2.1.1. Nonsteroidal Anti-inflammatory Drugs (NSAIDs). NSAIDs are a class of pain relievers that work by blocking cyclooxygenase (COX) enzymes, which catalyze the synthesis of different prostaglandins from arachidonic acid.[63] Among the traditional NSAIDs, the most widely used are ibuprofen and naproxen (Figure 5) in the U.S. and diclofenac in Europe.[64] Ibuprofen is also one of the top-ten drugs sold worldwide. Although it has been shown that only the S enantiomer has the desired biological activity, it is currently sold as the racemate. Diclofenac has been reported to partially degrade in artificial river biofilms.[65] Diclofenac, ibuprofen, and metabolites have been detected in surface waters.[66]

Ibuprofen is metabolized through oxidation and glucuronic acid conjugation of the inactive metabolites and excreted in the urine with <10% unchanged ibuprofen. Approximately 65% of diclofenac is eliminated as the glucuronide and the sulfate conjugates of the hydroxylated metabolites in the urine and approximately 35% via the bile to the feces as conjugates of diclofenac plus metabolites.

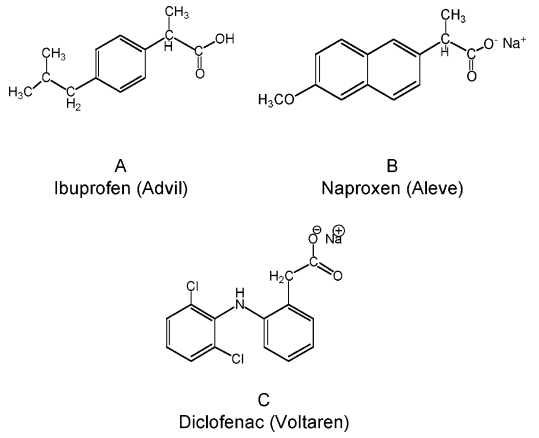


Figure 5. Nonsteroidal anti-inflammatory drugs (NSAIDs).

In 1991, two different isoforms of the enzyme cyclo-oxygenase (COX) were discovered.[67] COX-1 enzymes are involved in the production of prostaglandins that protect the stomach lining and play a crucial role in reducing blood clotting. COX-2 enzymes create prostaglandins that trigger inflammation and contribute to pain. NSAIDs inhibit both COX-1 and COX-2 enzymes. Consequently inflammation, pain, and fever are reduced, but inhibition of COX-1 enzyme can result in gastrointestinal ulcerogenic effects that can be life-threatening.

2.2.1.2. Coxibs (COX-2 Inhibitors; Antiarthiritics). Recognition of the two distinct cyclooxygenase (COX) isoforms prompted development of newer drugs (coxibs) that selectively block the activity of COX-2, thus providing pain relief and reducing inflammation while sparing COX-1, the enzyme where NSAID interference is apparently able to cause ulcers.[68] The blockbuster drugs rofecoxib (Vioxx), celecoxib (Celebrex), and valdecoxib (Bextra; Figure 6) are selective COX-2 inhibitors that have been popular arthritis drugs. The sulfonamidophenyl structural group is a key feature of these compounds that bind in a side-pocket of the enzyme's active site formed by conformational change. These drugs were/are marketed as safer alternatives to the general COX inhibitors and traditional pain relievers and took over 40% of the market share from the first year of inception. However, Vioxx, which had sales of $2.55 billion in 2003, was withdrawn from the market in September 2004 because of cardiovascular side effects.[69]

Celecoxib, which ended up with sales of $3.3 billion in 2004,[70] has also been reported to pose significant risks of heart attacks in cancer patients. Celecoxib, a diaryl-substituted pyrazole with a sulfonamide moiety, is excreted primarily as its carboxylic acid metabolite in the feces

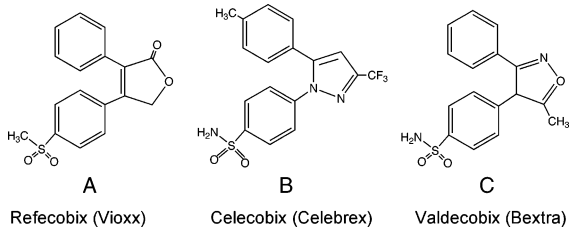


Figure 6. COX-2 inhibitors.

(~57%) and urine (~27%) with low amounts of the glucuronide appearing in the urine, both metabolites being inactive as COX-1 or COX-2 inhibitors.[71]

Another variant of cyclooxygenase enzyme named COX-3 was discovered in 2002. It plays a role in the alleviation of pain and possibly fever, but unlike COX-1 and COX-2, it appears to have no role in inflammation.[72] Aspirin and acetaminophen, the two major drugs often distinguished from the NSAIDs, are known to be reversible active site inhibitors.[67] Acetaminophen or paracetamol (Tylenol), the most widely used over-the-counter common pain reliever, inhibits COX-3, but it has little effect on the other two COX enzymes. Acetaminophen has been identified as one of the most frequently detected anthropogenic compounds in a survey of 139 streams in the United States.[21] It is excreted as labile sulfate and glucuronide conjugates.

2.2.2. Cardiovascular Drugs

Chronic cardiovascular disease represents a huge proportion of human illness (30% of projected total worldwide deaths in 2005). It has led to the world's largest therapeutic drug sector with annual sales of about $70 billion (IMS Health report 2006).

2.2.2.1. Cholesterol and Triglyceride Reducers. Lipid abnormalities are among the key risk factors for cardiovascular disease. It has been shown that interventions that lower low-density lipoprotein (LDL) cholesterol concentrations can significantly reduce the incidence of coronary heart disease (CHD) and other major vascular events in a wide range of individuals. In the decade 1995−2005, the rate of cholesterol-lowering drugs prescribed by physicians to men in the 55−64 years age group almost tripled.[73]

2.2.2.1.1. Fibrates. Fibric acid derivatives are a class of drugs that has been shown to inhibit the production of very low-density lipoprotein (VLDL) and reduce levels of plasma triglycerides. Many analogues of clofibrate, such as gemfibrozil and fenofibrate, have been developed and marketed in the U.S.

Gemfibrozil (GEM; Figure 7B) is oxidized in the liver to four main metabolites including formation of hydroxymethyl- and carboxyl-GEM by oxidation of the ring methyl group.[74] Fenofibrate is hydrolyzed to fenofibric acid, subsequently undergoing carbonyl reduction to result in reduced fenofibric acid. Both fenofibrate and reduced fenofibric acid are active pharmacologically.[74] Gemfibrozil was detected in 4% of monitored streams.[21] Unmodified GEM is reported at concentrations as high as 2.1 μg/L in treated wastewaters and 0.5 μg/L in surface waters. Reports on the amount of unmodified GEM excreted in human urine range widely from 5% to 70%.[74,75]

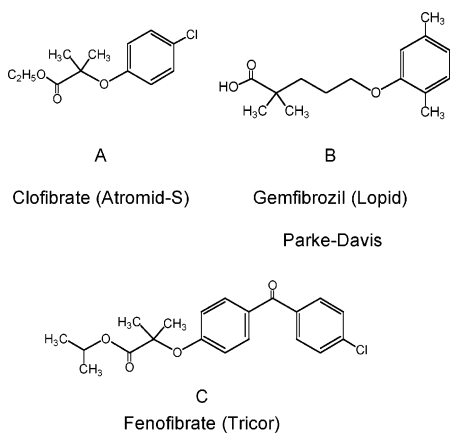


Figure 7. The fibrates available in the United States.

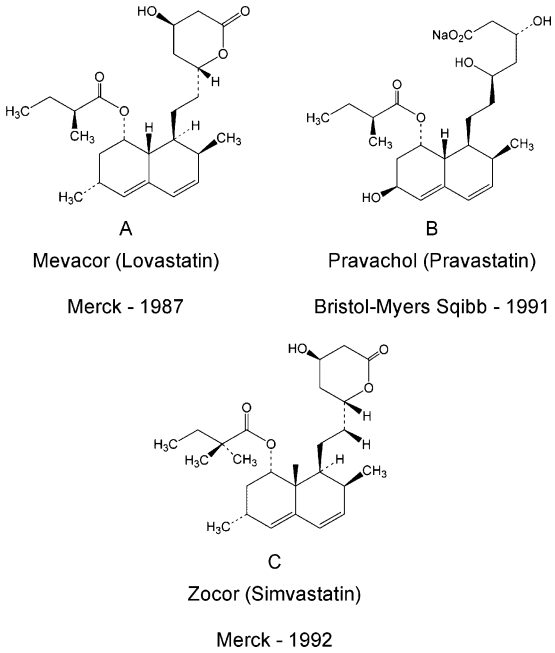


Figure 8. Natural statins, Merck's Mevacor and Zocor and Bristol Myer's Pravachol, are all produced in fermentation by fungal microorganisms.[78]

Clofibric acid, the main metabolite of clofibrate, is one of the most frequently detected chemicals in environmental monitoring of pharmaceuticals. It has been found in wastewaters, surface waters, groundwater, and seawater.[17,76] It has been found in a groundwater reservoir being tapped for Phoenix's drinking water,[77] having arrived there in treated sewage water that is used by the city to replenish the aquifer. Clofibric acid is regarded as one of the most persistent drug residues with an estimated persistence in the environment of 21 years.[18,76]

2.2.2.1.2. Statins. The statins are a class of lipid-lowering drugs that primarily lower plasma levels of low-density lipoprotein (LDL) cholesterol and triglycerides to considerably reduce the risk of cardiovascular events. All statin drugs inhibit the enzyme 3-hydroxy-3-methylglutaryl-coenzymeA (HMG-CoA) reductase, a key enzyme in the biosynthesis of cholesterol. HMG-CoA reductase catalyzes the conversion of hydroxymethylglutaryl-CoA to mevalonic acid, an early rate-limiting step in cholesterol biosynthesis. When this enzyme is blocked, the liver manufactures less cholesterol and blood cholesterol levels fall. As cholesterol production falls, the liver takes up more cholesterol from the blood, so levels drop even further. Statins increase levels of high-density lipoprotein (HDL), or so-called "good cholesterol". The normal treatment regimen for these drugs involves daily intake over a period of many years and this heightens the potential for PIE problems.

Statins were introduced clinically for their hypolipidemic effects (or ability to promote the reduction of lipid concentrations in the serum) with the launch in 1987 of lovastatin (Mevacor; Merck). This was followed in 1991 by simvastatin (Zocor) and pravastatin (Pravachol), in 1993 by fluvastatin (Lescol), in 1996 by atorvastatin (Lipitor), and in 2003 by rosuvastatin (Crestor).[78] There are three natural statins (lovastatin, pravastatin, and simvastatin), which are products of fungal fermentation, that contain decalin rings (Figure 8). The other three statins (fluvastatin, atorvastatin, and rosuva-

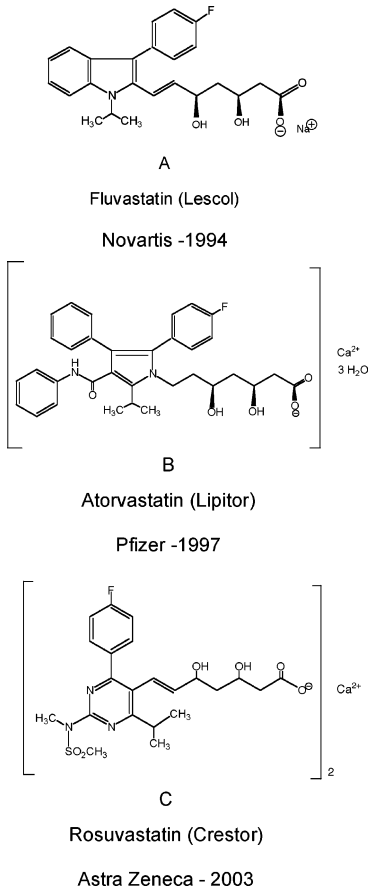


Figure 9. Synthetic cholesterol-lowering statins.[78]

statin) are synthetic APIs that contain aromatic rings (Figure 9). The potency of rosuvastatin, advertised as "superstatin", is more than twice that of atorvastatin. It has also been found to reverse atherosclerosis, a form of arteriosclerosis characterized by the deposition of atheromatous plaques containing cholesterol and lipids on the innermost layer of the walls of large and medium-sized arteries. Lovastatin, simvastatin, and atorvastatin are fat soluble, while fluvastatin, rosuvastain, and pravastatin are water-soluble compounds.[78]

Atorvastatin is extensively metabolized to *ortho*- and *para*-hydroxylated derivatives as well as a glucuronide conjugate of *ortho*-hydroxy atorvastatin.[79] The in vitro inhibition by the hydroxylated derivatives of HMG-CoA reductase is equivalent to that of atorvastatin. A biotransformation pathway reported for all statins is β-oxidation of the dihydroxy heptanoic or heptanoic acid side chain, a structural feature found in all statins. Metabolites shortened by two- or four-carbon units to the pentanoic derivatives or propanoic derivatives, respectively, have been observed in vivo for atorvastatin and pravastatin (both contain the same dihydroxy heptanoic side chain) and analogous metabolites have been found in animals and humans for cerivastatin and fluvastatin. These metabolites have been referred to as β-oxidation products because they resemble those observed in the β-oxidation of fatty acids that are characterized by stepwise oxidation of the carbon chain at two carbons for each cycle.[80,81] In clinical trials and postmarketing surveillance, the two statins fluvastatin and pravastatin have been found not to be metabolized by the cytochrome P450 3A4 system.[78]

Just about 2% of the dose of atorvastatin is recovered in urine following oral administration. Detection of atorvastatin has been reported in samples of sewage treatment plant (STP) effluent and surface water near the point of sewage discharge into the Little River in Ontario, Canada.[82]

Atorvastatin and simvastatin were the world's no. 1 and no. 5 drugs in 2005, with annual global sales of $12.9 billion (U.S sales $7.4 billion) and $5.3 billion, respectively.[83] Global sales of AstraZeneca's Crestor in 2005 are reported to be $1.3 billion.[84]

2.2.2.2. Vasodilators: Antihypertensives. *2.2.2.2.1. Angiotensin-Converting Enzyme (ACE) Inhibitors.* The angiotensin-converting enzyme inhibitors (ACE inhibitors) lower blood pressure causing blood vessels to dilate by effectively blocking the conversion of angiotensin I to angiotensin II. Currently, there are 11 ACE inhibitors available for clinical use in the United States, including enalapril (Figure 10A) and quinapril (Figure 10B), which are used to treat hypertension (high blood pressure) and congestive heart failure. Following absorption, both enalapril and quinapril are deesterified to their major active metabolites, enalaprilat and quinaprilat, respectively. Enalapril, which has an oral bioavailability of about 60%, is nearly all eliminated via the kidneys either intact or as enalaprilat. Quinaprilat is 3-fold more potent than the parent compound, and it is eliminated primarily by renal excretion (up to 96%).[85]

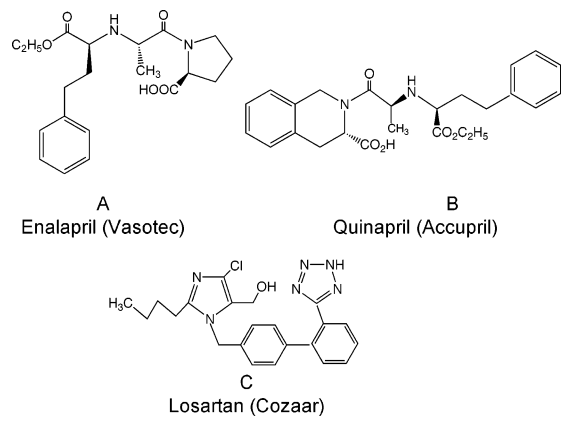


Figure 10. Anti-hypertensive ACE-inhibitor and ACE-blocker drugs.

2.2.2.2.2. Angiotensin II Blockers. Losartan (Cozaar; Merck; Figure 10C) is the first of a new class of blood pressure medications called angiotensin II receptor blockers (ARB). It is also marketed under the name Hyzaar, as a combination drug with hydrochlorothiazide, a low-dose diuretic. Other drugs in this class are irbesartan (Avapro; not shown) and valsartan (Diovan; not shown). All three drugs block angiotensin II, a chemical that causes the arteries and veins to narrow, from attaching to the arteries and veins. As a result, the arteries and veins dilate, reducing blood pressure. Valsartan (Diovan; Novartis) sales are reported to have crossed $4 billion in 2006.[86]

In case of enalapril, approximately 94% of the dose is recovered in the urine and feces as enalaprilat, a deesterified active metabolite (40%), with the remainder as intact enalapril. Similarly, 38% of the quinalapril dose is deesterified to the active metabolite, quinaprilat, which is eliminated primarily by renal excretion. Losartan is converted in part to an active carboxylic acid metabolite. When losartan is administered orally, about 4% of the dose is excreted unchanged in the urine and about 6% is excreted in the urine as active metabolite.[85]

2.2.2.3. Beta Blockers. One of the most important groups of prescription drugs is the beta blockers. Beta blockers slow

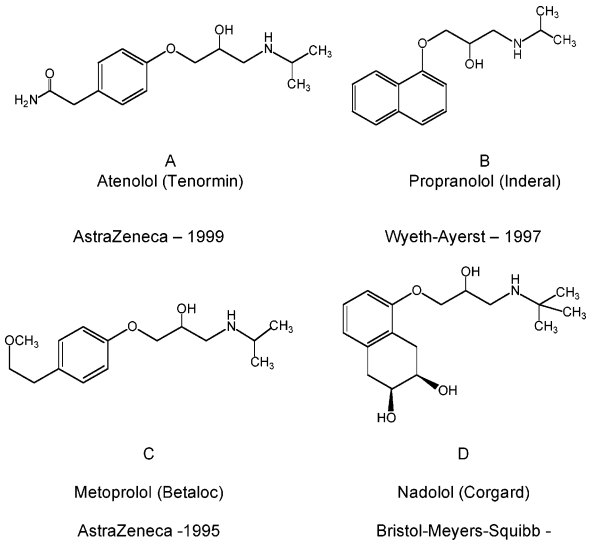


Figure 11. Heart's adrenaline-responsive beta-receptor blocker drugs.

the heart rate and check excessive heart stimulation, thus playing a significant role in the therapy of cardiovascular diseases. These drugs work on the heart and circulatory system to lower hypertension, relieve angina (chest pain), correct arrhythmias (irregular heartbeats), and, in heart attack patients, help prevent additional heart attacks.

Some beta blockers, such as propranolol (Figure 11), cross the blood−brain barrier particularly well. Propranolol is extensively metabolized and less than 10% is excreted as the parent drug, mostly in the feces. The net elimination of propranolol is largely due to oxidative metabolism via side chain oxidation (41%) and aromatic ring oxidation (42%).[87] About 90% of propranolol, converted to 12 metabolites, was recovered in the urine.[87] The dominant metabolites are 4-hydroxypropranolol and naphthoxylactic acid. In the case of metoprolol, <5% of an oral dose is recovered unchanged in the urine. The remainder is excreted via the kidneys as metabolites that do not have any clinical significance. In contrast, the hydrophilic atenolol is hardly biotransformed and almost equal fractions of the parent compound are excreted via the feces and the urine. The hydroxylated and conjugated metabolites make up less than 10%.

Beta blockers are not highly persistent, but as a consequence of their high volume of usage, they are likely to have a constant presence in the aquatic environment, that is, to be pseudopersistent. Several beta blockers have been identified in wastewater, surface water, and groundwater.[17,88,89]

2.2.2.4. Calcium Channel Blockers. Amlodipine (Norvasc; Figure 12) is a long-acting calcium channel blocker drug, which relaxes artery muscles and dilates coronary

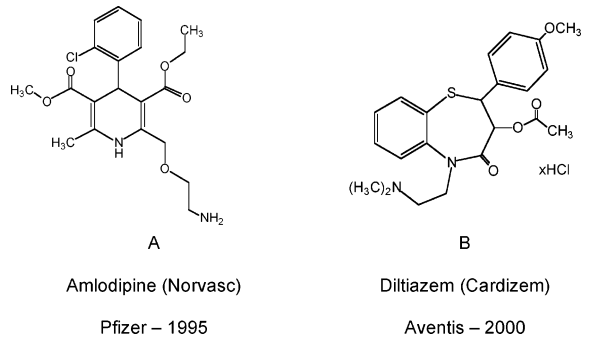


Figure 12. Calcium channel blocker drugs.

arteries and other arteries of the body. It is used to treat high blood pressure and angina. The latter arises when the heart does not get enough blood to the body. Amlodipine is extensively (~90%) converted to inactive metabolites with 10% of the intact amlodipine and 60% of the metabolites excreted in the urine. Norvasc was the sixth largest selling drug worldwide in 2004 and 2005 and generated revenues of $4.8 and $5.0 billion, respectively.[83]

Diltiazem (Cardizem) is another drug in this class, which is metabolized to desacetyldiltiazem, manifesting 25−50% of the activity of diltiazem, and which is excreted through both the bile and urine. Diltiazem was detected in raw drinking water samples in the Chattahoochee River watershed in Metropolitan Atlanta in the U.S.[46] It is considered to be a pharmaceutical of environmental concern because of its relative persistence (<50% removal in STP)[90] and high aquatic toxicity (LC_{50} = 8.2 mg/L) for *Daphnia magna*.[91]

2.2.2.5. Antiplatelet Clustering Drug (Stroke Prevention Drug). Plavix (clopidogrel; Figure 13) prevents blood platelets from clustering and avoids excessive blood clotting to reduce the risk of stroke or heart attack. It is an inactive prodrug, which requires activation by cytochrome P450 to a metabolite that inhibits adenosine diphosphate (ADP)-induced platelet aggregation. However, only a small proportion of administered clopidogrel is metabolized by P450, while the majority is hydrolyzed to an inactive carboxylic acid derivative.

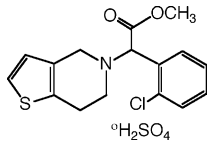


Figure 13. Clopidogrel (Plavix) from Bristol-Myers Squibb, 1997.

Clopidogrel's carboxylic acid and glucuronide derivatives are observed in plasma and urine. Plavix was the second largest selling drug worldwide in 2005 generating revenues of $5.9 billion.[83]

2.2.3. CNS Drugs

Drugs that affect the CNS can selectively relieve pain and reduce fever and can be used to treat anxiety, depression, mania, or schizophrenia.[92,93]

2.2.3.1. Antidepressants and Mood Stabilizers. Depressive disorders are a leading cause of disability in the United States, with about 9.5% of the population affected in a given year.[94,95] By 2020, depression is expected to be the second leading cause of worldwide health care morbidity.[96] The pathophysiology of depression is complex and has not been definitively characterized.[97] Several theories on the cause involve biological amines such as norepinephrine, serotonin, and dopamine. One such theory is known as the "permissive amine hypothesis" and states that decreased levels of serotonin lead to depression. Another theory involves changes in the amine receptors on a chronic basis.[97]

2.2.3.1.1. Selective Serotonin Reuptake Inhibitors (SSRIs). SSRIs are the most commonly used class of antidepressants for treating depression, anxiety, panic disorder, obsessive-compulsive disorder, eating disorders, and social phobia. Low levels of the neurotransmitter serotonin (5-hydroxytryptamine) can cause clinical depression. SSRIs block the function of the serotonin transporter on cell membranes leading to elevated levels of serotonin. Transmission by serotonergic neurons is increased. The three well-established SSRIs, —

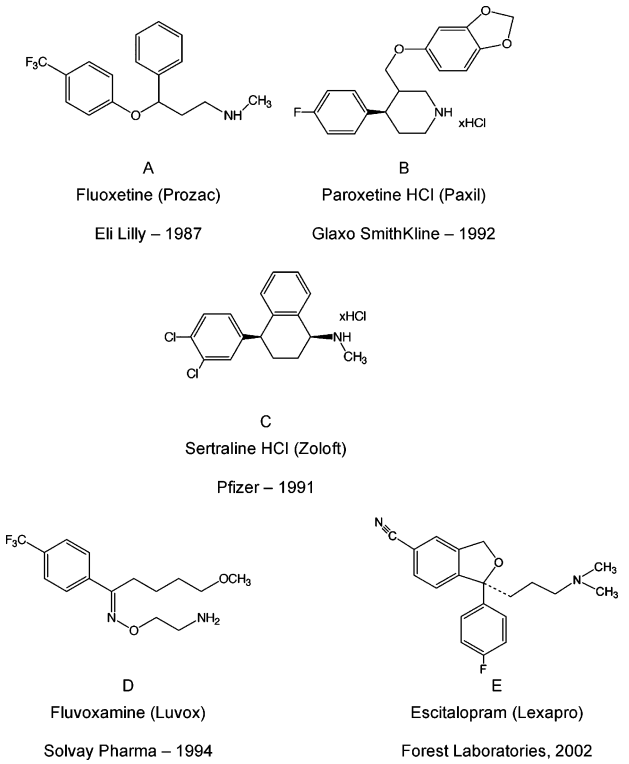


Figure 14. Selective serotonin reuptake inhibitors introduced in the mid-1980s.

fluoxetine, Prozac (Figure 14A); paroxetine, Paxil (Figure 14B); and sertraline, Zoloft (Figure 14C), are similar in efficacy and safety, although Paxil has more extensive indicators on its label and Prozac has more convenience in a weekly dosage form.[98] Prozac, Zoloft, Paxil, and Celexa (Figure 14E, a recemic mixture) have been found in low concentrations in surface water, particularly wastewater.[93]

In 1997, antidepressants led by Prozac held three spots in the top-ten best-selling U.S. drugs list, and Prozac was the second highest-selling drug of any kind in the United States. Prozac is known to transform a patient from a state of sadness and agitation to one of calm and tranquility, albeit with a diminished intellectual passion and reduced sex drive.[99] Fluoxetine (Prozac) is extensively metabolized in the liver to norfluoxetine, an N-demethylated metabolite, and other unidentified metabolites. Norfluoxetine is also a serotonin reuptake inhibitor with its pharmacological activity being similar to that of the parent drug. Norfluoxetine contributes to the long duration of action of fluoxetine. Fluoxetine was detected in STP effluent samples in Canada[82] and in U.S. streams.[21]

The principle initial pathway of sertraline (Zoloft) metabolism is N-demethylation to N-desmethylsertraline, which shows negligible pharmacological activity. Both sertraline and N-desmethylsertraline undergo oxidative deamination and subsequent reduction, hydroxylation, and glucuronide conjugation. Biliary excretion of metabolites is significant.[100] Similarly, the majority of paroxetine (Paxil) appears to be oxidized to a catechol intermediate, which is converted to highly polar glucuronide and sulfate metabolites through methylation and conjugation reactions.

