Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change?

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During the last three decades, the impact of chemical pollution has focused almost exclusively on the conventional "priority" pollutants, especially those acutely toxic/carcinogenic pesticides and industrial intermediates displaying persistence in the environment. This spectrum of chemicals, however, is only one piece of the larger puzzle in "holistic" risk assessment. Another diverse group of bioactive chemicals receiving comparatively little attention as potential environmental pollutants includes the pharmaceuticals and active ingredients in personal care products (in this review collectively termed PPCPs), both human and veterinary, including not just prescription drugs and biologics, but also diagnostic agents, "nutraceuticals," fragrances, sun-screen agents, and numerous others. These compounds and their bioactive metabolites can be continually introduced to the aquatic environment as complex mixtures via a number of routes but primarily by both untreated and treated sewage. Aquatic pollution is particularly troublesome because aquatic organisms are captive to continual life-cycle, multigenerational exposure. The possibility for continual but undetectable or unnoticed effects on aquatic organisms is particularly worrisome because effects could accumulate so slowly that major change goes undetected until the cumulative level of these effects finally cascades to irreversible change—change that would otherwise be attributed to natural adaptation or ecologic succession. As opposed to the conventional, persistent priority pollutants, PPCPs need not be persistent if they are continually introduced to surface waters, even at low parts-per-trillion/parts-per-billion concentrations (ng-μg/L). Even though some PPCPs are extremely persistent and introduced to the environment in very high quantities and perhaps have already gained ubiquity worldwide, others could act as if they were persistent, simply because their continual infusion into the aquatic environment serves to sustain perpetual life-cycle exposures for aquatic organisms. This review attempts to synthesize the literature on environmental origin, distribution/occurrence, and effects and to catalyze a more focused discussion in the environmental science community. *Key words*: aquatic, drugs, ecologic health, ecologic risk assessment, emerging risk, pharmaceuticals, pollution, sewage. — *Environ Health Perspect* 107(suppl 6):907–938 (1999).
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Summary

Risks associated with previously unknown, unrecognized, unanticipated, or unsuspected chemical pollutants in the environment have long been a major concern of environmental scientists. The importance of identifying such emerging risks is reflected in one of the top five goals of the Strategic Plan 2000 for the U.S. Environmental Protection Agency's (U.S. EPA) Office of Research and Development. Early identification and investigation of potential environmental pollution issues before they worsen are critical for protecting ecologic and human health. It is also important to rule out issues that could be of concern but prove otherwise, so that limited resources can be redirected. Ecosystem change is effected by human activities primarily via three routes: habitat fragmentation, alteration of community structure (e.g., via nonindigenous species), and chemical pollution. The scope of the former two is highly delineated and obvious compared with the latter. During the last three decades, the impact of chemical pollution has focused almost exclusively on the conventional "priority" pollutants. This group of chemicals, however, is only one piece of the larger puzzle.

One large class of chemicals receiving comparatively little attention comprises the pharmaceuticals and active ingredients in personal care products (PPCPs), which are used in large amounts throughout the world; quantities of many are on par with agrochemicals. Escalating introduction to the marketplace of new pharmaceuticals is adding exponentially to the already large array of chemical classes, each with distinct modes of biochemical action, many of which are poorly understood. In contrast to agrochemicals, most of these products are disposed or discharged into the environment on a continual basis via domestic/industrial sewage systems and wet-weather runoff. The bioactive ingredients are first subjected to metabolism by the dosed user; the excreted metabolites and unaltered parent compounds can then be subjected to further transformations in sewage treatment facilities. The literature shows, however, that many of these compounds survive biodegradation, eventually being discharged into receiving waters; metabolic conjugates can even be converted back to their free parent forms. Many of these PPCPs and their metabolites are ubiquitous and display persistence in, and bioconcentration from, surface waters on par with those of the widely recognized organochlorine pollutants. Additionally, by way of continual infusion into the aquatic environment, those PPCPs that might have low persistence can display the same exposure potential as truly persistent pollutants since their transformation/removal rates can be compensated by their replacement rates.

Although certain biochemical actions of many drugs in humans have been elucidated, these actions are not necessarily always the ones responsible for the purported physiologic target effects. Sometimes the known pathways of action may have nothing to do with the actual desired effect, as the actual mechanism remains totally unknown. Understanding of the complex biochemical signaling pathways is currently too limited to design drugs that act only via targeted routes, and even then, if their activity can be limited to a single type of receptor, the tissue distribution of the receptor may not be fully known. Unpredicted and unknown side effects are often the norm. The possible actions and biochemical ramifications on nontarget aquatic biota are even less understood; many are totally unknown. The few that are known to elicit subtle but dramatic effects on aquatic life at very low concentrations, however, may point to an ill-defined vulnerability in aquatic ecosystems. A major concern is not necessarily acute effects to

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nontarget species (effects amenable to monitoring once they are understood), but rather the manifestation of perhaps imperceptible effects that can accumulate over time to ultimately yield truly profound changes—those whose causes would be obscured by time and that would not be distinguishable from natural events. The specter of subtle, cumulative effects could reduce the usefulness of current toxicity-directed screening methods in testing waste effluents for toxicologic end points due to PPCPs. Subtle effects, from low concentrations of bioactive PPCPs, whose continual expression over long periods of time in certain nontarget populations, could lead to cumulative, insidious, adverse impacts that would otherwise be attributed to natural change/adaptation or ecologic succession—any "signal" would be lost among the noise. Current comprehensive environmental risk assessments and epidemiologic studies do not factor in exposures/body burdens from PPCPs and therefore may be flawed by over simplicity.

It is useful to note that the data reported and evaluated in this review reflect the diverse and uneven nature of the PPCP literature published for source/origin, occurrence, distribution, transport, transformation, ecologic exposure and effects, risk assessment, and test strategies. The comprehensiveness of the published literature in each of these areas and across the broad spectrum of PPCP classes is very unequal. This review therefore does not present an exhaustive and rounded view of this emerging topic but rather summarizes most of the significant papers in an integrated, comprehensive manner, and thereby elucidates many of the questions that still need to be addressed by the environmental science community. This review aims to catalyze a discussion on the potential importance of PPCPs in the environment and presents recommendations for focusing further research (Table 1).

Introduction

For the purposes of this discussion, pharmaceutical (and veterinary and illicit) drugs (and the ingredients in cosmetics, food supplements, and other personal care products), together with their respective metabolites and transformation products, will collectively be referred to as pharmaceuticals and personal care products. PPCPs are continually infused into the environment via sewage treatment facilities and wet weather runoff. In many instances, untreated sewage is discharged into receiving waters (e.g., flood overload events, domestic "straight-piping," or sewage waters lacking municipal treatment). In the United States alone, possibly more than a million homes do not have sewage systems but instead rely on direct discharge of raw sewage into streams by straight-piping or by outhouses not connected to leach fields (*1*). A number of Canadian cities are reported to discharge 3.25 billion liters per day (over 1 trillion liters per year) of essentially untreated sewage into surface waters and the ocean (*2*). Raw/treated sewage is also disposed of from some locales in the deep ocean where it may possibly remix with upper waters.

We hope that this overview of PPCPs in the environment will *a*) catalyze a concerted effort among environmental chemists and ecotoxicologists to survey sewage treatment effluents, surface waters/groundwaters, and potable water for the presence of PPCPs and their bioactive transformation products and to determine their origins; *b*) elucidate the spectrum of possible physiologic effects of PPCPs on nontarget species, especially those that are aquatic; and *c*) promote discussion of whether this is an environmental issue deserving further investigation. We believe that a scientific debate on this topic is warranted given the evidence that has been accumulating over the last two decades on the occurrence of various pharmaceuticals in sewage effluent and in both surface waters and groundwaters. The big unknown is whether the combined low concentrations from each of the numerous PPCPs and their transformation products have any significance with respect to ecologic function, while recognizing that immediate effects could escape detection if they are subtle and that long-term cumulative consequences could be insidious. Another question is whether the pharmaceuticals remaining in water used for domestic purposes poses long-term risks for human health after lifetime ingestion via potable waters multiple times a day of very low, subtherapeutic doses of numerous pharmaceuticals; this issue, however, is not addressed in this review.

The hypothesis is further complicated by the fact that while the concentration of individual drugs in the aquatic environment could be low (sub-parts per billion or sub-nanomolar, often referred to as micropollutants), the presence of numerous drugs sharing a specific mode of action could lead to significant effects through additive exposures. It is also significant that drugs, unlike pesticides, have not been subjected to the same scrutiny regarding possible adverse environmental effects. They have therefore enjoyed several decades of unrestricted discharge to the environment, mainly via sewage treatment works. This is surprising especially since certain pharmaceuticals are designed to modulate endocrine and immune systems and cellular signal transduction and as such (as opposed to pesticides and other industrial chemicals already undergoing scrutiny as endocrine disruptors) have obvious potential as endocrine disruptors in the environment. Exposure to PPCPs in the environment, especially for aquatic organisms, may differ from that of pesticides and other industrial chemicals in one significant respect—exposures may be of a more chronic nature because PPCPs are constantly infused into the environment wherever humans live or visit, whereas pesticide fluxes are more sporadic and have greater spatial heterogeneity. It is quite apparent that little information exists from which to construct comprehensive risk assessments for the vast majority of PPCPs having the potential to enter the environment.

Although little is known of the occurrence and effects of pharmaceuticals in the environment, more data exist for antibiotics than for any other therapeutic class. This is a result of their extensive use in both human therapy and animal husbandry, their more easily detected effects end points (e.g., via microbial and immunoassays), and their greater chances of introduction into the environment, not just by sewage treatment plants, but also by run-off and groundwater contamination, especially from confined animal feeding operations (CAFOs). The literature on antibiotics is much more developed because of the obvious issues of direct effects on native microbiota (and consequent alteration of microbial community structure) and development of resistance in potential human pathogens. Because of the considerably larger literature on antibiotics, this review only touches on the issue; for the same reason, this discussion only touches on steroidal drugs (those purposefully designed to modulate endocrine systems).

For the purposes of this document, pharmaceuticals will refer to nonbiologic drugs (i.e., those that do not comprise proteinaceous or nucleotide material). The number of biologics approved by the U.S. Food and Drug Administration (FDA) is growing, and their fate in the environment is unknown. This overview covers only a subset of the commercially available classes of pharmaceuticals and active ingredients in personal care products. The subset of classes discussed in this review comprises the primary classes for which the limited data on environmental occurrence and effects on nontarget species can be found, in a highly fragmented, disjointed, and disparate literature.

Pharmaceutical drugs are chemicals used for diagnosis, treatment (cure/mitigation), alteration, or prevention of disease, health condition, or structure/function of the human body. The definition is extended to veterinary pharmaceuticals and can also be applied to illicit (recreational) drugs. It also must be noted that the active ingredient in a drug may or may not be the actual formulated parent compound. For example, prodrugs such as the esters of clofibric acid, a metabolite of certain lipid regulators, are converted from pharmacologically inactive parent

Table 1. Conclusions, potential research needs, and recommendations.

Conclusion/finding	Research needs and recommendations
Chemical identification	
Of all the aspects of pharmaceuticals in the environment, the one that is perhaps the best developed is chemical identification and quantitation. The trend in pharmaceuticals toward higher potency (e.g., enantiomerically pure drugs) while serving to reduce the burden of pharmaceuticals in the environment will add an additional challenge to the analytical effort required to characterize environmental samples because the required detection levels will be even further lowered from the current ppt-ppb levels. Identification of nontarget (unsuspected) toxicants in complex waste streams by toxicity-directed assay of fractions is insufficient (because of the exponential complexity of stressor-receptor combinations). Direct, rigorous chemical characterization of problematic samples must play a role in identifying toxicants that might present previously unrealized (e.g., subtle) effects in nontarget organisms.	A feature distinguishing PPCPs from the currently recognized persistent organic pollutants (POPs) is the higher polarity of the parent PPCPs. This, coupled with their low concentrations, necessitates more work in the area of analysis, especially preconcentration. More development is also required for sensitive chemical analysis approaches to polar pollutants, which are not directly amenable to conventional protocols. The environmental monitoring community would benefit from additional analytical methods, including improved cleanup/preconcentration techniques, possibly based on highly specific approaches such as immunochemical or molecular imprinting [a highly sensitive, specific, and cost-effective technique that has already shown promise for nerve gas hydrolysis products, e.g., (*147*)]. In the absence of comprehensive ecotoxicity tests that can accommodate the wide range of PPCPs and broad spectrum of possibly subtle effects, screening must also rely on rigorous chemical characterization—often for nontarget analytes. The results can then be used to direct subsequent toxicologic testing. Standard reference materials for pharmaceuticals and their metabolites need to be made more widely available at lower cost to environmental researchers to aid in monitoring activities. The NIST/EPA/NIH Mass Spectral Library (*148*) needs to be expanded to encompass a larger set of pharmaceuticals (those that are directly amenable to gas chromatography) as well as their derivatives; these spectra are currently available only in specialty databases such as Pfleger/Maurer/Weber (*3,4*). Non-EI (electron ionization) spectra need to be produced for the nonvolatile PPCPs (e.g., see *http://www.chemicalsoft.de/a.htm*).
Source and occurrence	
Wide arrays of PPCPs representing a diverse spectrum of modes of action are used throughout the world in large quantities, rivaling those of agrochemicals. The major sources of PPCPs in the environment are primarily STW effluent and, secondarily, terrestrial run-off (e.g., from CAFOs). Some PPCPs (e.g., blood lipid regulators such as clofibric acid, X-ray contrast media, and musks) are ubiquitous and extremely persistent in the environment. Only a very small percentage of commercially used PPCPs have even been investigated for their occurrence in the environment. Drug classes that will experience huge usage rates (e.g., impotence drugs such as sildenafil citrate) have no associated environmental occurrence or exposure data. Although the genotoxic potency of industrial wastewaters is often the highest, the overall loadings of genotoxic compounds to surface waters are far greater (up to several orders of magnitude) from municipal treatment plants—and antineoplastic drugs might play the largest role. Aquatic monitoring efforts that focus on accumulation of pollutants in filter feeders may be grossly underestimating the levels of many pollutants, simply because functional MXR systems keep these pollutants at abnormally low concentrations within their cells. The corollary to this is that many aquatic organisms may be more susceptible to more hydrophilic toxicants (those that MXR systems are less effective at dealing with).	A systematic survey of potential drugs in waterways (especially those receiving hospital effluents) and their sources should be undertaken for those PPCPs that are most persistent or that elicit effects on aquatic life at very low concentrations (e.g., clofibric acid, antineoplastics, amino-nitro musks, SSRIs, chemosensitizers). To date (and very roughly), occurrence data for only about 50 nonantibiotic drugs (of the thousands in use today) have been published; numerous others may be present in the aquatic environment. Screening: Given the large numbers of pharmaceuticals that could be present in receiving waters, a rough screening approach is needed for assessing the potential of pharmaceuticals to occur. Samples with high potential could then be subjected to more rigorous analysis for individual targets. A possible approach might rely on analyses for only two widely used PPCPs/metabolites/inactive ingredients. The first would serve as a "conservative" indicator, one that is relatively easily biodegraded and whose presence would indicate that the possibility is high that many other (less degradable drugs) are also present. The second would also be ubiquitously used in large quantities but would be relatively persistent and relatively easily analyzed (e.g., musks). By monitoring its presence in receiving waters (and determining subsequent concentration gradients), the dilution of any drug (from the source) could be determined. Monitoring programs focusing on aquatic systems should consider that bioaccumulated tissue concentrations may be aberrant, depending on the degree of MXR induction or inhibition.
Fate	
The low concentrations of individual PPCPs (possibly exceeding the catabolic enzyme affinities of sewage microbiota), coupled with their metabolic "novelty," leads to incomplete removal from STWs. Compared with POPs, there is a paucity of information on the fate, especially, biotransformation and phototransformation, of PPCPs. The low volatility of PPCPs means that their distribution through the environment will primarily occur through aqueous transport and food-chain dispersal. The polar, nonvolatile nature of most drugs prevents their escape from the aquatic realm. ("Global distillation" presumed to occur with POPs would not be a factor.) Drug conjugates potentially act as storage "reservoirs" from which the free parent drug can later be released (e.g., via hydrolysis) in the environment.	The nearly unknown ramifications of PPCPs in the environment (fate, transport, effects) warrant a more precautionary view on their environmental disposition. Environmental scientists need to focus more attention on this concern. An effort similar to that which was invested in elucidating the environmental transformation and fate of pesticides and industrial "toxics" (especially POPs) may need to be made for PPCPs. Fate studies that simply follow disappearance (removal) of a PPCP will underestimate the level of parent compound (e.g., because of reservoirs of conjugates) and completely miss any bioactive metabolites.

Table 1. *Continued.*

Conclusion/finding	Research needs and recommendations
Exposure	
An extreme diversity of stressor–receptor possibilities (most of which have yet to be identified) exists for nontarget species exposed to PPCPs and their metabolites entering the environment and serves to exacerbate an already complex problem. The bioconcentration/bioaccumulation potential for at least some PPCPs (e.g., nitro musks) matches that for many of the more persistent organohalogen POPs. Because the main source of PPCPs in the environment (STWs) allows for continual, year-long introduction of these chemicals into the environment, outright persistence of an individual PPCP does not play the overwhelming role ordinarily found in governing exposure. Even relatively short-lived PPCPs could effect significant chronic exposures, as they are continually infused to the aquatic environment. Aquatic organisms are captives of their environment and therefore suffer perpetual exposure. Organisms in less polluted waters may be at more risk from newly introduced chemicals than those in more polluted areas simply because their levels of MXR are not as fully developed. Even naturally occurring PPCPs (e.g., nutraceuticals) could present risks to nontarget species because their usage serves to redistribute and extend their normal occurrence in the environment, promoting exposure to nontarget organisms that otherwise would never occur, and possibly resulting in higher concentrations in surface waters than would normally occur at their geographic sites of origin. Aquatic exposure can be increased in receiving waters having lower flows (e.g., smaller streams or during dry weather). On the other hand, wet weather and seasonal transitions can disrupt STWs and lead to poor removal efficiencies. Discharge of untreated sewage maximizes exposure.	Guidance is needed to determine those aquatic (and to a lesser extent, certain nonaquatic) organisms most susceptible to exposure to PPCPs. Although little is known regarding nontarget effects in the aquatic environment, the SSRIs have the most data pointing to the potential for subtle behavioral/reproductive effects (at low concentrations), and the musks (nitro/amino) for acute effects, but nothing is known about their occurrence or fate in the environment. Much more research is needed to establish whether aquatic exposures are significant for PPCPs. Although the introduction of PPCPs to STWs might remain relatively constant, wet weather and seasonal transitions (leading to overflows or upsets) can lead to increased aquatic exposures that must be accounted for in determining exposure ranges. Monitoring MXR activity in aquatic organisms should be pursued as a means of measuring overall health due to exposure. Perhaps more concern should be directed at exposure of organisms in more pristine aquatic locations than those in areas receiving established, known pollutant loads because the former are more at risk to effects from the introduction of a new pollutant since they have lower MXR activity. Similarly, the introduction of a pollutant to a pristine aquatic environment may pose more toxicological significance than for a more polluted environment. Detection of exposure of fish to many drugs can be facilitated through the analysis of bile.
Effects	
Some PPCPs (e.g., nitro and amino-nitro musks) show very high acute aquatic toxicity. Others (e.g., SSRIs) can elicit constellations of significant but subtle effects across numerous species. These effects are not necessarily readily detectable but have the potential to lead to ecologic change that would be erroneously attributed to natural change. Although pharmaceuticals with broad modes of action (e.g., antineoplastics) may pose cause for concern in nontarget species, recent evidence shows that those with highly specific mechanisms (e.g., SSRIs) can elicit profound effects at extremely low concentrations. It is clear that aquatic life can be exquisitely sensitive to at least some PPCPs (e.g., SSRIs). Between-species, between-sex, and between-drug effects can also vary widely. Gross within-class differences regarding aquatic effects possibly make the approach of assessing ecologic risk on a class-by-class basis untenable. For example, some SSRIs are extremely potent, whereas others have almost no effect. A trend among individual drugs of a given class concerning effects on one species may not hold for other end points in the same species. Simple extrapolations of aquatic effects from higher concentrations do not necessarily have any predictive value for lower concentrations. Antineoplastics harbor potential concern for environmental effects, not just for their acute toxicity but for their ability to effect subtle genetic changes, the cumulative impact of which over time could lead to more profound ecologic change. Chemosensitizers—those chemicals that inhibit multixenobiotic transporters—may play key roles in potentiating the effects of PPCPs. Little is known, however, as to how prevalent this ability is among pollutants. The capacity of MXR can be overwhelmed by nonspecific agents that simply competitively overwhelm the MXR mechanism, but which otherwise would not be toxic. Since many drugs are relatively polar (in contrast to most "conventional" pollutants), the defensive utility of MXR may not be effective for many PPCPs. The EDSTAC screening strategy will focus initially on only the three primary hormone systems—estrogen, androgen, and thyroid—hormone systems of relatively unknown importance to invertebrates.	Practically no aquatic toxicity data, especially behavioral effects, exists for PPCPs, even for those known to occur ubiquitously (e.g., blood lipid regulators, musks). This is a major unaddressed area. Although some studies have been done peripherally (e.g., MEIC) (*125*), none have been dedicated to PPCPs in the aquatic environment. Ecotoxicity tests need to better accommodate subtle end points (e.g., behavioral/genetic modifications), whose continued expression over long periods of time in certain populations could lead to adverse impacts that would otherwise be attributed to natural change. These tests need to address the higher levels of organization as expressed on the population/community structure level. Ecotoxicity screening procedures must be developed that take into consideration the modes of action (currently largely unknown) of PPCPs on nontarget species. Research is particularly needed to identify those PPCPs that act as chemosensitizers for aquatic organisms. Quick assays for multixenobiotic resistance/inhibition (e.g., those using dyes) (*34*) would be particularly valuable. More attention is required to identify those PPCPs that modulate the endocrine systems of, or act as behavioral/developmental signaling agents in, aquatic species (e.g., retinoid receptors).

