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Occurrence and Removal of Antiviral Drugs in Environment: A Review

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Abstract Antiviral drugs have been recently recognized as one of the emerging contaminants in the environment. These are discharged after therapeutic use through human excretion. Effluent containing high concentration of antiviral drugs discharged from production facilities is also a cause of concern to nearby aquatic bodies. There is an increased interest in their removal because they are highly bioactive. Some antiviral drugs are resistant to conventional methods of degradation, and there is a risk of development of antiviral resistance in humans and animals if exposed repeatedly for long periods. To date, the potential human, animal, and ecological risks associated with the discharge of these antiviral compounds to the environment are not well documented. This study presents a brief summary on occurrence, ecotoxicological risks, and physicochemical properties of antiviral drugs in the environment. The needs regarding removal, disposal, and treatment of antiviral drugs are also addressed.

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1 Introduction

Antiviral drugs have recently drawn interest among the general public due to the outbreak of swine influenza around the globe (Prasse et al. 2010). In August 2010, the World Health Organization (WHO) reported swine influenza H1N1 cases in more than 214 countries with over 18,000 deaths (WHO 2011). There are approximately 6.8 million people all over the world that received antiretroviral therapy (treatment used in HIV infection) in 2010 (WHO 2010). Over the past few years, continuous release and persistence of antiviral drugs in the environment even at trace concentrations has become an emerging environmental problem which may impose toxicity to the organisms present in the surroundings.

With reference to the toxicity towards daphnids, fishes, and algae, antiviral drugs are reported to be among the most predicted hazardous therapeutic classes (Al-Rajab et al. 2010; Kummerer 2008). These drugs, through various routes when introduced to the environment, find their way into the food chain and can hinder or interfere with natural biological systems of living organisms. Antiviral drugs in the environment have gained attention due to the fact that they escape degradation route in wastewater treatment plants (WWTPs) and find their way into the surface and groundwater sources (De Clercq 2007; Kahn 2005; Osborn et al. 2008). Due to

their refractory nature, they escape degradation in conventional wastewater treatment or sewage treatment plants (STPs; Al-Rajab et al. 2010; Kummerer 2008). Antiviral drugs in aquatic environment have also raised an alarm due to their role in growing antiviral drug resistance among influenza viruses (Tyring 2004).

After the administration, antiviral drugs are partially metabolized or excreted as active metabolites in urine and feces, and subsequently enter into STPs where these compounds are treated, along with other constituents of wastewater. Large amounts of oseltamivir carboxylate (OC), an active metabolite of Tamiflu®, used in the treatment against H5N1 and H1N1 influenza viruses, were excreted by humans during pandemic and entered WWTPs in biologically active forms (Slater et al. 2011). In addition to toxic effects, these drugs may cause long-term and irreversible change to the viral genome, making them resistant in their presence at low concentrations.

Presently, antiviral drugs are in the state of development, and there are more than 40 compounds formally licensed for clinical use against viral infections (De Clercq and Field 2006). Currently, one-half of all antiviral agents are antiretroviral drugs, and the rest are general antiviral drugs which are in various phases of clinical trials (De Clercq 2007). As depicted in Fig. 1, viral diseases can be treated with vaccines or drugs depending upon their availability at a particular time and the extent of exposure. There is an increased demand of antiviral drugs due to unavailability of vaccines at desired places. A key difference between a vaccine and antiviral drug is that vaccines can be given long before exposure to the virus and can provide protection over a long period of time, whereas antiviral drugs can be used in the treatment of those living beings that have already been infected by a virus. Use of vaccines in prophylaxis of viral diseases may reduce the load/flux of antiviral drugs into the environment. Extensive use of antiviral drugs has not been very common in veterinary applications, and therefore, only a few studies have been reported on animals (Durand et al. 2009; Giese 1998; Kahn 2005).