The newest of antidepressants, introduced by Forest Laboratories in 2002, is named escitalopram (Lexapro; Figure 14E). It is the active (*S*)-isomer of citalopram (Celexa, 1998), a racemic mixture of (*S*)- and (*R*)-enantiomers. The (*R*)-isomer is totally inactive. Escitalopram is metabolized to (*S*)-desmethylcitalopram ((*S*)-DCT) and (*S*)-didesmethyl-citalopram ((*S*)-DDCT). These metabolites are reported to be pharmacologically far less active than the parent compound, suggesting that the metabolites do not contribute significantly to the antidepressant actions of escitalopram.[101] Elimination of metabolites occurs primarily in the urine with <10% unchanged parent compound.[102] For escitalopram, the fraction of unchanged drug and (*S*)-DCT recovered in the urine are about 8% and 10%, respectively.

2.2.3.1.2. Selective Serotonin and Norepinephrine Reuptake Inhibitors (SSNRIs). Wyeth-Ayerst's venlafaxine (Effexor; Figure 15A) became the first agent in a category of antidepressants known as the SSNRIs. It is a phenethylamine derivative, chemically unrelated to tricyclic, tetracyclic, or other available antidepressant agents. It is thought to work by inhibiting the reuptake both of serotonin and norpinephrine, two chemicals for which imbalance in the brain is linked to depression. It is extensively metabolized in the liver by O-demethylation. The O-desmethyl metabolite is active but is conjugated with glucuronic acid and excreted. Effexor was the 10th largest selling drug worldwide in 2005 yielding a revenue of $3.8 billion.[83] Eli Lilly's duloxetine HCl (Cymbalta; Figure 15B), approved by the FDA in 2004 for the treatment of major depressive disorders, is a new addition to the SSNRIs. It appears to exhibit a greater balance in the relative reuptake inhibition of serotonin and norepinephrine.

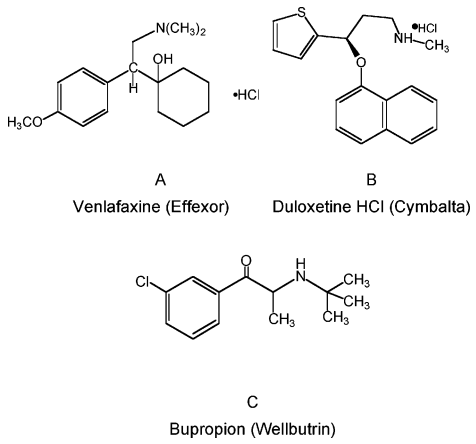


Figure 15. The newer SSNRI class of antidepressants.

Bupropion (Wellbutrin; Figure 15C) is unrelated to other antidepressants and is unique in that it works by inhibiting the reuptake of norepinephrine and dopamine. It has no clinically significant impact on serotonin uptake. It has at least three active metabolites; hydroxybupropion, threohydrobupropion, and erythrohydrobupropion. These active metabolites are further metabolized to inactive metabolites and eliminated through excretion in the urine. The U.S. revenue for Wellbutrin in 2005 was $1.48 billion.[83]

2.2.3.2. Antipsychotic Drugs. Schizophrenia is a major health problem worldwide. Antipsychotic drugs such as olanzapine (Figure 16A), quetiapine (Figure 16B), risperidone (Figure 16C), and aripiprazole (Figure 16D) represent a new generation of drugs prescribed for schizophrenia and delusional disorders. These drugs work by regulating serotonin and dopamine levels in the brain. Olanzapine was the world's seventh largest selling drug in 2005 with a revenue of $4.7 billion.[83] Risperidone (Risperdal) was the eighth largest selling drug with global sales of $4.0 billion, while quetiapine (Seroquel) and aripiprazole (Abilify) are also

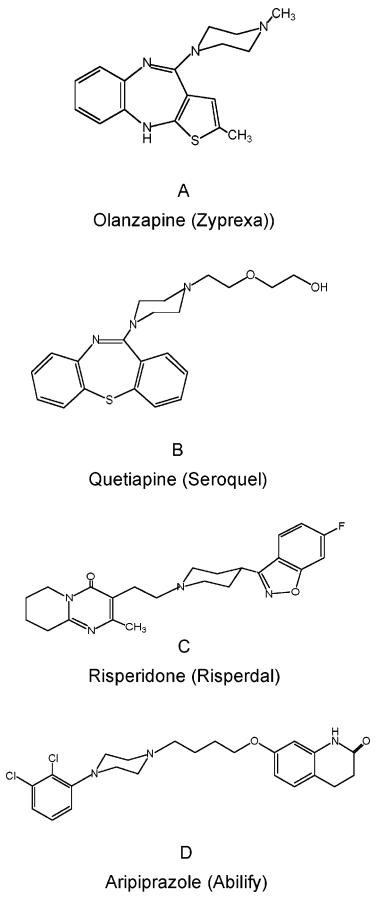


Figure 16. Antipsychotic drugs for schizophrenia and delusional disorders.

blockbuster drugs with U.S. 2005 sales of $2.6 and $1.54 billion, respectively.[83] Paliperidone (Invega), a 9-hydroxy derivative of risperidone developed by Janssen Pharmaceutica, is expected to be another blockbuster drug in this class.[60]

All the discussed schizophrenia drugs extensively metabolize in the liver. Following a single oral dose of ^{14}C-labeled olanzapine and quetiapine, 7% and 1%, respectively, of the individual doses were recovered unchanged in the urine. Quetiapine's major metabolites are pharmacologically inactive sulfoxide and carboxylic acid derivatives, and olanzapine's major metabolites are inactive glucuronide and des-methyl products. On the other hand, hydroxyrisperidone and dehydro-aripiprazole, the main metabolites of risperidone and aripiprazole, had pharmacological activities similar to the parent compounds. Less than 1% of unchanged aripiprazole was excreted in the urine. Approximately 18% of the oral dose was recovered unchanged in the feces.

2.2.3.3. Antiallergic Drugs. *2.2.3.3.1. Antihistamines (Histamine H1 Receptor Antagonists).* Antihistamines comprise a broad class of pharmacologic agents that are used in the treatment of patients with allergic disorders. The primary action of the drugs is to block histamine H1 at the receptor site. The newer piperidine group of antihistamines, such as fexofenadine (Figure 17A), cetirizine (Figure 17B), and loratadine (Figure 17C), are peripherally selective H1-receptor antagonists that eliminate many adverse effects of the older drugs.

Human mass balance studies have documented a recovery of approximately 80% and 11% of a ^{14}C-labeled fexofenadine hydrochloride dose in the feces and urine, respectively.[103] Approximately 5% of the total dose was metabolized. A mass

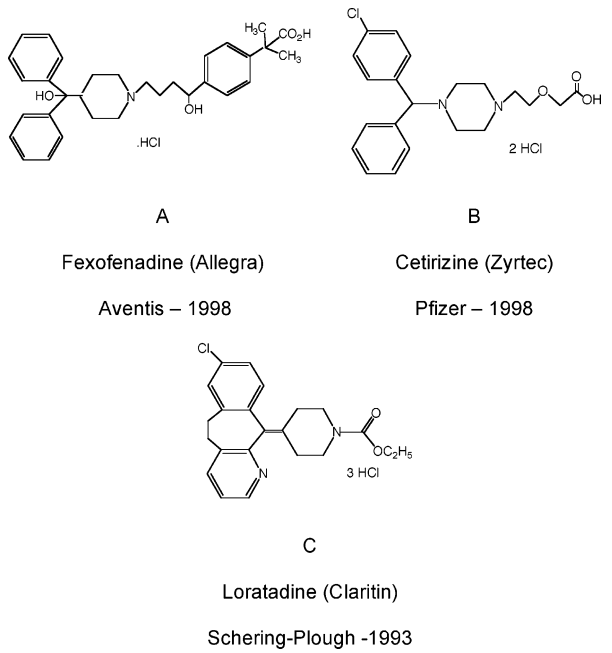


Figure 17. Antihistamine compounds.

balance study for cetirizine indicated that 70% of the administered radioactivity was recovered in the urine and 10% in the feces. Approximately 50% of the radioactivity was identified in the urine as unchanged drug. Cetirizine is metabolized to a limited extent by oxidative O-dealkylation to a metabolite with negligible antihistaminic activity.

Metabolic studies of loratadine have demonstrated that this drug is rapidly absorbed and undergoes extensive first-pass metabolic transformation by human liver cytochrome P450 enzymes to descarboethoxyloratadine, which has 2.5−4 times more pharmacological potency than its parent drug. Schering-Plough patented this active metabolite and launched it as an OTC drug, Clarinex, at the end of 2002.

2.2.3.4. Gastrointestinal (Heartburn) Drugs. *2.2.3.4.1. Acid-Reducing Drugs: Histamine (H2) Blockers.* Antagonists for H2 histamine receptors, such as cimetidine (Figure 18A), ranitidine (Figure 18B) and nizatidine (Figure 18C), relieve symptoms and promote ulcer healing by reducing the production of stomach acid. They are commonly used in the

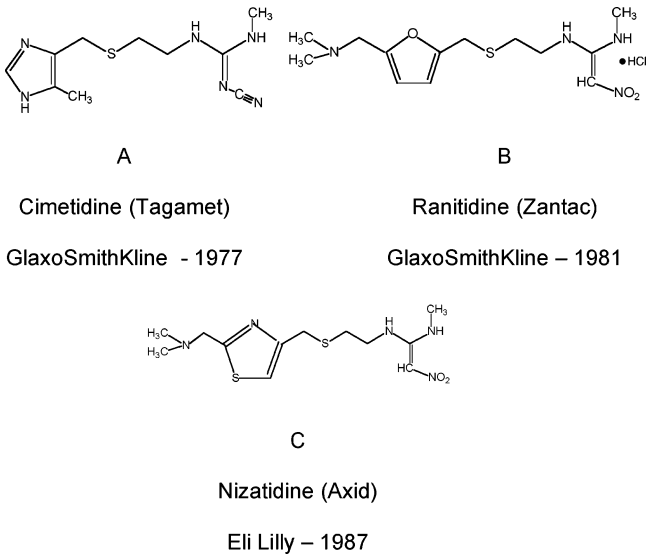


Figure 18. Histamine H_2-blockers.

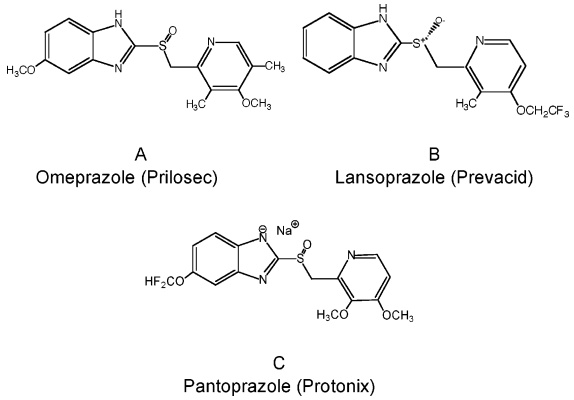


Figure 19. Proton-pump inhibitors (PPIs).

treatment of peptic ulcer and gastroesophageal reflux disease. Ranitidine, developed in 1981 by what is now GlaxoSmith-Kline, was the largest selling prescription drug by 1988. Cimetidine and ranitidine have been regularly detected in U.S streams.[21] Proton pump inhibitors such as omeprazole (Figure 19A) have now largely superseded these antacids.

2.2.3.4.2. Proton-Pump Inhibitors (PPIs). Proton-pump inhibitor drugs consist of substituted benzimidazoles that inhibit gastric acid secretion by blocking the H^+K^+-ATPase in the proton pump. Because the proton pump is the final pathway for secretion of hydrochloric acid by the parietal cells in the stomach, its inhibition dramatically decreases the secretion of hydrochloric acid into the stomach and alters gastric pH.

The three FDA approved drugs in this class include omeprazole (Prilosec; Figure 19A), lansoprazole (Prevacid; Figure 19B), and esomeprazole (Nexium, 2001; Figure 19A, (*S*)-isomer). Esomeprazole is a single (*S*)-isomer version of the older drug Prilosec (omeprazole).[104] Prilosec was conceptually a new pharmaceutical that became the world's best selling drug in the late 1990s with sales of about $6 billion a year. Esomeprezole has been found to be significantly superior in terms of higher bioavailability and became the third highest selling drug in 2005 with $5.7 billion global sales.[83] Lansoprazole, a 2,2,2-trifluoroethoxy derivative, was ninth in global sales in 2005 with revenues of $4.0 billion. Pantoprazole generated U.S. sales of $2.38 billion in 2005.[83]

Esomeprazole is excreted <1% unchanged and ~80% as inactive metabolites in the urine. The remainder is found as inactive metabolites in the feces. Virtually no unchanged lansoprazole was excreted in the urine. A single oral dose of ^{14}C-lansoprazole resulted in approximately one-third of the radiation excreted in the urine and two-thirds in the feces, implying a significant biliary excretion of the metabolites. Similarly, administration of ^{14}C-pantoprazole resulted in approximately 71% of the dose excretion in the urine and 18% in the feces through biliary excretion.

2.2.3.5. Antiepileptics. Antiepileptics and anticonvulsants such as carbamazepine (Tegretol; Figure 20A) and gabapentin (Neurontin; Figure 20C) are CNS drugs that help to quiet the abnormal firings of nerves in the brain and central nervous system. Carbamazepine acts by blocking voltage-dependent sodium channels of excitatory neurons and gabapentin is a GABA analog that binds to an auxiliary protein of voltage-gated calcium channels and, as a result, modulates the action of calcium channels and neurotransmitter release. Gabapentin is approved for treatment of neuropathic pain. Novartis came up with an oxo-analog of carbamazepine in 2000, a second level improved drug Trileptal (Figure 20B).

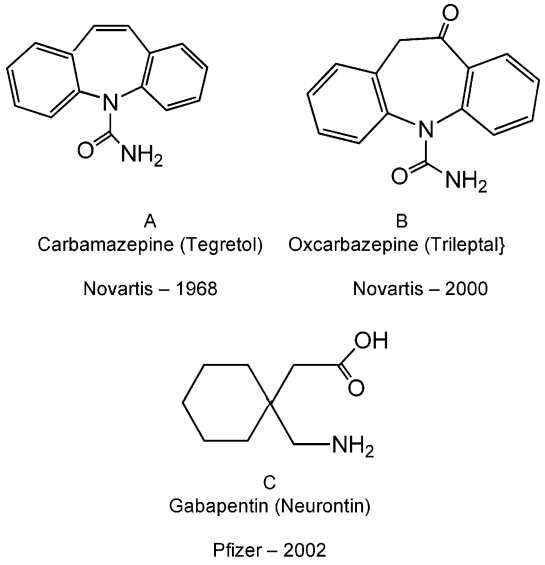


Figure 20. Antiepileptic drugs.

About 3% carbamazepine is excreted unchanged. Thus, at a daily dosage of 1200 mg, a single patient excretes at least 36 mg of carbamazepine into the environment every day.[105] Carbamazepine is predominantly metabolized into carbamazepine-10,11-epoxide, which also has strong anticonvulsant properties. The remainder comprises hydroxylated and conjugated metabolites, such as 10,11-dihydro-10,11-dihydroxycarbamazepine. Carbamazepine and its metabolites have been detected most frequently in wastewaters and in groundwater and have been found to occur ubiquitously in rivers in the U.S.A., Canada, and Germany.[105−107]

Carbamazepine has a mean 50% dissipation time (DT_{50}) of 82 ± 11 days under semifield conditions, making it one of the most persistent pharmaceuticals detected in the environment.[108] For this reason, it has been proposed as a marker of anthropogenic urban contamination.[109,110] Similarly, gabapentin is not appreciably metabolized in humans; 75−80% of an oral dose is recovered unchanged in the urine and 10−20% in the feces.

2.2.4. Cytostatics: Cancer Drugs

Cancer is a chronic disease that represents a huge proportion of human illness (13% of projected total worldwide deaths in 2005). Bristol Myers' carboplatin (Paraplatin) is a major cancer drug that is offered for chemotherapy to patients with advanced lung cancer. It is one of the few coordination compound drugs, diammineplatinum-*O*,*O*′-[(1-cyclobutane-dicarboxylate)] (Figure 21).

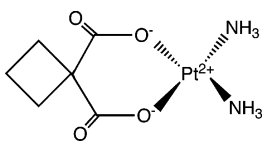


Figure 21. Cancer drug Carboplatin (Paraplatin), Bristol Myers.

Carboplatin produces predominantly interstrand DNA cross-links, rather than DNA−protein cross-links, causing lesions and biological effects. It is not bound to plasma proteins. However, platinum from carboplatin becomes irreversibly bound to plasma proteins and is slowly eliminated with a minimum half-life of 5 days. The major route of carboplatin elimination is through renal excretion. Patients

excrete about 71% of the dose within 24 h, and the platinum in 24 h urine is present as carboplatin.[111]

2.2.4.1. Protein Kinase Inhibitors. Imatinib (Gleevac; Figure 22A) is a new type of cancer drug called a signal transduction inhibitor, which acts by turning off an enzyme, tyrosine kinase inhibitor, that causes cells to become cancerous and multiply. It has proven to be effective in fighting a form of leukemia called chronic myeloid leukemia (CML), a rare slowly progressing cancer of the blood and the bone marrow. The bone marrow produces an excessive number of abnormal (leukemic) white blood cells that suppress the production of normal white blood cells, which act to protect the body against infection. Imatinib is largely eliminated as metabolites, the main metabolite an N-demethylated piperazine derivative being also active; only 25% is eliminated unchanged. Gleevac sales in 2005 are reported to have reached $2.2 billion.[112]

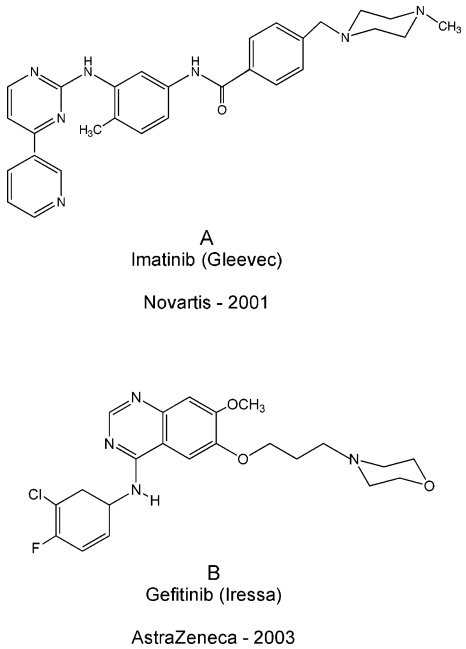


Figure 22. Anticancer protein-kinase inhibitor drugs.

Gefitinib (Iressa; Figure 22B) blocks growth signals caused by tyrosine kinase present in lung cancer cells, as well as other cancers and normal tissues, which appears to be important to the growth of cancer cells. However, recently it has been found that only for 10% of patients on Iressa did lung tumors shrink rapidly. The FDA barred its sale to new patients for lack of efficacy. Gefitinib excretion occurs predominantly via the feces (86%), and metabolites account for less than 4% of the administered dose. *O*-Desmethylgefitinib was the only active metabolite with activity similar to gefitinib.

Several other drugs approved in this class include Avastin, Herceptin, and Erbitux (monoclonal antibodies), geldanamycins, and flavopitidols.[113]

2.2.5. Respiratory Drugs

2.2.5.1. Asthma Drugs: Lucasts (Leukotriene Receptor Antagonists). In 2003, it was estimated that 20 million Americans had chronic asthma. The largest group among the inhaled asthma drugs is the bronchodilators, which open up the airways of the lungs by relaxing the muscles in the

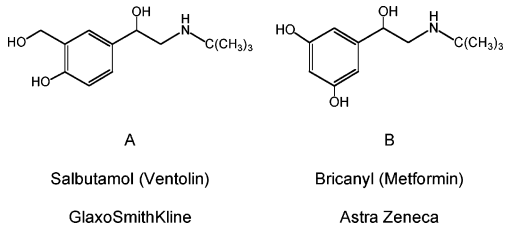


Figure 23. Bronchodilator drugs for asthma.

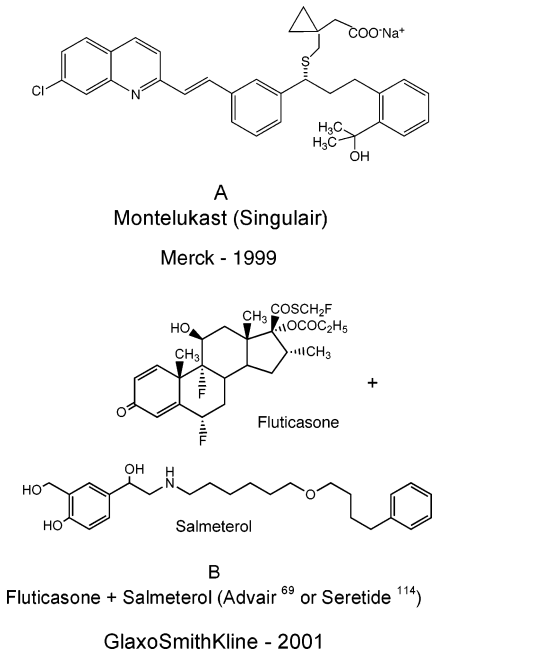


Figure 24. Leukotriene blockers and combination asthma drugs.

air tubes. Salbutamol (known as albuterol in the U.S.) and bricanyl (Figure 23) have been detected in sewage in several cases.[17]

A new class of asthma medicines called leukotriene blockers, of which Singulair (montelukast) is the key member, work by blocking leukotrienes involved in the inflammatory process associated with asthma. In 2003, worldwide sales of Singulair (Figure 24A) touched $2.2 billion. Accolate (not shown) is another drug in this class. The inhaled combination asthma drug Advair consists of the fluoridated synthetic corticosteroid fluticasone and the long-acting bronchodilator salmetrol (Figure 24B). Advair was the fourth largest selling drug worldwide in 2005 with a revenue of $5.6 billion.[83] Another combination drug, Symbiocort by AstraZeneca, consists of the corticosteroid budesonide and the brochodialator formoterol.