Table 1. *Continued.*

Conclusion/finding	Research needs and recommendations
Risk assessment	
The approach of assessing ecologic risk on a class-by-class basis (either by chemical or by mode of action) may not be feasible given that some drugs within the same class (e.g., SSRIs) display effects at concentrations differing by many orders of magnitude. Evidence that the persistence and bioaccumulative potential of at least some PPCPs can be similar to the problematic organohalogen POPs should necessitate their consideration in comprehensive risk assessments. Over the decades, innumerable epidemiologic studies have purported correlations of various disease states with the body burdens of particular pesticides/industrial pollutants. The findings of these studies may well be flawed, as they made no attempt to also consider the possible effects of PPCP body burdens. Any comprehensive risk assessment must factor in the exposures/body burdens of all pollutants, regardless of origin—and PPCPs are perhaps the most ignored remaining major class of pollutants.	The approval of pharmaceuticals needs to be better coupled with meaningful ecologic risk assessments (and followed up with confirmatory environmental survey ERA studies after market introduction). When determining ecologic risk, consideration must be given to both additive effects (drugs of like-mode of action) and to synergistic effects (adverse interactions between drugs of different classes). Even though the concentration of any one drug might be very low, the additive effects of multiple drugs sharing a like mode of action must be considered. This approach is already adopted under the Food Quality Protection Act (FQPA), in which the exposure risks for humans from pesticides having common mechanisms of action must be combined in calculating total risk; dioxins and PCBs are also assessed this way (e.g., via TEFs). Epidemiologic studies (both ecologic and human) should start to give equal consideration/weight to the body burdens/fluxes of PPCPs. Comprehensive risk assessments may not be possible without considering the simultaneous presence of pesticides, PPCPs, and other industrial chemicals. Assessment of risk should proceed on two fronts: *a*) studies focused on PPCPs already in wide use, and *b*) requirement for studies prior to registration of new PPCPs.
Mitigation, pollution prevention, and regulation	
The removal efficiencies of most PPCPs from STWs is poorly understood. And then, in those instances where efficiencies have been determined, only the disappearance of the parent compound has been tracked—this approach ignores the issue of fate (e.g., bioactive metabolites, and conjugates of the parent PPCP). Direct discharge of untreated sewage to surface waters would probably be the major source in the environment for those PPCPs that are otherwise easily removed by conventional STW processes. As such, individual direct discharge sources possibly have the most profound impact on the loading of the more easily degraded PPCPs in the environment. Highly bioactive nonprescription chemicals are used in huge quantities and represent an unregulated source of (hormonally) active agents. The continued development of optically pure pharmaceuticals may eventually serve to reduce both the burden of pharmaceuticals in the environment and the exposure to daughter enantiomers that might have untoward effects. Dosages of drugs could be reduced by the co-administration of inhibitors of microsomal oxidases and multi-drug transporters to enhance intestinal uptake. The advent of gene therapy might help to ease the use of pharmaceuticals.	Prevention of direct discharge of untreated sewage to the environment would have the greatest impact on reducing the discharge of less persistent PPCPs. Small, unregulated sources (e.g., "straight-piping") may have the largest impacts (analogous to the overall smog impact of exhaust emissions from a small number of vehicles not in compliance). Reuse of treated wastewater would reduce impacts on surface waters. The disposal of pharmaceuticals (e.g., unwanted/expired drugs) to the domestic waste system (sewage and garbage) should be discouraged (this could be addressed with a new labeling requirement). More attention may be needed in ensuring that the degradation of pharmaceuticals to innocuous products in waste treatment plants is maximized. This could entail the development of new or improved treatment technology. Drugs should be screened for MXR inhibitory activity. Land disposal (or use) of sewage sludge may need to be carefully monitored for release of PPCPs. Physicians should resist the temptation to over-prescribe in response to unfounded patient demands. Prescriptions should be written for no more than the requisite course. More emphasis should be placed on patient education with respect to prescribing unneeded medications. Sales of prescription drugs over the Internet may need to be regulated.
Research planning	
No coordinated effort aimed at studying PPCPs in the environment yet exists. While resources continue to be focused on environmental fate/toxicology of conventional POPs, yielding only incremental enhancement of our knowledge base, a fraction of these same resources could yield significant advancements in the analogous understanding of PPCPs in the environment.	The multifaceted nature of PPCPs in the environment will require the collaborative efforts of different regulatory and scientific agencies, such as the U.S. EPA, the FDA, the National Institute of Environmental Health Sciences, and the OECD. A single agency should be responsible for research coordination and facilitating interorganization communication. An interagency strategic research plan covering occurrence, exposure, and effects for nontarget species, ecologic risk assessment, and mitigation would be very useful. The PPCP industry, university partners, and other stakeholders should be actively involved. The literature on the occurrence, fate, and effects of PPCPs in the environment is sometimes hard to access and is highly fragmented, uneven, and difficult to assess and integrate . A concerted effort will be required to bring this disparate literature together into a useful body of knowledge. A web-based electronic database on occurrence, concentrations, and ecotoxicologic data (peer reviewed) for PPCPs in the environment would be highly useful.

compounds to the physiologically active form. With the exception of antibiotics and antineoplastics, the objective for most drug classes is simply to control symptoms and not to actually cure conditions. As such, many drugs are taken for very long periods, sometimes a good portion of the user's lifetime.

Although drugs are usually designed with a specific mode of action in mind (e.g., methotrexate universally affects all organisms in the same manner—by inhibiting nucleic acid synthesis), they can also have numerous effects on nontarget, or as yet unknown, receptors and possibly cause side effects in the target organism. Furthermore, and of equal importance, nontarget organisms can have receptors, or receptor tissue distributions, that do not exist in the target organisms, and therefore unexpected effects can result from unintentional exposure. This is a primary basis for the hypothesis of this paper.

Pharmaceuticals in the Environment

Sources and Origins

The possibility that pharmaceuticals can enter the environment from a number of different routes and possibly cause untoward effects in biota has been noted in the scientific literature for several decades, but its significance has gone largely unnoticed. This probably results in large part from the international regulation of drugs by human health agencies, which usually have limited expertise in environmental issues. Traditionally, drugs were rarely viewed as potential environmental pollutants; there was seldom serious consideration as to their fates once they were excreted from the user. Then again, until the 1990s, any concerted efforts to look for drugs in the environment would have met with limited success because the requisite chemical analysis tools with sufficiently high separatory efficiencies, to resolve the drugs from the plethora of other substances—native and anthropogenic alike, and low detection limits (i.e., nanograms per liter or parts per trillion), were not commonly available. Other obstacles, which still exist to a large degree, are that many pharmaceuticals and cosmetic ingredients and their metabolites are not available in the widely used environmentally oriented mass spectral libraries. These are available in specialty libraries such as Pfleger (e.g., *3,4*), which are not frequently used by environmental chemists. Analytical reference standards, when available, are often difficult to acquire, and are quite costly. The majority of drugs are also highly water soluble. This precludes the application of straightforward, conventional sample clean-up/preconcentration methods, coupled with direct gas chromatographic separation, that have been used for years for "conventional" pollutants, which tend to be less polar and more volatile.

Drugs in the environment did not capture the attention of the scientific or popular press until the last couple of years, with some significant overviews/reviews presented by Halling-Sørenson et al. (*5*), Montague (*6*), Raloff (*7*), Roembke et al. (*8*), Ternes et al. (*9*), and Velagaleti (*10*), among others. The evidence supports the case that PPCPs refractory to degradation and transformation [see Halling-Sørenson et al. (*5*) for summary of published transformation studies] do indeed have the potential to reach the environment. What is not known, however, is whether these chemicals and their transformation products can elicit physiologic effects on biota at the low concentrations (ng-µg/L) at which they are observed to occur. Another unknown is the actual quantity of each of the numerous commercial drugs that is ingested/disposed. With respect to determining the potential extent of the problem, this contrasts sharply with pesticides in which usage is much better documented and controlled.

A list of the PPCPs covered in this review, together with their chemical names, structures, and some representative environmental occurrence/effects data, is presented in Table 2. These chemicals, together with their synthetic precursors and transformation products, are continually released into the environment in enormous quantities as a result of their manufacture, use (via excretion, mainly in urine and feces), and disposal of unused/unwanted drugs and those that have expired, both directly into the domestic sewage system and via burial in landfills. Although largely unknown, there is evidence that large quantities of prescription and nonprescription, "over-the-counter" (OTC) drugs are never consumed (for any number of reasons) (*11*), and many of these are undoubtedly eventually disposed down toilets or via domestic refuse.

A striking difference between pharmaceuticals and pesticides with respect to environmental release is that pharmaceuticals have the potential for ubiquitous direct release into the environment worldwide—anywhere that humans live or visit. Even areas considered relatively pristine (e.g., national parks) are subject to pharmaceutical exposures, especially given that some parks have very large, aging sewage treatment systems, some of which discharge into park surface waters and some of which overflow during wet weather events and infrastructure failures (e.g., Yellowstone National Park) (*12,13*). Other possible sources include disposal of unwanted illicit drugs and synthesis byproducts into domestic sewage systems by clandestine drug operations; disposal of raw products and intermediates (e.g., ephedrine) via toilets is not uncommon in illegal laboratories. Also, in contrast to pesticides, pharmaceuticals in any stage of clinical testing (not yet approved for dispensing by the FDA) are subject to release into the environment, although their overall concentrations would be very low.

Some drugs are excreted essentially unaltered in their free form (e.g., methotrexate and platinum antineoplastics), often with the help of active cellular "multidrug transporters" for moderately lipophilic drugs. Others are metabolized to various extents, which is partly a function of the individual patient and the circadian timing of the dose (the P450 microsomal oxidase system is a major route of formation of more polar, more easily excreted metabolites). Still others are converted to more soluble forms by formation of conjugates (with sugars or peptides). The subsequent transformation products—metabolites and conjugates from eukaryotic and prokaryotic metabolism, and from physicochemical alteration—add to the already complex picture of thousands of highly bioactive chemicals. The FDA refers to all metabolites and physicochemical transformation products, for example, those that range from the dissociated parent compound to photolysis products, for a given drug as structurally related substances (SRSs), which can have greater or lesser physiologic activity than the parent drug.

As in mammals, the metabolic disposition of lipophilic xenobiotics, such as numerous drugs, in vertebrate aquatic species is largely governed by what is referred to as Phase I and Phase II reactions (*14*); less is known about invertebrate metabolism. Phase I makes use of monooxygenases (e.g., cytochrome P450), reductases, and hydrolases (for esters and epoxides) to add reactive functional groups to the molecule. Phase II uses covalent conjugation (glucuronidation) to make the molecule hydrophilic and more excretable. These reactions are catalyzed by glycosyltransferases and sulfotransferases (for hydroxyaromatics and carboxy groups), glutathione *S*-transferases (for electrophilic functional groups such as halogens, nitro groups, or unsaturated/conjugated sites), acetyltransferases (for primary amines or hydrazines), and aminoacyltransferases (for forming peptides from carboxy groups using free amino acids). This metabolic strategy creates metabolites successively more polar than the parent compound, thereby enhancing excretion (Figure 1). Considerable interspecies and intraspecies diversity, however, can be observed in actual metabolic potentials. Many drugs and metabolic products, especially those over 400 Da, are concentrated in the bile of fish (vs blood or fat) (*15*). Although the total amount excreted via the urine may be higher, Guarino and Lech (*15*) recommend bile analysis to maximize the chance of detecting drugs, especially their conjugates, in fish in order to confirm exposure. They also report that the

Table 2. PPCPs identified in environmental samples—or having significance with respect to aquatic life.

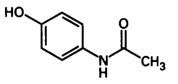
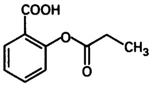
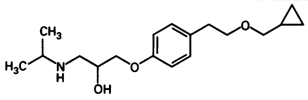
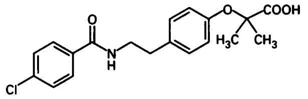
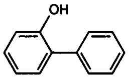
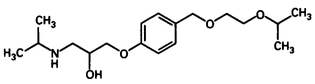
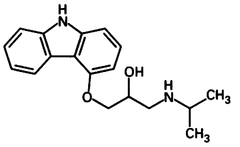
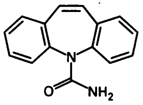
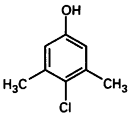
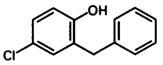
Compound	Structure and CAS name	CAS RN MW Formula	Use/origin	Environmental occurrence	Trade names, comments, and nontarget species effects
Acetaminophen	 *N*-(4-Hydroxyphenyl)acetamide; (Paracetamol)	103-90-2 151.17 $C_8H_9NO_2$	Analgesic/anti-inflammatory	Efficiently removed by POTW (*18*); POTW max. effluent: 6.0 µg/L; not detected in surface waters (*18*)	e.g., Tylenol; *Daphnia* immobilization EC_{50} 0.27–0.90 mM (*125*)
Acetylsalicylic acid	 2-(Acetyloxy)benzoic acid; (Aspirin)	50-78-2 180.16 $C_9H_8O_4$	Analgesic/anti-inflammatory	Ubiquitous. One of first pharmaceuticals identified in sewage influent/effluent; POTW removal efficiency 81% (*18*); POTW max. effluent: 1.5 µg/L; max. in surface waters: 0.34 µg/L. Sewage effluent: 1 µg/L (*40*)	Efficiently removed by POTWs; *Daphnia* immobilization EC_{50} 0.9–8.2 mM (*125*)
Betaxolol	 1-[4-[2-(Cyclopropylmethoxy) ethyl]-phenoxy]-3-[(1-methyl-ethyl)amino]-2-propanol	63659-18-7 307.43 $C_{18}H_{29}NO_3$	Beta-blocker (antihypertensive, antiglaucoma)	POTW max. effluent: 0.19 µg/L; max. in surface waters: 0.028 µg/L (*73*)	e.g., Betoptic
Bezafibrate	 2-[4-[2-[(4-Chlorobenzoyl)-amino]ethyl]phenoxy]-2-methylpropanoic acid	41859-67-0 361.82 $C_{19}H_{20}ClNO_4$	Lipid regulator	Loading of ~ 300 g/day in German POTW (18); POTW removal efficiency 83% (18); POTW max. effluent: 4.6 µg/L; max. in surface waters: 3.1 µg/L. Influent concentration of 1.2 µg/L in Brazilian STWs (*69*) with removal efficiencies ranging from 27–50%	Among highest reported values for occurrence in STW effluent and surface waters; e.g., Befizal
Biphenylol	 2-Biphenylol 2-Hydroxydiphenyl	90-43-7 170.21 $C_{12}H_{10}O$	Antiseptic, fungicide	POTWs in Germany: biphenylol routinely found in both influents (up to 2.6 µg/L) and effluents (*70*), but removal was extensive	e.g., Dowicide A
Bisoprolol	 1-[4-[[2-(1-Methylethoxy)-ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]-2-propanol	66722-44-9 325.45 $C_{18}H_{31}NO_4$	Beta-blocker (antihypertensive)	POTW max. effluent: 0.37 µg/L.; max. in surface waters: 2.9 µg/L (*73*)	e.g., Concor
Carazolol	 1-(9*H*-Carbazol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol	57775-29-8 298.38 $C_{18}H_{22}N_2O_2$	Beta-blocker (antihypertensive, antianginal, antiarrhythmic)	POTW max. effluent: 0.12 µg/L; max. in surface waters: 0.11 µg/L (*73*)	e.g., Conducton
Carbamazepine	 5*H*-Dibenz[*b,f*]azepine-5-carboxamide	298-46-4 236.27 $C_{15}H_{12}N_2O$	Analgesic; antiepileptic	Loading of over 100 g/day in German POTW (*18*); but load in effluent can be 114 g/day; POTW removal efficiency 7% (*18*); POTW max. effluent: 6.3 µg/L; max. in surface waters: 1.1 µg/L	e.g., Tegratal; only 1–2% excreted free (*18*); 10,11-epoxy-carbamazepine major metabolite; also excreted as glucuronides
4-Chloro-3,5-xylenol (Chloroxylenol)	 4-Chloro-3,5-dimethylphenol	88-04-0 156.61 C_8H_9ClO	Antiseptic	POTWs in Germany: 4-chloroxylenol occasionally found in both influents and effluents (< 0.1 µg/L) (*70*)	e.g., Benzytol
Chlorophene	 4-Chloro-2-(phenylmethyl)phenol; (*o*-Benzyl-*p*-chlorophenol)	120-32-1 218.68 $C_{13}H_{11}ClO$	Antiseptic	POTWs in Germany: chlorophene routinely found in both influents (up to 0.71 µg/L) and effluents (*70*); removal not as extensive as for biphenylol.	e.g., Santophen 1

Table 2. *Continued.*

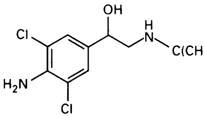
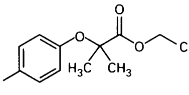
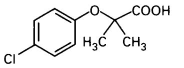
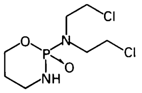
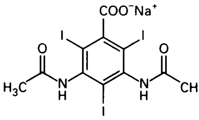
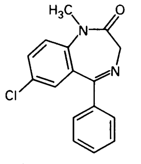
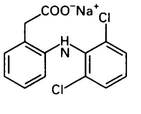
Compound	Structure and CAS name	CAS RN MW Formula	Use/origin	Environmental occurrence	Trade names, comments, and nontarget species effects
Clenbuterol	 4-Amino-3,5-dichloro-α-[[(1,1-dimethylethyl)amino]-methyl]benzenemethanol	37148-27-9 277.19 $C_{12}H_{18}Cl_2N_2O$	β_2-Sympathomimetic (bronchodilator)	POTW max. effluent: 0.08 µg/L; max. in surface waters: 0.05 µg/L (*18*)	e.g., Monores
Clofibrate	 2-(4-Chlorophenoxy)-2-methyl-propanoic acid ethyl ester	637-07-0 242.70 $C_{12}H_{15}ClO_3$	Lipid regulator	Not detected in POTW effluent (*18*); not detected in surface waters. River water: ~ 40 ng/L (*40*)	e.g., Bioscleran; rapidly hydrolyzed upon ingestion
Clofibric acid	 2-(4-Chlorophenoxy)-2-methylpropanoic acid; e.g., Regulipid	882-09-7 214.66 $C_{10}H_{11}ClO_3$	Polar, active metabolite of lipid regulators (clofibrate, etofyllin clofibrate [theofibrate], etofibrate)	One of first prescription drugs/metabolites ever reported in sewage influent/effluent: Missouri STW effluent avg. 2.1 kg/day (*38*); 0.8–2.0 µg/L in raw sewage and activated sludge effluent (*37*). Loading of over 50 g/day in German POTW (*18*); POTW removal efficiency 51% (*18*); POTW max. effluent: 1.6 µg/L; max. in surface waters: 0.55 µg/L. Swiss rural/urban lakes: 1–9 ng/L (ppt); North Sea (up to 7.8 ng/L) (*67*). Influent concentration of 1 µg/L in Brazilian STWs (*69*) with removal efficiencies ranging from 15–34%. Up to 270 ng/L in German tap waters (*23*)	Active metabolite of clofibrate; formed via hydrolysis very soon after ingestion; excreted primarily as glucuronide (very little as the free acid); presence in POTWs indicates hydrolysis of conjugate (*18*)
Cyclophosphamide (Cyclophosphane)	 *N,N*-Bis(2-chloroethyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide; e.g., Cycloblastin	50-18-0 261.09 $C_7H_{15}Cl_2N_2O_2P$	Antineoplastic	POTW max. effluent: 0.02 µg/L; not detected in surface waters (*18*). Hospital sewage 146 ng/L (*149*) and 19 ng/L–4.5 µg/L (*82*); POTW receiving hospital waste: influent up to 143 ng/L, effluent up to 17 ng/L	Oxazaphosphorine (structural isomer of ifosfamide); high dosages (over 100 mg/kg); up to 50% excreted unaltered; mutagen/carcinogen; resistant to microbial degradation
Diatrizoate (Na)	 COO⁻Na⁺ I I O O H3C N H N H CH3 I 3,5-Bis(acetylamino)-2,4,6-triiodobenzoic acid sodium salt	737-31-5 635.90 $C_{11}H_8I_3N_2NaO_4$	X-Ray contrast media (radiopaque medium)	Resistant to biodegradation and yields refractory, unidentified metabolites (*91*). In German surface waters, median concentration of 0.23 µg/L (*92*; isolated maximum values above 100 µg/L indicate that locally very high concentrations can occur, especially in small streams containing a high percentage of STW discharges.	e.g., Hypaque Sodium; very high annual worldwide usage rates
Diazepam	 7-Chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one	439-14-5 284.74 $C_{16}H_{13}ClN_2O$	Psychiatric drug (anxiolytic; muscle relaxant)	POTW max. effluent: 0.04 µg/L; not detected in surface waters (*18*). Groundwater from a Superfund site near Atlantic City, New Jersey: 10–40 µg/L (*88*).	e.g., Valium; *Daphnia* immobilization EC_{50} 0.015–0.049 mM (*125*)
Diclofenac-Na	 2-[(2,6-Dichlorophenyl)-amino]benzeneacetic acid-Na	15307-79-6 318.13 $C_{14}H_{10}Cl_2NO_2Na$	Analgesic/anti-inflammatory	Loading of ~100 g/day in German POTW (*18*); POTW removal efficiency 69% (*18*); POTW max. effluent: 2.1 µg/L; max. in surface waters: 1.2 µg/L. Influent to Swiss STWs 500–1800 ng/L and effluents more than 50% as much; Swiss lakes/rivers 1–12 ng/L, with lower order streams 11–310 ng/L (*71*). Influent concentration of 0.8 µg/L in Brazilian STWs (*69*) with removal efficiencies ranging from 9–75%.	e.g., Voltaren; lab data show rapid and extensive photodegradation to multiple products (*71*)

Table 2. *Continued.*

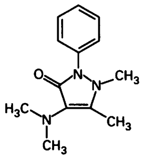
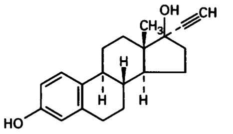
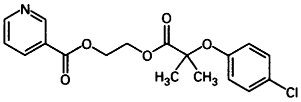
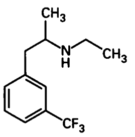
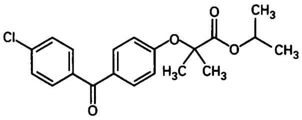
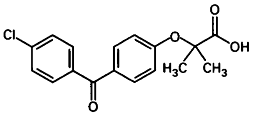
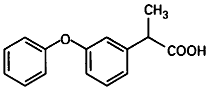
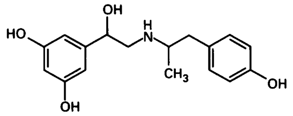
Compound	Structure and CAS name	CAS RN MW Formula	Use/origin	Environmental occurrence	Trade names, comments, and nontarget species effects
Dimethylaminophenazone (Aminopyrine)	 4-Dimethylaminoantipyrine	58-15-1 231.30 $C_{13}H_{17}N_3O$	Analgesic/anti-inflammatory	Loading of over 50 g/day in German POTW (*18*); POTW removal efficiency 38% (*18*); POTW max. effluent: 1.0 µg/L; max. in surface waters: 0.34 µg/L	e.g., Piridol
17α-Ethinyl estradiol	 (17α)-19-Norpregna-1,3,5 (10)-trien-20-yne-3,17-diol	57-63-6 296.41 $C_{20}H_{24}O_2$	Oral contraceptive (in combination with progestogens)	Up to 7 ng/L in POTW effluent (*26*). Not detected in German surface water above 0.5 ng/L (*9*), but found in Dutch Rhine water up to 4.3 ng/L (*150*).	Prime synthetic suspect regarding estrogenic effects in fish; the natural estrogen is 17β-estradiol; e.g., Oradiol
Etofibrate	 3-Pyridinecarboxylic acid 2-[2-(4-chlorophenoxy)-2-methyl-1-oxopropoxy]ethyl ester	31637-97-5 363.80 $C_{18}H_{18}ClNO_5$	Lipid regulator	Not detected in POTW effluent (*18*); not detected in surface waters.	e.g., Lipo-Merz; rapidly hydrolyzed upon ingestion
Fenfluramine	 *N*-Ethyl-α-methyl-3-[trifluoromethyl] benzeneethanamine	458-24-2 231.26 $C_{12}H_{16}F_3N$	Sympathomimetic amine (anorexic)	While no one has looked for fenfluramine in sewage, it is known to enhance the release of serotonin (5-HT), and in the crayfish, 5-HT in turn triggers release of ovary-stimulating hormone—resulting in larger oocytes with enhanced amounts of vitellin (consequences unknown) (*74*). Similarly, in fiddler crabs, fenfluramine (dose of 125 nmol) stimulates (through 5-HT) the production of gonad-stimulating hormone—accelerating testicular maturation (*75*).	Popular diet (anorectic) drug removed from the U.S. market in 1998 by the FDA because of heart valve damage; e.g., hydrochloride: Pondimin
Fenofibrate	 2-[4-(4-Chlorobenzoyl)-phenoxy]-2-methylpropanoic acid 1-methylethyl ester	49562-28-9 360.84 $C_{20}H_{21}ClO_4$	Lipid regulator	Efficiently removed by POTW (*18*); POTW max. effluent: 0.03 µg/L; not detected in surface waters.	e.g., Fenobrate; rapidly hydrolyzed upon ingestion
Fenofibric acid	 2-[4-(4-Chlorobenzoyl)phenoxy]-2-methylpropanoic acid	42017-89-0 318.84 $C_{17}H_{15}ClO_4$	Polar, active metabolite of fenofibrate	Loading of over 50 g/day in German POTW (18); POTW removal efficiency 64% (18); POTW max. effluent: 1.2 µg/L; max. in surface waters: 0.28 µg/L. Influent concentration of 0.4 µg/L in Brazilian STWs (*69*) with removal efficiencies ranging from 6–45%.	Formed via hydrolysis very soon after ingestion; excreted primarily as glucuronide (very little as free acid); presence in POTWs indicates hydrolysis of conjugate (*18*)
Fenoprofen	 α-Methyl-3-phenoxybenzeneacetic acid	31879-05-7 242.27 $C_{15}H_{14}O_3$	Analgesic/anti-inflammatory	Not detected in POTW effluent or surface waters (*18,69*).	e.g., Fenopron
Fenoterol	 5-[1-Hydroxy-2-[[2-(4-hydroxyphenyl)-1-methylethyl] amino]ethyl]-1,3-benzenediol	13392-18-2 303.36 $C_{17}H_{21}N_4O$	β_2-Sympathomimetic (bronchodilator)	POTW max. effluent: 0.06 µg/L; max. in surface waters: 0.061 µg/L (*18*)	e.g., Airum

Table 2. *Continued.*

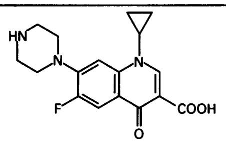
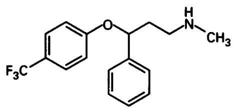
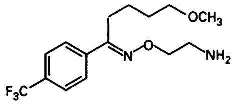
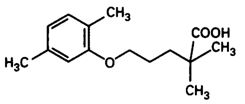
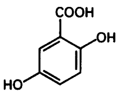
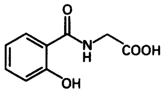
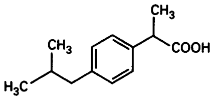
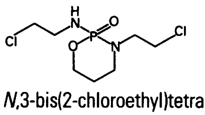
Compound	Structure and CAS name	CAS RN MW Formula	Use/origin	Environmental occurrence	Trade names, comments, and nontarget species effects
Fluoroquinolone carboxylic acids	 Large class; e.g., ciprofloxacin	e.g., 85721-33-1 331.35 $C_{17}H_{18}FN_3O_3$	Antibiotics	As one of only many classes of pharmaceuticals, antibiotics in general have been investigated for their occurrence in the environment more than any other class of PPCPs. Their ubiquitous occurrence in the environment is a leading proposed cause of the rise in resistance among pathogenic bacteria. Strongly sorbs to soil (*151,152*). Highly active in hospital wastewaters (*62,153*)	Gyrase inhibitors (needed for DNA replication); excreted mainly as parent compound
Fluoxetine	 *N*-Methyl-γ-[4-(trifluoro-methyl)phenoxy]benzene-propanamine	54910-89-3 309.33 $C_{17}H_{18}F_3NO$	Antidepressant (SSRI)	Not yet searched for in environmental samples	e.g., Prozac; Fluoxetine elicits significant spawning in male mussels at 10^{-7} M (~150 µg/L) and in females at 10^{-6} M (*76*)
Fluvoxamine	 5-Methoxy-1-[4-(trifluoro-methyl)phenyl]-1-pentanone *O*-(2-aminoethyl)oxime	54739-18-3 318.34 $C_{15}H_{21}F_3N_2O_2$	Antidepressant (SSRI)	Not yet searched for in environmental samples	e.g., Luvox; Fluvoxamine elicits significant spawning in male mussels at 10^{-9} M (~0.318 µg/L) and in females at 10^{-7} M. Fluvoxamine is the most powerful spawning inducer ever identified for bivalves (*76*)
Gemfibrozil	 5-(2,5-Dimethylphenoxy)-2,2-dimethylpentanoic acid	25812-30-0 250.34 $C_{15}H_{22}O_3$	Lipid regulator	Loading of over 50 g/day in German POTW (*18*); POTW removal efficiency 69% (*18*); POTW max. effluent: 1.5 µg/L; max. in surface waters: 0.51 µg/L. Influent concentration of 0.3 µg/L in Brazilian STWs (*69*) with removal efficiencies ranging from 16–46%	e.g., Lopid
Gentisic acid	 2,5-Dihydroxybenzoic acid	490-79-9 154.12 $C_7H_6O_4$	Hydroxylated metabolite of acetylsalicylic acid	Efficiently removed by POTW (*18*); POTW max. effluent: 0.59 µg/L; max. in surface waters: 1.2 µg/L. Average gentisic acid concentrations in POTW influents of 4.6 µg/L (*70*) with no detectable amounts in the effluents	A minor ultimate metabolite
o-Hydroxyhippuric acid	 *N*-(2-Hydroxybenzoyl)glycine	487-54-7 195.17 $C_9H_9NO_4$	Metabolite of acetylsalicylic acid	Efficiently removed by POTW (*18*); not detected in POTW effluent or surface waters (*18*); average *o*-hydroxyhippuric acid concentrations in POTW influents of 6.8 µg/L; no detectable amounts in effluents (*70*)	
Ibuprofen	 α-Methyl-4-(2-methylpropyl)-benzeneacetic acid	15687-27-1 206.28 $C_{13}H_{18}O_2$	Analgesic/anti-inflammatory	Loading of over 200 g/day in German POTW (*18*); POTW removal efficiency 90% (*18*); POTW max. effluent: 3.4 µg/L; max. in surface waters: 0.53 µg/L. Influent concentration of 0.3 µg/L in Brazilian STWs (*69*) with removal efficiencies ranging from 22–75%. STW influents up to 3.3 µg/L, POTW removal >95%, surface waters up to 8 ng/L; one of few studies to look at metabolites (*72*)	e.g., Advil; excreted substantially by humans in free form or conjugated (*72*)
Ifosfamide	 *N*,3-bis(2-chloroethyl)tetra-hydro-2*H*-1,3,2-oxazaphos-phorin-2-amine 2-oxide; e.g., Holoxan	3778-73-2 261.09 $C_7H_{15}Cl_2N_2O_2P$	Antineoplastic	POTW max. effluent: 2.9 µg/L; not detected in surface waters (*18*). Hospital sewage 24 ng/L (*149*). Hospital effluent: max 1.91 µg/L, median 109 ng/L; POTW influent/effluent max 43 ng/L, median 6.5–9.3 ng/L (*83*). Found to be totally refractory to removal by POTW (*83*)	Oxazaphosphorine (structural isomer of cyclophosphamide); high dosages (over 100 mg/kg). Up to 50% excreted unaltered, but generally ~20%; fate of metabolites unknown (*83*)

Table 2. *Continued.*

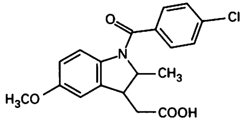
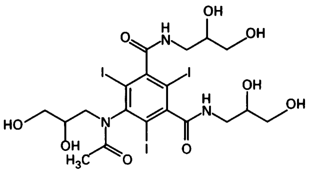
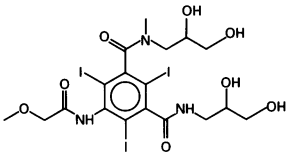
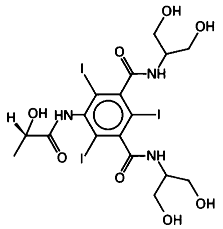
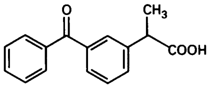
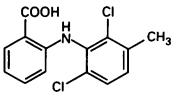
Compound	Structure and CAS name	CAS RN MW Formula	Use/origin	Environmental occurrence	Trade names, comments, and nontarget species effects
Indomethacine	 1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indole-3-acetic acid	53-86-1 357.79 $C_{19}H_{16}ClNO_4$	Analgesic/anti-inflammatory	Loading of ~10 g/day in German POTW (*18*); POTW removal efficiency 75% (*18*); POTW max. effluent: 0.60 µg/L; max. in surface waters: 0.20 µg/L. Influent concentration of 0.95 µg/L in Brazilian STWs (*69*) with removal efficiencies ranging from 71–83%	e.g., Amuno
Iohexol	 5-[Acetyl(2,3-dihydroxypropyl)-amino]-*N,N'*-bis(2,3-dihydroxy-propyl)-2,4,6-triiodo-1,3-benzene-dicarboxamide	66108-95-0 821.14 $C_{19}H_{26}I_3N_3O_9$	X-Ray contrast (radiopaque) media; e.g., Omnipaque	Very low aquatic toxicity reported by Steger-Hartmann et al. (*93*)	Very high annual worldwide usage rates. Parent compounds possibly low toxicity (*93*). Metabolites have unknown aquatic toxicology. Extremely persistent
Iopamidol	*N,N'*-Bis[2-hydroxy-1-(hydroxy-methyl)ethyl]-5-[(2-hydroxy-1-oxopropyl)amino]-2,4,6-triiodo-1,3-benzenedicarboxamide	60166-93-0 777.09 $C_{17}H_{22}I_3N_3O_8$	X-Ray contrast (radiopaque) media; e.g., Isovue	Concentrations as high as 15 µg/L in municipal STW effluents (*92*), and median concentration of 0.49 µg/L	
Iopromide	 *N,N'*-Bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-[(methoxy-acetyl)amino]-*N*-methyl-1,3-benzenedicarboxamide	73334-07-3 791.12 $C_{18}H_{24}I_3N_3O_8$	X-Ray contrast (radiopaque) media; e.g., Ultravist	Resistant to biodegradation and yields refractory, unidentified metabolites (*91*). Reported by Ternes et al. (*92*) in rivers. Concentrations as high as 11 µg/L in municipal STW effluents (*92*)	
Iotrolan	 5,5'-[(1,3-Dioxo-1,3-propane-diyl)bis(methylimino)]-bis[*N,N'*-bis[2,3-dihydroxy-1-(hydroxy-methyl)propyl]-2,4,6-triiodo-1,3-benzenedicarboxamide]	79770-24-4 1626.2 $C_{37}H_{48}I_6N_6O_{18}$	X-Ray contrast (radiopaque) media; e.g., Isovist	Very low aquatic toxicity reported by Steger-Hartmann et al. (*93*)	
Ketoprofen	 3-Benzoyl-α-methyl-benzeneacetic acid	22071-15-4 254.28 $C_{16}H_{14}O_3$	Analgesic/anti-inflammatory	POTW max. effluent: 0.38 µg/L; max. in surface waters: 0.12 µg/L (*18*). Influent concentration of 0.5 µg/L in Brazilian STWs (*69*) with removal efficiencies ranging from 48–69%	e.g., Oruvail
Meclofenamic acid	 2-[(2,6-Dichloro-3-methyl-phenyl)amino]benzoic acid	644-62-2 296.15 $C_{14}H_{11}Cl_2NO_2$	Analgesic/anti-inflammatory	Not detected in POTW effluent or surface waters (*18,69*)	Used mainly in veterinary medicine; e.g., Arquel

Table 2. *Continued.*

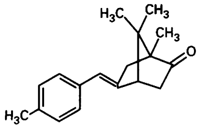
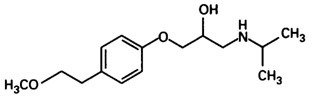
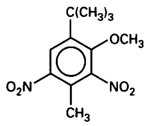
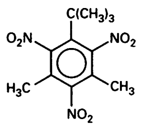
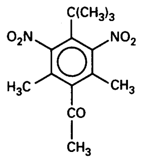
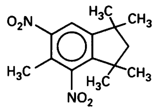
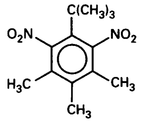
Compound	Structure and CAS name	CAS RN MW Formula	Use/origin	Environmental occurrence	Trade names, comments, and nontarget species effects
Methylbenzylidene camphor	 3-(4-Methylbenzyliden) camphor	36861-47-9 254.37 $C_{18}H_{22}O$	Sunscreen agent	Bioconcentrated in roach from German lakes (*115*)	e.g., Eusolex 6300
Metoprolol	 1-[4-(2-Methoxyethyl) phenoxy]-3-[(1-methylethyl) amino]-2-propanol	37350-58-6 267.37 $C_{15}H_{25}NO_3$	Beta-blocker (antihypertensive)	Loading of nearly 400 g/day in German POTW (*18*); POTW removal efficiency 83% (*18*); POTW max. effluent: 2.2 µg/L; max. in surface waters: 2.2 µg/L	Tartrate: e.g., Lopressor; principal metabolite: metaprolol acid
Musk ambrette (a nitro musk)	 2,6-Dinitro-3-methoxy-4-*tert*-butyl toluene	83-66-9 268.27 $C_{12}H_{16}N_2O_5$	The first of two major classes of synthetic musks–the "nitro" musks. Widely used in a wide array of fragrances for cosmetics and other personal care products. Introduced to commerce in late 1800s	Synthetic musks first began to be identified in environmental samples almost 20 years ago. Yamagishi et al. (*100,101*) performed the first comprehensive monitoring effort, identifying musk xylene and musk ketone in freshwater fish, marine shellfish, river water, and STW wastewater. Musk xylene was found in all samples, and musk ketone was found in 80% of the 74 samples analyzed. Concentrations in STW effluents ranged from 25 to 36 ng/L (musk xylene) and from 140 to 410 ng/L (musk ketone). Concentrations of musk xylene in fish muscle were in the tens of ppb, while those for musk ketone were less than 10 µg/kg, with highest values in fish downstream of STWs. In contrast, for shellfish, the concentrations ranged lower, between 1 and 5.3 µg/kg, presumably because of their lower lipid contents. In river water, musk xylene occurred in all samples, whether upstream or downstream of STWs, and ranged from 1 to 23 ng/L; musk ketone was generally in the same range, but in distinct contrast, was not detectable in upstream samples Winkler et al. (*104*) measured musks in 31 particulate matter and water samples from the Elbe River (Germany). In all particulate matter samples were found musk ketone (4–22 ng/g), Galaxolide (148–736 ng/g), and Tonalide (194–770 ng/g); Celestolide was found in 23 of the particulate matter samples (4–43 ng/g). The values for the three most prevalent musks were within the same magnitude as	The nitro musks are being phased out of use in many parts of the world because of toxicity concerns. Musk xylene was introduced in 1888
Musk xylene (a nitro musk)	 1-*tert*-Butyl-3,5-dimethyl-2,4,6-trinitrobenzene (MX)	81-15-2 297.27 $C_{12}H_{15}N_3O_6$			
Musk ketone (a nitro musk)	 1-*tert*-Butyl-3,5-dimethyl-2,6-dinitro-4-acetylbenzene (MK)	81-14-1 294.31 $C_{14}H_{18}N_2O_5$			
Musk moskene (a nitro musk)	 4,6-Dinitro-1,1,3,3,5-pentamethylindane	116-66-5 278.31 $C_{14}H_{18}N_2O_4$			
Musk tibetene (a nitro musk)	 1-*tert*-Butyl-2,6,-dinitro-3,4,5-trimethylbenzene	145-39-1 266.30 $C_{13}H_{18}N_2O_4$			