2.2.6. Endocrinology Treatments

2.2.6.1. Antidiabetics. The hallmark of diabetes is an abnormally high level of glucose in the blood, a condition known as hyperglycemia. An injection of insulin helps keep the glucose in check by preparing cells to admit sugar. In a healthy body, the pancreas normally secretes as much insulin as needed to keep the blood sugar level steady. In diabetic patients, the pancreas produces little or no insulin (type 1) or the body does not adequately respond to insulin (type 2).

The antihyperglycemic drugs for type 2 diabetes include sulfonylureas, such as glyburide (Figure 25A), and thiazolidinediones or TZDs (insulin sensitizers), such as rosiglitazone (Figure 25B) and pioglitazone (Figure 25D).

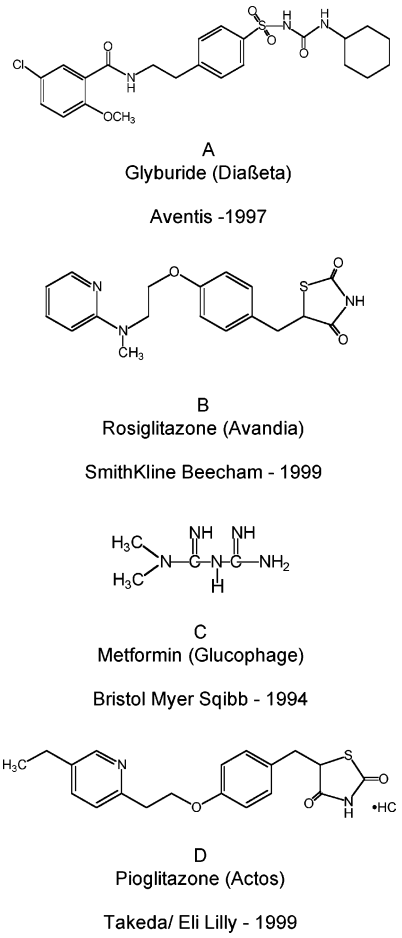


Figure 25. Antidiabetic drugs for type 2 diabetes.

Biguanides are another antidiabetic drug class for treatment of type 2 diabetes. Metformin (Figure 25C) of this class, approved in 1994, has been detected in streamwater samples.[21]

Glyburide is excreted as metabolites, mainly 4-*trans*-hydroxy and 3-*cis*-hydroxy derivatives, in the bile and urine, approximately 50% by each route. These metabolites are thought to contribute no significant hypoglycemic action since they are only weakly active in rabbits, 1/400th and 1/40th, respectively, compared with glyburide.

Rosiglitazone is extensively metabolized with no unchanged drug excreted in the urine. The major metabolites were formed from N-demethylation and hydroxylation, followed by conjugation with sulfate and glucuronic acid, all considerably less potent than the parent compound. Pioglitazone was recovered in 15−30% of the oral dose in urine, while renal elimination primarily contained metabolites, hydroxy and keto derivatives, and their conjugates. Metformin is excreted unchanged in the urine and does not undergo hepatic metabolism (no metabolites have been identified in humans) nor biliary excretion. Approximately 90% of the absorbed drug is eliminated via the renal route.

2.2.6.2. Steroid Hormones. *2.2.6.2.1. Ovulation Inhibitors (Estrogen Agonist).* Synthetic analogs of 17β-estradiol (17α-ethinylestradiol, EE_2 (Figure 26D), mestranol (Figure 26E), and 19-norethisterone (Figure 26F)) or 17β-estradiol (Figure 26A) itself are primarily used as oral contraceptives and for substitution therapy during menopause. Low levels are being found of natural and synthetic steroid estrogens in the aquatic environment, such as 17β-estradiol and its more persistent oxidation product estrone, and the synthetic oral contraceptives. The biological effects on aquatic organisms have issues

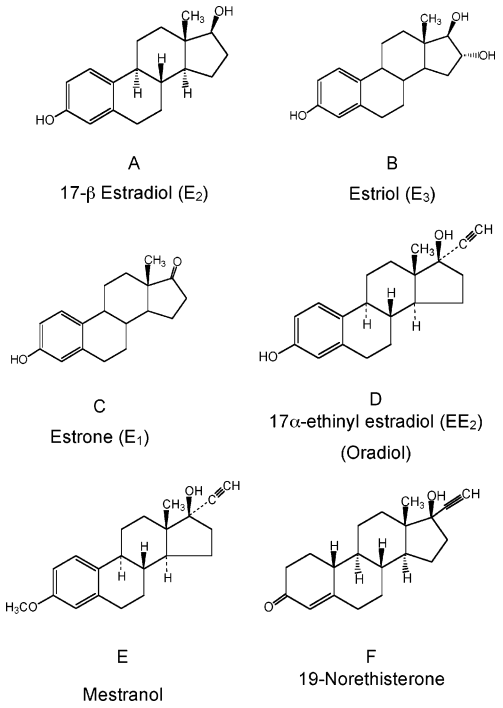


Figure 26. Oral contraceptives and hormone substitutes. Estriol and estrone are oxidative degradation products of estradiol.

of considerable concern.[115] Oral contraceptives contain typically 20−40 μg of 17α-ethinylestradiol, which has been found to be approximately 11−27 times more potent than the female sex hormone 17β-estradiol by in vivo tests.

Ethinylestradiol is excreted in urine as free EE_2 or as a glucuronide-EE_2 conjugate and is usually found to undergo little degradation in wastewater treatment facilities. Bacterial deconjugation of E_2- and EE_2-glucuronides during waste treatment releases free E_2 and EE_2, increasing free EE_2 concentrations.[116] Thus, estrogenic pharmaceutical products may significantly contribute to the total estrogenic load from humans in the environment.[117]

2.2.6.2.2. Cattle Growth Hormones. Aquatic effects from animal medicinal products are now also coming to light following recent environmental studies involving cattle growth hormones.[118] We regard the issues to be so important that we have added this brief animal section. These anabolic steroids are widely used in the United States (although banned in Europe) to promote weight gain in beef cattle. Female fathead minnows taken downstream from several Nebraska feedlot effluent outlets were found to have male characteristics; the male minnows were found to have abnormally small testes and certain female characteristics.[119] Water from the same locations produced androgenic effects similar to male sex hormones by in vitro assays. One specific growth promoter, trenbolone acetate (Figure 27), the androgen receptor agonist, has been shown by laboratory tests to affect the reproductive endocrine functions of fathead minnows in several ways. These effects occurred at water concentrations as low as 0.03 μg/L, or 30 ppt.[120]

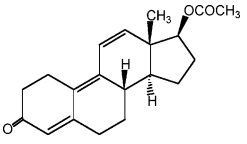


Figure 27. Trenbolone acetate.

2.2.7. Antimicrobials

2.2.7.1. Antibiotics. About 23 000 tons of antibiotics are produced in the U.S. each year, of which about 40% are fed to livestock as growth enhancers in agriculture.[121] Large quantities of antibiotics are administered to humans and animals to treat diseases and infection. This usage may result in their presence in environmental waters because up to 90% can be excreted without undergoing metabolism.[122] By use of the closed bottle test for biodegradability, some of the most important groups of antibiotics used in human and veterinary therapy were found to be not readily biodegradable.[122] The results indicated that many antibiotics might not be biodegraded efficiently in treatment plants and in surface water.[123] The presence of antibiotics in environmental waters is troubling because antibiotic contaminants could perturb microbial ecology, increase the proliferation of antibiotic-resistant pathogens, and pose threats to human health.[16,19,124] Aquatic antibiotic contamination also presents challenges for the water industry for water reuse and water resource planning.[122]

Antibiotics are also commonly used at subtherapeutic levels in livestock to prevent diseases and promote growth.[125] The large feedlots for livestock have proved to be another source of drug pollution. As farmers feed livestock low doses of antibiotics to boost growth, some bacteria in the animals, in manure-tainted fields, and in local waters evolve to coexist with the drug.[126] A number of studies find that U.S. rivers have become a major reservoir of microbes that have developed resistance to antibiotic drugs, posing a large and growing threat to the success of modern medicine.

Among more than 10 antibiotic classes, six (aminoglycoside, β-lactam, macrolide, fluoroquinolone, sulfonamide, and tetracycline) are important in both human medicine and animal husbandry.[122] The β-lactam antibiotics (such as amoxicillin, not shown) account for the most antibiotic usage in human therapy, followed by macrolides (such as azithromycin, Figure 28B, and clarithromycin), sulfonamides (such as sulfamethoxazole; Figure 28C), and fluoroquinolone antibiotics (such as ciprofloxacin; Figure 28A).

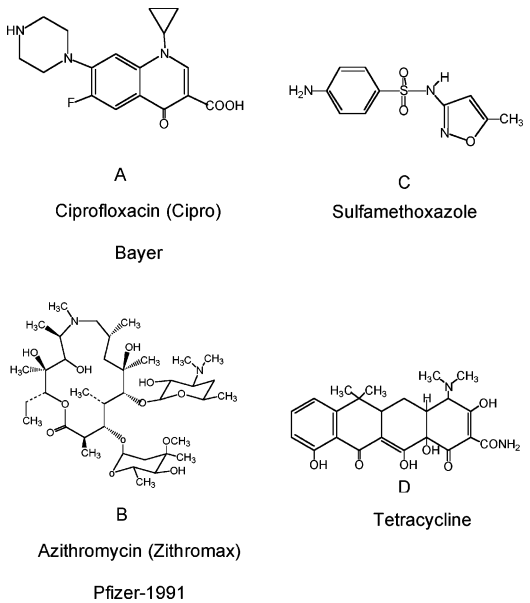


Figure 28. Important classes of antibiotics: fluoroquinolone ciprofloxacin; macrolide azithromycin; sulfonamide sulfamethoxazole.

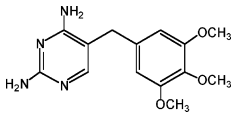


Figure 29. Trimethoprim (Trimpex, Proloprim).

Although antibiotics are used in quantities similar to those of many agrochemicals and other organic micropollutants, they are not required to undergo the same level of testing for possible environmental effects. Test system studies indicate that a number of antibiotics are not biodegradable in aquatic environments. Since these products can have long half-lives, they accumulate to reach detectable and biologically significant concentrations.

Trimethoprim (Figure 29), a synthetic antibiotic, is commonly used in combination with sulfamethoxazole. The FDA first approved it in 1973 in combination with sulfamethoxazole (Septra) and in 1980 as a stand-alone drug. Trimethoprim inhibits production of tetrahydrofolic acid by inhibiting the enzyme responsible for making tetrahydrofolic acid, a chemical that is necessary in order for bacteria and human cells to produce proteins. However it is also causing concern due to its relatively high persistence with little removal being effected by municipal wastewater plants.[127]

2.2.7.2. Antifungals. Fungal infections such as *Cryptococcal meningitis* and *C. candidia* infections are treated with the bis-triazole antifungal drug fluconazole (Figure 30). Fluconazole is a highly selective inhibitor of fungal cytochrome P-450 that enacts sterol C-14 α-demethylation. Fluconazole disrupts key cellular processes in yeast and fungi slowing cellular growth and reproduction. It is a blockbuster in terms of annual sales. The unchanged drug in urine was found to account for 80% of the administered dose with only 11% excreted as metabolites.[128]

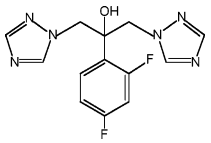


Figure 30. Antifungal fluconazole (Diflucan), Pfizer, 1985.

Conventional quantitative structure−activity relationship (QSAR) models have been used to prioritize the environmental risk of pharmaceuticals in surface waters. It has been predicted that antifungals, along with sex-hormones, sunscreens, and antimalaria compounds are the most frequent hazardous therapeutic pharmaceuticals in the environment.[129]

2.2.7.3. Antiviral Drugs. There are only a few clinical antiviral APIs because of the nature of viruses as intracellular parasites. Acyclovir, a purine analog (Figure 31A), is an antiviral drug that acts against the herpes viruses. It is used to treat cold sores, genital herpes, shingles, and chickenpox by inhibiting herpes DNA replication, thereby slowing the growth and spread of the viruses. The EPA has identified

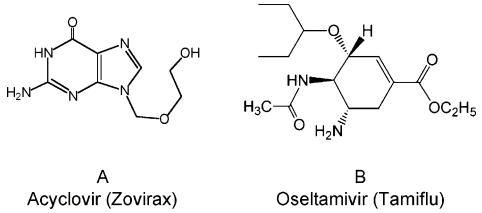


Figure 31. Antiviral drugs.

acyclovir as a pharmaceutical of environmental concern because of its fate and safety.[90,130]

Oseltamivir phosphate (Tamiflu; Figure 31B) has emerged as an important antiviral drug. It is recommended for the treatment and prevention of pandemic influenza. It is a selective inhibitor of influenza virus A and B neuraminidase, which stops the virus emerging from the infected cell. Oseltamivir is primarily (~70%) excreted in the active antiviral form oseltamivir carboxylate (OC) mostly in the urine. Less than 20% of an oral radiolabeled dose was found to eliminate in feces.[131] No observed oxidative metabolites of OC have been reported in humans, indicating that OC is resistant to cytochrome P450 mixed-function oxidases and glucuronosyltransferase, and thus it has the potential to be maintained in rivers receiving treated wastewater.[132]

3. Fluorinated Pharmaceuticals

As we describe below, fluorine substituents bring many useful pharmacodynamic and pharmacokinetic properties to APIs. Thus, it is highly likely that the pharmaceutical industry will continue to expand in the fluorinated drug area. But because of the increased lipophilicity and degradation resistance that accompanies the introduction of fluorine into APIs, understanding how to deal with fluorinated APIs in the environment is becoming a major challenge, and we therefore discuss the background of the appeal of fluorine to the pharmaceutical industry. It is often a major challenge to convert a compound binding with high affinity to a biological target (i.e., a hit, lead, or candidate molecule) into a successful drug on the market. A lead compound with desired pharmacological activity may have undesirable characteristics that limit its bioavailability or structural features that adversely influence its metabolism and excretion from the body. Bioisosterism represents one approach that medicinal chemists use for the rational modification of lead compounds into safer and more clinically effective agents.[133] Bioisosteres are compounds related by the exchange of an atom or group of atoms with another roughly similar atom or group of atoms. The objective of a bioisosteric replacement is thus to create new compounds with similar biological properties to a parent compound. The classical bioisosteres are those that have similar steric and electronic features. Bioisosteric replacement groups often, but not always, have the same number of atoms as the substituent targeted for replacement. The substitution of a hydrogen atom or hydroxyl group by a fluorine atom is among the most commonly employed bioisosteric replacements. Fluorine and trifluoromethyl groups are important bioisosteric replacement entities.

In 1990, there were 220 fluorinated drugs on the market representing ca. 8% of all synthetic drugs.[134] In 1996, more than 1500 fluorine-containing drugs were under development[134] to further refine compounds for improved potency, higher selectivity, and superior pharmacokinetics, such as better ADME (absorption, distribution, metabolism, and excretion) properties. It is now estimated that as many as 20% of pharmaceuticals on the market contain fluorine, including half of the top 10 drugs sold in 2005.[135]

A wide range of pharmaceuticals across therapeutic categories contain fluro groups, including antidepressants, anti-inflammatory agents, antimalarial drugs, antipsychotics, antiviral agents, steroids, and anaesthetics.[136] The fluorine-containing antidepressants citalopram, escitalopram (Figure 14E), and paroxetine (Figure 14B) have shown impressive market growth in the past few years. Anticholesterol drugs, such as the blockbuster drugs atorvastatin and fluvastatin, are also fluorinated compounds.

3.1. The Role of Fluorine in the Stability and Bioavailability of Pharmaceuticals

Fluorine as a substituent has played a significant and increasingly important role in the development of modern pharmaceuticals and agrochemicals. The underlying reasons are that the incorporation of fluorine into a drug allows simultaneous modulation of electronic, lipophilic, and steric parameters, all of which can influence both the API's pharmacodynamic and its pharmacokinetic properties.[136] The pharmacological superiority of fluorinated compounds over their non-fluorinated analogues derives from the involvement of such features in critically increasing the intrinsic activity, the chemical and metabolic stability, and the bioavailability.[134] This versatility has been used to great effect in many of the most active pharmaceuticals and agricultural chemicals on the market. The rationale behind incorporating fluorine atoms in active substances is manifold, and the various benefits have been summarized as follows:

- The van der Waals radius for fluorine (1.47 Å) lies between that of oxygen (OH; 1.52 Å) and hydrogen (1.20 Å), and the CF_3 (2.20 Å) group is sterically at least as large as the $-CH(CH_3)_2$ (2.20 Å) group. The CH_3 group van der Waals radius is 1.80 Å. Despite the fact that fluorine has a greater size than hydrogen, several studies have demonstrated that it is a reasonable hydrogen mimic.[136]
- Fluorine incorporation leads to a significantly enhanced lipophilicity, with functional groups such as $-CF_3$, CF_3O-, and CF_3S- being the most lipophilic groups known. This may result in different pharmacokinetic behavior such as enhanced passive diffusion of compounds across membranes leading to improved bioavailability. This also reduces clearance by the liver.[136]
- In general, carbon−fluorine bonds (C−F, ~116 kcal/mol) are stronger than carbon−hydrogen bonds (C−H, ~99 kcal/mol), providing an increased oxidative and thermal stability of carbon−fluorine compounds compared with the carbon−hydrogen isosteres. Because of the electronegativity difference between carbon and fluorine (2.5 vs 4.0), C−F bonds are more polar, and this contributes to the difference in C−F versus C−H bond strengths. Thus, fluorine substituents have the potential to improve the metabolic stability of drugs.[136]
- The singularly high electronegative character of fluorine often has a significant impact on a nearby ionizable group. This can be used to adjust the pK_a value of a compound, for example, toward the physiological pH of 7.4, which can further translate into improved absorption properties.[136]
- Oxidative metabolism of the phenyl rings of APIs is a common problem, but fluorine substitution, usually in the 4-position, has become a widespread practice for increasing stability and enhancing central nervous systems (CNS) penetration in various drug classes. The presence of the fluorine can alter the oxidation potential of an aromatic system and thus alter the rate of autoxidation and formation of quinones and quinone-imines.[136]
- Another outcome of adding fluorine can be an enhancement of an API's binding affinity to a target

protein. For example, a 3,5-di(trifluoromethyl)phenyl group increases binding affinity in NK1 agonists compared with the phenyl isosteres.[137]

- Fluorine substituents can exert substantial effects on molecular conformations. For example, methoxybenzenes without *ortho* substituents favor a planar conformation. However, spectroscopic studies and quantum-mechanical calculations indicate that trifluoromethylanisole favors an orthogonal conformation, which could be preferred in lead optimization.[137]

3.2. Fluorine Substitution in the Development of Pharmaceuticals

Fluorine substitution has often been used to extend the biological half-life of synthetic compounds and sometimes to eliminate the formation of toxic metabolites. Aryl methyl groups are also prone to phase I metabolism by CYP450 oxidases. The CF_3 group has often been employed in such cases to enhance metabolic stability. Decreasing the metabolic degradation rates and increasing the absorption properties of APIs enables the use of a lower medial dose to achieve successful treatments.

Rohypnol (flunitrazepam; Figure 32B), a fluorophenyl-substituted diazepine, provides an example of a very potent drug, similar in nature to valium (diazepam; Figure 32A) but considered to be about 10 times more potent as a tranquilizer. These drugs are depressants that act on the part of the brain associated with anxiety to reduce tension and induce sleep.

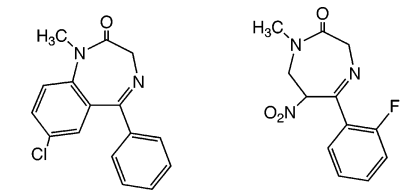


Figure 32. Fluoro-substituted flunitrazepam is a far more potent tranquilizer than non-fluorinated diazepam.

An aminopyrazinone acetamide (Scheme 1) developed by Merck to perform as a potent thrombin inhibitor (anticoagulant) did not have satisfactory persistence.[134] Three principal sites of metabolism were identified—oxidation at the benzylic position, oxidation of the methyl group attached to the pyrazinone ring, and conjugation of the aminipyridyl group. The introduction of a CF_2 group at the benzylic position and replacement of the aminopyridyl group by a fluoropyridyl moiety improved the metabolic stability. Further modification, directed toward the improvement of the aqueous stability at physiologic pH, led to formation of the N-oxide, all of which translated into sufficient performance enhancement that the molecule advanced to Phase I clinical trials (Scheme 1).[134] The introduction of the pyridine-*N*-oxide in place of pyrazinone overcame metabolic liability associated with the pyrazinone ring while retaining the activity.[138,139]

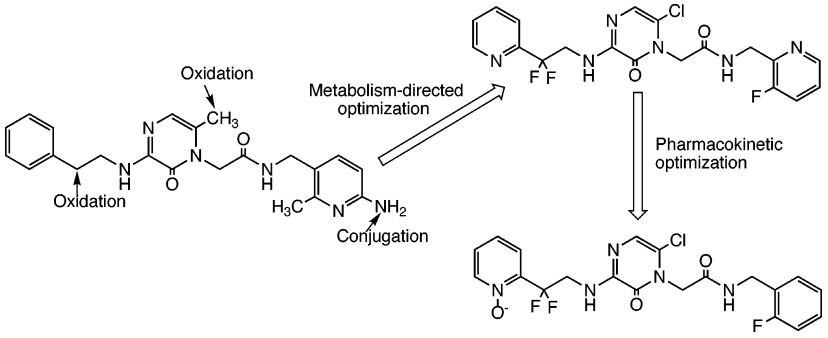
Schering-Plough's development of the oral cholesterol absorption inhibitor ezetimibe (Zetia) involved introduction of fluorine (SCH58235 from SCH48461) to block undesirable metabolic transformations. The three principal identified sites of metabolism were oxidation at the benzylic position, hydroxylation of the aromatic ring, and demethylation of methoxyphenyl ring. Metabolic activation by cytochrome P450 is generally blocked at carbon atoms bearing fluorine substituents. The lead compound had a 50-fold greater potency in vivo than its non-fluorinated analogue (Scheme 2).[136]

Sertraline (Zoloft; Figure 14C), a Pfizer SSRI antidepressant that does not contain a fluorine atom in its structure, has the shortest half-life (26 h) of the drugs in this class. It undergoes almost total metabolic conversion, principally through presystemic elimination, which is a performance that may be associated with the lack of fluorine substitution.[136] On the other hand, the presence of the trifluoromethyl group on the fluoxetine (Prozac; Figure 14A) molecule appears to contribute to its high selectivity and potency, possibly as a result of its electron-withdrawing effect and lipophilicity.[140] The *para* location of the trifluoromethyl group in fluoxetine is also critical for serotonin transporter potency[92] and probably assists in preventing phase I metabolism *para* to the ether linkage.[141]

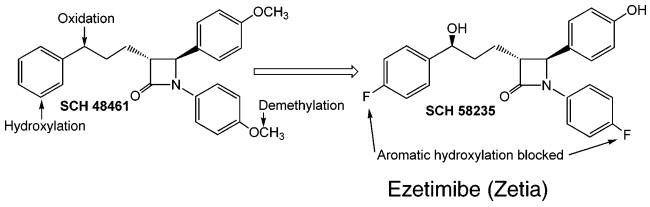
It has been proposed that the presence of the 5-fluoro group in the antibiotic ciprofloxacin is important for both cell penetration and gyrase affinity.[136] Similarly, the non-fluorinated version of AstraZeneca's anticancer drug, Iressa (gefitinib; Figure 22B), a tyrosine kinase inhibitor, has a half-life of just 1 h, but the addition of a fluorine atom to a phenyl ring allows the drug to persist at high levels in the bloodstream for 24 h.[142] Sometimes a fluorine substituent can make a molecule too stable and lead to problems for dosing schedules. For example, a fluorine-substituted version of Pfizer's anti-inflammatory drug Celebrex (celecoxib), a COX-2 inhibitor, was found to be stable for days in rat studies. When the fluorine was replaced with a methyl group, the drug's half-life was reduced to about 4 h in rats, which was subsequently found to be 11 h in humans.[137,142]

HDL-cholesterol levels have a strong genetic component and are inversely correlated with the risk of coronary heart disease. Approximately half of the patients with coronary heart disease have low levels of HDL cholesterol. It has been

Scheme 1. Fluorine Substitution Improves the Metabolic Stability of a Potent Thrombin Inhibitor Developed by Merck[134]



Scheme 2. Fluorine Substitution Blocked Undesirable Metabolic Transformations in the Cholesterol Absorption Inhibitor Ezetimibe (Zetia) Developed by Schering-Plough[136]



shown that there is a reduced incidence of coronary events in association with an increase in plasma HDL levels in patients treated with fibrate drugs, indicating a potential for HDL-raising therapies to reduce the risk of cardiovascular disease.