Table 2. *Continued.*

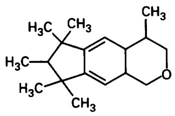
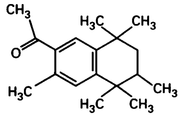
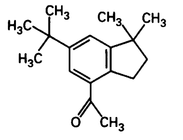
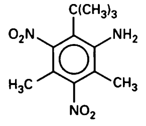
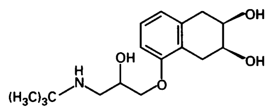
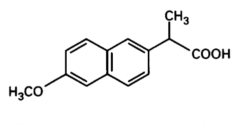
Compound	Structure and CAS name	CAS RN MW Formula	Use/origin	Environmental occurrence	Trade names, comments, and nontarget species effects
Galaxolide (HHCB) (a polycyclic musk)	 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-(g)-2-benzopyrane	1222-05-5 258.40 $C_{18}H_{26}O$	The second of two major classes of synthetic musks—the "polycyclic" musks. Widely used in a wide array of fragrances for cosmetics and other personal care products. Introduced to commerce in 1950s	that for 15 PAHs, and exceeded those for 14 common polychlorinated organic pollutants (only HCB and *p,p'*-DDT were of similar concentration). Also found in all the water samples were musk ketone (2–10 ng/L), Galaxolide (36–152 ng/L), and Tonalide (24–88 ng/L); Celestolide was only found at 2–8 ng/L. These higher values exceeded those for all the polychlorinated organics and the PAHs Draisci et al. (*106*) examined freshwater fish in Italy and identified two of five targeted musks in most fish samples; Galaxolide and Tonalide were identified at levels ranging from less than 4 ng/g to 105 ng/g (ppb) in fish muscle tissue. Eschke et al. (cited in *107*) identified Galaxolide, Tonalide, and Celestolide in the fatty tissue of bream and perch from the Ruhr River (Germany) at average concentrations ranging from 2.5 to 4.6 mg/kg (ppm). Müller et al. (*98*) identified in the Swiss river Glatt, Galaxolide, Tonalide, and Celestolide at ng/L concentrations (136, 75, and 3.2, respectively); they also found the nitro-musks (tibetene, ambrette, moskene, ketone, xylene) at ng/L concentrations (0.04, <0.03, 0.08, 8.3, and 0.62, respectively)[a]	The nitro musks are being phased out of use in many parts of the world because of toxicity concerns. Musk xylene was introduced in 1888
Tonalide (AHTN) (a polycyclic musk)	 7-Acetyl-1,1,3,4,4,6-hexamethyltetraline	1506-02-1 258.40 $C_{18}H_{26}O$			
Celestolide (ADBI) (a polycyclic musk)	 4-Acetyl-1,1-dimethyl-6-*tert*-butylindane	13171-00-1 244.38 $C_{17}H_{24}O$			
Musk xylene derivatives reduced (aminated)	 1-*tert*-Butyl-3,5-dimethyl-2-amino-4,6-dinitrobenzene 1-*tert*-Butyl-3,5-dimethyl-4-amino-2,6-dinitrobenzene 1-*tert*-Butyl-3,5-dimethyl-2,4-diamino-6-nitrobenzene 1-*tert*-Butyl-3,5-dimethyl-2,4,6-tri-aminobenzene		Transformation products of nitro musks, resulting from microbial reduction of the nitro groups.	Behecti et al. (*111*) tested the acute toxicity of four reduced analogs of musk xylene on *Daphnia magna*. The *p*-aminodinitro compound exhibited the most toxicity of the four, with EC_{50} values averaging 0.25 µg/L (0.25 ppb). Gatermann et al. (*96*) identified in sewage influent/effluent and in Elbe River (Germany) musk xylene and musk ketone together with their amino derivatives: 4- and 2-amino-musk xylenes and 2-amino musk ketone. Sewage influent: musk xylene and musk ketone at 150 and 550 ng/L, respectively; in the effluent, concentrations 10 and 6 ng/L, respectively. Amino derivatives not detectable in influent, but concentrations in the effluents dramatically increased: 2-amino musk xylene (10 ng/L), 4-amino musk xylene (34 ng/L), and 2-amino musk ketone (250 ng/L	The amino musks show greater toxicity than the parent nitro musks
Nadolol	 5-[3-[(1,1-Dimethylethyl)-amino]-2-hydroxypropoxy]-1,2,3,4-tetrahydro-2,3-naphthalenediol	42200-33-9 309.40 $C_{17}H_{27}NO_4$	Beta-blocker (antihypertensive)	POTW max. effluent: 0.06 µg/L; not detected in surface waters (*18*)	e.g., Corgard
Naproxen	 (*S*)-6-Methoxy-α-methyl-2-naphthaleneacetic acid	22204-53-1 230.26 $C_{14}H_{14}O_3$	Analgesic/anti-inflammatory	Loading of over 50 g/day in German POTW (*18*); POTW removal efficiency 66% (*18*); POTW max. effluent: 0.52 µg/L; max. in surface waters: 0.39 µg/L. Influent concentration of 0.6 µg/L in Brazilian STWs (*69*) with removal efficiencies ranging from 15–78%.	e.g., Naprosyn

Table 2. *Continued.*

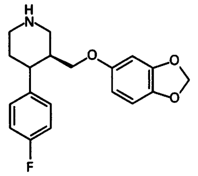
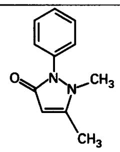
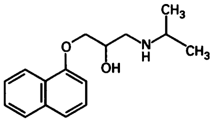
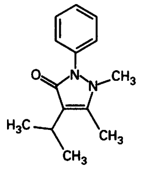
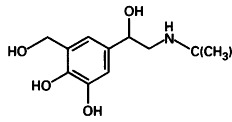
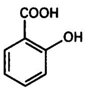
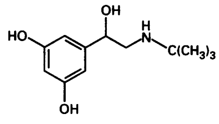
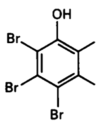
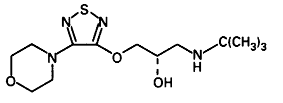
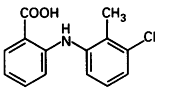
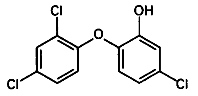
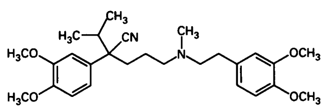
Compound	Structure and CAS name	CAS RN MW Formula	Use/origin	Environmental occurrence	Trade names, comments, and nontarget species effects
Paroxetine	 (3*S*-*trans*)-3-[(1,3-Benzodioxol-5-yloxy)methyl]-4-(4-fluorophenyl)piperidine	61869-08-7 329.37 $C_{19}H_{20}FNO_3$	Antidepressant (SSRI)	Not yet searched for in environmental samples	Compared with fluoxetine and fluvoxamine, paroxetine does not elicit spawning behavior in molluscs; e.g., Paxil
Phenazone (Antipyrine)	 1,2-Dihydro-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one	60-80-0 188.23 $C_{11}H_{12}N_2O$	Analgesic	Loading of ~10 g/day in German POTW (*18*); POTW removal efficiency 33% (*18*); POTW max. effluent: 0.41 µg/L; max. in surface waters: 0.95 µg/L	e.g., Parodyne
Propranolol	 1-[(1-Methylethyl)amino]-3-(1-naphthalenyloxy)-2-propanol	525-66-6 259.35 $C_{16}H_{21}NO_2$	Beta-blocker (antihypertensive)	Loading of over 500 g/day in German POTW (*18*); POTW removal efficiency 96% (*18*); POTW max. effluent: 0.29 µg/L; max. in surface waters: 0.59 µg/L	e.g., Avlocardyl; principal metabolite: 4-hydroxypropanolol; *Daphnia* immobilization EC_{50} 0.01–0.06 mM (*125*)
Propyphenazone	 4-Isopropylantipyrine	479-92-5 230.31 $C_{14}H_{18}N_2O$	Analgesic/anti-inflammatory	Grinsted (Denmark) landfill leachates: 0.3–4.0 mg/L directly beneath and declining depending on depth and distance along plume (*21*); prevalent in Berlin waters (*23*)	e.g., Isoprochin
Salbutamol albuterol (in U.S.)	 α^1-[[(1,1-Dimethylethyl)-amino]methyl]-4-hydroxy-1,3-benzenedimethanol	18559-94-9 239.31 $C_{13}H_{21}NO_3$	β_2-Sympathomimetic (bronchodilator)	POTW max. influent: 0.17 µg/L; max. in surface waters: 0.035 µg/L (*18*)	e.g., sulfate: Ventolin
Salicylic acid	 2-Hydroxybenzoic acid; e.g., Duofilm	69-72-7 138.12 $C_7H_6O_3$	Primary hydrolytic metabolite of acetylsalicylic acid, keratolytic, dermatice, preservative of food	Up to 54 µg/L in POTW effluent but efficiently removed in effluent (*18*); POTW max. effluent: 0.14 µg/L; max. in surface waters: 4.1 µg/L. Average salicylic acid concentrations in POTW influents of 55 µg/L and in effluents of 0.5 µg/L (*70*)	Efficiently removed by POTWs; the free form of salicylic acids represents only one (minor) of several ultimate metabolites
Sulfonamides	Large class	NA	Antibiotics	Grinsted (Denmark) landfill leachates: 0.04–6.47 mg/L directly beneath and declining depending on depth and distance along plume (*21*)	
Terbutaline	 5-[2-[(1,1-Dimethylethyl) amino-1-hydroxyethyl]-1,3-benzenediol	23031-25-6 225.29 $C_{12}H_{19}NO_3$	β_2-Sympathomimetic (bronchodilator)	POTW max. effluent: 0.12 µg/L; not detected in surface waters (*18*)	e.g., sulfate: Brethaire

Table 2. *Continued.*

Compound	Structure and CAS name	CAS RN MW Formula	Use/origin	Environmental occurrence	Trade names, comments, and nontarget species effects
3,4,5,6-Tetrabromo-*o*-cresol		576-55-6 423.72 $C_7H_4Br_4O$	Antiseptic, fungicide	POTWs in Germany: tetrabromo-*o*-cresol found in both influents and effluents (<0.1 µg/L) (*70*)	
Timolol	 (*S*)-1-[(1,1-Dimethylethyl)-amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol	26839-75-8 316.42 $C_{13}H_{24}N_4O_3S$	Beta-blocker (antihypertensive)	POTW max. effluent: 0.07 µg/L; max. in surface waters: 0.01 µg/L (*18*)	e.g., hemihydrate: Betimol
Tolfenamic acid	 2-[(3-Chloro-2-methylphenyl)-amino]benzoic acid	13710-19-5 261.71 $C_{14}H_{12}ClNO_2$	Analgesic/anti-inflammatory	Not detected in POTW effluent or surface waters (*18*); in Brazilian STW effluent 1.6 µg/L (*69*)	Veterinary NSAID e.g., Tolfedine
Triclosan	 5-Chloro-2-(2,4-dichloro-phenoxy)phenol	3380-34-5 289.54 $C_{12}H_7Cl_3O_2$	Antiseptic	0.05-0.15 µg/L in water (*113*). Antibacterial widely used for 30 years in a vast array of consumer products. Its usage as a preservative and disinfectant continues to grow. Triclosan's use in commercial products spans footwear (in hosiery and insoles of shoes called "odor-eaters"), hospital handsoap, acne creams (e.g., Clearasil), and rather recently as a slow-release product called Microban, which is incorporated in a wide variety of plastic products (from children's toys to kitchen utensils, such as cutting boards)	e.g., Irgasan DP 300
Verapamil	 α-[3-[[2-(3,4-Dimethoxyphenyl)-ethyl]methylamino]propyl]-3,4-dimethoxy-α-(1-methyl-ethyl)benzeneacetonitrile); e.g., hydrochloride: Vasolan	52-53-9 454.61 $C_{27}H_{38}N_2O_4$	Cardiac drug (calcium ion influx inhibitor) (antihypertensive)	No occurrence data	µM concentrations and lower greatly increase toxicity of certain drugs for many aquatic organisms (*32*) because of inhibition of "multidrug transporters"; removal of other drugs is reduced, lengthening the exposure time; *Daphnia* immobilization EC_{50} 0.11–0.67 mM (*125*)

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; max, maximum; MW, molecular weight; NA, not applicable. [a]In the first survey of Canadian aquatic life (*154*), maximum musk lipid-concentrations from populated areas in Canada showed values in distinct contrast with those from Europe: musk ketone (2.2–17.7 µg/g lipid; mussels, winter flounder, clams); 0.2–0.7 (trout, eel, lobster); Galaxolide: 0.01–3.0 µg/g in the same samples. For a given sample, the lipid concentrations of musk ketone (the dominant nitro musk) were several-fold to over an order of magnitude greater than the concentrations for the predominant polycyclic (Galaxolide); most samples contained lower concentrations of musk xylene and Tonalide. These data contrast with those from Europe—the concentrations of nitro and polycyclics are comparable in Canada, whereas the nitro musks are 1–3 orders of magnitude lower than the polycyclics in Europe (presumably reflecting different usage patterns and the fact that the nitro musks have not been restricted in the West). In comparison with Europe, insignificant quantities of amino metabolites of nitro musks were found in Canadian aquatic life, presumably a reflection of the lower degree of sewage treatment in Canada [e.g.,(*2*)].

ratio of drug concentrations in bile to that in the surrounding water can increase many orders of magnitude as exposure duration increases (*15*). Detection of exposure of fish to many drugs can thereby be facilitated through the analysis of bile.

The introduction of drugs into the environment is partly a function of the quantity of drugs manufactured, the dosage frequency and amount [the 200 most frequently prescribed drugs, representing about two-thirds of all prescriptions filled in the United States for the most recently documented year, are listed in RxList (*16*)], the excretion efficiency of the parent compound and metabolites, propensity of the drug to sorb to solids, and the metabolic transformation capability of subsequent sewage treatment (or landfill) microorganisms. Publicly owned wastewater treatment plants (POTWs) receive influent from both domestic, municipal, and industrial (including pharmaceutical manufacture) sewage systems. The processed liquid effluents from primary and secondary treatments are then discharged to surface waters and the residual solids (sludge) to landfills/farms; land disposal, including manure from treated animals at CAFOs, creates the potential for introduction into groundwaters or surface waters (via wet weather run-off). Theoretically, PPCPs in sewage sludge applied to crop lands could be taken up by plants.

Compounds surviving the various phases of metabolism and other degradative or

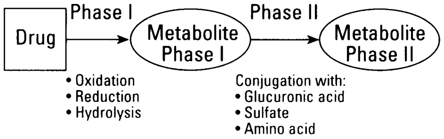


Figure 1. Metabolic approach to increasing the polarity (and excretability) of drugs.

sequestering actions (i.e., display environmental persistence) can then pose an exposure risk for organisms in the environment. Even the less/nontoxic conjugates (glucuronides) can later be converted back to the original bioactive compounds via enzymatic (β-glucuronidases) or chemical hydrolysis (e.g., acetylsalicylic acid can be hydrolyzed to the free salicylic acid). Some degradation products can even be more bioactive than the parent compound. Therefore, conjugates can essentially act as storage reservoirs from which the free drugs can later be released into the environment. Up to 90% of certain drugs can become conjugated (*17,18*), conjugation varying as a function of chemical class. These pathways of introduction into the environment have been summarized by Velagaleti (*10*).

Sewage treatment plants. Treatment facilities, primarily POTWs or sewage treatment works (STWs), which include privately owned works as well, play a key role in the introduction of pharmaceuticals into the environment [see Rogers (*19*) for a review of the fate of synthetic chemicals in sewage treatment plants]. STWs were designed to handle human waste of mainly natural origin, primarily via the acclimated degradative action of microorganisms (the efficiency of metabolism of a given drug can increase with duration of treatment because of enzyme induction and cellular adaptation) and the coagulation/flocculation of suspended solids; sometimes, tertiary treatment (e.g., chemical/ultraviolet [UV] oxidation) is used. Most anthropogenic chemicals introduced along with this normal waste suffer unknown fates. Two primary mechanisms remove substances from the incoming waste stream: *a*) microbial degradation to lower molecular weight products, leading sometimes to complete mineralization—CO_2 and H_2O; and *b*) sorption to filterable solids, which are later removed with the sludge.

Although the microbiota of sewage treatment systems may have been exposed to many PPCPs for a number of years, two factors work against the effective microbial removal of these substances from STWs. First, the concentrations of most drugs are probably so low that the lower limits for enzyme affinities may not be met. For example, the daily loadings of PPCPs into STWs are largely a function of the serviced human population, the dosages/duration of medications consumed, and the metabolic/excretory half-lives, which are all large variables. As an example, the daily load of a subset of pharmaceuticals to a particular POTW near Frankfurt/Main, Germany, ranged from tens to hundreds of grams, with approximate individual removal efficiencies varying widely from 10 to 100% but trending to around 60% (*18*). This particular POTW serviced about a third of a million people at a flow rate of roughly 60,000 m^3/day. Despite the number of studies on treatment efficiencies, a widespread investigation is still lacking for the differences in removal efficiencies for distinct types of STWs as well as for individual treatment techniques. The extent to which a particular plant uses primary, secondary, and tertiary technologies will greatly influence removal efficiencies; the technologies employed vary widely among cities. The biodegradative fate of most compounds in STWs is governed by nongrowth-limiting (enzyme-saturating) substrate concentrations (copiotrophic metabolism). In contrast, PPCPs are present in STWs at concentrations at enzyme-subsaturating levels, which necessitates oligotrophic metabolism. These micropollutants might be handled by only a small subset of specialist oligotrophic organisms whose occurrence is probably more prevalent in native environments characterized by low-carbon fluxes (e.g., sediments and associated pore waters, where desorption mass transfer is limiting) than in STWs. This means that degradation of PPCPs may occur more prevalently in the receiving waters/sediments than in STWs.

Second, many new drugs are introduced to the market each year; some of these drugs are from entirely new classes never seen before by the microbiota of an STW. Each of these presents a new challenge to biodegradation. A worst-case scenario may not be unusual—the concentration of a drug leaving an STW in the effluent could essentially be the same as that entering. Only the several-fold to multiple order of magnitude dilution when the effluent is mixed into the receiving water, assuming a sufficiently high natural flow, serves to reduce the concentration; obviously, smaller streams have increased potential for having higher concentrations of any PPCP that has been introduced. In general, most pharmaceuticals resist extensive microbial degradation (e.g., mineralization) (*10*). Although some parent drugs often show poor solubility in water (*10*), leading to preferential sorption to suspended particles, they can thereby sorb to colloids and therefore be discharged in the aqueous effluent. Metabolites, including breakdown products and conjugates, will partition mainly to the aqueous effluent. Some published data demonstrate that many parent drugs do make their way into the environment (see references cited in Table 2 under "Environmental Occurrence").

The efficiency of removal of pharmaceuticals by STWs is largely unknown. Currently, the most extensive study of treatment efficiency (*18*) reports removal from German STWs of 14 drugs representing five broad physiologic categories. Removal of the parent compound (keep in mind that possible subsequent metabolites were not accounted for) ranged from 7% (carbamazepine, an antiepileptic) to 96% (propranolol, a beta-blocker); most removal efficiencies averaged about 60%. Fenofibrate, acetominophen, and salicylic acid, *o*-hydroxyhippuric acid, and gentisic acid (acetylsalicylic acid metabolites) could not be detected in effluent; salicylic acid was found in the influent at concentrations up to 54 µg/L. It is important to understand that absent the stoichiometric accounting of metabolic products, one cannot distinguish between the three major fates of a substance: *a*) degradation to lower molecular weight compounds, *b*) physical sequestration by solids (and subsequent removal as sludge), and *c*) conjugates that can later be hydrolyzed to yield the parent compound (e.g., clofibric and fenofibric acid conjugates) (*18*). Therefore, by simply following disappearance (removal) of a substance, one cannot conclude that it was structurally altered or destroyed—it may simply reside in another state or form. Identifying metabolic products is difficult not only because of the number of metabolites (sometimes several per parent compound) but also because standard reference materials are difficult to obtain commercially and can be costly.

Despite high removal rates in STWs for some drugs, upsets in the homeostasis of a treatment plant can result in higher than normal discharges. For example, Ternes (*18*) found that wet weather runoff dramatically reduced the removal rates for certain drugs (e.g., several nonsteroidal anti-inflammatory drugs [NSAIDs] and lipid regulators) in a facility located close to Frankfurt/Main. During the increased period of influent flow, the removal rate dropped to below 5% from over 60% previously; several days were required for the removal rates to recover. Clearly, even for drugs efficiently removed, the operational state of the STW can have a dramatic effect on the removal efficiencies. Other transients that could affect removal include transitions between seasons and sporadic plug-flow influx of toxicants from various sources. Overflows from STW failure or overcapacity events (e.g., floods, excessive water use) lead to direct, untreated introduction of sewage into the environment. In efforts to improve tributary conditions (by increasing stream flow), some cities have considered increasing the percentage of annual overflow events (e.g., see the Portland, Oregon, proposal (*20*). The highest concentration in an STW effluent reported by Ternes (*18*) was for bezafibrate (4.6 µg/L); the highest concentration in surface water also was for bezafibrate (3.1 µg/L ppb).

Landfills. PPCPs can be introduced to landfills both directly via domestic and industrial routes and indirectly via sewage sludge. Holm et al. (*21*) first reported leachates carrying pharmaceuticals from a landfill. Large amounts of numerous sulfonamides (antibiotics) and barbiturates from domestic waste and from a pharmaceutical manufacturer were disposed of at a Danish landfill over a 45-year period. High concentrations (ppm) of many of these drugs were found in leachates close to the landfill; these compounds even accounted for 5% of the total nonvolatile organic carbon found in the leachate. It was also found that the concentrations dropped off dramatically tens of meters down gradient, presumably a result of microbial attenuation.

Drinking water. Few pharmaceuticals have been identified in domestic drinking water, probably because of the dearth of monitoring efforts and because the required detection limits are too low for current routine analytical technology. In Germany, however, clofibric acid concentrations up to 165 ng/L (*22*) and 270 ng/L (*23*) have been measured in tap water; the presumed source was from recharged groundwaters that had been contaminated by sewage. Stumpf et al. (*24*) and Ternes et al. (*9*) found several pharmaceuticals in German drinking water in the lower nanograms-per-liter range, with a maximum of 70 ng/L for clofibric acid. Additionally, these investigators found that diclofenac, bezafibrate, phenazone, and carbamazepine were sometimes present. In the majority of the samples analyzed, however, no drugs were observed. The investigations performed to date therefore indicate that contamination of drinking water does not appear to be a general problem. Depending on the water source for drinking water production, however, certain facilities can experience contamination, especially if the source is polluted groundwater and if polishing technology does not remove the PPCP [e.g., see Heberer et al. (*23*) and Stumpf et al. (*24*)]. A major unaddressed issue regarding human health is the long-term effects of ingesting via potable waters very low, subtherapeutic doses of numerous pharmaceuticals multiple times a day for many decades. This concern especially relates to infants, fetuses, and people suffering from certain enzyme deficiencies (which can even be food-induced, e.g., microsomal oxidase inhibition by grapefruit juice).

Drinking water regulations. Regulations designed to safeguard receiving waters (from sewage treatment) and drinking water were historically designed to protect the consumer from the obvious threats of pathogens, widely used industrial chemicals, and certain radionuclides. The treatment processes used by state-of-the-art POTWs evolved from the need to remove these limited sets of contaminants. In areas of water scarcity, the future will see more and more reuse of treated sewage to meet drinking water needs. This will impose a severe burden on water providers to ensure that all chemical contaminants have been removed to the greatest extent possible. It will also require the ability to identify as many of the plethora of potential chemicals in the upgraded water as possible.

According to the National Research Council (NRC) (*25*), more than two dozen major U.S. utilities release so much effluent to receiving waters that when the natural flows are low, the discharged waste composes 50% of the eventual flow. Any residual, unidentified contaminants therefore are diluted 2-fold at best. In more densely populated countries (e.g., United Kingdom), this figure can rise as high as 90% of flow during times of low rainfall (*26*).

Domestic animals. Whereas the concentration of many drugs is greatly attenuated through sewage treatment plants, larger quantities of many pharmaceuticals are used in various animal husbandry operations, especially CAFOs. With aquaculture in particular, which uses many anti-infectives and anesthetics, the chance for introduction into the immediate environment is greatly enhanced, and the possibility of direct human consumption of therapeutic quantities is correspondingly heightened. Even in the United States the extremely large populations of pet dogs and cats are recipients of numerous drugs (e.g., tranquilizers and antidepressants)—some prescribed by veterinarians and others intended for their owners' use as pet owners sometimes administer medications to their pets to test off-label uses for themselves. PPCPs (both veterinary drugs and OTC products) used with terrestrial domestic animals can be dispersed into the environment through the same routes as those PPCPs used for humans, with the added major route of run-off/leaching of on-ground fecal material.

Significant Aspects of Ecotoxicology

Shortcomings of effluent toxicologic screening: comprehensive chemical characterization cannot be replaced—chemical characterization and toxicity screening must be better integrated. There are two debates in the realm of ecotoxicology, both of which have ramifications with respect to performing ecologic risk assessments (ERAs) for PPCPs. The first is the relevance of purposefully simplified, defined-species toxicity tests to predicting/extrapolating pollutant impacts on the more highly organized and complex structural/functional levels of communities or ecosystems (processes) [see Boudou and Ribeyre (*27*)]; this is truer for PPCPs than for pesticides, as the former were generally never designed to have any intended effects on wildlife and therefore any knowledge as to what types of effects to look for is clearly more limited. Can changes in a complex system be predicted from knowledge of a small subset of the underlying components? The second is the question of whether it is necessary to know the spectrum of possible physiologic effects, given a multitude of organisms, or possible mechanisms (modes) of action before looking for and ascribing causation to changes at the population level and higher. Considering this, one can only pose at this time the rhetorical question as to whether the risk posed by the presence of pollutants in complex waste streams (e.g., PPCPs in STW effluents) can be detected/quantified by the use of current toxicity screening tests never designed to embrace the spectrum of end points (some exquisitely subtle) that may be involved. The most conservative approach would be one that captures the coordinated use of toxicity-directed screening and chemistry-directed characterization, feeding the results of each to the other, to better reveal the nature of any stressors.

Although most pharmaceuticals are designed to target specific metabolic pathways in humans and domestic animals, they can have numerous often unknown effects on metabolic systems of nontarget organisms, especially invertebrates. Although many nontarget organisms share certain receptors with humans, effects on nontarget organisms are usually unknown. It is important to recognize that for many drugs, their specific modes of action even in the target species are also unknown. For these drugs, it is impossible to predict what effects they might have on nontarget organisms. Without knowing the mode of action, coupled with not knowing the possible receptors, it is impossible to design rational toxicity testing procedures at the organism level. In the final analysis, given the vast array of mechanisms of drug action and side effects, the total number of different toxicity tests possibly required to screen the effluent from a typical STW could be impractically large. The current batteries of acute/chronic toxicity tests used for ecotoxicity screening merely supply gross indications of directly measurable acute effects. Even if the known mode of action is considered when selecting ecotoxicity tests [as recommended by Henschel et al. (*28*)], this falsely presupposes that other modes of action are nonexistent or nominal.

Regulatory agencies only in the last few years have recognized that pharmaceuticals should be screened to determine possible effects on nontarget species. The world's first requirement for ecotoxicity testing as a prerequisite for registration of a pharmaceutical was established in 1995 and first implemented in Germany according to European Union (EU) guideline 92/18 EWG for veterinary pharmaceuticals. For a more in-depth discussion, see Henschel et al. (*28*), and for a general discussion of the issues in aquatic ecotoxicology, see Boudou and Ribeyre (*27*).

Screening waste effluent and receiving waters for toxicologic effects can at best be only partially effective because the range of physiologic effects is too broad and relevant to a vast array of aquatic and terrestrial organisms, spanning everything from acute toxicity to very subtle behavioral or genetic changes, of which the consequences are not immediately manifested and can be detected only over long periods of time. There are too many scenarios to discuss in an efficient, comprehensive manner. The complexity of accounting for a wide range of mechanisms of action was made clear in the National Research Council's recent report on endocrine disruptors (*29*). Although for this class of pollutants the number of modes of action is very large, they represent only a subset of those for PPCPs in general. Quite clearly, any successful toxicity-directed methodology for risk assessment of complex effluents or environmental samples should also make use of a well-developed knowledge of the chemical constituents and their modes of action; current approaches are not yet sufficiently comprehensive. The complexity of this task is further magnified when the effect and necessarily its mode of action have not even been elucidated.

A popular means of attempting to identify the toxic constituents, using toxicity identification and evaluation, in complex waste such as sewage effluent is that of bioassay-directed fractionation screening (*30*), in which chemical separation techniques yield distinct chemical-class fractions that are then subjected to toxicity testing. Those fractions showing activity against the selected end point are then subjected to chemical identification protocols. Even if one accepts the limitations of selecting appropriate end points (the number with environmental relevance would be enormous), this extremely time-consuming approach would miss any combined effects, whether antagonistic or synergistic, of multiple chemicals. Direct, rigorous chemical characterization of problematic samples clearly must play a role in the identification of toxicants that might elicit previously unrealized toxic effects in nontarget organisms.

The trend toward optically pure pharmaceuticals: fewer side effects and lower concentrations. Most pharmaceuticals are racemic mixtures. For a specific optically active drug, it is theorized that only one of its optical isomers is responsible for the desired physiologic, therapeutic effects; the other isomers are at best inactive, or even worse, responsible for many of the untoward side-effects that most drugs display. A recent trend in the pharmaceutical industry, and now supported by the FDA, is to produce only the optically pure therapeutic isomer (*31*). This has the potential to not only lessen side effects, but for some drugs, the total dosage can be lessened by at least 50%. This could help in reducing the burden on sewage treatment plants. The significance of the industry's switch to optically pure isomers is that the number of metabolites and other SRSs entering the environment will be reduced at least by half, and the use of the active ingredient will also be reduced by at least 50% because the potency will effectively increase. At the same time, however, the trend of pharmaceuticals toward higher potency will increase the difficulty of environmental monitoring because the required detection levels will be lowered.

Synergistic effects and potentiation: the potentially critical role of "multixenobiotic resistance." The biochemical interactions of drugs, often leading to adverse effects, is well known in humans. Little is known, however, of this interplay in aquatic organisms. The following is provided as an example of the complex potential for adverse drug interactions (one actually leading to increased exposure), as it also illuminates the interwoven pathways that ultimately determine exposure. Mostly during this decade, a new mechanism for elimination of xenobiotics from organisms (first observed in tumor cells) has been elucidated—multidrug transporters. This excretory system, also called multixenobiotic transporters, comprises proteins that facilitate the active export of potentially toxic substances, primarily those of moderate lipophilicity, from inside cells. The best-known transporters are the *P*-glycoproteinlike (*P*gp) transporters (*P* is for permeability altering), or P170 (because of their 170-kDa mass), which have been well characterized in mammals, especially tumor cells, and bacteria.

The toxicologic significance of these nonspecific transporters in maintaining a first line of defense against exposure to multiple xenobiotics in aquatic species has been largely pioneered and reviewed by Epel (*32*) and by Kurelec and co-workers (*33–35*); this system confers what has become known as multidrug or multixenobiotic resistance (MDR or MXR). Although these protective proteins have not been found in all aquatic organisms, they have been found in many, especially filter feeders and bottom dwellers (those having potentially high exposures to xenobiotics). This extrusion pump protein system, and possibly others as yet identified, facilitate the removal and prevent the entrance of those compounds not metabolized or conjugated. They seem to have nonspecific recognition, working for many pesticides, drugs, and natural toxins alike. The action of this transporter system can be inhibited by certain substances such as verapamil (α-[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl)benzene acetonitrile), a cardiac drug—calcium ion influx inhibitor—that directly binds to the active site of *P*gp. Exposure to verapamil at micromolar concentrations and lower greatly increases the toxicity of a number of drugs or other xenobiotics for many aquatic organisms (*32*), as the toxicant cannot be readily removed from the exposed organism; exposure time is thereby lengthened by its intracellular accumulation. This elimination system does not function for highly hydrophobic substances (e.g., DDT, polychlorinated biphenyls [PCBs]) and as such might play a more critical role in eliciting effects from exposure to the less hydrophobic PPCPs. Xenobiotics may irreversibly inhibit (cyclosporine A inhibits ATPase), competitively inhibit (verapamil, quinidine, reserpine at low concentrations or high concentrations of general lipophilic compounds such as petroleum oil), or indirectly modulate (e.g., via phosphorylation) MXR regulation or expression (staurosporine inhibits protein kinase C *P*gp regulator), resulting in its reversal.

The slow escalation, by induction or genetic enrichment, of MXR occurrence and activity among aquatic organisms can give the illusion that the toxicity potential in the aquatic environment is stable or even decreasing when in reality it may be increasing. The introduction of a new substance, at what would normally be a no-effect level, that disrupts the activity of MXR could thereby lead to a profound cascade of unanticipated and unaccounted-for toxic events—a phenomenon akin to what is being termed toxicant-induced loss of tolerance in humans. Organisms in an aquatic environment that have adapted via MXR to certain levels of a suite of toxicants could experience widespread interspecies toxic events should their MXR be inhibited by the addition of a single agent capable of inhibiting MXR, even one that ordinarily would elicit no effect on its own. The resulting effects would be inexplicable if considered solely on the basis of exposure to the new toxicant.

Little is known about which xenobiotics have activity within this relatively newly identified class of chemicals, referred to as chemosensitizers, or their frequency of occurrence in the environment. Smital and Kurelec (*35*), however, showed that unidentified agents in samples from polluted waters enhance the accumulation of aromatic amines in clams, mussels, snails, and sponges. Some examples of known MXR inhibitors (*34,35*), other than verapamil include trifluoroperazine (Stelazine, which is a calmodulin antagonist and an antipsychotic tranquilizer), reserpine (antihypertensive), quinidine and amiodarone (anti-arrythmics), cyclosporins (immunosuppressants), anthracyclines (noncytotoxic cytotoxin analogs), and progesterone (steroid); some natural substances such as agent(s) in grapefruit juice are also known to inhibit the *P*-glycoprotein system (*36*).

Environmental Studies on Pharmaceuticals

Given the numbers and quantities of pharmaceuticals manufactured and used throughout the world and that many of these chemicals are designed to have profound physiologic effects, comparatively little research has been published on their occurrence in the environment, effects on nontarget organisms, or assessment of environmental impact. Literally thousands of distinct drugs are approved for use throughout the world. Many of these are manufactured and used in very large quantities. The world's combined literature (the vast majority of these studies have originated in Europe, but the issue applies equally worldwide) has addressed only a very small percentage of these compounds, and the huge array of associated metabolites and other transformation products, many of which undoubtedly have strong physiologic activity, simply compounds the magnitude of the problem.

When drugs are detected in the environment (e.g., surface waters), their concentrations are generally in the ng/L–µg/L (ppt–ppb) range. Although parts-per-billion concentrations may not pose much acute risk, it is completely unknown whether other receptors in nontarget organisms are sensitive. It must also be recognized that even though individual concentrations of any drug might be low, the combined concentrations from drugs sharing a common mechanism of action could be substantial. Exposures in the aquatic environment are of particular concern, since aquatic organisms (as opposed to those spending at least some time in terrestrial settings) are subject to continual, unabated lifecycle exposures. This is a highly significant consideration for pharmaceuticals (or bioactive metabolites) that are refractory to structural transformations and are continually introduced into surface waters from sewage treatment plants. Moreover, the polar, nonvolatile nature of most drugs prevents their escape from the aquatic realm. Effectively, even PPCPs with relatively short environmental half-lives assume the qualities of highly persistent pollutants because they are continually replenished by infusion to the aquatic environment from STWs.

Environmental Occurrences

(Note: The names, structures, Chemical Abstracts Service Registry Numbers, and some of the data for environmental occurrences cited in this paper are summarized in Table 2.) Probably the first report of a prescription drug in the environment (sewage treatment effluent) was made over 20 years ago by Garrison et al. (*37*), who reported clofibric acid (the active metabolite from the lipid regulators clofibrate, etofibrate, and theofibrate) concentrations of 0.8–2.0 µg/L in raw sewage and activated sludge effluent. They also found the ubiquitous caffeine and nicotine to be the two most prevalent compounds in influent and effluent from activated sludge, but they did not find the parent clofibrate in any sample. In parallel, Hignite and Azarnoff (*38*) reported salicylic acid and clofibric acid in the influent and effluent from a Kansas City, Missouri, municipal sewage treatment plant [the history of clofibric acid identified in the environment has been summarized by Stan and Heberer (*39*)]. Clofibric acid was routinely detected in the effluent of this Missouri STW at an average effluent rate of 2.1 kg/day; over a 10-month period its loading remained in the tight range of 0.76–2.92 kg/day. Similarly, salicylic acid, a hydrolytic metabolite of aspirin, averaged 8.6 kg/day but ranged more widely from 0.55 to 28.7 kg/day. Stan and Heberer also observed that the influent concentrations of clofibric acid were only 20% higher than the effluent concentrations, showing that this chemical resisted removal by the STW. In contrast, for salicylic acid, the influent concentration was about an order of magnitude higher than the effluent, showing more efficient removal.

It therefore was clearly recognized over 20 years ago that the continual, daily introduction of kilogram quantities of drugs from a given STW into receiving waters could result in sustained concentrations with the potential to lead to exposures in aquatic organisms. Little more transpired in the literature, however, during the next 15 years, although clofibric acid continued to appear in a number of monitoring efforts that did not target PPCPs. The most complete investigation to date of the occurrence of pharmaceuticals in both the influent and effluent of POTWs (and also in various surface waters) has been published by Ternes (*18*).

The distribution of pharmaceuticals is a large function of their production volumes, which can rival those for many pesticides. There are thousands of registered drugs that are dispensed both as prescriptions and OTC; this makes it difficult to estimate usage rates for those pharmaceuticals sold via both routes (e.g., many analgesics). In Germany, roughly 2,900 drugs are permitted in human medicine alone (*18*). Many countries dispense drugs in the absence of prescriptions. The two primary sources for release into the environment are from human and veterinary applications. Ternes (*18*) states that at least for lipid regulators and NSAIDs the source is almost entirely from human usage, as these drugs are infrequently (or never) used in veterinary medicine. In general, the literature shows that most pharmaceuticals, when detected, are present in surface waters in a concentration range of 1 ng/L–1 µg/L. To put this in perspective, Richardson and Bowron (*40*) state that 1,000 kg of a chemical distributed evenly among the rivers in England and Wales would yield a concentration of about 0.1 µg/L. Many pharmaceuticals are consumed in amounts far exceeding this; in fact, Richardson and Bowron report 170 pharmaceuticals used annually in excess of this amount.

Terrestrial and Atmospheric Exposure

Minor route for PPCPs in contrast to pesticides. The majority of PPCPs introduced into the environment is undoubtedly into aquatic systems; the terrestrial environment receives only a secondary input. Although the primary source for terrestrial exposure is probably from disposal of biosolids from STWs and from animal wastes both applied to land and stored in open-air pits (waste lagoons), other possible sources for veterinary pharmaceuticals result from animal dips and direct deposition of dung from medicated animals. To date, most attention has been focused on the application of animal wastes to land, primarily because of the suspected introduction of antibiotics and nutrients, not because of PPCPs other than veterinary antibiotics, which are used in comparatively smaller amounts. It should be noted, however, that even though the introduction of veterinary antibiotics into the environment, both terrestrial and aquatic, via animal wastes is widely discussed, the topic has experienced little attention in the peer-reviewed literature (*41,42*). This topic also relates directly to the human health concern of introducing/promoting antibiotic resistance in bacteria, both native to and introduced into the environment [see section on "Antibiotics" and Williams and Heymann (*43*)].

The polar nature of the majority of drugs/metabolites leads to facile leaching from land disposal areas into groundwater or wet weather runoff into surface waters. The remainder (largely those designed to pass the blood–brain barrier) have lipophilic character, rendering them prone to bioconcentration from consumption of water or bioaccumulation from consumption of tissue.

Dung-feeding fauna such as birds, beetles, worms, flies, and microorganisms could experience immediate exposure to excreted terrestrial veterinary pharmaceuticals and metabolites.

These organisms in turn could suffer effects themselves from exposure or, alternatively, pass on accumulated residues further up the food chain. All other routes of dispersal to other environmental compartments also play roles, with the distinct exception of direct volatilization, because nearly all PPCPs, with the exception of medical gases and fragrances in contrast with many other anthropogenic compounds are polar or otherwise nonvolatile. The major volatile pharmaceuticals are the inhalable anesthetics (e.g., halothane); these hydrofluoroalkanes are known to oxidize in the atmosphere, like the conventional hydro[chloro]fluorocarbon refrigerants, to yield the highly persistent, toxic, and ubiquitous product trifluoroacetic acid (TFA). This source of TFA is believed to be minor (*44*).

Drug Classes and Environmental Occurrences

Hormones/Mimics

Potential for receptor interaction may not be rare. An excellent overview of hormone systems is given by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) (*45*). Steroids were the first physiologic compounds to be reported in sewage effluent (*46–49*) and as such were the first pharmaceuticals to capture the attention of environmental scientists. Estrogenic drugs, primarily synthetic xenoestrogens, are used extensively in estrogen-replacement therapy and in oral contraceptives, in veterinary medicine for growth enhancement, and in athletic performance enhancement. A special issue of *The Science of the Total Environment* (*50*) is devoted to drugs (especially hormones) as pollutants in the aquatic environment.

Although the synthetic oral contraceptive (17α-ethynylestradiol) occurs generally at low concentration (< 7 ng/L) in POTW effluent, it is still suspected, in combination with the steroidal estrogens 17β-estradiol and estrone [*30*], of causing vitellogenin production (feminization) in male fish. Feminization is a phenomenon first observed for fish in sewage treatment lagoons in the mid-1980s (*26*). An overview of pharmaceutical hormones in the environment is presented by Arcand-Hoy et al. (*51*). The estrogenic activity of various waters (from sewage to drinking water) has been shown to vary dramatically, spanning six orders of magnitude. Some other widely used synthetic hormone modulators include Proscar/Propecia (finasteride: an androgen hormone inhibitor) and various thyroxine analogs (thyroid hormones); nothing is known of the environmental fates of these compounds. In general, the lipophilicity of these hormones is sufficiently great that at least a large portion are removed via sorptive processes in sewage treatment (*52,53*) and therefore partition to the sludge; but even the low concentrations that remain in the effluents may be capable of exerting physiologic effects in aquatic biota.

In addition to these synthetic steroids and xenoestrogens is a suite of naturally occurring estrogen hormones, for example, phytoestrogens such as the complex series of leguminous isoflavonoids, including genistein, daidzein, and glycitein in soy. Further complicating the picture are a host of newly suspected endocrine-disrupting compounds (EDCs), more recently referred to as hormonally active agents (HAAs) by the NRC (*29*), which have gained attention in the last few years, primarily as a result of the 1996 publication *Our Stolen Future* by Colburn et al. (*54*). These inadvertent EDCs include such commonly recognized industrial pollutants and products as halogenated dioxins/furans, PCBs, organohalogen pesticides, phthalates, and bisphenol A.

The issue of screening many of the major commercial chemicals (over 87,000 total) for endocrine disruption potential has been formalized with the creation of the EDSTAC, which had been charged by the U.S. EPA with the task of implementing a screening and testing program by August 1999 (*45*). The Chemical Manufacturers Association (CMA) also has launched an intensive health effects investigation for over 3,000 high-volume chemicals (called the Health and Environmental Research Initiative) (*55*). It is significant, however, that pharmaceuticals are not specifically targeted by the EDSTAC (or the CMA) in its tiered screening program that focuses on pesticides, commodity chemicals, naturally occurring nonsteroidal estrogens (phytoestrogens and mycotoxins), food additives, cosmetics, nutritional supplements, and representative mixtures (for possible synergistic effects). Even though the strategy gives top priority to "chemicals with widespread exposure at the national level" (*55*), PPCPs are not specially targeted. It is also significant that the screening strategy will initially focus on only the three primary hormone systems—estrogen, androgen, and thyroid—hormone systems of relatively unknown importance to invertebrates (*45*).