Vytorin (2004), a combination drug by Merck combining its statin drug simvastatin (Zocor; Figure 8C) with Schering-Plough's cholesterol absorption inhibitor drug ezetimibe (Zetia; Scheme 2) is the first product to treat the two sources of cholesterol by inhibiting the production of cholesterol in the liver and blocking the absorption of cholesterol in the intestine, including cholesterol from food. Ezetimibe has been shown to achieve higher efficacy at lower dosages.[143]

4. Ecotoxicity of Human Pharmaceuticals

It will be our purpose in this section to highlight that pharmaceuticals in the environment are impairing aquatic lifeforms, sometimes profoundly, and are producing changes that threaten the sustainability of the ecosphere on which our chemocentric civilization depends. Pharmaceutical compounds are designed either to be highly active and interact with receptors in humans and animals or to be toxic for many infectious organisms, including bacteria, fungi, and parasites. However, many lower animals have receptor systems similar to humans and farm animals. Furthermore, many groups of organisms that negatively affect human and animal health, and are therefore targeted by pharmaceuticals, play crucial roles in the functioning of ecosystems. Thus, pharmaceuticals are often found to cause adverse ecological effects on aquatic and terrestrial organisms.[144] These effects are often not highlighted by the standard studies aimed at securing human safety. As human medicines are almost continuously released to the environment, aquatic organisms in particular, and also wildlife, are becoming exposed over their entire lifecycle. This is usually much longer than the durations of exposures employed in standard safety testing.[145] The ecotoxicities of several human pharmaceuticals have been reviewed recently.[146,147] A review has also appeared on chronic aquatic environmental risks from exposure to human pharmaceuticals.[148]

Increasingly, we are finding that APIs persist in the environment with a wide range of pharmaceuticals being found in fresh and marine waters in low concentrations. It has been shown recently that even in these infinitesimal concentrations, some pharmaceuticals have the potential to interfere detrimentally with the normal development of aquatic life. The low concentrations of pharmaceuticals are unlikely to elicit acute health toxic effects. What is not well enough known is whether more subtle effects such as growth, fertility, sex ratios of higher organisms,[149] or reproductive behavior are potentially being impacted in aquatic life by the long-term, low-level exposure to pharmaceutically active compounds.[145,147,150,151] Localized changes in biogeochemical cycles[152] are causing subtle modifications in plant growth,[153] failure of larvae to molt or hatch,[154] and anatomical deformities in a wide range of organisms.[155] It has been reported that in many rivers in Switzerland, a 50% decline in fish catch has been observed over the last 15 years, and there is some evidence that organic micropollutants could be contributing to this effect.[156]

In ecotoxicology, research is usually focused upon harmful effects at the levels of the population, community, and entire ecosystem, which differs from the focus on health risks to individuals in human testing.[157] Effects upon individuals in the former studies are only important insofar as they can be related to consequent effects at the levels of populations and above. Moreover, in ecotoxicity testing, a few surrogates are used to represent the diversity of wild species, while several different species, for example, rat, mouse, rabbit, and guinea pig, are being used as surrogates for human risk assessment.[157] This raises considerable uncertainities when extrapolating data obtained in the laboratory on a few surrogates to species exposed in the field, which are often not closely related to them and may differ considerably in their sensitivity to the chemical being tested. Not surprisingly, large safety margins are often invoked when carrying out environmental risk assessment using such data.[157]

4.1. Environmental Risk Assessment

In making an environmental risk assessment (ERA), a risk quotient (RQ) is usually calculated by comparing estimated environmental exposure with estimated environmental toxicity, the latter being obtained from ecotoxicity tests.[158] One way of doing this is to divide the predicted environmental concentration (PEC) by the predicted no-effect concentration (PNEC). When the concentration of a compound in a particular environmental compartment is unknown, it may be predicted from a combination of estimates of the amount of consumption or sales, expected route of entry into the environment, and physicochemical properties.[159] A PNEC is obtained by dividing the lowest no observed effect concentration (NOEC) for the most sensitive species by an appropriate safety factor. The resulting PEC/PNEC ratio has been widely used in ERA, which is dependent on the amount used, the environmental fate, and the ecotoxicity of the specific compound. Because there are large uncertainties in such calculations, it is usual to incorporate large safety factors, sometimes as much as 1000, when calculating the ratio. The PNEC is divided by the safety factor, which is typically 10, 100, or 1000. If after compensation, the PEC value falls well below the PNEC value, the risk is taken to be low. On the other hand, if the value equals or exceeds unity, the risk is deemed significant. It is not surprising that given the uncertainties involved, a cautious approach has been adopted, which may greatly overestimate the risk that a chemical presents. This also makes it unlikely that wild species will experience those toxic effects (e.g., lethality) that have been employed as endpoints in the ecotoxicity testing.[157]

Since 1980, the US Food and Drug Administration (FDA) has required environmental risk assessments be performed for human and veterinary medicines on the effects on aquatic and terrestrial organisms before a product can be marketed.[160] The EU introduced similar requirements in 1997. These environmental impact studies investigate potential negative effects on fish, daphnids, algae, bacteria, earthworms, plants, and dung invertebrates.[161] Risk assessments usually use standard ecotoxicity tests, which often employ short time

scales and focus predominantly on mortality as the endpoint. Moreover, aquatic tests tend to focus on the water compartment and do not take into account pharmaceuticals residing in sediments. In general, the effects observed in these studies occur at much higher concentrations than those that are measured in the environment. Currently, no single assessment factor appears to apply to all aquatic species across a wide diversity of pharmaceuticals.[162]

Of course, there are many benefits to the current risk assessment approaches. For example, the use of a single toxicity test or a common battery of tests can produce reliable indices of the toxic impact of effluents in the aquatic environment for the endpoint employed. Acute lethality tests also allow for the building of comparative databases in which species can be compared in terms of their sensitivity to the same chemical or by which chemicals can be compared with one another using the same species. Invertebrate tests have become increasingly important to detect contaminants and their effects on aquatic biota. Such effects include disruptions to food-chain dynamics at lower trophic levels, such as unicellular algae, aquatic invertebrates (mostly daphnids), and fish, which represent key elements of aquatic food chains.[163] The effects can translate into severe ecological consequences for top predators and species of economic as well as ecological concern.[164] Because of their widespread distribution, short life cycle, and sensitivity, daphnids are commonly used for assessing the toxicity of contaminants in freshwaters.[165] Acute tests include determining the survival of water fleas after a 48-h exposure to contaminants compared with controls. Freshwater shrimps are also used for conducting 96-h acute tests. A toxicity ranking is assigned to water samples, as harmful (EC_{50} 10−100 mg/L), toxic (EC_{50} 1−10 mg/L), and very toxic (EC_{50}, <1 mg/L) to aquatic organisms. An EC_{50} value >100 mg/L is assigned as not harmful to aquatic organisms.[166]

4.2. Human Health Risk Assessment of Pharmaceuticals

Pharmaceuticals are designed to stimulate or inhibit physiological responses in humans, animals, and plants, the only exceptions being diagnostic and cosmetic drugs. However, they can have adverse effects on nontarget ecological species when released to the environment. Continuous releases and chronic exposure can result in subtle effects on aquatic species and could pose a risk to human health associated with consuming contaminated drinking water over a lifetime.[167] Currently there is no specific regulatory guidance as to how the significance of the presence of pharmaceuticals at trace concentrations in drinking water supply may be assessed. Also, there is scant information in the literature addressing the potential effects to human health from the presence of trace levels of pharmaceuticals found in surface and drinking water.[162,168−171] Some of the existing studies claim that the concentrations of many drugs and their metabolites in surface waters compared with their acceptable drinking water intake are so low that they do not pose appreciable risk to human health.[170,171] However, it was acknowledged that there are exceptions, such as antibiotics that have non-human target effects, estrogens that were developed for just one gender, genotoxic antineoplastics that have high potential for allergic responses, or compounds that have very high bioaccumulation potential, such as the synthetic estrogen 17α-ethinylestradiol[172] and the NSAID diclofenac,[173] which may need to be individually evaluated.[171] Another study concluded that while risk for acute toxic effects in the environment with the current use of pharmaceuticals is unlikely, chronic environmental toxic effects cannot be excluded due to lack of chronic ecotoxicity data.[174]

The widespread and unrestricted use of prophylactic antibiotics to forestall bacterial infections in fish rearing[175] and as an additive in animal feeds[176] has resulted in the emergence of antibiotic-resistant bacteria. A high proportion (as much as 90%) of the antibiotics added to animal feed is excreted unchanged in urine or manure and enters surface or groundwater through nonpoint source pollution from manure-applied lands.[176] The transfer of these resistance determinants is known to occur to the bacteria of land animals and to human pathogens and to cause alterations of the bacterial flora both in sediments and in the water column.[175]

Similarly, widespread use of the antiviral oseltamivir phosphate (Tamiflu; Figure 31B) to fight avian flu in birds as a means of avoiding a pandemic in humans has been predicted to lead to drug-resistant strains of the virus in wild birds.[132] It has been surmised that in the event of a pandemic, hundreds of millions of courses of Tamiflu will be rapidly deployed resulting in excretion of oseltamivir carboxylate (OC), the active antiviral metabolite of Tamiflu, into surface waters from sewage treatment plants for several weeks.[132] OC is expected to withstand biodegradation. It is believed that when birds drink OC-laced water from catchments receiving treated wastewater, they could produce Tamiflu-resistant strains and pass them on to other birds.[132]

4.3. Aquatic Ecotoxicity from Chronic Exposure

It is widely recognized that chronic effects arising from long-term exposure to pharmaceuticals at much lower concentrations may follow different toxicodynamic mechanisms than extrapolated from short-term acute studies.[177] Most human pharmaceuticals have low acute aquatic toxicity[178] by design, but to be truly effective medicines, they have targeted, chronic pharmacological activity. Therefore, it has been suggested that in contrast to mortality as the only measured effect in acute toxicity tests, ecologically relevant endpoints such as impairment of growth, development, and reproduction should be used to assess ecotoxicologic effects.[148] Most chronic aquatic toxicity data for human pharmaceuticals are available for algae, which have been found to be sensitive to several different therapeutic classes, including fluoroquinone and sulfonamide antibiotics, antidepressants, beta blockers, and estrogens.[178] Cyanobacteria such as *Microcystis aeruginosa* are considerably more sensitive to antibiotics than standard algal toxicity test species such as a single-celled, freshwater green alga, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*), and can function as sensitive surrogates for algae and other unicellular microorganisms for chronic toxicity tests.[148] Green algae and higher aquatic plants such as duckweeds have somewhat similar sensitivity.

Aquatic vertebrates, such as fish and amphibians, are highly sensitive to endocrine modulation, particularly through exposure to both natural and synthetic steroid estrogens. Fish are also sensitive to beta blockers,[179] but there is little evidence for adverse effects of antibiotics at environmentally realistic concentrations.[167,180] The need for chronic, sublethal tests for determining approximate effects that are more likely to occur in nature have led to the development of full life cycle (from birth until the organism reproduces) fish tests. While these are time-consuming and expensive, they provide

more realistic models for assessing risks associated with endocrine disruption. As an example of the tests, fathead minnows are freshwater fish that are adaptable to laboratory conditions for full life cycle testing.[164] Their complete life cycle is typically 3−6 months depending on broodstock and test conditions. Consequently, partial life cycle testing focused upon sensitive life stages surrounding growth and reproduction can be carried out with comparative ease, increasing the potential for obtaining data that is more relevant to endocrine disrupting compound-related risks.

In addition to ecotoxicity testing in the laboratory for the purpose of environmental risk assessment, ecological effects that pharmaceuticals may have when released into ecosystems are assessed by monitoring of surface waters, such as rivers where there is discharge from sewage outfalls or runoff from agricultural land. For example, male fish may be studied downstream from sewage outlets to detect the presence of estrogenic modulators. This biomarker assay relies on the fact that exposure to low levels of environmental estrogens can elevate vitellogenin concentrations in the blood of the male fish; vitellogenin is a variety of primary lipoprotein produced by egg-laying vertebrates, including fish.

An increasingly popular test method is quantitative structure−activity relationships (QSARs), which attempt to correlate structure with activity using statistical approaches. Using an EPIWIN program, an estimation software for prediction of physical properties and environmental fate, based on (Q)SAR models, relative hazards toward algae, daphnids, and fish have been ranked for a large number of pharmaceuticals. Cardiovascular, gastrointestinal, antiviral, anxiolytic sedatives, hypnotics, antipsychotics, corticosteroid, and thyroid pharmaceuticals have been predicted to be the most hazardous therapeutic classes.[129]

4.4. Aquatic Ecotoxicity of Pharmaceuticals

Our current understanding of the ecotoxicology of pharmaceutical compounds is poor and limited to only a few substances in a limited number of species. A summary of the reports on ecotoxicological studies of diverse pharmaceutical classes of environmental concern is provided in the following paragraphs.

4.4.1. Steroid Hormones

A study of 139 streams in the United States found that 5.7% had concentrations >5 ng/L (ppt) of estrogenic 17α-ethinylestradiol (EE_2; Figure 26D), a component of the contraceptive pill.[21] In the yeast estrogen screen (YES) test, the estrogenic activity of EE_2 and the reference compound 17β-estradiol (E_2; Figure 26A; log(1/EC_{50} (M))) was found to be 9.6 and 9.54 ± 0.04, respectively.[181] The lowest reported concentrations of estrogens able to induce intersex changes are 10 ng/L for E_2 or estrone (E_1; Figure 26C) and 0.1 ng/L for EE_2.[115] Surface water concentrations of these estrogens is often near or equal to the lowest concentrations that were found to induce reproductive disturbances in male fish in controlled laboratory studies.

A partial life-cycle exposure of juvenile zebrafish (*Danio rerio*) to EE_2 concentrations ranging from 1 to 25 ng/L showed a dose-dependent increase in vitellogenin concentrations. At exposure levels of 1 ng/L, significant changes in sex ratios in favor of females were found, and at 2 ng/L EE_2, complete sex reversal and no intersex fish were seen. On the other hand, an exposure to 17α-methyltestosterone (MT) ranging from 26 to 1000 ng/L decreased vitellogenin concentrations and induced complete sex reversal at all the concentrations of MT tested. On exposure to 1000 ng/L MT, a large proportion of intersex fish was also observed.[182]

Life-long exposure of breeding populations of zebrafish and other species to EE_2 at very low environmentally relevant concentrations has been shown to exert severe deleterious effects on their reproductive success.[116,183−186] Zebrafish mature adults (F_0 generation) on long-term exposure (up to 40 days) at 5 ng/L showed no impact on reproductive success.[183] But in contrast, the F_1 generation on life-long exposure at this level exhibited a 56% reduction in fecundity (the state of being capable of producing offspring), with some males showing complete reproductive failure associated with disrupted sexual differentiation leading to either the absence of functional testes or undifferentiated or intersex gonads. A partial recovery in reproductive capacity was reported on depuration (removal of xenobiotics from the body) after 5 months. Even though F_1 males lacked functional testes, they showed male-pattern reproductive behavior, inducing the spawning act and competing with healthy males to disrupt fertilization.[183]

A full life-long exposure of fathead minnows (*Pimephales promelas*) at lower doses of 0.2 and 1 ng/L EE_2 caused reductions in the offspring's hatching success of 20% and 35%, respectively, but had no impact on fecundity.[186,187] It appeared that very low concentrations of EE_2 could decrease sperm production and viability. Fish exposed to EE_2 concentrations >3.5 ng/L laid no eggs and were 100% female fish. At lower concentrations, male fish were present, but the sex ratios skewed toward females, in significant contrast with the controls.[116] Male fathead minnows were demasculinized by lifetime exposure to ∼1 ng/L EE_2 mainly by decreases in the number of their tubercles.[116,187] The findings indicate that low EE_2 concentration exposures mainly affect male fish, whereas higher concentrations of EE_2 exposure also affect female fish. Similarly, exposure of sheephead minnow (*Cyprinodon variegates*) to 200 ng/L EE_2 for 59 days caused reduced hatching success in the progeny of the exposed fish. Histological examination (examination of the microscopic structures of tissues) revealed generalized edema, damage to gill epithelia, hepatic toxicity, fibrosis of the testis, and evidence of sex reversal, including testis−ova and spermatagonia-like cells in ovaries.[188]

Life-long exposure of Japanese medaka (*Oryzias latipes*) showed no significant effects at lower doses of EE_2 (0.2 and 1 ng/L), but complete reproductive failure was found for 10 ng/L exposure.[185,189] A significant increase in vitellogenin concentrations has been found in male medaka exposed to EE_2 at different concentrations for 21 d under flow-through conditions. It was found that the physiological and histological measurements were approximately 8-fold more responsive to the EE_2 exposure than were overt reproductive effects.[190] Similarly, male rainbow trout (*Oncorhynchus mykiss*) exposed to 2 ng/L EE_2 exhibit induced vitellogenin and inhibited testis growth.[191]

The effects of exposure for 21 d under flow-through conditions of the estrogen 17β-estradiol (E_2; Figure 26A) and an androgen 17β-trenbolone (TB; Figure 27) on three small fish species, medaka (*O. latipes*), fathead minnow (*P. promelas*), and zebrafish (*D. rerio*), have been investigated. Significant increases in estrogenic activity were observed in both sexes of all three fish species for E_2 exposures. The lowest-observed-effect concentrations (LOECs) of E_2 for vitellogenin (VTG) induction in males of medaka, fathead

minnow, and zebrafish were 8.94, 28.6, and 85.9 ng/L, respectively. For TB exposures, VTG reduction in females of all three fish species was observed along with masculinization of secondary sex characteristics in medeka with a LOEC of 365 ng/L and in fathead minnow with a LOEC of 401 ng/L. The results suggested a higher susceptibility of medaka to estrogenic chemicals than fathead minnow and zebrafish.[192] 17α-Trenbolone, like 17β-trenbolone has also been found to be a potent reproductive endocrine toxicant in the fathead minnow (*P. promelas*).[193]

In another study, Japanese medaka (*O. latipes*) exposed to E_2 during early life stages at concentrations of 33.5 and 140.6 ng/L were found to exhibit histological changes in the gonad. In control fish, differentiation of the ovary and testis was apparent 12 days posthatch (dph). Normal testes were observed at 12 dph in male fish exposed to 33.5 ng/L E_2, but at 14 and 20 dph, testis−ova was recognized. Male fish exposed to 140.6 ng/L E_2 had testis−ova at 12 dph, and the gradual transformation of the testis to an ovary was observed until 20 dph. 17β-Estradiol induced testis−ova in male medaka during the larval period, and sex transformation was seen more frequently at higher concentration (140.6 ng/L).[194]

4.4.2. Antibiotics

An often-cited concern regarding PIE is the spread of antibiotic-resistant bacterial strains. The genetic selection of resistant bacteria is a potentially irreversible effect that is postulated to be induced by low concentrations of antibiotics, such as are being found in the environmental waters.[19,195] Sulfonamide- and trimethoprim-resistant bacteria have been found in U.S. rivers, and this resistance is likely to develop further because low river concentrations can be anticipated for the foreseeable future.[196] Antibiotic residues from human waste and from animal husbandry can be expected to apply selective pressures on environmental bacteria, increasing the prevalence of genes conferring antimicrobial resistance. The use of antibiotics as a growth promoter for livestock is of particular concern, especially in cases where the same or similar drugs are used for human therapies. Antibiotics in sewage effluent, most likely a result of therapeutic use, have the potential to negatively impact organic matter degradation. Most studies have shown that bacteria in raw sewage are significantly more resistant to antibiotics than bacteria elsewhere.[150]

One mechanism of cell defense is to actively pump or efflux toxic substances out of the cell. Efflux pump-inhibiting drugs can threaten aquatic organisms by promoting the accumulation of xenobiotics in their cells.[16,197] It has been speculated that broad-spectrum antiseptics such as triclosan may increase antimicrobial resistance in bacteria by applying population pressure to produce more efflux pumps.[150] In one study, three strains of *Escherichia coli* and an unidentifiable bacterium were isolated from a sewage treatment plant and assessed for resistance to antibiotics: erythromycin, ampicillin, tetramycine, trimethoprim, ciprofloxacin, and sulfamethoxazole. The predominant resistance was observed to erythromycin in which all the bacteria displayed resistance. This was followed by ampicillin (resistance in three bacteria), tetracycline (resistance in two bacteria), and ciprofloxacin, trimethoprim, and sulfamethoxazole (resistance in one bacterial strain for each).[198] A similar observation was reported on *E. coli* isolates from a sewage treatment plant, which were found to be resistant to several antibiotics with the highest resistance being for tetracyclines.[199] Numerous pseudomonad isolates from nonpolluted groundwater wells in the vicinity of leaking sewers have been reported to contain multiple antibiotic resistances.[200]

Several commonly used antibiotics, including erythromycin, sulfamethoxazole, and sulfamethazine, can persist in the environment for more than a year. The reported data on the occurrence of antibiotics in the aquatic environment confirm the persistence of sulfonamides and fluoroquinolones followed by macrolides (Figure 28); the studies derive from focuses on both municipal wastewater and agricultural runoff.[122] The hydrolysis of macrolides and sulfonamides in the neutral pH range is known to be very slow. On the other hand, β-lactam antibiotics are readily hydrolyzed under mild acidic or basic conditions and have not been detected in the environment despite the fact that they are used in the greatest amounts.

Many antibiotics are harmful to aquatic organisms such as algae and crustaceans in freshwater and marine environments. Amoxicillin, a broad spectrum aminopenicillin antibiotic widely used in human and veterinary medicine, was tested for toxicity on microalgal species and found to be nontoxic toward the green alga, *Ps. subcapitata*, and the phytoplankton, *Cyclotella meneghiniana*, but showed marked toxicity toward the cyanobacterium, *Synechococcus leopoliensis* (EC_{50} 2.22 μg/L).[201] Pomati reported that the macrolide erythromycin induced growth inhibition in the cynobacterium *Synechocystis* sp. at concentrations of 10 μg/L and higher.[202] Ciprofloxacin was reported to potentially influence both the structure and the function of suspended and attached natural freshwater algal communities.[203]

Different classes of antibiotics were assessed for phytotoxicity to the aquatic higher plant *Lemna gibba* (7 d static-renewal test). Fluoroquinolone, sulfonamide, and tetracycline antibiotics displayed significant phytotoxicity below 1000 μg/L (1 ppm).[204] Levofloxacin (LVFX) and clarithromycin (CAM) on microtox and *Daphnia* immobilization testing did not display any acute toxicity. An algal growth inhibition test revealed that both LVFX and CAM have high toxicity to the microalga, CAM being about 100-fold more phytotoxic than LVFX from a comparison of EC_{50} values.[205]

Tetracyclines are used for both human and veterinary applications and are the biggest volume therapeutic class of antibiotics in the United States, accounting for approximately 29% of the total antibiotic usage. Chlortetracycline, oxytetracycline, and tetracycline were detected in U.S. surface waters at maximum concentrations of 0.69, 0.34, and 0.11 μg/L, respectively, while doxycycline was not detected.[21] Toxicity studies of tetracyclines on the freshwater cyanobacteria *Microcystis aeruginosa* and the green alga *Selenastrum capricornutum* indicated differential toxicity (Table 2).[206,207] The higher sensitivity of the cyanobacteria was explained by their closer relationship with bacteria that the antibiotics were originally intended to kill, but having not been routinely exposed to these compounds in the past they still have much lower resistance.[206]

The effects of tetracycline exposure on the growth of cynobacterium, *Synechocystis* sp., and the duckweed *Lemna minor* at doses of 1, 10, 100, and 1000 μg/L have been reported. At the dose levels of 10 and 100 μg/L, growth inhibition in *Synechocystis* was observed with reduced cell densities of 20% and 22%, respectively. A tetracycline dose of 1000 μg/L actually promoted growth by 9% compared with the controls.[202] In contrast, the effect of tetracycline on the growth of *Lemna* was markedly stimulatory at 1−10 μg/L

Table 2. Differential Toxicities of Tetracycline Antibiotics in Phytoplankton[206,207]

		Antibiotics EC_{50}, 7 d growth (μM)		
organism	species	tetracycline	chlorotetracycline	oxytetracycline
Cyanophyta (freshwater cynaobacteria)	*M. aeruginosa*	0.20	0.10	0.45
Chlorophyta (green alga)	*Se. capricornutum*	4.95	6.46	9.77

Table 3. Ecotoxicity of Fluoroquinolone Antibiotics, Ciprofloxacin, Levofloxacin, Ofloxacin, Lomefloxacin, Flumequin, and Enrofloxacin[216]

organism	species	test	EC_{50}
cyanobacterium (blue-green algae)	*M. aeruginosa*	5 d growth, reproduction	7.9−1960 μg/L (med. 49 μg/L)
green alga	*Ps. subcapitata*	3 d growth, reproduction	1.100−22700 μg/L (med. 7400 μg/L)
duck weed	*L. minor*	7 d reproduction	53−203 μg/L[a] (med. 106 μg/L)
crustacean	*Da. magna*	48 h survival	10 mg/L
fathead minnow larvae	*P. promelas*	7 d early life stage survival, growth	10 mg/L[b]

[a] Flumequin was least toxic at EC_{50} of 2470 μg/L. [b] Clinafloxacin was found most toxic to *Ps. subcapitata* and caused some mobility impairment to *Da. magna*. It was also found to have deleterious effects on *P. promelas*, producing nearly 100% mortality at 10 mg/L.

resulting in 18−26% increase compared with untreated plants. However, at a higher tetracycline dose of 1000 μg/L, the *Lemna* culture was strongly inhibited (−43%).[202]

The acute toxicity toward the freshwater crustacean *Daphnia magna* was determined for oxytetracycline (48 h, $EC_{50} \approx 1000$ mg/L) and tetracycline (NOEC 340 mg/L). The acute toxicity to the aquatic vascular plant *Lemna minor* (7 d, growth inhibition, EC_{50} 4.92 mg/L) was also reported for oxytetracycline.[208] In a microcosm study, oxytetracycline exposure for two aquatic macrophytes, *Egeria densa* and *Ceratophyllum demersum*, over a 6-week period at 250 μg/L led to a significant decline in growth.[209] The chronic toxicity (EC_{50} values) in the *Da. magna* reproduction tests was 44.8 mg/L for tetracycline and 46.2 mg/L for oxytetracycline.[210,211] Oxytetracycline chronic toxicity (EC_{50}) in the growth inhibition of the green alga *Ps. subcapitata*, population growth inhibition of the rotifer *Brachionus calyciflorus*, and reproduction inhibition of microcrustacean *Ceriodaphnia dubia* has been reported to be 0.17, 1.87, and 0.18 mg/L, respectively.[212] The toxicity of a mixture of tetracyclines consisting of oxytetracycline, chlortetracycline, tetracycline, and doxycycline observed for *Myriophyllum sibiricum* and *Lemna gibba* exhibited a significant concentration-dependent reduction in the resulting dry mass. The microcosm study suggested that the ecological risks of these compounds in aquatic environments are likely to be low.[213,214]

In addition to abiotic reactions, biological organisms may also degrade pharmaceutical substances in treatment systems, water bodies, and soils. Generally, these processes reduce the potency of medicines; however, some breakdown products of tetracyclines have shown similar toxicity to their parent compounds toward environmentally relevant bacteria.[215]

Fluoroquinolone antibiotics, including two of the most widely used human-use antibiotics in the U.S., ciprofloxacin and levofloxacin, have been detected in various stream and river waters in low microgram per liter (ppb) to nanogram per liter (ppt) ranges. Because of their wider use and resistance to biodegradation, these are of environmental concern. Fluoroquinolones inhibit key bacterial enzymes involved in unwinding the DNA helix for replication and transcription. The toxicity of several fluoroquinolone antibiotics including ciprofloxacin, lemofloxacin, ofloxacin, levofloxacin, and an experimental drug with wider microbial activity called clinafloxacin, as well as two veterinary drugs, enrofloxacin and flumequine, have been evaluated on five aquatic organisms (Table 3). The study found only levofloxacin at 1 μg/L exceeding the benchmark value of hazard quotient of 0.1 for *M. aeruginosa*.[216] Ofloxacin exhibited significant inhibition of a mixed culture of nitrite-oxidizing bacteria isolated from activated sludge.[217]

A structure−phototoxicity relationship has been reported for eight fluoroquinolone antibiotics using female albino Balb/c mice, determined by administering a fixed dose of 100 mg/kg to mice intravenously, followed by UV-A irradiation for 4 h (21.6 J/cm^2).[218] The increase in predose (0 h) auricular thickness was compared at 96 h postdose, revealing that a fluorine substituent at the C-8 position of the quinolone ring (fleroxacin, lomefloxacin, or sparfloxacin) introduced moderate to sever phototoxicity, where drugs with a hydrogen substituent in this position (ciprofloxacin or norfloxacin) had comparatively mild impacts on the lesion.[218]

Sulfonamide antibiotics rank among the top 200 prescribed human drugs in the U.S., and a large quantities are used in animal husbandry. These are excreted as the unaltered parent compounds or as acetylated metabolites, which can be reactivated by bacterial cleavage of the acetyl moiety. As noted above, sulfonamides are not readily biodegraded, persist in soils, and are among the most frequently detected pharmaceuticals in U.S. streams. The ecotoxicity of these sulfa drugs was evaluated using microalgae. They were found to have some growth inhibitory activity on the green algae *Selenastrum capricornutum* and *Chlorella vulgaris* with the EC_{50}'s of sulfamethoxazole, sulfadiazine, and sulfadimethoxine being 1.5, 2.2, and 2.3 mg/L, respectively.[219] The following sulfamethoxazole EC_{50} values were reported: Microtox-30 min, >84 mg/L; *Ps. subcapita*-96 h growth, 146 μg/L; blue-green alga *Synechococcus leopolensis*-96 h growth, 26.8 μg/L; *Da. magna*-48 h, >100 mg/L; and *Ce. dubia*-48 h, >100 mg/L.[220] Sulfamethoxazole was found to significantly inhibit the performance of a mixed culture of nitrite-oxidizing bacteria isolated from activated sludge.[217]

The sulfonamide antibiotic sulfochlorpyridazine was reported to be toxic to *Lemna minor* (7 d, growth inhibition, EC_{50} 2.33 mg/L), but it exhibited no toxicity to green alga *Chlorella vulgaris* at the highest tested concentration of 2000 mg/L.[208]

Sulfonamides probably engage in covalent cross-coupling to soil organic matter as their residues cannot be extracted from soils. In the presence of surrogate humic acid constituents, phenoloxidases and manganese dioxide mediate cross-coupling of sulfonamides, thereby diminishing the mobility, bioavailability, and biological activity.[221]

The macrolide antibiotic erythromycin, which blocks bacterial protein synthesis as its mode of action, inhibits the growth of cynobacterium, *Synechocystis* sp., and the duckweed *Lemna minor* at doses of 1, 100, and 1000 μg/L, but at 10 μg/L it stimulated cynobacterial growth. It has been suggested that the antibiotic can be degraded by the cynobacteria and duckweed into metabolites that are no longer toxic and may even be growth stimulating.[202] Chronic toxicity data of erythromycin shows that it affects algae, rotifers, and crustaceans at EC_{50}'s in the range of 10−100 μg/L and that long-term negative effects cannot be excluded at environmental concentrations.[212]

Isidori and colleagues (2005) have estimated the risk for six antibiotics and found an acceptable risk (PEC/PNEC < 1) for oxytetracyclin, sulfamethoxazole, and ofloxacin. However, the three macrolides, erythromycin, lincomycin, and clarithromycin, were found to have PEC/PNEC ratios of 1, 3.6, and 10, respectively, indicating that this class of antibiotics should be considered harmful to the aquatic environment.[212]

4.4.3. Neuroactive Compounds: Antidepressants

The SSRI drugs in waste flowing from sewage-treatment plants end up in fish, reflecting their bioaccumulation properties. Fluoxetine (Figure 14A) and sertraline (Figure 14C) and their metabolites, namely, norfluoxetine and desmethylsertraline, have been detected at levels greater than 0.1 ng/g in every tissue tested from the fish residing in a municipal effluent dominated stream.[222] The mean EC_{50} values reported for three SSRIs, fluoxetine, fluvoxamine, and citalopram, on *Vibrio fischeri* luminescence were 724, 1368, and 1371 μg/L, respectively.[223] Fluoxetine has antimicrobial properties and potentially exerts its toxicity by inhibiting cellular efflux pumps.[224] The aquatic toxicity of fluoxetine toward various organisms is given in Table 4. The hazard quotient (HQ) derived from the ratio of PEC/PNEC has been calculated to be $\ll 1$.[225]

Table 4. Aquatic Toxicity of Fluoxetine

organism	species	test	LC_{50}/EC_{50}	ref
green alga	*Ps. subcapitata*	120 h, growth	24 μg/L[a]	366
crustacean	*Ce. dubia*	48 h, survival	234 μg/L[b]	366
crustacean	*Da. magna*	48 h, survival	820 μg/L[b]	366
fathead minnow	*P. promelas*	48 h, survival	705 μg/L[b]	366
midge larvae	*Ch. tentans*	48 h, survival	15 mg/L[b]	366
rainbow trout	*On. mykiss*	48 h, survival	2 mg/L[b]	370

[a] EC_{50}. [b] LC_{50}.

Serotonin is said to be the most potent and ubiquitous neuromodulator in vertebrates. It is synthesized in cells lining the gut, in neurons of the hypothalamus that regulate pituitary activity, and in the brainstem. Many of these neurons release serotonin into the synaptic cleft where it acts as a neurotransmitter.[222] Because of the diversity and critical nature of the functions regulated by serotonin, environmental SSRIs could alter the appetite, the immune system, and reproduction, as well as other behavioral functions.[226] Serotonin acts directly on the immune system by modulating cellular function and indirectly through actions on the central nervous system. Serotonin is also an important neuromodulator of sexual function in vertebrates and invertebrates.[189]

Long-term exposure to minute quantities of common antidepressants that can pass through sewage treatment systems into rivers and streams has been found to delay development in fish and delay metamorphosis in frogs. Low concentrations of fluoxetine, particularly, slowed male sexual development by 2−4 weeks in *Gambusia affinis* (Western mosquitofish; 7 d, LC50 614 ppb), which is often used to study toxicity on aquatic organisms.[93] A chronic exposure of *Daphnia* (30 d, 36 μg/L) to fluoxetine significantly increases fecundity, and a mixture of fluoxetine (36 μg/L) and clofibric acid (100 μg/L) causes significant mortality; the same fluoxetine concentration mixed with 10 μg/L clofibric acid resulted in significant deformities, including malformed carapaces and appendages.[227] Thus, where risk quotients on individual drugs may lead to acceptable hazard quotient determinations, it can be a very different story when the risk associated with mixtures of drugs are considered as here with fluoxetine and clofibric acid.

Exposure of the coral reef fish bluehead wrasse (*Thalassoma bifasciatum*) to fluoxetine at 6 μg/(g·d) for 2 weeks or a single, acute treatment of 10 μg/g via ip injection decreased the male territorial aggression behavior in response to an introduced intruder, both in laboratory and in field studies.[228] Exposure of Japanese medaka (*Oryzias latipes*) to fluoxetine at aqueous nominal concentrations of 0, 0.1, 0.5, 1, and 5 μg/L for 4 weeks led to a low incidence of developmental abnormalities in offspring for all fluoxetine treatments. Circulating plasma estradiol levels in females were significantly increased by 0.1 and 0.5 μg/L treatments.[189] Exposure of the crustacean *Gammarus pulex* to concentrations of fluoxetine at 10−100 ng/L produced a significant decrease in activity, whereas at higher concentrations (1 μg/L to 1 mg/L), it was found to be similar to the control.[229] These findings and many other like them in this review, where lower doses exhibit more deleterious effects than higher doses, partly belies a fundamental maxim of toxicology that "the dose makes the poison". This statement is an adaptation of one by Paracelsus (1493−1541) that "all things are poison and nothing is without poison, only the dose permits something not to be poisonous."

A study of acute and chronic toxicity of five SSRIs, namely, fluoxetine, fluvoxamine, paroxetine, citalopram, and sertraline, evaluated on the daphnid *Ce. dubia* found that they can impact both survival and reproduction. Only sertraline was found to be more toxic than fluoxetine in the study.[230]

Paroxetine has been assessed for environmental risks.[231] The main paroxetine metabolite (PM), formed by oxidation at the methylenedioxyphenyl carbon atom, was measured to have a Microtox EC_{50} value of 33.0 mg/L, while the *Da. magna* EC_{50} value was 35.0 mg/L. Microtox studies of the

Table 5. Acute Toxicity of NSAIDS Evaluated with Crustaceans and Algal Tests

organism/ species test	crustacean *Da. magna* mortality 48 h	green alga *De. subspicatus* growth inhibition 3 d	crustacean *Ce. dubia* growth inhibition 48 h	crustacean *Th. platyrus* mortality	duck weed *L. minor* growth inhibition 7 d
measure	EC_{50} mg/L	EC_{50} mg/L	EC_{50} mg/L	LC_{50} mg/L	EC_{50} mg/L
diclofenac	68.0[a]	71.9[b]	22.7[c]		7.5[b]
ibuprofen	101.2[a]	342.2			22[b]
naproxen	166.3[a]	625.5	66.37[d]	84.09[d]	24.2[b]
acetylsalicylic acid	88.1[c]	106.7[c]			
	1293.05[e]				

[a] Reference 202. [b] Reference 233. [c] Reference 220. [d] Reference 236. [e] Reference 371.

PM biodegradation byproducts indicated no detectable residual toxicity.

4.4.4. Neuroactive Compounds: Antiepileptics

An evaluation of the aquatic environmental impact of carbamazepine (CBZ) toward bacteria, algae, invertebrates, and fish suggest it is a hazardous compound. Carbamazepine (Figure 20A) EC_{50} values were reported for Microtox-30 min, >81 mg/L; *Da. magna*-48 h, >13.8 mg/L; and *Ce. dubia*-48 h, 77 mg/L.[154] A risk quotient (PEC/PNEC) >1 calculated for carbamazepine suggests that risk for the water compartment is to be expected.[154] Life cycle and reproduction tests have been reported on invertebrates, *Lumbriculus variegates* and *Chironomus riparius*, for sublethal effects.[232] An endocrine disruption activity of carbamazepine has been suggested following the observation of inhibition of the formation of *Chironomus* pupa in the test. CBZ presence on a mixed culture of nitrite-oxidizing bacteria isolated from activated sludge caused only minor, if any, inhibition on their performance.[217]

A combination of CBZ with clofibric acid showed a much stronger effect than either of the individual compounds, in line with the concept of concentration addition.[233] On the other hand, in a study of the effects of lifetime CBZ exposure at 1 μg/L on the cladoceran *Daphnia pulex*, a stimulatory effect was identified. At this concentration level, *Daphnia* matured and reproduced slightly earlier and produced more offspring than did the control population. However, chronic adverse effects were observed for CBZ at 200 μg/L with the retardation of juvenile somatic growth resulting in delayed maturity and a consequent lower rate of population growth.[234]

The cnidarian *Hydra attenuata* is adversely affected by pharmacetical products but at higher concentrations in studies to date than are found in the environment. It undergoes morphological changes that are normally associated with lethality. *Hydra* exposed to CBZ showed signs of lethal toxicity at high (0.6 and 6 mM) concentrations. An inducible threshold concentration for heme oxidase was found to be around 0.03 μM at 48 h exposure.[235]

4.4.5. Nonsteroidal Anti-inflammatory Drugs

The ecotoxicity of nonsteroidal anti-inflammatory drugs (NSAIDs), ibuprofen (Figure 5A), naproxen (Figure 5B), diclofenac (Figure 5C), and acetylsalicylic acid, has been evaluated using acute exposure tests of the crustacea (*Da. magna, Ce. dubia, and Thamnocephalus platyrus)*, a rotifer *(B. calyciflorus*), and a green alga (*Desmodesmus subspicatus*; Table 4).[202,220,233,236] All the NSAIDs tested are considered to have the same mode of action in humans; that is, they inhibit the cyclooxygenases, the key enzymes catalyzing prostaglandin biosynthesis, and have relatively low acute toxicities.[63] Individual EC_{50} values of diclofenac and acetylsalicylic acid are <100 mg/L (Table 5), and based on a scheme used by the European Union, only these would be classified as potentially harmful to aquatic organisms.[237]

The QSAR approach has shown that all substances act by a mechanism called nonpolar narcosis (baseline toxicity),[238,239] which is entirely dependent on their hydrophobicity—the higher an NSAID's *n*-octanol/water partitioning coefficient (log K_{OW}), the higher is its toxicity.[237]

Ibuprofen (1−1000 μg/L) stimulated the growth of cynobacterium, *Synechocystis*, over a 5 d exposure. The highest increase (72%) was observed at 10 μg/L. In contrast, ibuprofen inhibited the growth of duckweed, *L. minor*, after 7 d exposures at all concentrations tested. The strongest effect was observed at 1000 μg/L where 25% reduction over the control was observed.[202] Diclofenac exposure in concentration ranges commonly found in the environment has been reported to cause adverse effects to brown trout (*Salmo trutta f. fario*), affecting kidney and grill integrity as well as selected immune parameters.[240]

The bioaccumulation potential of NSAIDs in biota or food webs are not known with the exception of diclofenac, which accumulates in treated livestock when used as a veterinary medicine. Vultures that fed on carrion of diclofenac-treated domestic livestock and cattle were found to have a massive population decline.[241] The unusually high death rate among three species of vulture (*Gyps bengalensis, Gyps indicus*, and *Gyps tenuirostris*) across the Indian subcontinent[242,243] was found to correlate with diclofenac exposure, leading to renal failure. Diclofenac bioconcentration factors in rainbow trout (*Oncorhynchus mykiss*) fish are reported to be 10−2700 in the liver and 5−1000 in the kidney depending on exposure concentrations, indicating that prolonged diclofenac exposure in environmentally relevant concentrations leads to a general health deterioration in fish.[173,244]

4.4.6. Blood Lipid-Lowering Agents: Fibrates

The acute toxicities of clofibrate and its metabolite clofibric acid were evaluated in three aquatic species, namely, the euryhaline fish *Gambusia holbrooki*, the crustacean *Artemia parthenogenetica*, and the green alga *Tetraselmis chuii* (Table 6).[245] A 30 min luminescence test on *Vibrio fisheri* was also reported.[220] Clofibrate was considered to be moderately toxic to *A. parthenogenetica* and to *T. chuii* and very toxic to *G. holbrooki*. Nevertheless, clofibrate readily metabolizes into clofibric acid. Clofibric acid has been found to be nonhazardous by these tests, but as noted above, a mixture with fluoxetine produces developmental impairments in *Daphnia*. Moreover, Rebecca Klaper of the University of Wisconsin at Milwaukee reported that fathead minnows (*Pimephales promelas*) exposed to 1 ppb clofibric acid produce a milky mucous response, have difficulty with

Table 6. Acute Toxicity of Clofibrate and Clofibric Acid on Aquatic Species

species	organism	test	clofibrate	clofibric acid
bacterium	*V. fisheri*	30 min, luminiscence		IC_{50} 91.8 mg/L[a]
crustacean	*Da. magna*	24 h, mortality	EC_{50} 28.2 mg/L[b]	
crustacean	*A. parthenogenetica*	48 h, mortality	LC_{50} 36.6 mg/L[c]	LC_{50} 87.22 mg/L[c]
green alga	*T. chuii*	96 h, growth inhibition	IC_{50} 39.7 mg/L[c]	IC_{50} 318.2 mg/L[c]
green alga	*Sc. subspicatus*	72 h, growth inhibition		EC_{50} 89 mg/L[d]
fish	*G. holbrooki*	96 h, mortality	LS_{50} 7.7 mg/L[c]	LC_{50} 526.5 mg/L[c]

[a] Reference 220. [b] Reference 370. [c] Reference 245. [d] Reference 372.

Table 7. Acute Toxicity of Beta Blockers Evaluated on Algae, Crustacean, and Fish

organism/ species	test	propranolol EC_{50} mg/L	oxprenolol EC_{50} mg/L	atenolol EC_{50} mg/L	metoprolol EC_{50} mg/L	nadolol EC_{50} mg/L
bacterium *V. fischeri*	bioluminiscence 60 min	81.0[a]		130[a]	144[a]	
green alga De. subspicatus	growth rate 24 h	4.1[a]		1335[a]	40[a]	
	3 d	0.73[b]		620[b]	7.9[b]	
green alga *Ps. subcapitata*	growth inhibition 96 h	7.4[c]				
crustacean *Da. magna*	mortality 48 h	7.7,[b] 1.6[d]		313,[b] 200[e]	438,[b] 63.9[d]	≥100[f]
crustacean *Ce. dubia*	inhibition of mobility 48 h	1.4,[d] 1.5,[c] 0.85[d]	10.1[g]	33.4[g]	45.3,[g] 8.8[d]	163.4[d]
fish *O. letipes*	mortality 48 h	29.8,[b] 24.3[d]				

[a] Reference 253. [b] Reference 252. [c] Reference 220. [d] Reference 179. [e] Reference 166. [f] Reference 223. [g] Reference 254.

respiration, and exhibit severe motility inhibition within 24 h, physical manifestations of stressed and dying fish.[246] Also, clofibric acid produced a synergistic toxic effect in the presence of carbamezapine.[233] However, clofibric acid did not significantly affect cell density or growth of the alga *Dunaliella tertiolecta* or survival of the crustacean *Palaemonetes pugio*. The fish *Fundulus heteroclitus* was also unaffected by clofibric acid concentrations of ≤1000 μg/L. Measured concentrations of clofibric acid in the environment have not exceeded 10 μg/L.[247]

Exposure of goldfish (*Carassius auratus*) to environmentally relevant levels of gemfibrozil, detected at microgram per liter concentrations in domestic wastewater and nanogram per liter concentrations in surface waters, leads to the drug's bioconcentration in the blood serum, reducing circulating testosterone to around 50%, signaling the potential of a manifestation of endocrine disruption.[248]

4.4.7. Blood Lipid-Lowering Agents: Statins

Statins are known inhibitors of sterol biosynthesis in plants and have displayed phytotoxicty in radish[249] and aquatic plants of *Lemna* genus.[214] A study with *Lemna gibba* indicated that statins caused concentration-dependent toxicity via reduction of mevalonate (HMG-CoA mediated) derived products. Both lovastatin (Figure 8A) and atorvastatin (Figure 9B) significantly decreased stigmasterol and β-sitosterol concentrations in *Lemna gibba* by 50% on 7 d daily static renewal exposure of 64 and 36 μg/L, respectively.[214,250] However, statin hazard quotients are at <0.1, indicating there is little risk to *Lemna gibba* at environmentally relevant concentrations.[250] Nevertheless, the hazard quotient makes no allowance for ED effects as it is based on lethality tests.

The acute toxicity of atorvastatin toward the midge *Chironomus tentans* and the fresh water shrimp *Hyalella azteca* using standard 10-d acute toxicity tests showed that atorvastatin was approximately 10 times more toxic to *H. azteca* compared to *Ch. tentans*.[251] The measured toxicity thresholds were several orders of magnitude higher than current environmental concentrations, indicating that this compound poses little risk to benthic invertebrates.

4.4.8. Beta Blockers

As noted above, beta blockers constitute one of the most important families of prescription drugs, and they play a significant role for the therapy of cardiovascular diseases. The ecotoxicity of several beta blockers has been evaluated using the marine bacterium *Vibrio fischeri*, the water flea *Daphnia magna*, the green alga *Desmodesmus subspicatus*, and duck weed *Lemna minor*.[252,253] Propranolol (Figure 11B) was found to be more toxic than metoprolol (Figure 11C), followed by atenolol (Figure 11A). Propranolol with an EC_{50} <1 mg/L would be classified as very toxic, and metoprolol with an EC_{50} of 7.9 mg/L in the algal test would be classified as toxic to aquatic organisms according to an EU directive (1996). Propranolol is characterized by a membrane-stabilizing activity, whereby it reduces membrane permeability for ions such as Na^+, K^+, and Ca^{2+}.[254] Atenolol with an EC_{50} of 313 mg/L in the *Daphnia* test would be classified as being nontoxic (Table 7). *Lemna minor* was found to be the least sensitive test species with an EC_{50} of 114 mg/L obtained with propranolol and no effect with atenolol and metoprolol.[233] An acute toxicity study of beta blockers on *Ce. dubia* led to the determination that it is twice as sensitive as compared with *Da. magna*.[254] Huggett[179] reported a study

Table 8. Subchronic Toxicity of Beta Blockers Evaluated on *Da. magna*[255]

	LOEC mg/L		
beta blockers	growth inhibition	reproduction	heart rate
propranolol	0.44	0.11	0.055
metoprolol	12.0	6.0	3.1

using a fish, *Oryzias latipes*, an amphipod, *Hyallela azteca*, and two cladocerans, *Da. magna* and *Ce. dubia*.