A controversial hypothesis regarding multiple toxicants (sharing a common mode of action), when each is present at a low level, is that of synergism. Evidence of synergism among estrogenic mimics (where the effect can be elicited at orders-of-magnitude lower concentration than predicted by additive action) was reported by Arnold et al. (*56*). This study created much controversy by purporting synergistic action of low-level chemical mixtures. Subsequent studies by Gaido et al. (*57*) and others rebutted this hypothesis. They did not find any evidence of synergism in mixtures of mild estrogenic pollutants. McLachlan (*58*) later withdrew the article by Arnold et al. (*56*), but the issue has not been put to rest, especially given Arnold's other publications on this subject including Arnold et al. (*59*) and references cited therein. Another controversial issue is that of inverted (U-shaped) dose–response in which toxicity diminution tracks lower concentrations down to a certain level, at which point toxicity again increases. Consequently, higher dose effects might not be useful in predicting the type or magnitude of effects from lower doses (*29*). This unresolved issue, coupled with the controversy of whether toxicity thresholds necessarily exist, could severely impede EDSTAC's ability to reach its objective because the concentration ranges that must be investigated would be greatly expanded.

Low molecular weight nonpeptidyl molecules can mimic hormones. Another subclass of hormonelike substances includes those that are being purposefully designed to mimic the activity of therapeutically significant hormones. A long-sought objective has been to obviate the need for hormone-replacement therapy (e.g., insulin) by designing small synthetic (nonpeptidyl) molecules that mimic the hormone's effect yet can be ingested orally, taken up by the gut, and remain stable for a sufficiently long period of time in the blood. The first report of a "designer" hormone mimic (*60,61*), a polybenzimidazole that activates the receptor for a cytokine that regulates white blood cell production, perhaps portends the advent of many synthetic hormone mimics in therapeutic medicine. If the finding can be generalized, it could mean that the possible routes of hormone disruption by simple molecules could extend beyond that of the estrogen/androgen system.

With the exception of estrogenic mimics, the possibility of disrupting the activity of proteinaceous hormones by lower molecular weight anthropogenic chemicals has been held in low regard. This view has been based on the fact that a relatively large, complex proteinaceous molecule (the hormone) neatly "fits" within the complex three-dimensional domain of its target receptor, whereas in contrast a much smaller nonproteinaceous molecule would have little to offer in terms of recognition specificity. It has been believed that the complexity of larger proteins such as insulin was required to enable recognition by the corresponding receptors; smaller compounds simply did not convey enough three-dimensional information to have high-binding constants for one or multiple receptors.

The report by Tian et al. (*60*) demonstrates for the first time that a relatively small nonpeptide molecule can bind to a receptor normally dedicated to a proteinaceous hormone. While this has high therapeutic significance (this research might catalyze concerted attempts to develop the first protein-mimicking and therefore perhaps hormone-mimicking low molecular weight drugs), it also alludes to the possibility that existing anthropogenic compounds might have a greater chance of interacting with hormone receptors than was previously believed. Although the synthetic substance was three to six orders of magnitude less potent, its ability to bind to the receptor was undisputed (in the mouse *in vitro* and, more importantly, *in vivo*).

Antibiotics

In addition to pathogen resistance, genotoxicity may be a concern. A large body of literature exists on antibiotics in the environment. Veterinary and animal husbandry, especially aquaculture, usage plays a major role in their introduction into the environment. In one study of hospital effluent, fluoroquinolones was the chemical class contributing the major portion to overall DNA toxicity (*62*); ciprofloxacin, for example, was identified at 3–87 μg/L. Hirsch et al. (*41*) analyzed German STW effluents and groundwaters/surface waters for 18 antibiotics representing macrolides, sulfonamides, penicillins, and tetracyclines. Although the penicillins (susceptible to hydrolysis) and the tetracyclines (can precipitate with calcium and similar cations) were not found, the others were detected in the microgram per liter range. Indeed, the rampant, widespread (and sometimes indiscriminate) use of antibiotics, coupled with their subsequent release into the environment, is the leading proposed cause of accelerated/spreading resistance among bacterial pathogens, which is exacerbated by the fact that resistance is maintained even in the absence of continued selective pressure (an irreversible occurrence). Sufficiently high concentrations could also have acute effects on bacteria. Such exposures could easily lead to altered microbial community structures in nature and thereby affect the higher food chain. Their use in aquaculture results in eventual human consumption. For a discussion of promotion of antibiotic resistance, see the policy article by Witte (*63*). Hartmann et al. (*62*) propose that genotoxicity in hospital effluent may result more from antibiotics than from antineoplastics.

Recently, a number of stream surveys documented the significant prevalence of native bacteria that display resistance to a wide array of antibiotics including vancomycin (*64*). Isolates from wild geese near Chicago, Illinois, are reported to be resistant to ampicillin, tetracycline, penicillin, and erythromycin (*65*). All these reports could simply indicate that the natural occurrence of antibiotic resistence in native bacterial populations is much higher than expected or that these bacteria are being selected for by the uncontrolled release of antibiotics into the environment. If the latter is true then, excluding the significance of antibiotics themselves in the environment, their occurrence can be viewed as marking or indicating the possible presence of other PPCPs.

Blood Lipid Regulators

Fibrates—high usage. Fibric acid metabolites—ubiquitous, persistent pollutants. Clofibric acid was the first prescription drug (actually an SRS) reported in a sewage effluent (*37,39*), and it continues to be one of the most frequently reported PPCPs in monitoring studies. Clofibric acid (2-[4]-chlorophenoxy-2-methyl propanoic acid), the active metabolite from a series of widely used blood lipid regulators, and which also happens to be structurally related to the phenylalkanoic acid herbicide mecoprop (the methylphenoxy structural analog), has captured much attention from investigators in Europe. Stan et al. (*22*) first reported clofibric acid in Berlin tap water at concentrations between 10 and 165 ng/L. Heberer and Stan (*66*) found clofibric acid at levels up to 4 μg/L in groundwater under a sewage treatment farm; they also found clofibric acid concentrations up to 270 ng/L in drinking water samples. They concluded that it is not removed by sewage/water treatment processes.

Buser et al. (*67*) report finding clofibric acid in various Swiss waters ranging from rural to urban lakes. Concentrations ranged from 1–9 ng/L (ppt), whereas the parallel concentrations for mecoprop were higher at 8–45 ng/L; little of either compound was found in a relatively remote mountain lake, indicating no atmospheric deposition. Because this drug is not manufactured in Switzerland, its route of introduction into the environment had to be through medical use and subsequent excretion/disposal. Although these concentrations are very low, they are significant in that they are similar to the concentrations found for any of the conventional ubiquitous and persistent pollutants, sometimes referred to as persistent organic pollutants (POPs) or persistent bioaccumulative toxicants (PBTs) such as lindane [see Jones and de Voogt (*68*) for an overview]. In one of the lakes studied, Buser et al. (*67*) calculated steady-state amounts of clofibric acid to be roughly 19 kg (with export and import amounts balancing each other). Perhaps more significantly, they also found amounts of clofibric acid up to 7.8 ng/L in the North Sea; the parallel concentrations of mecoprop in the same North Sea samples were lower, up to only 2.7 ng/L, indicating that mecoprop was less persistent than clofibric acid.

Stumpf et al. (*24*) and Ternes (*18*) reported bezafibrate, gemfibrozil, and clofibric/fenofibric acids in river waters at the nanogram per liter level. Stumpf et al. (*69*) reported that the removal efficiencies from Brazilian STWs for clofibric/fenofibric acids, bezafibrate, and gemfibrozil ranged from only 6–50%, verifying extremely limited degradation for these compounds. This chemical class is ubiquitous because the daily human dosages are generally high (grams per day). Buser et al. (*67*) concluded that the concentrations seen in urban Swiss and German rivers, coupled with essentially the same concentrations in the North Sea, lead to an annual input of 50–100 tons of clofibric acid into the North Sea. The concentration of clofibric acid in the environment is more a function of dilution than of degradation. Clofibric acid is the most widely and routinely reported drug found in open waters. It would be expected that its occurrence in other parts of the world would parallel these studies.

Nonopioid Analgesics/Nonsteroidal Anti-Inflammatory Drugs

Stumpf et al. (*24*) were the first to identify diclofenac, ibuprofen, acetylsalicylic acid, and ketoprofen in sewage and river water. Ternes (*18*) reported levels of diclofenac, indometacine, ibuprofen, naproxen, ketoprofen, and phenazone in POTW effluent exceeding 1 μg/L; all these except ketoprofen were also found in surface waters at concentrations severalfold lower. In another study, Ternes et al. (*70*) reported average concentrations of acetylsalicylic acid generally less than 1 μg/L in most POTW effluents as well as less than 0.14 μg/L in rivers. They also reported salicylic acid concentrations of 54 μg/L in POTW influents, with two other acetylsalicylic metabolites, gentisic acid (4.6 μg/L) and *o*-hydroxyhippuric acid (6.8 μg/L). While low levels (0.5 μg/L) of salicylic acid appeared in the effluents, no detectable amounts of the metabolites could be found. Ternes et al. (*70*) also found naproxen in all POTW effluents examined and in river waters (~0.05–0.4 μg/L); two veterinary NSAIDs, meclofenamic and tofenamic acids, were not detectable in any river sample. In their screening of waters in Berlin, Heberer et al. (*23*) found that the most prevalent drugs, other than clofibric acid, were the NSAIDs diclofenac, ibuprofen, and propyphenazone. In groundwater from a drinking water plant, they found diclofenac, ibuprofen, and *N*-methylphenacetin (from phenacetin) (*23*). In the influent to Swiss STWs, Buser et al. (*71*) found diclofenac at concentrations of 0.5–1.8 μg/L, whereas the concentrations in the respective effluents were only moderately reduced (at most 50%). In the receiving water (Swiss lakes/rivers), they found 11–310 ng/L but only 1–12 ng/L in exiting waters. They concluded that photolysis was the major cause of the diminished concentrations of diclofenac in surface waters

(*71*). Buser et al. (*72*) showed that ibuprofen, while present in influents at 1–3.3 µg/L, was easily degraded to yield low effluent concentrations (nanograms/liter) in contrast to other NSAIDs, which were more refractory. This study is also one of the few that examined the enantiomeric selectivity in the degradation of the parent optical isomers as well as the production of metabolites.

Beta-Blockers/β_2-Sympathomimetics

Hirsch et al. (*73*) and Ternes (*18*) identified the beta-blockers metoprolol and propranolol, with lesser amounts of betaxolol, bisoprolol, and nadolol, in POTW effluent. Only metoprolol and propranolol were found in surface waters at concentrations just above the limit of detection. The β_2-sympathomimetics (bronchodilators) terbutalin and salbutamol (albuterol in the United States), but rarely clenbuterol and fenoterol, were detected in POTW effluent and only at low concentrations, less than 0.2 µg/L. They were rarely seen in surface waters. It may be significant to note that medications delivered by inhalers could result in portions of the dose being deposited externally because of improper dosing technique.

Fenfluramine (*N*-ethyl-α-methyl-3-[trifluoromethyl] benzene ethanamine hydrochloride), known as Pondimin in addition to other brand names, is a sympathomimetic amine, which was used as a popular diet (anorectic) drug and was removed from the U.S. market in 1998 by the FDA because of heart valve damage. Although no one has looked for fenfluramine in sewage, it is known to enhance the release of serotonin (3-(2-aminoethyl)indol-5-ol or 5-hydroxytryptamine creatinine sulfate [5-HT]); in the crayfish, 5-HT in turn triggers release of ovary-stimulating hormone, resulting in larger oocytes with enhanced amounts of vitellin (consequences unknown) (*74*). Similarly, in fiddler crabs, fenfluramine at a dose of 125 nmol stimulates (through 5-HT) the production of gonad-stimulating hormone, which accelerates testicular maturation (*75*).

Antidepressants/Obsessive–Compulsive Regulators

Subtle but possibly profound effects on nontarget [aquatic] species. Selective serotonin reuptake inhibitors (SSRIs) are a major class of widely prescribed antidepressants that includes Prozac, Zoloft, Luvox, and Paxil. These drugs enjoy widespread and heavy use. One of the few series of studies reported in the literature that addresses the effects of drugs on nontarget organisms (albeit not the intent of the studies) was performed in a quest for more effective spawning inducers for economically important bivalves (*76*). Fong's studies and those of other physiologists studying the function of serotonin in a wide array of aquatic creatures could prove highly significant in any discussion of the importance of low levels of pharmaceuticals in the environment. Fong's work is perhaps the most significant to date for showing the potential for dramatic physiologic effects on nontarget species (in this case invertebrates) by low (ppb) concentrations of pharmaceuticals.

Serotonin is a biogenic amine common in both vertebrate and invertebrate nervous systems. SSRIs increase serotonin neurotransmission by inhibiting its reuptake at the synapses by inhibiting the transporter enzymes. In addition to playing a key role in mammalian neurotransmission, serotonin is involved in a wide array of physiologic regulatory roles in molluscs, among most other creatures. For bivalves, reproductive functions including spawning, oocyte maturation, and parturition are regulated by serotonin, (*76*). Serotonin controls a wide spectrum of additional behaviors and reflexes in molluscs, including heartbeat rhythm, feeding/biting, swimming motor patterns, beating of cilia, and induction of larval metamorphosis (*77*). It also stimulates release of various neurohormones in crustaceans (hyperglycemic hormone, red pigment-dispersing hormone, neurodepressing hormone, and molt-inhibiting hormone) and ovarian maturation (*78*).

It has long been known that serotonin at concentrations of 10^{-4} to 10^{-3} M (~0.18–1.8 g/L) induces spawning in bivalves. Some commercial farmers make use of this by adding serotonin to induce spawning. Fong (*76*) found that Prozac (fluoxetine) and Luvox (fluvoxamine) are the most potent inducers ever found, eliciting spawning behavior in zebra mussels at aqueous concentrations many orders of magnitude lower than serotonin. Fluoxetine elicited significant spawning in male mussels at concentrations of 10^{-7} M (~150 µg/L); females were an order of magnitude less sensitive at 10^{-6} M. Fluvoxamine was the most potent of the SSRIs, eliciting significant spawning in male mussels, at 10^{-9} M (~0.318 µg/L); females were two orders of magnitude less sensitive, at 10^{-7} M. In males, spawning was complete in the first hour, while females were slower (within 2 hr). Paxil (paroxetine) was the least potent of these three SSRIs, eliciting male spawning, but to a lesser degree, at 10^{-6} M, and having no inducing effect on females at any concentration. It should be noted that Fong states that the evidence is not clear whether these compounds are indeed acting as SSRIs, or via some other mechanism. It is also unknown how these compounds are taken up by molluscs (*76*).

In another study, Fong et al. (*79*) showed that fluvoxamine induces significant parturition in fingernail clams at 1 nM; 1 nM fluvoxamine also potentiated the effect of 10 µM 5-HT by almost 5-fold. Paroxetine was less potent, requiring a concentration of 10 µM to effect significant parturition. In contrast, even at concentrations of 100 µM, fluoxetine displayed no effect, although it was capable at 5 µM of potentiating 5-HT at concentrations that were otherwise subthreshold. It is interesting that the order of potency for inducing parturition in clams differs from the order for induction of spawning in mussels (above). This points to the complexity of considering any approach involving extrapolations from one species to another or from one drug to another within a given class.

In crustaceans, Kulkarni et al. (*74*) found that fluoxetine significantly potentiates the effect of 5-HT in crayfish, enhancing the release of ovary-stimulating hormone, which results in larger oocytes with enhanced amounts of vitellin; any ecologic consequences of higher vitellin protein levels are unknown. Similarly, in fiddler crabs, fluoxetine at a dose of 125 nmol stimulates (through 5-HT) the production of gonad-stimulating hormone, which accelerates testicular maturation (*75*).

It is clear that aquatic life can be exquisitely sensitive to at least some of this class of compounds. Although some SSRIs are extremely potent, others have almost no effect, which possibly makes the approach of assessing ecologic risk on a class-by-class basis infeasible.

Concentration of SSRIs plays a complicated role with respect to effects. For example, Couper and Leise (*77*) found that while injected fluoxetine induced significant metamorphosis in a gastropod, 10^{-4} M induced less metamorphosis than 10^{-6} M. Simple extrapolations of effects from higher concentrations do not necessarily have any relevance to effects at lower concentrations.

The potential for SSRIs to elicit subtle effects on aquatic life is further extended by serotonin reuptake mechanisms that also are a factor in snails and squids (*76*), particularly in the regulation of aggression (*80*). Yet another example of a subtle effect that would go unnoticed is the fighting behavior of lobsters, in which serotonin causes behavior reversal by stimulating subordinates to engage in fighting against dominants by reducing their propensity to retreat (*80*).

Antiepileptics

Antiepileptics are ubiquitous and prevalent due to poor STW removal. Carbamazepine was the drug detected most frequently and in highest concentrations during a study by Ternes (*18*). This drug was detected in all POTWs and receiving waters, with a maximum concentration of 6.3 µg/L. Ternes hypothesized that the ubiquitous occurrence resulted from the very low removal efficiency from POTWs, which was calculated to be only 7%. Sacher et al. (*81*) found carbamazepine levels in the river Rhine in Germany up to 0.90 µg/L and always above 0.1 µg/L.

Antineoplastics

Antineoplastics are highly [geno]toxic compounds, primarily from hospitals, with poor removal from STWs. Antineoplastic agents, antitumor agents primarily used only within hospitals for chemotherapy, are found sporadically and in a range of concentrations, probably because only small amounts are introduced to STWs via domestic sewage because of their long-lived physiologic retention. These compounds act as nonspecific alkylating agents (i.e., specific receptors are not involved) and therefore have the potential to act as either acute or long-felt stressors (mutagens/carcinogens/teratogens/embryotoxins) in any organism. The fact that two oxazaphosphorines, ifosfamide and cyclophosphamide, were found in certain effluents in the low microgram-per-liter range indicates that these highly toxic compounds, which are probably refractory to microbial degradation at POTWs (*82*), can find their way into the environment. Indeed, Steger-Hartmann et al. (*82*) found levels of cyclophosphamide in sewage influent from servicing hospitals ranging from undetectable to 143 ng/L; the levels in the effluent reached 17 ng/L.

Additional evidence pointing to the refractory nature of ifosfamide is presented by Kümmerer et al. (*83*), who found that concentrations of ifosfamide in hospital effluent matched the predicted values of up to 1.91 µg/L; also the concentrations in the influent and effluent of POTWs that serviced chemotherapy hospitals were essentially unchanged (influent/effluent maximum, 43 ng/L; median, 6.5–9.3 ng/L). Kümmerer et al. (*83*) found ifosfamide to be totally refractory to removal by POTWs and to totally resist alteration during a 2-month bench-scale POTW simulation.

Another class of antineoplastics, the platinates, includes carboplatin and cisplatin. Although the stability of these compounds in sewage systems is unknown, Kümmerer et al. (*84*) calculated that if they were present in hospital sewage effluents as the intact parent compound, they could be present at daily average concentrations of up to 600 ng/L (on the basis of total platinum). Although the majority of the dose for these compounds is excreted in the urine in the first day, a large amount (~30%) resides in the body and is slowly excreted over a period of years and therefore could be excreted to residential sewage systems. Falter and Wilken (*85*) showed that while these compounds are difficult to determine analytically, their potential to remain in the aqueous phase after sewage treatment is high.

White and Rasmussen (*86*), in the most detailed overview to date on the genotoxicity of wastewaters, elaborate that while the genotoxic potency of industrial wastewaters is often the highest, the overall loading of genotoxic compounds to surface waters is far greater, up to several orders of magnitude, from municipal treatment plants. They present a striking correlation between the occurrence of direct-acting mutagens in surface waters and the human population served by the discharging STWs. This correlation points to the activities/metabolism of humans, not industrial activities, as the origin for these mutagens. A number of possible sources for the mutagens are discussed, an obvious one of which is antineoplastic drugs.

These data point to antineoplastics as a class of drugs of potential concern for environmental effects, not just for their acute toxicity but perhaps more for their ability to effect subtle genetic changes, the cumulative impact of which over time can lead to more profound ecologic change. Hospitals are the major source of genotoxic drugs. POTWs that service hospitals, especially multiple hospitals, are likely candidates for releasing these chemicals into surface waters.

Impotence Drugs

This class of drugs displays widespread use, new modes of action, and unknown effects on nontarget organisms. Even though a number of drugs from various chemical classes have been used over the years for treating impotence, the emergence of Viagra (sildenafil citrate) has focused tremendous attention on this market. The significance of this therapeutic class of drugs, with new ones awaiting FDA approval, is that they all tend to have distinct modes of action, most of which differ from those of traditional drugs. While potential effects on wildlife are totally unknown, the fact that Viagra, for example, works by inhibiting a phosphodiesterase responsible for regulating the concentration of cyclic guanosine monophosphate, which indirectly relaxes muscles and increases blood flow (*87*), gives cause for concern regarding the disruption of this common phosphodiesterase in unintended target species. Impotence drugs will prove to have very high usage rates, especially since they are one of the most common drugs available without prescription over the Internet, yielding high potential for environmental exposure and possibly nontarget effects.

Tranquilizers

Little is known about possible occurrence of tranquilizers. Ternes (*18*) reported diazepam in almost half of the POTWs but only in low concentrations of less than 0.04 µg/L; it could not be detected in surface waters. Genicola (*88*) reports diazepam in the groundwater from a monitoring well at a Superfund site near Atlantic City, New Jersey. Concentrations were approximately 10–40 µg/L and probably originated in a landfill in which pharmaceutical manufacturers disposed of chemicals.