An evaluation of chronic exposure of *Da. magna* to the beta blockers propranolol and metoprolol for three ecotoxicological endpoints has been reported.[255] The *Da. magna* endpoint sensitivity to chronic expoure for both of the beta blocker drugs was found to be ordered with mortality < growth < fecundity (reproduction) < heart rate. The lowest observed effect concentrations (LOEC) for the endpoints for 9 d chronic exposures are given in Table 8. The significantly lower LOECs for heart rate on exposure to both propanolol and metoprolol indicated sublethal toxicity to *Da. magna* at lower concentrations than those observed for more classical endpoints.[255] Long-term exposure of *Ce. dubia* and *H. azteca* to propranolol affects sex hormone blood plasma concentrations and reduces fecundity.[179] *Ce. dubia* exhibited a reproduction LOEC for propranolol on 7-d subchronic exposure of 0.25 mg/L, while *H. azteca* exhibited a reproduction LOEC similar to that of *Da. magna* as shown in Table 8.

When scrutinized via a 24 h exposure chlorophyll fluorescence assay, propranolol also inhibited photosynthesis in the green alga *Desmosdesmus subspicatus* ($\log(1/EC_{50}(M)) = 5.61$).[256] In a 72 h growth inhibition test for propranolol, an EC_{50} value of 20 μM reported earlier[233] was modified to 7.8 μM.[253] A value of 1 or above between the predicted environmental concentration (PEC) and the predicted no effect concentration (PNEC) is seen as a trigger value for environmental risk assessment requiring further testing.[252] The study found PEC/PNEC for all tested beta blockers to be <1 with the propranolol ratio being 0.81, indicating the environmental risk to be relatively low from the lens of this assay.[252]

4.5. Aquatic Ecotoxicity of Pharmaceutical Mixtures

Pharmaceuticals occur in the environment in combination with other pharmaceutical compounds together with their own metabolites and other environmental pollutants.[181] Because current environmental risk assessments focus on single substances, it is virtually certain that the prevailing assessments underestimate the real environmental impacts, and some examples of this have been pointed out above.

Thus, cumulative exposures need be considered for pharmaceuticals. This should clearly entail mixtures of APIs that share a common mode of toxic action, as well as mixtures with diverse modes of action. For APIs that share a common mode of action, the toxicity of any given mixture where the individual components act on the same or similar physiological targets may be able to be predicted by applying the concept of concentration addition (CA). It has been suggested that the CA concept is applicable to nonreactive, nonionized organic chemicals, which show no specific mode of action but whose toxicity toward aquatic species is governed by hydrophobicity.[233,237] According to Cleuvers et al., CA could provide a good prediction of toxicity of mixtures at the EC_{50} and EC_{80} level, but at lower levels, the mixture toxicities might be underestimated.[252]

By the EC_{50} measure, the toxicity of a mixture of beta blockers (acebutolol, atenolol, metoprolol, nadolol, oxprenolol, and propranolol) has been shown to fit well with a CA model.[253,254] Estrogenic activity of mixtures of estradiol and ethinylestradiol could also be predicted based on the EC_{50} values of individual chemicals for vitellogenin induction in rainbow trout.[257] Similarly, using vitellogenin induction in male fathead minnows as the end point, the combined effects of a multicomponent mixture of estradiol, ethinylestradiol, and nonylphenol, octylphenol, and bisphenol A revealed that these estrogenic chemicals acted together in an additive manner in this case. When these five estrogenic compounds were mixed in concentrations below levels at which their individual effects can be detected, their cumulative impact was detrimental.[258] In an algal test up to EC_{20} level, propranolol, metoprolol, and atenolol had practically no effect when applied singly, but the mixture of all three beta blockers has been reported to cause about 36% growth inhibition.[252]

The effect of a mixture of APIs at environmental levels (ng/L) on human embryonic kidney cells (HEK293) has been reported to inhibit the growth in vitro by affecting both the physiology and morphology. The API cocktail of 13 drugs was produced to mimic both the association and low concentration (nanogram per liter) profiles detected in the environment. It included atenolol, bezafibrate, carbamazepine, cyclophosphamide, ciprofloxacin, furosemide, hydrochlorothiazide, ibuprofen, lincomycin, ofloxacin, ranitidine, salbutamol, and sulfamethoxazole. The mixture stimulated the expression of cell cycle regulation genes and kinase proteins that signal cell stress, indicating a slower rate of cell division and less cell proliferation.[259,260]

Many mixtures produce higher toxic effects than would be predicted by the additive contribution of the single compounds. Interactive and synergistic effects are therefore occurring. Some seemingly harmless compounds can enhance the toxicity of others. These synergistic effects indicate amplified risk of pharmaceuticals with respect to the aquatic environment when used in combination.[166] The research carried out to date clearly establishes that much more work in this area must be done to learn how to protect society and the pharmaceutical industry from the ramifications of the impacts of APIs in the environment.

5. Natural Elimination of Pharmaceuticals in the Environment: Photodegradation

The persistence of pharmaceuticals in the environment plays a major role in determining their potential for producing adverse environmental effects. The bad news side of this is that the industry is actively working to make pharmaceuticals more resistant to degradation chemistries suggesting that the problems will only get worse with time. The good news is that this overarching fact presents society with opportunities to obviate the problems. Either by restricting or by better controlling pharmaceutical releases and by developing more effective technologies for their rapid destruction in water, we should be able to substantially ameliorate the problems. The latter strategy is a major opportunity for green chemists.

5.1. Photolysis as a Natural Removal Mechanism of Pharmaceuticals

In surface water, the main elimination processes are biodegradation, sorption, and photodegradation. As noted,

in general pharmaceuticals have a designed resistance to biodegradation, thereby inhibiting one of the major removal mechanisms. The sediment type has been shown to significantly affect the sorption of pharmaceuticals such as ibuprofen and diclofenac, thus implying that the efficiency of elimination by sorption is site specific[261] and cannot be relied upon on a global basis. However, many pharmaceuticals are expected to be photoactive, because many of these compounds feature aromatic rings, heteroatoms, and other functional groups that can either absorb solar radiation or react with photogenerated transient species in natural waters. Thus, photolysis and photochemical processes should be considered as important removal mechanisms of APIs in surface waters.[262,263]

Recent studies have shown that certain APIs are indeed susceptible to photodegradation in environmental settings. Therefore, if they are not removed by other chemical means, these species could be degraded by photochemical reactions in sunlit water. In some cases, however, the photodegradation of APIs gives rise to products that are also of environmental concern. The measured half-lives of several pharmaceuticals in sunlight-exposed pond water and autoclaved pond water revealed no significant differences, suggesting that both direct and indirect photodegradation are important in limiting their persistence and that biodegradation was not an importantant loss process in surface water.[108]

5.2. Direct and Indirect Photolysis

Direct photolysis occurs when a compound absorbs light, becomes unstable, and subsequently decomposes. However, direct photolysis of organic compounds is often inefficient because there is poor overlap between its absorption spectra and the spectrum of sunlight. Chemicals that cannot absorb light above 290 nm are resistant to direct photodegradation. Indirect (or sensitized) photolysis occurs through reactions with reactive intermediates generated by another light-absorbing molecule. The interaction with transients produced upon the photoexcitation of dissolved organic matter, such as the highly reactive, nonselective hydroxyl radical, •OH, has been shown to limit the persistence of many compounds that degrade only relatively slowly by direct photolytic means.

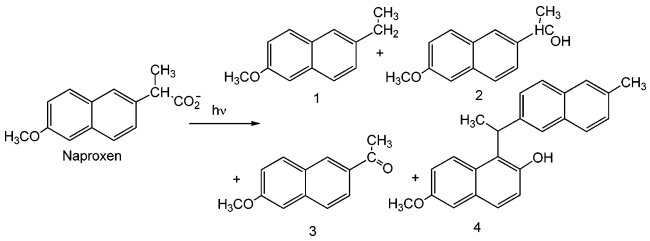
Multiple studies have identified the presence of the three acidic NSAIDs (naproxen, ibuprofen, diclofenac) and clofibric acid, the metabolite of lipid-lowering drugs clofibrate, etofibrate, and theofibrate, in American and European river water samples, emphasizing the need to study the fate of these drugs.

5.2.1. Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Ibuprofen does not absorb sunlight; hence direct phototransformation can be neglected.[264] Naproxen, a widely used anti-inflammatory drug, is susceptible to photodegradation in both distilled water and river water.[263] A number of studies have identified the photoproducts of naproxen. Three principal products (**1**−**3**) arise from the photoionization and decarboxylation of the parent compound (Scheme 3).[265−268] A coupling product (**4**) was also identified.[268] Phototransformation products of naproxen showed higher toxicities than the parent comound, while no genotoxicity was found.[236]

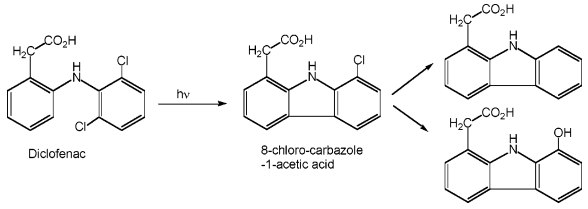
Diclofenac reacts rapidly via direct irradiation. Direct photolysis provides the dominant degradation mechanism and the removal process for diclofenac in surface water.[109,266,269,270] The degradation has the same half-life of 39 min in both

Scheme 3. Photodegradation Products of Naproxen[265,268]



natural water and demineralized water.[267] Under direct solar irradiation, there are 13 diclofenac phototransformation products identified in both demineralized water and reconstructed standard freshwater. The photolysis has been suggested to proceed by two routes. One results from initial photocyclization of diclofenac into carbazole derivatives. The other proceeds via an initial decarboxylation and further oxidation of the alkyl chain. The major photoproduct 8-chloro-9*H*-carbazole-1-ylacetic acid, comes from the first route (Scheme 4).[270−272]

Scheme 4. Photodegradation Products of Diclofenac[271,272]



A photo-Fenton reaction of diclofenac by solar photodegradation shows complete oxidation of the starting compound in 60 min and total mineralization after 100 min exposure to sunlight.[273,274] The proposed degradation route involves an initial hydroxylation of the phenylacetic acid moiety in the C-4 position leading to formation of a quinone imine, which undergoes multistep degradation. An alternative suggested degradation pathway involves oxidative C−N bond cleavage of the biphenyl amino moiety.

Wastewater treatment plants do not remove mefenamic acid, a NSAID that is structurally related to diclofenac.[275] The direct photolysis solar quantum yield of mefanamic acid in pure water was found to be low, $(1.5 \pm 0.3) \times 10^{-4}$. Significant photosensitization was observed in solutions of Mississippi river water and with model photosensitization compounds.[275] Degradation of mefenamic acid in sunlit natural waters is expected to depend on slow direct photodegradation and rapid sensitized photodegradation in the presence of dissolved organic matter.[275] Ibuprofen is transformed only minimally by direct photolysis under a Hg-vapor lamp.[267] The obervation of accelerated (indirect) photodegradation in Mississippi river water and quenching of this reaction by isopropyl alcohol favors a radical-mediated mechanism.[267]

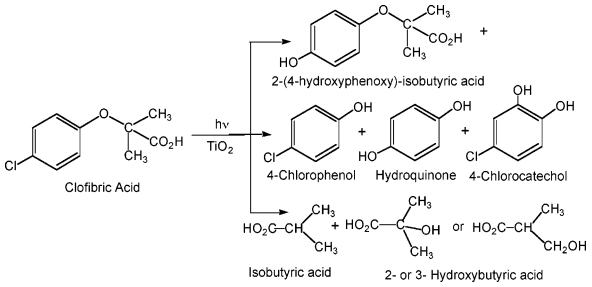
5.2.2. Clofibric Acid

Clofibric acid in sunlit natural water is reported to have a half-life of 50 h for a specific set of conditions.[267] The presence of nitrate and humic acids increased the degradation rate, implicating the involvement of radicals.[109] Separately, quenching of the degradation in Mississippi river water by isopropyl alcohol suggests involvement of an indirect photochemical process.[267] As only ∼20% of quenched transformation could be attributed to hydroxyl radicals, other

radical species were also implied participants. It has been suggested that both direct and radical-mediated indirect photolysis may play approximately equal roles in its transformation.[267]

The photocatalytic treatment of clofibric acid in aqueous TiO_2 suspensions facilitated degradation into several aromatic and aliphatic products (Scheme 5).[276,277]

Scheme 5. Photocatalytic Degradation of Clofibric Acid in Aqueous Solution Suspended with TiO_2[277]

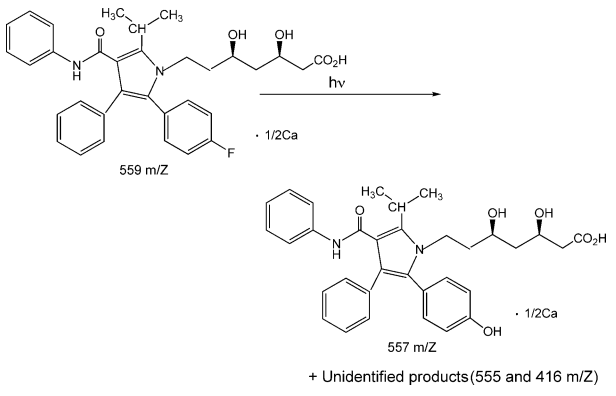


5.2.3. Atorvastatin

In a study of the persistence of eight pharmaceuticals from multiple classes, including atorvastatin, in aquatic outdoor field microcosms, no significant differences were observed between the measured half-lives of the pharmaceuticals in sunlight-exposed pond water and autoclaved pond water. This suggested that photodegradation limits their persistence and that biodegradation does not play an important role in removing them from surface water.[278]

The photodegradation products of atorvastatin have been characterized based on molecular ion peaks and fragments detected by liquid chromatography−mass spectrometry (LC−MS). One photoproduct has the F atom substituted by OH but with the amide group retained (Scheme 6). A molecular ion at 416 *m*/*z* results from N-dealkylation of atorvastain followed by water adduct formation.[278] In the photodegradation of the aqueous solution of atorvastatin in the presence of 1 mM H_2O_2, a molecular ion at 575 *m*/*z* was assigned to an oxygenated analogue of atorvastatin. This photoproduct, which has been proposed to be a hydroxylated form of atorvastatin, was not observed in direct photolysis experiments.[278]

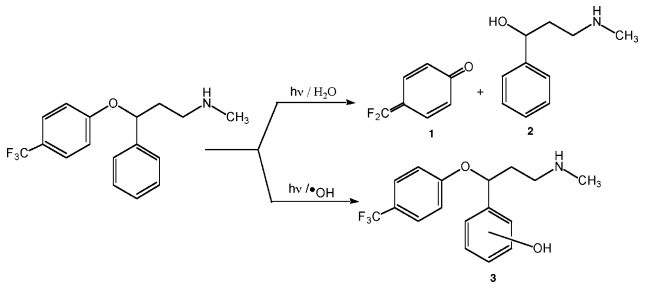
Scheme 6. Atorvastatin Photoproducts Include a Hydroxy Derivative by Substitution of F Atom by Hydroxy Group and Two Unidentified Products[278]



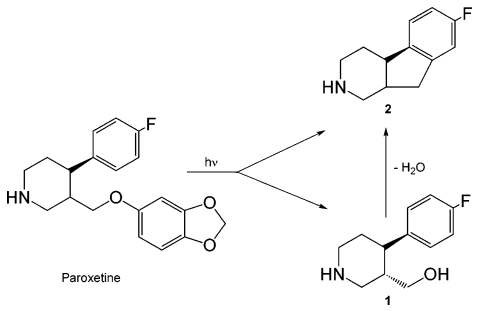
5.2.4. Selective Serotonin Reuptake Inhibitors (SSRIs)

SSRIs as a general class resist most forms of degradation in environmental systems and tend to partition to sediment where residues of these compounds would persist. Direct photodegradation products of fluoxetine in deionized water using a Xe lamp with a light intensity of 765 W/m^2 form via O-dealkylation to a semiquinone product, **1**, and a methylaminoethyl benzyl alcohol, **2** (Scheme 7). Indirect photolysis in synthetic field water was found to be considerably faster resulting in a possible photoproduct, **3**, from ring addition of •OH to the parent compound, in addition to **1** and **2** obtained from direct photolysis. Photoproduct **3** was not formed during direct photolysis experiments.[279] In another study using a different light source of light intensity 0.25 W/m^2 focused on the indirect photolysis of fluoxetine in a solution of synthetic humic water (SHW; pH 7), norfluoxetine, the demethylation product of fluoxetine, was identified as a photoproduct. This SHW study also reported a 13-fold photolysis rate enhancement compared with direct photolysis in buffered solution at the same pH.[280]

Scheme 7. Photodegradation Products of Fluoxetine by Direct and Indirect Photolysis[279]



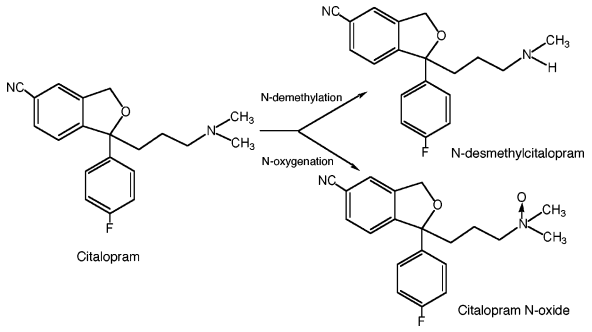
Scheme 8. Proposed Photodegradation Products of Paroxetine[281]



Photolysis of paroxetine, both in synthetic humic water and in lake water, resulted in formation of two photoproducts, **1** and **2** (Scheme 8), at molecular mass 209 and 191, respectively. The structure of **1** was proposed to arise from cleavage of the ether bond of the parent compound. Photoproduct **2** was proposed to result from loss of water from **1** with subsequent cyclization.[281] Another SSRI, fluvoxamine (Luvox; Figure 14D), has a C═N double bond and can exist as (*E*)- (*trans*) and (*Z*)- (*cis*) diastereomers; the (*E*)-diastereomer is clinically effective. UV irradiation of aqueous solutions of fluvoxamine generated the (*Z*)-diastereomer in both pure water[282] and synthetic humic water.[283]

The photolysis of citalopram (CIT) in water resulted in <0.5% degradation at pH 5 and pH 7 during the 30 d exposure period in a chamber outfitted with fluorescent lamps simulating the ultraviolet output of sunlight at 25 °C.[284] Citalopram degradation was faster in synthetic humic water (half-life 24 d) and in natural waters (half-lives 14 and 43 d) than in pH 9 buffer (half-life 65 d), indicating photosensitization by humic acid or other species. Two photo-

Scheme 9. Photodegradation Products of Citalopram[284]



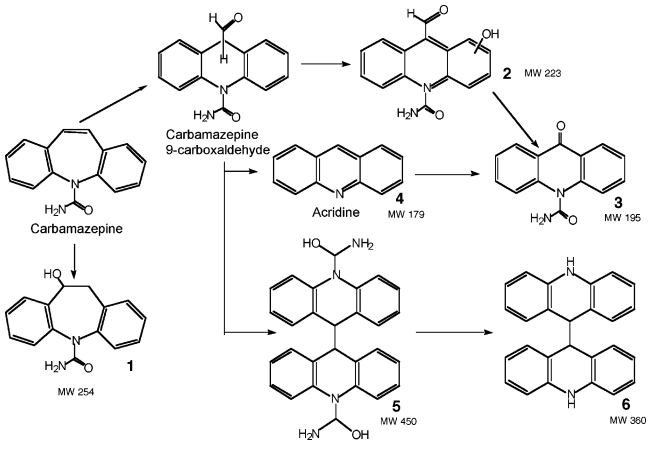
products were identified, *N*-desmethylcitalopram (DCIT), a major product, and CIT *N*-oxide as a minor product (Scheme 9).[284]

5.2.5. Carbamazepine

Several groups have studied the photochemical behavior of carbamazepine, and the major product is 10,11-epoxycarbamazepine.[109,276−278,285−287] The time required for conversion is matrix dependent. The epoxide yield is approximately 25% on carbamazepine, such that there are other unidentified photoproducts.[279]

Direct photolysis of carbamazepine (in ultrapure Milli-Q water) is reported to yield photodegradation intermediates **1**−**6** (Scheme 10), with acridine (**4**) as a major mutagenic and carcinogenic intermediate.[288] A UV/H_2O_2-induced degradation study of carbamazepine has also been reported to form acridine as the main product via the epoxycarbamazepine intermediate.[287] The photodegradation of carbamazepine was studied in reconstructed natural estuarine water (tap water, humic acids, Fe(III), nitrate, and chloride) under mercury lamp irradiation simulating natural light. This results in a substantially enhanced photodegradation rate with the formation of products **1**−**6** and two additional chlorinated compounds.[288]

Scheme 10. Carbamazepine Degradation Products on Direct Photolysis[288]



Photocatalysis with TiO_2 (λ < 413 nm) eliminates carbamazepine.[277] The influences of organic water constituents such as natural organic matter (NOM) and the behavior of mixtures of pharmaceuticals on the degradation rate of carbamazepine, clofibric acid, and iomeprol have been determined for TiO_2 photocatalytic degradation in aqueous suspensions.[276]

5.2.6. Steroid Hormones

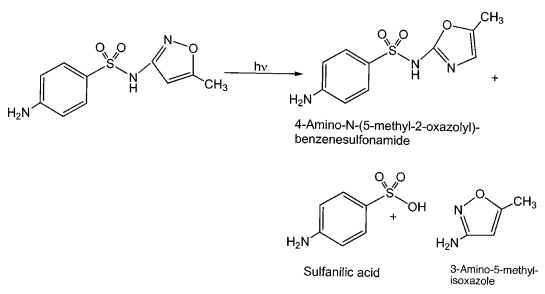
The natural estrogen estradiol (E_2) is a significant endocrine disruptor in the aquatic environment. Both E_2 and synthetic 17α-ethinylestradiol (EE_2) are reported to degrade on direct photolysis. A polychromatic medium-pressure UV radiation source was found to be more effective compared with a conventional low-pressure UV lamp. Degradation was found to be more effective on UV/H_2O_2 treatment compared with direct UV photolysis.[289] The titanium dioxide photocatalysis process has been shown to degrade 17β-estradiol via oxidation of the phenolic moiety to mineralized products.[290] Similar catalytic photodegradations of estrone (E_1), E_2, and EE_2 have been reported using titanium dioxide photosemiconductor thin films under UV light. Only 20% of the initial E_1 degraded on direct UV irradiation, but this could be increased to 90% by TiO_2 photocatalytic oxidation after 30 min irradiation.[291] Direct photodegradation of E_1 was observed under UV-lamp and high-pressure mercury lamp irradiations with degradation of the aromatic ring forming carbonyl group-containing oxidation products.[292] Photo-Fenton degradation involving UV−vis/Fe(III)/H_2O_2 resulted in 98.4% degradation of E_1 on 160 min irradiation. The relative degradability of different estrogenic compounds was $E_2 > EE_2 > E_1$.[293]

5.2.7. Antibiotics

Sulfonamides are used in aquaculture for veterinary applications and in the treatment of human respiratory and urinary tract infections. In the photolysis of sulfonamides containing five-membered heterocyclic rings, including sulfamethoxazole, sulfisoxazole, sulfamethizole, sulfathiazole, and sulfamoxole, sulfanilic acid and a small HPLC peak in each photolysate solution coinciding with the sulfanilamide standard have been identified.[294]

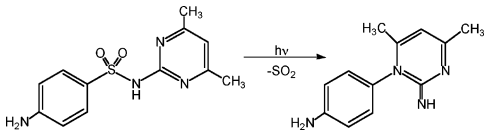
For sulfamethoxazole, five photoproducts have been characterized, including the structural isomer of sulfamethoxazole and two photoproducts formed by the clevage of the −SO_2−NH− linkage into 3-amino-5-methylisoxazole and sulfanilic acid (Scheme 11).[295] The rate of photodegradation of sulfamethoxazole in irradiated 1 mM H_2O_2 solution was significantly enhanced relative to the degradation in deionized water, which suggested that •OH-mediated reactions might be occurring alongside direct photolysis reactions. Similarly, the rate of degradation of the photoproduct, 3-amino-5-methylisoxazole, was found to be slow in pure water but faster in 1 mM H_2O_2.[278]

Scheme 11. Photoisomerization and Photodegradation Products of Sulfamethoxazole[295]



The direct photolysis of five sulfonamide antibiotics containing six-membered heterocyclic groups, namely, sulfamethazine, sulfamerazine, sulfadiazine, sulfachloropyridazine, and sulfadimethoxine, resulted in common loss of 86 mass

Scheme 12. Photolysis of Sulfamethazine and Other Structurally Similar Compounds Results in the Formation of Coupling Products of Aminobenzene through the Pyrimidine Nitrogen Atom *ortho* to the Original Connection Point, Such as in the Formation of 4-(2-Imino-4,6-dimethylpyrimidin-1(2*H*)-yl)aniline[296]



units, indicating SO_2 extrusion. NMR analysis of the photoproducts led to the identification of sulfamethazine as a product proposed to be formed by coupling of the aminobenzene ring through the pyrimidine nitrogen atom ortho to the original bond to the sulfonamide (Scheme 12). Similar photoproduct formations have been proposed for antibiotics with similar structures.[296] In the case of sulfachloropyridazine, an additional product has been reported showing the loss of the chlorine atom.[296]

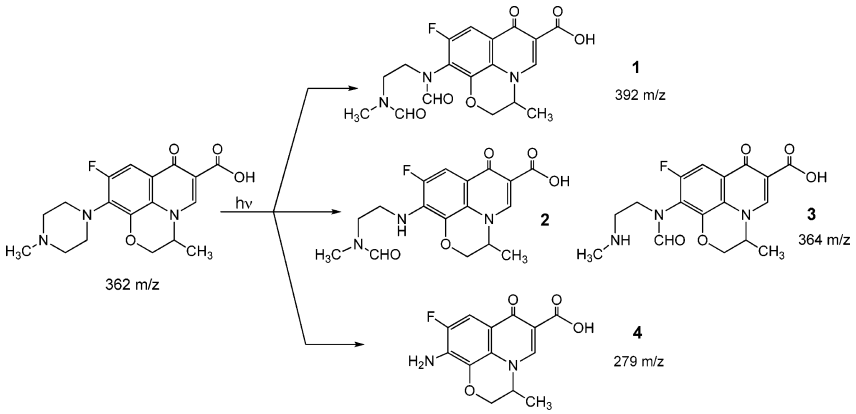
Antibiotics of the fluoroquinolone family are photosensitive, and the photodecomposition of some members of the class, including levofloxacin and clinofloxacin, has been studied extensively.[297−299] Four photoproducts of levofloxacin have been characterized. Three are formed by oxidation of the piperazine ring (**1**−**3**) and one derives from the loss of the piperazine moiety (**4**; Scheme 13).[278,299]

Photolysis of enrofloxacin, ciprofloxacin and norfloxacin produces products with altered piperazine rings.[297,300,301] Photolysis of enrofloxacin led primarily to abstraction of the ethyl group with formation of ciprofloxacin followed by oxidation of the piperazine ring (Scheme 14). Enrofloxacin and ciprofloxacin ended up with common photoproducts, **3** and **4**. Denofloxacin gave a single product, **4**, and norfloxacin had analogous products **5** and **6** (Scheme 14).[297]

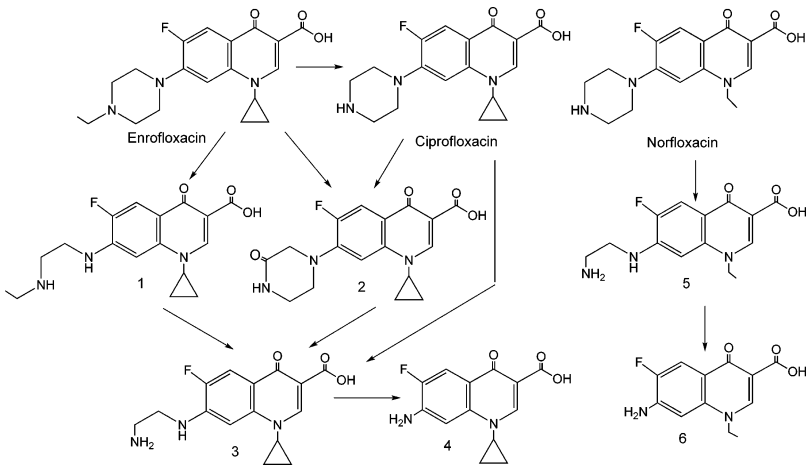
Clinafloxacin photodegradation proceeds through dechlorination followed by reactions involving the quinolone ring forming polar products.[298] Another route involves the degradation of the pyrrolidine side chain to yield nonpolar products. The structures of eight photoproducts (Scheme 15) are based on HPLC, LC-MS/MS, and NMR evidence.[298]

In sunlit natural surface waters, photodegradation reactions can occur by direct photolysis with organic compounds decomposing under the assault of actinic radiation in the 290−800 nm range. Indirect photolysis reactions proceed through the generation of reactive intermediates from other UV-absorbing material such as nitrate and dissolved organic matter, which are ubiquitous natural constituents of field water. Photogeneration of the highly reactive, nonselective hydroxyl radical has been shown to limit the persistence of many compounds that degrade relatively slowly by direct photolysis. The second-order rate constants for this oxidant with numerous organic compounds have been reported to approach diffusion-limited values (10^7−10^{10} M^{-1} s^{-1}).[302] The principal photolysis reactions involving •OH and other organic compounds include H-abstraction and addition to double bonds, and these can distinguish products resulting from direct and indirect photolysis.[278] Known second-order rate constants for the reaction between different pharma compounds and •OH are compiled in Table 9.

Scheme 13. Photoproducts of Levofloxacin[278,299]



Scheme 14. Photoproducts of Enrofloxacin, Ciprofloxacin, and Norfloxacin[297]



Scheme 15. Photoproducts of Clinafloxacin[297,298]

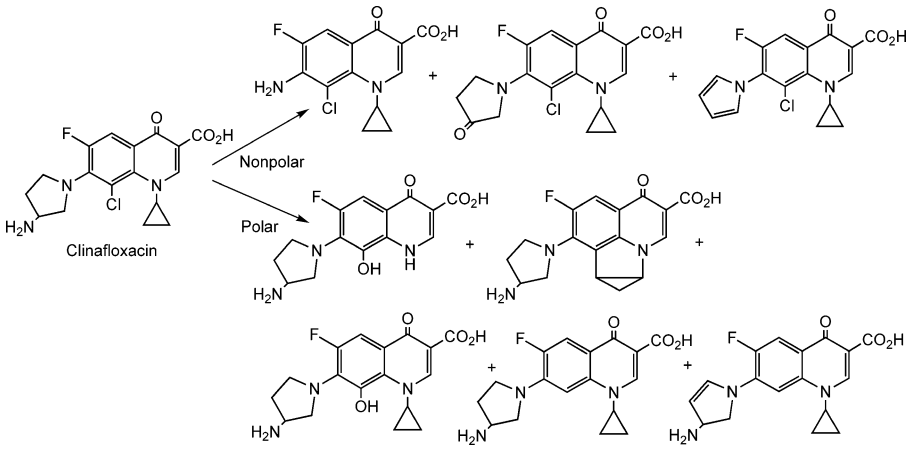


Table 9. Rates of Photodegradation of Pharmaceuticals in Sunlit Natural Surface Water

pharma compunds	second-order rate constant ($^{\bullet}$OH), M^{-1} s^{-1}	quantum yield (direct photolysis)	light source	ref
levofloxacin	$(6.6 \pm 0.6) \times 10^9$	0.05	sunlight simulator	278
ofloxacin		7.79×10^{-5}	sunlight	109
carbamazepine	$(9.4 \pm 0.4) \times 10^9$	1.3×10^{-4}	sunlight simulator	278
	$(8.8 \pm 1.2) \times 10^9$			303
	$(3.07 \pm 0.33) \times 10^9$		sunlight	287
		4.77×10^{-5}		294
sulfamethoxazole	$(3.7 \pm 0.1) \times 10^9$	0.02	sunlight simulator	278
	$(5.8 \pm 0.2) \times 10^9$			294
		4.29×10^{-3}	Hg lamp	109
atorvastatin	$(1.9 \pm 0.5) \times 10^{10}$	4.5×10^{-3}	sunlight simulator	278
fluoxetine	$(8.4 \pm 0.5) \times 10^9$		sunlight simulator	279
	$(9.6 \pm 0.8) \times 10^9$	$(4.2 \pm 1.5) \times 10^{-5}$		
naproxen	$(9.6 \pm 0.5) \times 10^9$	0.036		267
ibuprofen	$(6.5 \pm 0.2) \times 10^9$			267
clofibric acid	$(4.7 \pm 0.3) \times 10^9$			267

6. Oxidative Transformations of Pharmaceuticals in Water

Surface water is widely used for drinking water. Therefore, the occurrence of APIs in surface waters poses serious problems to society in general and water utilities in particular. Despite the ability of natural processes to break down many pharmaceuticals as discussed above, there is an increasing need for strategies to mitigate organic micropollutants in water as the demand for clean water is steadily rising. Up to now, there has been no proof that very low concentrations of APIs have adverse health effects on humans. But as we have already pointed out, it would be entirely premature to give these trace contaminants a stamp of approval. Low levels of EDCs in water are a major concern for society because EDCs exert physiological effects at very low concentrations that can translate into human impairment. Precautionary thinking ordains that drinking water should be free from trace APIs to minimize the unpredictable long-term risks.[303] In the face of uncertainty about what the effects will be of continuous exposure of the population to trace quantities of APIs, the only prudent course is to treat APIs in the water as an urgent issue for short- and long-term action with the strategic intent of protecting human health and the environment.

Drinking water treatment primarily relies upon adsorptive and oxidative processes to remove or transform organic materials. Persistent micropollutants can be removed by membrane filtration (nanofiltration and reverse osmosis) or filtration over activated carbon. However, the absorption or retention capacity of both approaches decreases with operation time as natural organic matter builds up and interferes.[304] Biofouling can also lead to clogging of filters.[304] The role of chemical oxidation depends on the treatment objectives and may vary from partial remediation to complete mineralization. In the case of partial treatment, chemical oxidation aims at the selective removal of the persistent components and their conversion to readily biodegradable intermediates that can subsequently be treated biologically. Compounds that are particularly susceptible to oxidation often contain heteroatoms with lone pairs of electrons (oxygen, nitrogen, sulfur), suggesting that APIs should be susceptible to oxidative treatment.

In drinking water treatment systems, chlorine, chlorine dioxide, and ozone are frequently used, while disinfection of wastewater effluent usually is limited to chlorine. Among the three oxidants, ozone tends to be the most reactive. All three oxidants are strong electrophiles that exhibit similar trends of reactivity with organic compounds. As a result, certain reactivity generalizations can be made. Oxidation with chlorine and ozone can result in the transformation of some compounds with reactive functional groups under the conditions employed in water and wastewater treatment plants. Chloramine is also finding expanded use in drinking water disinfection and some studies of its utility in decomposing APIs have been reported.

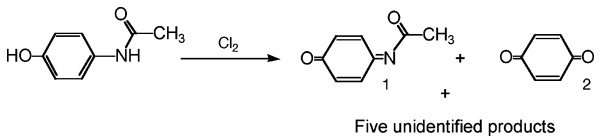
6.1. Non-Green-Chemistry Methods

6.1.1. Chlorination

The rate of transformation with free chlorine (i.e., HOCl/OCl^-) of compounds containing aromatic ether functional groups was strongly affected by the other substituents on the ring. The amine-containing pharmaceuticals undergo a rapid reaction to form chlorinated compounds.[305] Pharmaceuticals such as acetaminophen, sulfamethoxazole, diclofenac, and metoprolol all oxidized during chlorination. Metoprolol and sulfamethoxazole formed chloramines as one of their oxidation products. Gemfibrozil underwent chlorination with substitution of one chlorine atom.[306]

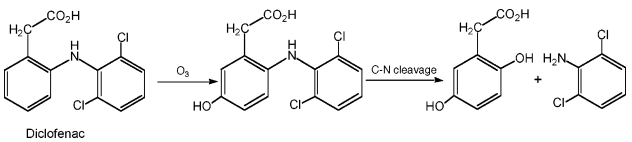
Acetaminophen reacts with chlorine to form multiple products, two of which have been identified as the toxic compounds *N*-acetyl-*p*-benzoquinine imine (**1**) and 1,4-benzoquinone (**2**; Scheme 16), the latter being a toxicant associated with lethality in acetaminophen overdoses. Other identified products included two ring chlorination products, chloro-4-acetamidophenol and dichloro-4-acetamidophenol.[306,307] A kinetic study of the chlorination of acetaminophen has been reported showing enhanced rates as the pH was decreased from 10 to 7, because HOCl is significantly more reactive than OCl^-.[305]

Scheme 16. Effect of Chlorination on Acetaminophen[307]



Diclofenac on treatment with chlorine forms at least five products, but it does not form a chloramine.[308] Diclofenac has also been investigated for its degradation in water on treatment with ozone and H_2O_2/UV. Both oxidant systems induced diclofenac degradation, completely converting the chlorine into chloride ions with degrees of mineralization of 32% for ozonation and 39% for H_2O_2/UV after 90 min treatments. The reactions were found to follow similar but not identical reaction pathways leading to hydroxylated intermediate, 2-[(2,6-dichlorophenyl)amino]-5-hydroxyphenylacetic acid, and C−N cleavage products, notably 2,5-dihydroxyphenylacetic acid, through competitive routes. Subsequent oxidative ring cleavage leads to carboxylic acid fragments via classic degradation pathways (Scheme 17).[309]

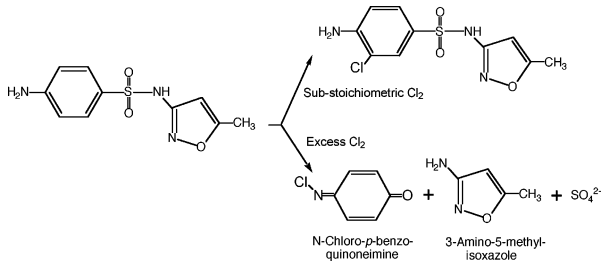
Scheme 17. Diclofenac Degradation Products on Treatment with Ozone[309]



Naproxen on chlorination is transformed into five intermediate products within 6 min, which further transform and degrade; two unidentified end products after 7 days were the only residuals.[310]

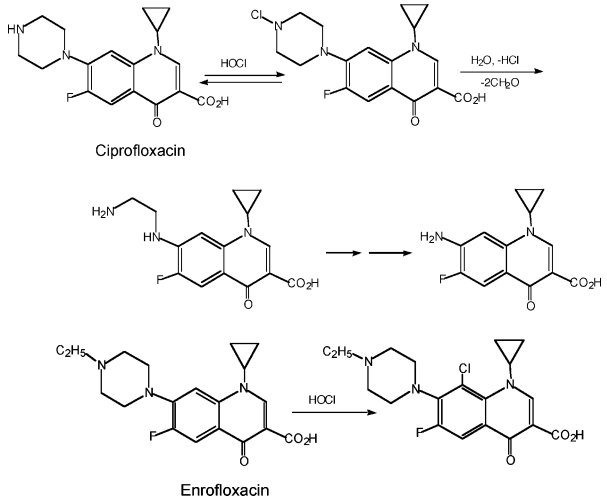
A detailed investigation of the chlorination reaction of sulfamethoxazole at substoichiometric concentrations of free chlorine revealed an unexpected aromatic amine chlorination reaction by direct chlorination of the aniline moiety. In excess chlorine, the reaction products identified include *N*-chloro-*p*-benzoquinoneimine, 3-amino-5-methylisoxazole, and SO_4^{2-} (via SO_2; Scheme 18).[311]

Scheme 18. Effect of Chlorination on Sulfamethoxazole in Municipal Wastewaters and Affected Drinking Waters[311]



Fluroquinolone antibiotics interact with aquous chlorine in markedly different manners depending upon whether they contain a secondary amine, for example, ciprofloxacin, or a tertiary amine, for example, enrofloxacin.[312] Ciprofloxacin reacts rapidly with HOCl at its secondary amine forming a chloramine intermediate that spontaneously decays, reportedly by a concerted piperazine fragmentation (Scheme 19). In contrast, enrofloxacin reacts slowly with HOCl at its tertiary amine, forming a reactive chlorammonium intermediate that has been reported to catalytically chlorinate enrofloxacin (Scheme 19) or other substrates present in solution. The finding suggests that tertiary amine-containing fluoroquinolones remain comparatively stable during water chlorination.[312]

Scheme 19. Transformation Products of Fluoroquinolones on Treatment with Aqueous Chlorine[312]



Oxidative removal of several sulfonamides, macrolides, and carbadox antibiotics in surface waters has been reported with free chlorine. An average of 88% of the antibiotics were removed on treatment with free chlorine (1 mg/L) in about 2 h over the pH range of 6.1−9.1 under ambient conditions. Monochloramine is less effective at typical drinking water dosage concentrations of 3 mg/L.[313]

The oxidative treatment of fluoroquinone antibiotics by manganese oxide results in dealkylation and hydroxylation at the piperazine moiety with the quinolone ring remaining intact.[314]

Oxidative degradation of both E_2 and EE_2 with chlorine is reported to result in >99% removal/transformation (2 log) of the parent compounds. The decrease in estrogenic activity paralleled the decrease in the estrogenic chemicals under the influence of free chlorine, investigated with the yeast two-

hybrid assay (YTA), estrogen receptor (ER) competition assay (ER-CA), and high-performance liquid chromatography/mass spectrometry (LC/MS).[114] In an estrogen receptor-positive human breast cancer cell line (MCF 7) assay, a lower stabilized estrogenic level was reached in 2 h.[315,316]

6.1.2. Treatment with Chlorine Dioxide

A number of environmentally relevant pharmaceuticals have been treated with chlorine dioxide (ClO_2) to assess its potential for the oxidation of pharmaceuticals.[317] It was found to be effective only for certain compounds such as the sulfonamide antibiotic sulfamethoxazole, the macrolide antibiotic roxithromycin, 17α-ethinylestradiol, and diclofenac. ClO_2 reacts selectively with functional groups with high electron density, such as neutral tertiary amines and phenoxide. It reacts at the tertiary amino group of roxithromycin and at the phenolic moiety of EE_2 (Figure 33).[317]

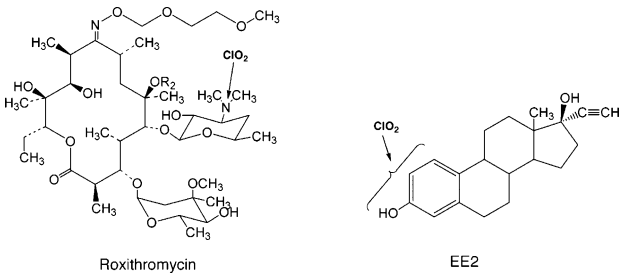


Figure 33. The sites of ClO_2 attack on reactive pharmaceuticals.[317]

Compared with ozone, ClO_2 reacts more slowly and with fewer compounds. However, ClO_2 reacts faster than chlorine with sulfonamide and macrolide antibiotics and estrogens, but in contrast with chlorine, it does not react with bezafibrate, carbamazepine, diazepam, and ibuprofen.[317]

6.2. Green-Chemistry Methods

Green chemists are just beginning to consider the PIE area as a place where they might focus upon the reducing or eliminating the use and generation of hazardous substances. Because of the many health and environmental uncertainities associated with pharmaceuticals in environment, this area is conceptually excellent for a green chemistry precautionary approach that may avoid known and possible problems. Green chemists will make a large contribution to the sustainability of the pharmaceutical industry if they develop highly effective, nonpolluting technologies for decomposing in water APIs and their various biologically active byproducts. Several chemical methods have been employed for the degradation of pharmaceuticals, including ozonation, advanced oxidation processes, such as H_2O_2/UV and O_3/H_2O_2, and a new catalytic oxidation process employing Fe-TAML/H_2O_2. In the last case, the work is being conducted with explicit green chemistry motives, although such are implicit in all the studies. Ikehata and colleagues have reviewed the degradation of pharmaceuticals in aqueous media where the kinetic parameters are known by ozonation and advanced oxidation processes.[318]

6.2.1. O_3, H_2O_2/UV, and O_3/H_2O_2 Oxidation

While the effectiveness of direct photolysis is governed by the absorption spectra of the contaminant and the quantum yield, the dominant mechanism on the addition of H_2O_2 involves highly reactive hydroxyl radicals, often significantly lowering the UV fluence (dose) required for oxidation compared with direct photolysis.[289]

Oxidative treatment of clofibric acid, ibuprofen, and diclofenac has been reported with O_3, H_2O_2/UV, and O_3/H_2O_2. Ozonation was effective in the degradation of diclofenac with complete conversion of the chlorine into chloride ions and 32% mineralization. A 90 min treatment of diclofenac with H_2O_2/UV resulted in 39% mineralization.[309] The combined application of O_3/H_2O_2 degraded all three compounds to more than 98% at 5.0 mg/L O_3 and 1.8 mg/L H_2O_2.[319]

Oxidative treatment with both UV/H_2O_2 and O_3 completely removes the toxicity of a mixture consisting of carbamazepine, clofibric acid, diclofenac, sulfamethoxazole, ofloxacin, and propranolol in a moderately hard synthetic medium within 1 min of treatment.[320] A longer treatment (3−5 min) did not lead to increased toxicity, but stimulated the algal growth of *Synechococcus leopoliensis.* This led to the suggestion that the degradation products of the APIs could be used as carbon sources by the alga.[320]

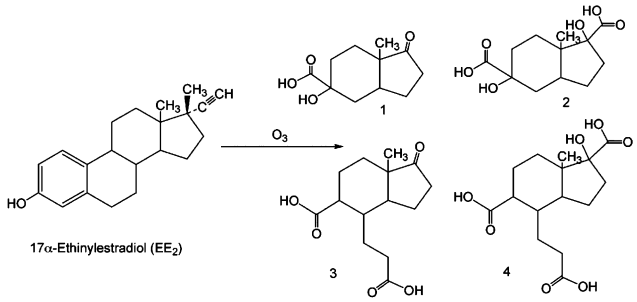
Ozone treatment of biologically purified water from wastewater treatment plants is reported to reduce concentrations of many pharmaceuticals below detection limits. The results are generally based on the disappearance of the parent compounds. This treatment would be useful in cases where the wastewater use poses ecotoxicological risks, such as irrigation in agriculture or dilution into surface waters.[321] The municipal wastewater effluents from conventional activated sludge treatment (CAS), as well as the effluent from a membrane bioreactor pilot plant, have been reported to be cleared of >90−99% of macrolide and sulfonamide antibiotics, estrogens, and the acidic pharmaceuticals diclofenac, naproxen, and indomethacin by O_3 doses of 2 mg/L in both effluents.[322] No degradation byproducts were identified.