Retinoids

High usage rates and profound activity in amphibians lends cause for concern. Retinoids, low molecular weight lipophilic derivatives of vitamin A, can have profound effects upon the development of various embryonic systems (*89*), especially amphibians in which retinoic acid receptors have been hypothesized to play a role in frog deformities. Although naturally occurring, retinoids have been used for a number of years for a wide array of medical conditions including skin disorders (e.g., Accutane [isotretinoin] for acne), antiaging treatments (e.g., Retin-A [tretinoin] for skin wrinkles), and cancer (e.g., Vesanoid [tretinoin] for leukemia). Isotretinoin (13-cis-retinoic acid) is related to both retinoic acid and retinol (vitamin A). Tretinoin is among the top 200 prescribed drugs in the United States. Methoprene, an insecticidal synthetic retinoic acid mimic, is photolabile and yields numerous photo-products, some of which also elicit strong retinoic acid activity (*90*). Although retinoic acids would also be expected to be photolabile (and therefore not persistent), their products may also still possess receptor activity.

Diagnostic Contrast Media

Diagnostic contrast media have very high usage rates, display considerable persistence, show no evidence for mineralization, and have low physiologic activity. Detailed X-ray images of soft tissues are routinely captured by the use of contrast media. Some of the more widely used members of contrast media are highly substituted and sterically hindered amidated, iodinated aromatics such as diatrizoate and iopromide (*91*), which are used worldwide at annual rates exceeding 3,000 tons. Kalsch (*91*) found these compounds to be quite resistant to transformation in STWs and in river waters. When transformations were effected, they merely terminated with unidentified resistant metabolites. Ternes et al. (*92*) recently reported significant amounts of iopromide in rivers.

In municipal STW effluents, Ternes et al. (*92*) found concentrations as high as 15 µg/L (iopamidol) and 11 µg/L (iopromide). In an STW close to Frankfurt/Main, they found two other contrast agents, diatrizoate and iomeprol, at concentrations up to 8.7 µg/L, as well as iothalamic acid and ioxithalamic acid in the nanogram-per-liter range. In rivers and streams, five iodinated diagnostics were repeatedly detected, with median values up to 0.49 µg/L for iopamidol and up to 0.23 µg/L for diatrizoate. Isolated maximum values above 100 µg/L for diatrizoate indicated that relatively high local concentrations can occur, especially in small streams containing a high percentage of STW discharges. Maximum groundwater concentrations for iodinated contrast agents ranged up to 2.4 µg/L and may well represent a worst case with respect to occurrence of pharmaceuticals in native waters. In Germany alone, individual contrast agents can experience annual usage rates of 100 tonnes. Such high usage, coupled with inefficient human metabolism (95% unmetabolized) and ineffective elimination of iodinated contrast agents by STWs, can lead to very high environmental accumulations and persistence. Despite these negative attributes, contrast agents have no bioaccumulation potential and low toxicity (*93*); Steger-Hartmann et al. (*93*) also found no acute toxicity for bacteria (*Vibrio fisheri*), algae (*Scenedesmus subspicatus*), crustaceaens (*Daphnia*), and fish (*Danio rerio, Leuciscus idus melanotus*) exposed to no more than 10 g/L of iohexol, iotrolan, diatrizaote, or iopromide.

Personal Care Products in the Environment

For the purposes of this review, personal care products are defined as chemicals marketed for direct use by the consumer (excluding OTC medication with documented physiologic effects) and having intended end uses primarily on the human body (products not intended for ingestion, with the exception of food supplements). In general, these chemicals are directed at altering odor, appearance, touch, or taste while not displaying significant biochemical activity. Most of these chemicals are used as the active ingredients or preservatives in cosmetics, toiletries, or fragrances. They are not used for treatment of disease, but some may be intended to prevent diseases (e.g., sunscreen agents). In contrast to drugs, almost no attention has been given to the environmental fate or effects of personal care products—the focus has traditionally been on the effects from intended use on human health. Many of these substances are used in very large quantities frequently more than recommended.

Table 3. Personal care products produced in Germany (1993).

Product category	Tons produced
Bath additives	162,300
Shampoos, hair tonic	103,900
Skin care products	75,500
Hair sprays, setting lotions, hair dyes	71,000
Oral hygiene products	69,300
Soaps	62,600
Sun screens	7,900
Perfumes, aftershaves	6,600
Total	559,100

Personal care products differ from pharmaceuticals in that large amounts can be directly introduced to the environment. For example, these products can be released directly into recreational waters or volatilized into the air (e.g., musks). Because of this direct release they can bypass possible degradation in POTWs. Also, in contrast to pharmaceuticals, less is known about the effects of this broad and diverse class of chemicals on nontarget organisms, especially aquatic organisms. Data are also limited on the unexpected effects on humans. For example, common sunscreen ingredients, 2-phenylbenzimidazole-5-sulfonic acid and 2-phenylbenzimidazole, can effect DNA breakage when exposed to UV-B (*94*).

The quantities of personal care products produced commercially can be very large. For example, in Germany alone the combined annual output for eight separate categories has been estimated (*95*) at 559,000 tons for 1993 (Table 3). A few examples are given below of common personal care products that are ubiquitous pollutants and that may possess substantial bioactivity.

Fragrances (musks) are ubiquitous, persistent, bioaccumulative pollutants that are sometimes highly toxic; amino musk transformation products are toxicologically significant. Synthetic musks comprise a series of structurally similar chemicals (which emulate the odor but not the structure of the expensive, natural product from the Asian musk deer) used in a broad spectrum of fragranced consumer items, both as fragrance and as fixative. Included are the older, synthetic nitro musks (e.g., ambrette, musk ketone, musk xylene, and the lesser known musks moskene and tibetene) and a variety of newer, synthetic polycyclic musks that are best known by their individual trade names or acronyms. The polycyclic musks (substituted indanes and tetralins are the major musks used today, accounting for almost two-thirds of worldwide production) and especially the inexpensive nitro musks (nitrated aromatics accounting for about one-third of worldwide production) are used in nearly every commercial fragrance formulation (cosmetics, detergents, toiletries) and most other personal care products with fragrance; they are also used as food additives and in cigarettes and fish baits (*96*).

The nitro musks are under scrutiny in a number of countries because of their persistence and possible adverse environmental impacts and therefore are beginning to be phased out in some countries. Musk xylol has proved carcinogenic in a rodent bioassay and is significantly absorbed through human skin; from exposure to combined sources, a person could absorb 240 µg/day (*97*). The human lipid concentration of various musks parallels that of other bioaccumulative pollutants such as PCBs (*98*). Worldwide production of synthetic musks in 1988 was 7000 tons (*96*); worldwide production for nitro musks in 1993 was 1,000 tons, two-thirds of which were musk xylene (*99*).

Synthetic musks first began to be identified in environmental samples almost 20 years ago (*100,101*). By 1981, Yamagishi et al. (*100*) had identified musk xylene and musk ketone in gold fish (*Carassius auratus langsdorfii*) in Japanese rivers and not much later (*101*) in river water, sewage, marine mussels (*Mytilus edulis*), and oysters (*Crassosterea gigas*). Yamagishi's studies comprised the first comprehensive monitoring efforts, identifying musk xylene and musk ketone in freshwater fish, marine shellfish, river water, and STW waters. Musk xylene was found in all samples, and musk ketone was found in 80% of the 74 samples analyzed. Concentrations in STW effluents ranged from 25 to 36 ng/L for musk xylene and from 140 to 410 ng/L for musk ketone. Concentrations of musk xylene in fish muscle were in the tens of parts per billion, whereas those for musk ketone were less than 10 µg/kg, with highest values occurring in fish downstream of STWs. In contrast, for shellfish the concentrations were lower, between 1 and 5.3 µg/kg, presumably because of their lower lipid contents. In river water, musk xylene occurred in all samples, whether upstream or downstream of STWs and ranged between 1 and 23 ng/L; those of musk ketone were generally in the same range, but in distinct contrast they were not detectable in upstream samples.

Geyer et al. (*102*) have published an excellent review on residues of nitro musk fragrances in fish and mussels as well as in breast milk and human lipids and the current ecotoxicologic and toxicologic knowledge for these personal care products. Residues of musk xylene and musk ketone found in the fillet of freshwater fish (e.g., pike, eel, brass, Zander, rainbow trout) from rivers of North Germany were between 10 and 350 µg/kg lipid and 10 and 380 µg/kg lipid for musk xylene and musk ketone, respectively. In mussels (*Mytilus edulis*) 10–30 µg/kg lipid of both fragrances were detected. In human breast milk from German women, musk xylene and musk ketone were detected between 10 and 240 mg/kg lipid (*102*). Recently, the literature has a number of additional publications from Europe, especially Germany and Switzerland. Rimkus et al. (*103*) give a brief overview of the occurrence of musks in the environment. Käfferlein et al. (*99*) and Geyer et al. (*102*) published the most thorough reviews to date on the occurrence (in the environment and in personal care products), transformation, and toxicology of the ubiquitous musk xylene; these reviews summarize many more occurrence studies (for musk xylene) than mentioned here.

Musks are refractory to biodegradation (other than reduction of nitro musks to amino derivatives), which explains why they have been measured in water bodies throughout the world (*96*). They also are very lipophilic [octanol–water partition coefficients are similar to those for DDT and hexachlorocyclohexane (*104*)] and therefore can bioconcentrate/bioaccumulate (*102,103*). Concern has been expressed regarding developmental toxicity in aquatic organisms. Musk ambrette (2,6-dinitro-3-methoxy-4-*tert*-butyl toluene) may play a role in damaging the nervous system (*105*).

Draisci et al. (*106*) examined freshwater fish in Italy and identified two of five targeted polycyclic musks in most fish samples; a hexahydro-hexamethylcyclopental-benzopyran (HHCB, trade name Galaxolide) and an acetylhexamethyltetralin (AHTN, trade name Tonalide) were identified at levels ranging from less than 4 ng/g (ppb) to 105 ng/g in fish muscle tissue. In the Swiss river Glatt, Müller et al. (*98*) identified Galaxolide, Tonalide, and Celestolide (ADBI, 4-acetyl-6-*tert*-butyl-1,1-dimethylindane) at concentrations of 136, 75, and 3.2 ng/L, respectively; they also found the nitro musks tibetene, ambrette, moskene, ketone, and xylene at concentrations of 0.04, <0.03, 0.08, 8.3, and 0.62 ng/L, respectively. Eschke et al. (cited in *107*) identified Galaxolide, Tonalide, and Celestolide in the fatty tissue of bream and perch from the Ruhr River, Germany, at average concentrations between 2.5 and 4.6 mg/kg (ppm), illustrating the extreme bioaccumulation potential for these compounds. Recently, Heberer et al. (*108*) investigated the contamination of surface waters in Berlin, Germany, (and vicinity) receiving high percentages of treated sewage and found maximum concentrations above 10 µg/L for the polycyclic musks Galaxolide, Tonalide, and Celestolide.

Winkler et al. (*104*) measured musks in 31 particulate matter and water samples from the Elbe River, Germany. In all particulate matter samples, concentrations for musk ketone were 4–22 ng/g, for Galaxolide 148–736 ng/g, and for Tonalide 194–770 ng/g; Celestolide was found at concentrations of 4–43 ng/g in 23 of the particulate matter samples. The values for the three most prevalent musks were within the same order of magnitude as those for 15 polycyclic aromatic hydrocarbons (PAHs) and exceeded those for 14 common polychlorinated organic pollutants (only hexachlorobiphenyl [HCB] and *p,p′*-DDT were of similar concentration). Also found in all the 31 water samples were musk ketone (2–10 ng/L), Galaxolide (36–152 ng/L), and Tonalide (24–88 ng/L); Celestolide was found only at 2–8 ng/L. These higher values exceeded those for all the polychlorinated organics and the PAHs. The occurrences of individual musks are sometimes correlated as a result of their use as mixtures in commercial products. In Germany, the nitro musks are being replaced by the polycyclic musks, therefore resulting in lower concentrations for musk ketone (*104*).

It is not surprising that musks have been detected in air. Kallenborn et al. (*109*) detected three polycyclic musks and two nitro musks in Norwegian outdoor air samples. The polycyclic musks were more prevalent. Concentrations of all these musks ranged from low picograms per cubic meter to hundreds of picograms per cubic meter. The most common was the polycyclic musk Galaxolide, but the relative ratios among the musks are a function of usage (which varies among countries) and photolability.

Although the significance of the aquatic toxicity of the nitro and polycyclic musks is debatable [genotoxicity from the polycyclics seems to not be a concern) (*110*), the aminobenzene (reduced) versions of the nitro musks can be highly toxic; these reduced derivatives are undoubtedly created under the anaerobic conditions of sewage sludge digestion. Behecti et al. (*111*) tested the acute toxicity of four reduced analogs of musk xylene on *Daphnia magna*. The *p*-aminodinitro compound exhibited the most toxicity of the four, with extremely low median effective concentration (EC_{50}) values averaging 0.25 µg/L (0.25 ppb).

Recently, the amino transformation products of nitro musks were identified in sewage treatment effluent and in the Elbe River, Germany. Gatermann et al. (*96*) identified musk xylene and musk ketone together with their amino derivatives 4- and 2-amino musk xylenes and 2-amino musk ketone. In sewage treatment influent, the concentrations of musk xylene and musk ketone were 150 and 550 ng/L, respectively. In the effluent, their concentrations dropped to 10 and 6 ng/L, respectively. In contrast, although the amino derivatives could not be detected in the influent, their concentrations in the effluents dramatically increased, showing extensive transformation of the parent nitro musks: 2-amino musk xylene (10 ng/L), 4-amino musk xylene (34 ng/L), and 2-amino musk ketone (250 ng/L). It was concluded that the amino derivatives could be expected in sewage effluent at concentrations more than an order of magnitude higher than the parent nitro musks. In the Elbe, 4-amino musk xylene was found at higher concentrations (1–9 ng/L) than the parent compound.

Given that the amino nitro musk transformation products *a*) are more water soluble than the parent musks, *b*) still have significant octanol–water partition coefficients (high bioconcentration potential), and *c*) are more toxic than the parent nitro musks, more attention should be focused on these compounds. Because synthetic musks are ubiquitous, used in large quantities, introduced into the environment almost exclusively via treated sewage effluent, and are persistent and bioconcentratable, they are prime candidates for monitoring in both water and biota as indicators for the presence of other PPCPs. Their analysis, especially in biota, has been thoroughly discussed by Gatermann et al. (*96*) and by Rimkus et al. (*103*).

Preservatives

Parabens (alkyl-*p*-hydroxybenzoates) are one of the most widely and heavily used suites of antimicrobial preservatives in cosmetics (skin creams, tanning lotions, etc.), toiletries, pharmaceuticals, and even foodstuffs (up to 0.1% wt/wt). Although the acute toxicity of these compounds is very low, Routledge et al. (*112*) report that these compounds (methyl through butyl homologs) display weak estrogenic activity in several assays. Although the risk from dermal application in humans is unknown, the probable continual introduction of these benzoates into sewage treatment systems and directly to recreational waters from the skin leads to the question of risk to aquatic organisms. Butylparaben showed the most competitive binding to the rat estrogen receptor at concentrations one to two orders of magnitude higher than that of nonylphenol and showed estrogenic activity in a yeast estrogen screen at 10^{-6} M .

Disinfectants/Antiseptics

Triclosan (Irgasan DP 300, a chlorinated diphenyl ether: 2,4,4′-trichloro-2′-hydroxydiphenyl ether) is an antiseptic agent that has been widely used for almost 30 years in a vast array of consumer products. Its use as a preservative and disinfectant continues to grow; for example, it is incorporated at < 1% in Colgate's Total toothpaste, the first toothpaste approved by the FDA to fight gingivitis. While triclosan is registered with the U.S. EPA as a pesticide, it is freely available OTC. Triclosan's use in commercial products includes footwear (in hosiery and insoles of shoes called Odor-Eaters), hospital handsoap, acne creams (e.g., Clearasil), and rather recently as a slow-release product called Microban, which is incorporated into a wide variety of plastic products from children's toys to kitchen utensils such as cutting boards. Many of these uses can result in direct discharge of triclosan to sewage systems, and as such this compound can find its way into receiving waters depending on its resistance to microbial degradation. Okumura and Nishikawa (*113*) found traces of triclosan ranging from 0.05 to 0.15 µg/L in water. Although triclosan has long been regarded as a biocide, a toxicant having a wide-ranging, nonspecific mechanism(s) of action—in this case gross membrane disruption, McMurry et al. (*114*) report that triclosan is rather an antibacterial having particular enzymatic targets (lipid synthesis). As such, bacteria could develop resistance to triclosan. As with all antibiotics in the environment, this could lead to development of resistance and change in microbial community structure (diversity).

A wide array of disinfectants are used in rather large amounts not just by hospitals, but also by households and livestock breeders. These compounds are often substituted phenolics as well as others such as triclosan. Biphenylol, 4-chlorocresol, chlorophene, bromophene, 4-chloroxylenol, and tetrabromo-*o*-cresol (*70*) are some of the active ingredients, at percentage volumes of < 1–20%. A survey of 49 STWs in Germany (*70*) routinely found biphenylol and chlorophene in both influents, up to 2.6 µg/L for biphenylol and up to 0.71 µg/L for chlorophene, and effluents. The removal of chlorophene from the effluent was less extensive than for biphenylol, with surface waters having concentrations similar to that of the effluents.

Sunscreen Agents

The occurrence of sunscreen agents (UV filters) in the German lake Meerfelder Maar was investigated by Nagtegaal et al. (*115*). The combined concentrations of six sunscreen agents (SSAs) identified in perch (*Perca fluviatilis*) in the summer of 1991 were as high as 2.0 mg/kg lipid and in roach (*Rutilus rutilus L*) in the summer of 1993, as high as 0.50 mg/kg lipid. Methylbenzylidene camphor (MBC) was detected in roach from three other German lakes. These lipophilic SSAs seem to occur widely in fish from small lakes used for recreational swimming. Both fish species had body burdens of SSA on par with PCBs and DDT. The bioaccumulation factor, calculated as quotient of the MBC concentration in the whole fish (21 µg/kg) versus that in the water (0.004 µg/L), exceeded 5,200, indicating high lipophilicity. The fact that SSAs (e.g., 2-hydroxy-4-methoxybenzophenone [oxybenzone] and 2-ethylhexyl-4-methoxycinnamate) can be detected in human breast milk [16 and 417 ng/g lipid, respectively (*116*)] shows the potential for dermal absorption and bioconcentration in aquatic species. No data have been published on newer SSAs such as avobenzene (1-[4-(1,1-dimethylethyl)phenyl]-3(4-methoxyphenyl)-1,3-propanedione).

Nutraceuticals/Herbal Remedies

During the last several years, the popularity of nutritional supplements was codified by the creation of a new term for the subclass of highly bioactive food supplements called nutraceuticals (*117*), also referred to as nutriceuticals. Nutraceuticals are a rapidly growing commercial class of bioactive compounds, usually botanicals, intended as supplements to the diet. Nutraceuticals and many herbal remedies can have potent physiologic effects. These are a mainstay of alternative medicine and have enjoyed explosive growth in use in the United States and other countries during the last decade. Many are used as food supplements that have either proven or hypothesized biologic activity but are not classified as drugs by the FDA, primarily because a given botanical usually has not one but an array of distinct compounds whose assemblage elicits the putative effect and because these arrays cannot be easily standardized. As such they are not regulated and are available OTC (heavily promoted via the Internet). Even in those cases in which the natural product is identical to a prescription pharmaceutical (e.g., the Chinese red-yeast product Cholestin newly introduced to the United States contains lovastatin, an active ingredient in the approved prescription drug Mevacor used to lower cholesterol levels), a recent ruling (*118*) prevented the FDA from regulation.

With the accelerating inverted age structure of our society, coupled with the U.S. 1994 Dietary Supplement Health and Education Act (DSHEA) (*119*) (which eases regulations on the introduction and marketing of supplements), the use of nutraceuticals could greatly escalate. The significance of dietary supplements in the United States was epitomized by the creation of the Office of Dietary Supplements (ODS) via the DSHEA in 1995 under the National Institutes of Health (NIH) (*119*). The ODS maintains a searchable database (International Bibliographic Information on Dietary Supplements [IBIDS]) of published scientific literature on dietary supplements (*120*). The NIH was also mandated to create the National Center for Complementary and Alternative Medicine (NCCAM, *121*) to "facilitate the evaluation of alternative medical treatment modalities to determine their effectiveness."

Although these substances are readily available OTC, albeit in poorly characterized/standardized forms, an effort is underway to patent various nutraceuticals by standardizing the extracts and thereby making them available only by prescription. This effort is being pioneered by PharmaPrint, Inc. (Irvine, CA) (*122*), which has applied to FDA for various investigational new drug applications. The patenting of hundreds of multiple-molecule nutraceuticals for therapeutic purposes could lead to more widespread use of these substances.

As an example, a recent addition to this class is a substance called huperzine A, an alkaloid extracted from a Chinese moss, which has been documented to improve memory. It is therefore experiencing strong demand for treating Alzheimer's disease and has captured the attention of those who follow the nutraceutical market because of its true pharmaceutical qualities. The significance of this particular compound is that it possesses acute biologic activity as a cholinesterase inhibitor identical to that of organophosphorus and carbamate insecticides. It is so effective that the medical community is concerned about its abuse/misuse, especially since it is legal. While huperzine A, and alkaloids in general (compounds with heterocyclic nitrogen, proton-accepting group, and strong bioactivity), are naturally occurring compounds, their susceptibility to biodegradation in STWs or in open waters is unknown. This is the case for almost all nutraceuticals.

Another example is Kava, which is prepared from the root of *Piper methysticum*, long used throughout the South Pacific because of its mild narcotic effect among a host of other effects. The active ingredients in Kava are believed to be a suite of lipophilic lactones comprising substituted α-pyrones (methysticin, kavain, yangonin, and others) (*123*). These compounds display a host of effects in humans, but nothing is known about their effects on other organisms or fate in STWs.