Ozonation of the macrolid antibiotic lincomycin results in reduction of the toxicity of the treated solution toward the alga *S. leopoliensis* compared with untreated solutions containing the antibiotic.[201] Similarly, aqueous ozone (O_3 + $^{\bullet}OH$) treatment of several antibacterial compounds from nine structural families has been reported to selectively oxidize their biochemically essential moieties with a second-order rate constant.[323] Ozone treatment in synthetic wastewater at a dose rate of 2.96 g/L has also been reported to degrade in 1 h the fluoroquinole veterinary antibiotic enrofloxacin.[324]

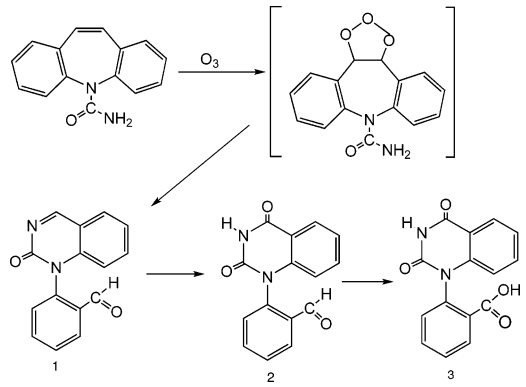
Ozonation of E_2 and EE_2 in distilled water results in transformation of >99% of the test compounds in 10 min to reach a stabilized estrogenic level assayed on a human breast cancer cell line (MCF-7).[315] Similarly, >99% degradation of EE_2 is reported on ozonation of Lake Zurich water to reduce the estrogenicity by 200-fold. The reduced estrogenicity has been attributed to the cleavage of the phenolic moiety of EE_2 (Scheme 20); the phenolic moiety is of particular importance for the binding of estrogens to the estrogen receptor.[325]

Unfortunately, full mineralization of pharmaceuticals is generally not achievable at the O_3 doses typically used in water treatment, resulting in the formation of oxidation byproducts. Ozonation completely transforms diclofenac, sulfamethoxazole, carbamazepine, and bezafibrate but not clofibric acid.[38] This treatment significantly reduces toxicity.[303] The ozonation products of carbamazepine in clean water have been identified as three quinazoline compounds (**1**−**3**; Scheme 21).[326]

Scheme 20. Ozonation of 17α-Ethinylestradiol (EE_2) Forms Products by Oxidation of the Phenolic Moiety by Ring-Opening and by Oxidation of Ethinyl Group; the Products I and III Are Also Obtained by Ozonation of Estradiol[325]



Scheme 21. Carbamazepine Ozonation Yields Three Quinazoline Compounds[326]



Different water treatment processes have been compared for their potential to eliminate pharmaceuticals. It has been demonstrated that among the classical drinking water treatment processes, only ozonation and filtration through granular activated carbon were effective in removing some pharmaceuticals.[38]

6.2.2. Catalytic Oxidation with Fe-TAML/Hydrogen Peroxide

Many pharmaceuticals found in the environment are fluoroaromatic compounds, which are relatively persistent both because of their engineered stability and because they are being continuously released. They constitute a worrying source of pollution because they are exceptionally bioactive and threaten toxicity and endocrine disruption potentials to humans, fish, algae, amphibians, and microorganisms. The development of Fe-TAML activators (Figure 34) of peroxide in Professor Collins' Institute for Green Oxidation Chemistry has made it feasible to begin attacking recalcitrant pollutants in the aquatic environment. In minute concentrations, Fe-TAML activators are able to unleash the oxidizing power of H_2O_2 in an efficient manner to rapidly oxidize a variety of substrates in water to environmentally acceptable end points.

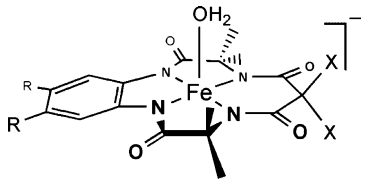


Figure 34. Fe-TAML activators of peroxide.

It is known that electron-withdrawing substituents, such as Cl and NO_2, can stabilize aromatic rings toward oxidative (or electrophilic) attack. In the course of Carnegie Mellon studies on the degradation of thiophosphate pesticides,[327] it was observed that nitrocresol undergoes facile degradation on Fe-TAML-activator/H_2O_2 treatment. Similarly, facile degradation of highly environmentally recalcitrant pentachlorophenol (PCP) and also 2,4,6-trichlorophenol (TCP) with Fe-TAML/peroxide has been reported earlier.[328] We have also observed total degradation of trichloropyridinol, 2-isopropyl-6-methyl pyrimidinol, and quinoxalinol.[329] The facile degradation of hydroxy-aromatics bearing electron-withdrawing chloro- and nitro-substituents suggests that Fe-TAML/H_2O_2 will be generally effective at degrading aromatic compounds bearing electron-withdrawing substituents, and this augers well for pharmaceutical degradations.

Several fluoro and other halogen-substituted aromatic ring containing pharmaceuticals are recalcitrant. Fe-TAML/peroxide has successfully degraded a number of these compounds as described in conference presentations. Degradation of atorvastatin (Figure 9B) with Fe-TAML/peroxide was found to result in 26 identified fragments separated by HPLC with partial mineralization. Similarly, sertralin (Figure 14C) underwent demethylation and further fragmentation.[330] The Fe-TAML/peroxide process has also been successful in degrading the antidepressant fluoxetine (Figure 14A).[331]

Other topical compounds studied in collaboration with Nancy Shappel of the USDA include 17β-estradiol (E_2; Figure 26A), estriol (E_3; Figure 26B), estrone (E_1; Figure 26C), and ethinylestradiol (EE_2; Figure 26D). Each compound in nanomolar concentrations is readily degraded ($\geq$95%, in minutes) on treatment with Fe-TAML/H_2O_2 (pH 10) at room temperature. Using LC MS−MS, neither estradiol metabolite, estriol nor estrone, was detected post-reaction.[332] In an earlier work, manganese peroxidase (MnP), a heme peroxidase enzyme or a laccase and 1-hydroxybenzotriazole (HBT) as mediator removed estrogenic activities (>80%) for both E_2 and EE_2 upon treatment for 1 h. Nearly all the estrogenicity was removed by extending the treatment time to 8 h.[333] Similar levels of estrogenic activity of E_2 could be removed in nearly 1/100th the process time by Fe-TAML/H_2O_2.[332]

7. Management of Human Pharmaceuticals in the Environment

Pharmaceuticals are inherently biologically active and often exquisitely potent. They are also designed to be resistant to biodegradation because metabolic stability usually improves pharmacological action. This contributes to their environmental persistence. As discussed earlier, pharmaceuticals often have physicochemical characteristics similar to harmful xenobiotics; for example, they are able to pass through membranes,[334] Human pharmaceuticals are ubiquitous water contaminants that have shown detrimental effects on aquatic organisms and may possibly affect human health also. The present PIE scientific programs are primarily toxicological, mostly aimed at measuring substances and their effects. Little has been done to prevent APIs from entering the environment in the first place. Various approaches have been advocated, including the control of pharmaceuticals at the source, the segregation of sources, the treatment of waste

products to remove pharmaceutical compounds, and the improvement of disposal systems for expired medicines[13,38,56]

In a recent survey on management of human pharmaceuticals in the environment, expert stakeholders in academia, government, and industry have acknowledged the high level of scientific uncertainty with regards to mixture effects, low-level chronic effects, and the lack of appropriate risk assessment methodology.[335] Low levels of EDCs in water are a major concern for society because EDCs exert physiological effects at very low concentrations that can translate into devastating human impairments. Our current knowledge of endocrine disruption makes it mandatory that we "guess an' fear" and work to rid drinking waters of all trace pharmaceuticals *that have not been proven to be safe for human consumption.* Given that obtaining such safety proof for any individual pharmaceutical would be extrordinarily difficult and perhaps impossible, we must restrict and regulate pharmaceuticals more aggressively, manage their use and disposal much more judiciously, and develop dramatically more effective technologies for removing them from water. Some ideas on prevention of the entry of pharmaceuticals in the environment and development of advanced wastewater treatment technology have been proposed. Other ideas that involve incentives for development of "green" pharmaceuticals and pharmaceutical-return programs have strong support.[335] Sweden has shown important leadership in this regard.[336]

7.1. Regulation of Pharmaceuticals

Detailed chemical analysis of water is a prerequisite for assuring the safety of water supplies. Limitations in our ability to identify contaminants in water also limit our ability to ensure water quality and to assess environmental impacts. The advances in analytical chemistry of recent years have dramatically improved our ability to measure trace pollutants in water, and it is a remarkable fact that only in recent times have we been able to easily detect and measure many endocrine disruptors at environmentally relevant concentrations that happen to correspond with concentrations capable of disrupting development. In 1999, the National Research Council determined that for typical natural water samples, the mass of compounds that is within the analytical window (i.e., that can be specified in terms of both a specific structure and concentration) is small compared with the total organic carbon. In studies using coupled gas chromatography/mass spectroscopy (GC/MS) in conjunction with derivatization, specifically identified compounds usually account for less than 12% (or <1 mg/L) of the organic carbon in different groundwater samples. The remaining 80% (or $2-10$ mg/L) of the total organic carbon typically remains uncharacterized other than in aggregate form (e.g., in terms of average chemical properties, functional group content, or size distribution).[337] While chemical analysis has advanced greatly in the intervening years, there is certainly still room for progress, both in the range of structures that can be easily identified and in the lowest concentrations that can be detected for any compound.

Although there is a great deal of uncertainty concerning the possible detrimental effects of pharmaceuticals on the aquatic ecosystems, the precautionary principal, which suggests that regulatory action against potential risk that is deemed unacceptable should be taken even when science has not established direct cause and effect relationships, may give rise to more stringent demands on wastewater treatment in the future. We believe that new scientific evidence will also advance the need for precautionary approaches. Substances such as natural estrogenic hormones, and especially synthetic female steroid hormones, are very biologically potent compounds. They are only partially eliminated during conventional wastewater treatment and have been measured in the effluent of wastewater treatment plants in United States and Europe.[338]

In the United States, water-quality standards do not regulate pharmaceuticals in reclaimed wastewater, drinking water, or natural waters. The National Research Council has identified pharmaceuticals as one of the several major classes of chemicals that have not received sufficient attention as potential water pollutants and recommended that they be considered for future versions of the EPA's Drinking Water Contaminant Candidate List.[339]

The U.S. FDA has required ecological testing and evaluation of a new pharmaceutical only if the predicted concentration when entering the environment in water or soil reaches or exceeds 1 μg/L or 100 μg/kg, respectively.[340,341] It has been suggested that this level was set to guard against acute effects (as opposed to chronic effects) on the basis of very limited toxicity information,[342] and the registration of pharmaceuticals is not dependent on the results of this assessment.[343] However, under current regulations, a company can obtain a "categorical exclusion" and not have to perform an environmental assessment if they manufacture less than 40 000 kg/year. Assuming that the drug is spread uniformly across the United States, 40 000 kg correlates to about 1 ppb in the aquatic environment.[105]

The European Union has embraced the paradigm of the "precautionary principle", which holds that a regulator responsible for, say, clean water, should respond to uncertainity about the toxic effects of a given chemical by setting a limit that EU holds to be safe in advance of more precise information.[344] The European threshold of 0.01 μg/L as a predicted environmental concentration (PEC), above which a more detailed assessment would be required, is 100 times more stringent than the U.S. FDA (1998) level for triggering a more detailed investigation.[345]

7.1.1. Safety Testing of Pharmaceuticals

Biologically, women have different vulnerabilities to chemicals than men, especially at certain times in their life cycle. Pregnancy is the most obvious time. Minute quantities of a drug taken by a pregnant woman at a particular stage in fetal development can cause deformities, cancer, and subtle cognitive effects. Some specialists believe no dose of synthetic hormones is safe for the developing embryo and fetus. As an example of the basis of the concerns, phthalate esters, which are not drugs, are potent EDCs. The global annual production is on the order of 10 billion lbs, and the entire US population is exposed virtually continuously; approximately 75% turn up as plasticizers in PVC. Phthalates have been unequivocally linked to severe impairment of the male reproductive tract in animals. Creative epidemiological studies by Shanna Swan's group of the University of Rochester have linked biomarkers of exposure in the mothers (singly hydrolyzed phthalates in urine) to biomarkers of effect in baby boys (anogenital distane) signaling that phthalates may also be potent impairing agents of the reproductive tract of human males.[346]

The cells in women's breasts appear to reach full maturity only at a first full-term pregnancy, at which time they become

more resistant to cancer-causing chemicals and radiation. Women of any age who have not had children may therefore have increased susceptibility to carcinogenic chemicals in the environment compared with women of the same age and health status who have had children. Furthermore, on average, women have more fatty tissue than men, so they can store more lipophilic endocrine disruptors in their bodies. That women have adverse reactions to drugs more often than men may derive from physiological differences that make women more susceptible to some drug-related health risks.[347]

Despite the evidence of the particular damage chemicals can have on women's health, safety standards for chemicals have often been based on healthy white adult males. Research on male animal models, and on men, is easier to conduct precisely because researchers do not have to contend with the hormonal fluctuations of monthly cycles, pregnancy, and menopause—processes that are strongly regulated by hormones and, therefore, possible targets of endocrine disruptors.[347]

More research is needed concerning the timing of exposures to EDCs as better testing for pharmaceuticals and PIE exposures are developed for humans. Endocrine disruption impairments can be associated with particularly short-lasting exposures during pregnancy. For example, in a recent study of the impact of the prostate cancer drug flutamide on reproductive endpoints in the male offspring of exposed pregnant rats, the impairments varied greatly and manifested in different components of the male reproductive tract and associated features of masculinity (nipples, hypospadias, seminal vesicles, prostate, bladder) depending on the day on which the mother was exposed.[348]

7.2. Preventing the Entry of Pharmaceuticals into the Aquatic Environment

The pharmaceutical industry is enormously important both to maintaining human health and to the economy. Control of pharmaceuticals by restricting or banning their use will likely be even more difficult to accept than banning other chemicals, especially when there is a clear benefit to suffering people. In this context, regulating the pathways by which the pharmaceuticals enter the environment may be a suitable alternative.[349]

Source separation aimed at preventing the entry of drugs and their metabolites into the aquatic environment may offer a more sustainable solution to the entire wastewater problem, including organic micropollutants.[350] For example, segregating sources of pharmaceuticals, such as hospital wastewater, which is likely to be heavily contaminated with pharmaceuticals and antibiotic-resistant bacteria, could make it possible to focus treatment resources on the most contaminated waters.[145] Another approach could be urine source separation, which has been suggested as an elegant solution to the problems of nutrients and pharmaceuticals alike and for minimized losses of untreated pollutants to the environment.[351−354] Since anthropogenic organic chemicals are in general metabolized to a polar water-soluble form to allow excretion by the kidney, this approach is of special interest for the question of pharmaceuticals in wastewater. Urine source separation would also prevent wasting of nutrients and potentially hazardous micropollutants from entering the wastewater stream.[353] It would be a major achievement if drug metabolites in urine, which often are bioactive and potentially toxic in the environment, could be prevented from entering waterways.

Collection of urine for use in evaluating biomarkers of exposure has become relatively common due to the rapid metabolism and excretion patterns of many environmental contaminants of concern. Analysis of urine samples for relevant metabolites can provide important information for estimation of human exposure to environmental contaminants and for evaluation of predictive models used in regulatory decision-making. Although few technologies for the separation of urine have been developed to date, the 100−500 times higher concentrations of micropollutants promise more efficient conditions for all removal technologies known for conventional wastewater treatment.[355]

7.2.1. Pharmaceutical Return Program

In a survey on the household disposal of unused and expired pharmaceuticals as a source of pharmaceutical compounds in the environment, it emerged that a prominent disposal route of out-of-date or unwanted medicines may be via the sink/toilet or in household waste that is then taken to landfill sites, which, via leaching, end up in groundwater.[54]

The U.S. Federal prescription drug disposal guidelines (2007)[356] issued in response to rising trends in prescription drug abuse and potential environmental concerns, include flushing when it is instructed to be safe to do so and return of unused, unneeded, or expired medicines to pharmaceutical take-back locations for safe disposal. It is important that time-expired or unused medines are kept out of landfill sites and water supplies. Through pharmaceutical-return programs, residual medications can be collected from the public at take-back locations and disposed of in an environmentally sound manner.

In Spain, pharmaceutical return programs have been set up by the pharmaceutical industry to collect unused and expired medicines. Similarly, in British Columbia, Canada, provincial waste management regulations require all brand owners of pharmaceutical products to fund and organize pharmaceutical-return programs involving efficient collection and safe disposal of leftover medicines returned by the public.[357] The stakeholder's survey also indicated strong support for the implementation of return programs for unused/expired medications, coupled with public education regarding the need to return drugs rather than flushing them down the toilet or disposing of them in the garbage.[335] In the U.S., the EPA has suggested the desirability of a national regulation for disposal of unwanted and expired pharmaceuticals and personal care products and of implementing an "extended producer responsibility" for manufacturers and distributors. The agency has listed these issues as outstanding research needs.[358]

7.2.2. Advanced Wastewater Treatment and Incineration of Solid Waste

Ozonation and membrane filtration have achieved removal rates of >95% for many pharmaceutical compounds[20,89,319,321,359] compared with an average of 60% for secondary wastewater treatment plants (WWTPs).[17] In the European Union, all cities are required to have a secondary WWTP as a minimum. Germany, Switzerland, and The Netherlands incinerate their municipal solid waste, rather than send it to landfills, thus preventing pharmaceuticals from leaching into groundwater. Switzerland also incinerates sewage sludge and prohibits its spreading on agricultural land to mitigate contamination of surface water.[335] This has been seen as an effective end-of-pipe strategy for removing

pharmaceutical contaminants that also addresses several sources of water contamination and scored highest (8 out of maximum 10) in the expert stakeholders survey in terms of effectiveness.[335]

7.3. Ecofriendly Pharmaceuticals

Sweden's Stockholm County Council has been working toward the assessment and classification of pharmaceuticals according to their environmental impact: persistence, ability to resist removal from or degradation in the aquatic environment; bioaccumulation, accumulation in adipose tissue of aquatic organisms; and toxicity, the potential to poison aquatic organisms. Each of these properties is assigned a value on a scale of 0 to 3. The sum of these values constitutes the PBT index for the pharmaceutical. A PBT value of 0 in each category would indicate that a drug is readily biodegradable, is not bioaccumulating, and has low ecotoxicity. At the other end of the scale, a value of 3 indicates the highest level for these unfavorable parameters for drugs in the environment. A drug with a low PBT index is environmentally preferable to one with a higher score, with 0 being best and 9 being worst. Where more than one drug of similar action and efficiency is available to treat an ailment, the idea is that the drug with the lowest PBT index should be chosen.[360] It is hardly likely that a medicine would be banned because it is not biodegradable in the environment. However, an environment label is being introduced in Sweden with the assistance of the chemical industry, which would enable the physician and the patient, where medications of similar action and efficiency are available, to select the treatment that is more environment friendly[336,361]

7.3.1. Development of "Green" Drugs

The ideal research trajectory for obviating PIE problems would be to replace persistent pharmaceuticals with a new suite of pharmaceuticals designed to incorporate an added criterion that would facilitate their rapid removal upon release to the environment. This could take the form of a built in chemical switch, as has been suggested by Paul Anastas in conversation with Terry Collins, that once activated would lead to rapid decomposition. Such switches would need to be not activated by any of the body's diverse chemistries but activated upon excretion. As a second attractive idea, removal might also be achieved via the attachment of affinity groups that could facilitate quantitative sorption on some support to be employed at treatment plants. However, the structure−function-related pharmacodynamics and pharmacokinetics of important pharmaceuticals entail numerous subtle and demanding requirements. Such searches for panaceas to persistent pharmaceuticals may simply be hoping for the impossible. But it is important to keep intellectually attractive solutions in mind and to allow creative young investigators the leeway to explore them. The development of green products and degradation technologies will also protect the industry from potential future liabilities.[342]

7.4. Placing the Onus of Responsibility on Industry

The EU White Paper "Strategy for a Future Chemicals Policy," is currently being implemented in EU legislation. It is designed to make industry responsible for the safety of its products. The White Paper specifies a mandatory authorization procedure for substances that are *carcinogenic, mutagenic or toxic to reproduction, or persistent, bioaccumulative, and toxic*; that is, special attention is paid to endocrine disrupters and substances with persistent organic pollutant (POP) characteristics. Under the proposed system, registration of basic information will be required for all substances with an annual production volume exceeding 1 ton, regardless of whether they are classified as new or existing, thus requiring the chemical industry to assume greater responsibility for its products.

In addition to a regulatory responsibility for safety of medicines, as more is discovered about the pharmaceutical effects in the evironment in the coming years, the pharmaceutical industry could become liable to class action lawsuits and targeted by environmental activists for being seen as the source of the problem.[342] where litigants will attempt to characterize the industry a negligent source. Biomonitoring, which measures the concentration of a chemical in a body fluid/tissue in humans and evolving analytical methods for detecting pharmaceuticals are likely to drive public concerns and increase the focus on ecotoxicity issues.

8. A Green Chemistry Perspective

With pharmaceuticals, a great deal of precommercialization effort is invested by the industry into ensuring that they are not unacceptably hazardous to health outside the sphere of the malady or condition they are aimed at treating. But the direct experimental human studies required to ensure that any particular pharmaceutical would not produce developmental impairment in humans could, in many cases, be very difficult and prolonged in their performance and also constrained on ethical grounds. Shanna Swan's work with phthalates is important in indicating that creative epidemiological studies can provide the information on the impacts of EDCs on humans in a relatively straightforward manner. All this is not to say that the pharmaceutical industry cannot more adequately protect the public from repeat performances of the thalidomide tragedy, but in the area of impairments that may not materialize until development is completed or later still, we have so much more to learn that it is entirely appropriate to "guess an' fear". For example, a recent Massachusetts study has shown that there has been a population-level decline in serum testosterone levels in American men of 17% since the late 1980s.[362] So what is causing this and is the increasing use of pharmaceuticals in the United States a factor? Could there be a connection with PIE APIs that have been ingested through drinking water? Of course, this is only one of numerous studies of observed human impairments where it is reasonable (and not alarmist because society must look everywhere for the answers) to ask such questions. We believe that it is definitely in the industry's long-term interest to acknowledge the need for independent studies to answer such questions. As with so many of the anthropogenic chemicals that we have introduced into the ecosphere, we would be duping ourselves to the total suppression of common sense were we to continue the 20th century practice of giving "the prophecy of bliss" precedence over the "prophecy of doom" in considering the balance of pluses and minuses that are likely to result from pharmaceuticals.[363] The addition to water of a cocktail of trace pharmaceuticals, compounds that are designed to exhibit potent physiological activity, is a very important emerging water issue calling for prudent examination and strategic planning to obviate potential negatives.

One of the great lessons of the last century of scientific analysis is that water purity has a huge impact on the welfare not only of humans but also of all other living things. A corollary is that when negative impacts of water-borne anthropogenic chemicals are observable in aquatic organisms, harmful impacts on humans are to be considered more probable. In our view, negative impacts on aquatic organisms present sufficient reason for corrective actions without the need for proof of human impairment. We argue this not from a precautionary point of view with respect to human health, but because we believe that impairment of other living things than humans represents a prima facie case for removing the source of the impairment.

We close as we started by reminding the reader that at the deepest level of concern lies the analysis originating from Dr. Theodora Colborn that certain anthropogenic chemicals can impair wildlife by disrupting components of the endocrine systems that control cellular development.[1] Since Theo Colborn's endocrine disruption insight first became public, the rapidly advancing research surrounding it has only added to the gravity of the case for both humans and animals. Certain xenobiotics have the ability to drastically impair living things including humans. This gives rise to a fundamental question that society and science must struggle with for the foreseeable future and with which our educational, industrial, economic, political, regulatory, and legal systems are currently ill-prepared to deal; "What is the ultimate value of a chemical technology, be it pharmaceutical or otherwise, that improves the welfare or comfort of existing adult generations, if at the same time it is known to be or may be capable of undermining the welfare of developing or future generations which are so much more sensitive to the impairments that derive from hormonal disruption?" With the international struggles that flash across TV screens on a daily basis, people worldwide are becoming far more aware of what life is like in our different cultures. It has not escaped our imagination that an ultimate irony for the peoples of developed countries could be that in decades to come, about the best thing that could happen to a human being is that he or she would be born in a community as isolated as possible by culture and geography from anthropogenic chemicals because that would guarantee the least impairment. To ensure this never happens, it makes sense for every section of the chemical enterprise to take developmental impairment seriously and to work energetically to understand how to avoid it.

It is in the context of these lessons and their hard-to-answer questions that our chemocentric civilization must struggle with the often-competing interests of short-term economic advancement and the sustainability wisdom that requires a much longer-term perspective. To define the struggle in which green chemists are rightful protagonists, we must search for a path to a vital chemical economy that can function in harmony with the welfare of living things. While this is a review about environmental aspects of pharmaceuticals, it also has implied relevance for chemistry in general, since chemicals are key to the dynamic context in which our civilization has been rapidly evolving in its technological dimension while arguably lacking effective rudders for directing its evolution toward sustainable trajectories. This is the stage upon which green chemistry must act out its mission to reduce or eliminate hazards in all areas of chemical products and processes including pharmaceuticals,[6] thus becoming the sustainability rudder for the chemical enterprise.

It is conceivable that the pharmaceutical industry could become a standard bearer for sustainable development, especially because its primary mission is to protect human health. The industry already has numerous achievements in greening its synthetic processes. By turning its intellectual and economic resources toward promoting internal and independent studies of PIE problems, its ability will be strengthened for improving the health of incumbent generations while consciously averting the environmental and transgenerational injustices that PIE problems do already or could represent.

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10. Note Added after ASAP Publication

This review was published ASAP on May 27, 2007 with errors in Figure 1 and the first paragraph of section 2.2.2. The corrected version was published ASAP on May 31, 2007.

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