There are countless other nutraceuticals, both new and ancient, experiencing vigorous consumption. These few examples illustrate the unknowns regarding whether these compounds are being excreted, surviving sewage treatment, and then eliciting effects on aquatic organisms. Nutraceuticals and herbal remedies would have the same potential fate in the environment as pharmaceuticals, with the added dimension that their usage rates could be much higher, as they are readily available and taken without the controls of prescription medication. Because these compounds are natural products, however, they would be expected to be more easily biodegraded.

Although the argument can be made that naturally occurring compounds would not pose an ecologic risk, this ignores that *a*) the concentrations of these compounds in effluents could be higher than they are in the environment in which they occur naturally, and *b*) many of these substances/mixtures come only from isolated parts of the world (e.g., Kava, huperzine), and their use/dispersal in other parts of the world would essentially make them anthropogenic. The use of these compounds serves to redistribute their normal occurrence in the environment, and even though they might be naturally occurring, this promotes exposure to organisms that normally would never occur.

Nontarget Organism Exposure to and Effects from PPCPs

Environmental Exposure

Persistence is not critical if the source is constant, leading to perpetual aquatic exposure. Many PPCP ingredients seem to have considerable persistence in the environment; blood lipid regulators and musks are examples. Although environmental persistence usually is a major determinant of exposure in the environment, for pollutants that are used on a continual basis and are introduced to the environment through STWs, the supply is continually replenished. In the absence of very short half-lives, exposures even to nonpersistent compounds could be significant. This is especially true for aquatic organisms, which are captives of their environment and perpetually exposed.

Seasonal variations in pharmaceutical infusion to surface waters from sewage treatment effluents may not be a factor. No seasonal variations were noted for musks; weekly variations of severalfold in concentration have been noted in the River Elbe in Germany (*104*).

Effects on Nontarget Organisms

Although acute data are lacking, subtle effects might be the major concern. The potential effects of PPCPs on nontarget species, especially on aquatic organisms, are mostly unknown. No concerted research effort has focused on the ecotoxicology of PPCPs. Some isolated studies, however, have included pharmaceuticals in various toxicity assays relevant to aquatic life. One study in particular was begun in Scandinavia.

In 1989 under the direction of the Scandinavian Society of Cell Toxicology (SSCT), an international toxicologic evaluation study was initiated—Multicenter Evaluation of In Vitro Cytotoxicity Tests (MEIC). While the main purpose of the MEIC was to thoroughly investigate a select list of chemicals for evaluating human toxicity by employing and benchmarking a wide battery of *in vitro* and *in vivo* tests, the MEIC generated a range of ecotoxicologic data for various species including fish, amphibians, crustaceans, and single-cell eukaryotes. Of the 50 selected chemicals, at least 18 were drugs. Although limited and focusing on the more common, obvious end points, the MEIC data sets are some of the only available that catalog the effects of certain drugs on aquatic life. The MEIC was concluded in 1996, and the data are still being evaluated. A database of SSCT's MEIC cytotoxicity data can be found at the web site for the Cytotoxicology Laboratory, Uppsala, Sweden (CTLU) (*124*).

An example of one of the MEIC studies relevant to aquatic effects is the study by Lilius et al. (*125*) that presents effects data for all 50 MEIC chemicals on two species of the crustacean *Daphnia.* Of 50 MEIC reference chemicals, 18 pharmaceuticals had immobilization EC_{50} values ranging from below 0.01 mM (e.g., 0.0037 for amitriptyline and 0.0017 for thioridazine) to less than 10 mM (e.g., 6 mM for phenobarbital and 8.2 mM for aspirin); 9 of the 18 drugs had EC_{50} values below 0.1 mM. In comparison, the EC_{50} values for a range of common industrial chemicals and pesticides were generally in the same millimolar range (e.g., phenol, 0.078; 2,4-dichlorophenoxyacetic acid, 0.65; nicotine, 0.023; potassium cyanide, 0.0086; lindane, 0.005). Another MEIC study (*126*) reports data from a number of tests relevant to the aquatic environment (several crustaceans, rotifer, and Microtox). Using the OECD (Organisation for Economic Cooperation and Development) Test Guideline 202, Part II (chronic *Daphnia* reproduction), Kalbfus and Kopf (*127*) reported results for clofibrate and salicylic acid: for clofibrate, NOEC (no observed effect concentration) of 10 µg/L and EC_{50} (24 hr) of 106 µg/L, whereas the NOEC for salicylic acid was three orders of magnitude higher (10 mg/L); the clofibrate NOEC concentrations for algae and luminescence bacteria ranged between 5 and 40 µg/L, whereas those for salicylic acid were between 15 and 60 mg/L.

Acute toxicity, the major type of end point investigated with nontarget species, is only one of many possible ecotoxicologic end points of concern. Investigation of multigenerational life-cycle effects is almost entirely lacking for any nontarget species. This is surprising, as in the aquatic environment—given that exposures to PPCPs would be more constant than episodic—organisms are exposed for their entire life cycles. Perhaps more important, however, subtle behavioral modifications or genetic alterations have the potential to lead to profound long-term ecologic effects for which it could prove extremely difficult to pinpoint the cause. Acute toxicity should not be a primary concern because it can be so easily detected, and mitigation measures can be designed in a timely manner. Rather, concern should be directed toward effects occurring undetected that can lead to long-term adverse impacts, which in turn are perceived simply as natural variation or evolution. This concern is amplified compared with that for pesticides, as nearly nothing is known about the effects of PPCPs on aquatic or terrestrial life. We do know, however, that these substances have the potential to be profoundly bioactive through a constellation of different modes of action. The toxicologic data that exist for nontarget species are almost exclusively focused on antibiotics and woefully lacking for fish [see Halling-Sørenson et al. (*5*) for a tabulated summary of effects data]. The studies of Fong et al. (*76,79*), presented earlier under "Antidepressants," are the best examples to date of subtle effects resulting from low concentrations. Another overlooked issue regarding effects is that of organisms from lower trophic levels whose presence is critical to community homeostasis.

Subtle Effects—Beyond Our Notice

Acute toxicity is only a small part of a larger puzzle. Many drugs that are used successfully to modify the behavior of humans could have unforeseen effects on nontarget organisms. These effects could be so subtle that their consequences may be imperceptible but nevertheless profound when elicited over long periods of time. For example, subtle behavioral effects could be responsible for changing any number of attributes for a particular species, resulting in changes over numerous generations that would otherwise be deemed part of normal adaption/evolution. Many drugs even have unpredicted or unanticipated effects in humans. Such unforeseen biologic effects could prove even more profound and unexpected with nontarget species.

The ability to elicit numerous subtle effects across a wide range of species is embodied by no single class of pharmaceuticals better than SSRIs. This class of drugs shows the potential for PPCPs to elicit a constellation of effects that would be hard to detect in natural settings, or to tease apart from what would otherwise be considered normal behavior.

Perhaps the most important concern regarding the exposure of aquatic and terrestrial organisms to PPCPs is that the effects could be so subtle that they would escape any effort to detect them, with the result that imperceptible changes could accumulate until they had a significant impact—not necessarily on the individual organisms but rather on the population or community, perhaps after generations of change. Subtle effects via regulation by any of the countless pathways/networks of signal transduction in aquatic species can range from modification or reversal of attraction and boldness to avoidance and shyness affecting all behavior characteristics spanning the range from feeding to mating to directional sensing (e.g., chemotaxis, gravitaxis). On the surface, the result would simply be attributed to natural adaptation or confounded by other natural changes.

Kurelec (*128*) has formalized a concept, genotoxic disease syndrome (GDS), that embodies the idea of nonobvious change as effected in the aquatic environment, especially as exacerbated by compromising the activity of MXR defense systems. As proposed, GDS is seen as the gradual accumulation of a wide spectrum of toxic events, none of which alone results in an obvious outcome. The cumulative effects fall under the general, diffuse, ill-defined alteration/impairment/inhibition of enzyme systems, protein turnover, metabolism, and cytotoxic repair, leading to reduced fitness, gradual degeneration/atrophy of tissues/organs, reduced growth, accelerated aging, impaired immunologic systems, impaired reproduction, higher incidence of disease, and impaired adaptation/survival/succession.

Kurelec (*33*) reported another example of behavioral alteration. The MXR inhibitor verapamil when added to polluted river waters (at a level of ~1 ppm that normally did not affect fish) elicited dramatic avoidance attempts by the fish, escalating to the point of frantic escape attempts when 2-aminoanthracene was also added at a concentration 0.53 µM, which would otherwise not elicit a response.

The significance of subtle and cumulative impacts is only beginning to be recognized by environmental toxicologists. Weiss (*129*) wrote that just as the predicted rise in "crack babies" whose pregnant mothers used cocaine was never observed—rather only a small but significant 3% reduction in IQ was observed—the same subtle effects are very likely occurring from environmental toxicants. Weiss points out that the effects on humans by lead (Pb), methyl mercury, PCBs, and endocrine disruptors in general probably are manifested in almost undetectable changes, and these may accumulate over time to yield truly profound changes that would not be distinguishable from natural events. The specter of subtle, cumulative effects could make current toxicity-directed screening largely useless in any effort to test waste effluents for toxicologic end points.

Abnormal behavior can masquerade as seemingly normal deviation within a natural statistical variation. Change can occur so slowly that it appears to result from natural events, with no reason to presume artificial causation. It is difficult to connect the issues of cause and ultimate effect, in part because of the ambiguous and subjective nature of subtle effects, but especially when these effects are confounded as aggregations of numerous, unrelated interactions. Weiss (*130*) points out that statistical tests based on means and *p* values are incapable of detecting the very subtle changes that low-level toxicants can impart. Slight shifts within the statistical distributions of any particular characteristic expressed among a population are not necessarily reflected by statistics of the mean/median; these changes may well be more obvious, however, when considering an individual organism rather than looking across a population.

Another aspect to gradual, undetected changes relates to community composition/organization, especially in microorganisms, in which the composition of species can be affected over time (i.e., successional effects) as a result of the presence of anthropogenic chemicals (*131*). The pressure for succession can result from adverse effects (e.g., toxicity preferentially to one species over another) or from conferring an advantage to one species (e.g., use of the toxicant as a nutrient source). Succession of community structure tends to occur on such a long time line that cause and effect issues are rarely considered.

A number of toxicity testing approaches have been developed over the years, some of which employ less obvious end points for the nonspecific detection of the presence, but not the identities, of toxicants. One of the best known rapid approaches uses the reduction in light output of a bioluminescent bacterium (e.g., Microtox) (*132*). Another more recent example (*133*) automatically detects changes in the gravitaxis of a unicellular flagellate—indeed, a subtle end point in itself and one that would probably go unnoticed and undoubtedly lead to die off. Such approaches are badly needed for detecting changes in toxicant concentrations in wastewater effluents and for directing subsequent chemical characterization to identify the putative toxicants.

Environmental Assessment

Although various levels of prospective ERA via standardized tests are required in the United States and Europe as part of the drug registration process (see "Approach of Regulatory Agencies" below), meaningful effort on this front is simply not possible with the currently limited state of knowledge on environmental fate, transport, and effects of pharmaceuticals; to date, retrospective studies (e.g., ERAs based on environmental survey) are rare. Examples of prospective ecologic assessments can be seen in Henschel et al. (*28*), who performed these assessments for four high-use pharmaceuticals in Germany: salicylic acid (the main metabolite of acetylsalicylic acid, aspirin), paracetamol (4-acetamidophenol, acetominophen—analgesic/antipyretic), clofibric acid (chlorophenoxyisobutyric acid; blood lipid regulator), and methotrexate (4-amino-10-methyl-folic acid; chemotherapy folic acid antagonist that disrupts nucleotide synthesis). Unmetabolized, the loading of these drugs into bodies of water in Germany could be hundreds of tons per year. Henschel et al. (*28*) found that although all four drugs would have passed traditional ecotoxicity screening, methotrexate would not have passed at least one nonstandard test. This showed that the current guidelines could be underestimating nontarget effects.

The OECD, an intergovernmental organization with representatives from 29 countries, publishes the *OECD Test Guidelines* (*134*), a collection of methods used to assess the hazards of chemicals and of chemical preparations such as pesticides and pharmaceuticals. Assessment of methodology for aquatic toxicity of chemicals has been recently reviewed by the OECD (*135*). General information on ecologic risk assessment is available from the U.S. EPA (*136*).

The enormous array of pharmaceuticals will continue to diversify and grow as the human genome is mapped. Today, there are about 500 distinct biochemical receptors at which drugs are targeted; U.S. private R&D investment in new pharmaceuticals in 1998 was nearly $18 billion. The number of targets is expected to increase up to 20-fold (yielding 3,000–10,000 drug targets) in the near future according to the Pharmaceutical Research & Manufacturers Association (*137*). In 1998, the FDA approved 30 new nonbiologic drugs, one of which was Viagra (*138*). The FDA Modernization Act of 1997 (*139*) will also help to accelerate this growth. Most of the new drugs have totally unpublished environmental transformation/fate/effects properties; two examples of highly prescribed new drugs are Viagra (sildenafil: 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]-pyrimidin-5-yl)-4ethoxyphenyl]-sulfonyl]-4-methylpiperazine]) and Propecia/Proscar (finasteride: [5α-17β-*N*-(1,1-dimethylethyl)-3-oxo-4-azaandrost-1-ene-17-carboxamide; used in treating male baldness and benign prostatic hyperplasia). This explosion in new drugs will severely exacerbate our limited knowledge of drugs in the environment and possibly increase the exposure/effects risks to nontarget organisms. Finally, the current proliferation of web sites offering prescription drugs by mail will only exacerbate the growing use/misuse of a wide array of drugs.

Approach of Regulatory Agencies

There are only two major activities with respect to managing the release of pharmaceuticals in the environment. One results from the research that has occurred in various European/Scandinavian countries over the last few decades, culminating in guidelines from the EU. The other comes from the FDA. It is important to understand, however, that responsibility in the United States for monitoring drugs in the environment does not currently rest with either the FDA or the U.S. EPA. Few other alternative approaches for assessing ecologic risk posed by pharmaceuticals have been proposed. In one of the more comprehensive approaches, Roembke et al. (*8*) used the basic ecologic risk assessment approach, upon which the U.S. EPA's current approach (*136*) is based, to discuss alternatives; in particular, they noted that acute effects testing cannot be relied upon by itself—chronic effects testing is extremely important.

European Union Activities

Agency for the Evaluation of Medicinal Products—EMEA/CVMP/055/96. In the early 1980s, government regulators [e.g., European Commission—Pharmaceuticals and Cosmetics (EEC) which sets rules governing medicinal products in the EU (*140*)] first showed concern over the release of veterinary pharmaceuticals and their metabolites into the environment and any untoward effects they might have on biota: "the potential risks for the environment resulting from the use of the medicinal product." Veterinary medicinals were targeted presumably because they were perceived as having a more direct route of introduction to the environment (e.g., fish farms, parasite dips, farm runoff). Only much later has concern been expressed with respect to human drugs, but no regulations/guidelines have been established. An analogous directive has yet to be published by the EU for human pharmaceuticals. A good overview of the approach used by the EEC was published by Henschel et al. (*28*) and Montforts et al. (*42*).

In EMEA/CVMP/055/96-final (*141*), the CVMP (European Committee for Veterinary Medicinal Products) sets forth its final guidance for risk assessments for veterinary medical products, excluding biologics. The guidance elaborates on the phased-assessments set forth in EEC Directive 92/18/EEC, where Phase I assesses the potential for release to the environment [derivation of predicted environmental concentrations (PECs)]; for more information on establishing PECs for exposure assessment, see OECD (*142*). Phase II is broken into two tiers: tier A evaluates possible fate and effects, and tier B (should cause for concern regarding specific biologic species result from tier A findings) looks at effects on specific biota that might receive exposure. ERAs are required for new veterinary drugs. The report must include potential for environmental exposure (considering patterns of use and routes of administration [internal vs external], metabolism/excretion [metabolites representing less than 20% of the dose are excluded from concern], and disposal), fate and effects, and any needed risk management strategies. The guidance mandates the use of worst-case exposure scenarios. EMEA's guidelines are also being developed specifically for environmental impact assessments of veterinary medicinals by EMEA (*143*).

Effects testing includes algal growth inhibition, fish acute/chronic/bioaccumulation exposure, avian dietary and reproductive, earthworm toxicity, terrestrial plant growth, and activated sludge respiration inhibition. The guidance document seems to recognize the incredible diversity of stressor–receptor possibilities that could result from pharmaceuticals and their metabolites entering the environment: "there may be considerable variation in receptor specificity/sensitivity between species" (*143*). This is complicated further by the fact that the mode(s) of action responsible for the desired therapeutic effect of many drugs is poorly understood (sometimes totally unknown). For example, although various modes of action for the disease-modifying agents used to treat rheumatoid arthritis (e.g., methotrexate and hydroxychloroquine, intended for use in chemotherapy and malaria, respectively) have been known for many years, the actual mechanisms by which the symptoms of this particular disease are alleviated are mostly unknown. It would therefore be impossible to forecast what type of effects could be anticipated.

Biocides. The new EU Biocide Directive (*144*) covers the commercialization of biocidal products (e.g., disinfectants), but few of these are used in personal care products. Significantly, however, the Directive emphasizes ecotoxicologic issues (on par with human health issues), including fate and ecologic effects.

FDA

Concern regarding introduction of pharmaceuticals to the environment in the United States is addressed by the FDA, which requires Environmental Assessments (EAs), as required under National Environmental Policy Act of 1969) (NEPA), and the specifics of which are set forth in "Guidance for Industry: Environmental Assessment of Human Drug and Biologics Application" (*145*) for all drug applications/actions meeting minimum criteria. As with the EU's approach, concern rests primarily on acute and chronic effects as measured by traditional toxicity tests. Much less concern is expressed for behavioral effects, whether avoidance, breeding, etc. The FDA does, however, recognize "extraordinary circumstances" where there is the potential for serious harm to the environment or for an action to "significantly affect the quality of the human environment" (*145*). This notion includes not just toxicity to environmental organisms but also "environmental effects other than toxicity, such as lasting effects on ecological dynamics" (*145*). Clearly this could cover subtle behavioral modifications from which effects accumulate over time/generations, eventually leading to measurable change but unrecognized as such. NEPA [40 CFR 1508.27; also see Appendix C in the FDA document (*145*)] also defines "significantly" around two issues—"context" and "intensity" (severity of impact). Among the 10 issues with respect to "intensity," one relates to:

> Whether the action is related to other actions with individually insignificant but cumulatively significant impacts. Significance exists if it is reasonable to anticipate a cumulatively significant impact on the environment.

The FDA's approach is very similar to that of the EU. The FDA requires an EA if the expected environmental concentration (EEC, analogous to the PEC—predicted environmental concentration) at the point of entry to the aquatic environment (the expected introduction concentration [EIC]) exceeds 1 ppb. The EIC is calculated assuming that all the drug product produced for 1 year enters POTWs, that the drug's usage is spread across the country in proportion to the population, and that none of the parent drug is metabolized or transformed; this can be altered if transformation data are available, but if metabolites or other SRSs are present at greater than 10% of the parent level, then toxicology must also be known. Its value is calculated as the product of *a*) kilogram per year of active product produced for use, *b*) reciprocal of the influent to POTWs (liters per day), *c*) reciprocal of 365 days/year, and *d*) 10^9 µg/kg.

Like the EMEA, the FDA uses a tiered approach to determine if regulatory actions are required. This approach centers around assessment factors used to determine when ecotoxicity testing is not needed. An assessment factor is calculated by dividing the test end point (e.g., lethal dose or EC_{50}) by the maximum EEC (which is equal to the greater of EEC or EIC). Lower factors necessitate more extensive testing. Further testing is also necessitated by drugs that bioaccumulate or with SRSs that are more toxic than the parent drug. The weakest aspect to this approach is that the toxicity of the SRSs is assessed from what is known for human toxicology rather than for potential nontarget organisms. Moreover, given the current knowledge of fate, transport, and ecotoxicity of anthropogenic chemicals, there are simply too many unknowns to be able to predict whether a pharmaceutical (or its transformation products) will find its way into the environment at a particular concentration. The FDA also requires EAs for drugs that also occur naturally in the environment if their usage and subsequent discharge (to POTWs, landfills, etc.) will alter the natural, ambient concentration.

A significant shortcoming of either of these two current regulatory approaches to determining ecologic risk results from not taking into account the cumulative (additive/synergistic/antagonistic) impacts of drugs affecting the same receptors. The EEC value for any given drug could easily be exceeded when the cumulative concentrations of like-mode-of-action drugs are considered, especially in those instances where numerous competing drugs are commercially available in any class. Needless to say, this approach also ignores the possibility of synergistic effects from drugs of other classes.

Conclusions and Recommendations

This review aims to catalyze a discussion in the environmental science community to determine the significance of PPCPs in the environment and to foster further research efforts, if warranted. The intent is not to outline a tiered approach for a research strategy, but rather to highlight where further research might be needed in each of various areas organized around the "risk paradigm," as set forth by the National Research Council (*146*) (Table 1). A step-wise strategy to determine if a major research effort needs to be launched would require efforts at establishing the incidence of PPCP occurrence in the environment coupled with parallel determinations of whether effects among a wide spectrum of aquatic organisms can occur at documented concentrations of PPCPs and whether cost-effective modifications of STW operation can dramatically improve removal efficiencies. The authors' personal recommendations and summary of significant conclusions are presented in Table 1. It is hoped that this broad overview presents a wide perspective and proper context for this emerging problem.

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