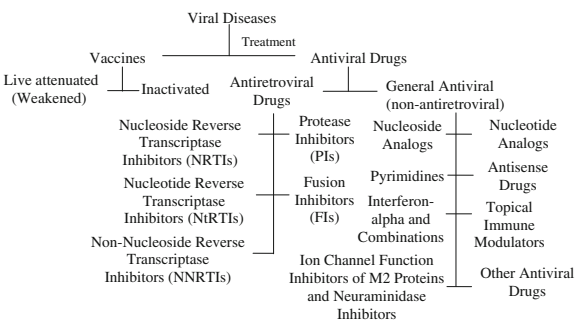


Fig. 1 Classification of vaccines and antiviral drugs

Except oseltamivir, the occurrence of antiviral drugs, their degradation products in STPs effluents, chemical or physical treatment methods for removal, and potential toxicity towards other organisms are to date far less documented. Moreover, there is a lack of available knowledge about the total worldwide use and release of antiviral drugs in the environment. Therefore, it is imperative to investigate the fate, effects, and impact of antiviral drugs in the environment. The objective of this paper is to present a review on occurrence, physicochemical properties, and removal methods used for antiviral drugs. Despite the increased research and regulatory interest in the occurrence of antiviral drugs and their degradation products in STP effluents and freshwater ecosystems, their distribution between different environmental systems is far less explored to date. A part of preliminary work on the fate and detection methods of antiviral drugs in the environment has already been published earlier (Jain et al. 2011).

2 Antiviral Drugs in the Environment: A Cause of Concern

The presence of antiviral drugs has been detected in different aqueous systems such as raw wastewater, WWTP effluents, groundwater, and surface water in different countries (Buchberger 2007; Prasse et al. 2010; Singer et al. 2008). After the introduction of any chemical into the environment, different structural changes can occur resulting from biotic and non-biotic processes including effluent treatment (Kummerer 2008). Some antiviral drugs like acyclovir, didanosine, and tenofovir can be excreted as largely unchanged parent compound (Al-Rajab et al. 2010; Galasso et al. 2002; Jjemba 2006). Presence of organic and inorganic constituents in wastewater may react with the parent compound and give rise to additional molecules, which may be persistent or difficult to remove from wastewater. Formation of such additional molecules after the excretion of parent compounds and metabolites into the water bodies is a serious environmental concern.

The drugs entering WWTPs are only partially removed (Prasse et al. 2010) and may reach the ecosystem via hierarchical levels. Figure 2 outlines the proposed alternatives through which antiviral drugs can enter into the environment via different sources and ultimately reach drinking water sources. Unused drugs are disposed off into the sewage system, drains, and sometimes to trash. There are three main sources for antiviral drugs to reach potable water sources through various pathways:

1. Effluent from pharmaceutical industries
2. Hospital wastes
3. Disposal of out-of-date, unused, or unwanted medicines.

Some of the antiviral drugs are reported to be persistent and recalcitrant (Goncalves et al. 2011; Mascolo et al. 2010a). Söderström et al. (2009) found that the active metabolite of oseltamivir was present in Japanese waterways at clearly detectable levels and also found that OC levels were high near the major STPs and downstream in a river system. The behavior of the majority of the antiviral compounds (metabolites or active form) present in STPs has been scarcely documented. When these drugs pass through the STPs, they are found to be highly bioactive, and may have serious health impact on non-target organisms (Ghosh 2009). Straub (2009) concluded on the basis of standard chronic toxicity tests that oseltamivir poses no significant risk to WWTPs or to surface water

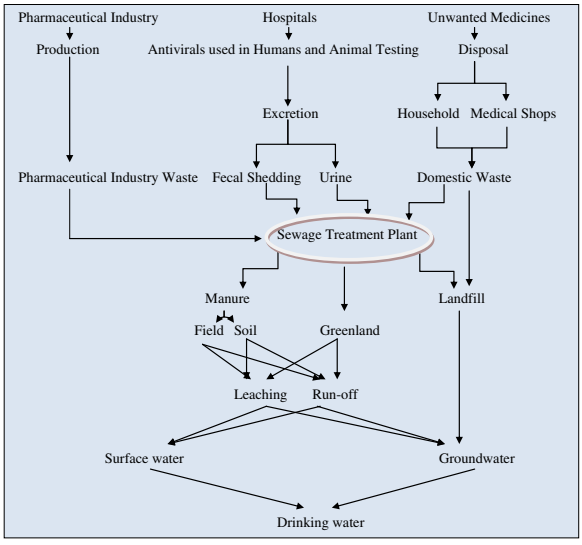


Fig. 2 Pathways of antiviral drugs from domestic wastes to the drinking water sources

environment and found that it was persistent. To date, no information is available on the fate and impact of antiretroviral drugs in the environment and WWTPs (Germer and Sinar 2010).

Singer et al. (2007) reported that an antiviral drug, relenza, which comes under neuraminidase inhibitors, can be used as a model for assessing the ecotoxicological risks and fate in the environment of other antiviral drugs due to lack of any other empirical evidence. The properties of relenza are as follows: (a) readily soluble in water, (b) chemically stable in water having a half-life greater than 1 year, (c) not readily volatile, (d) not likely to sorb on soil or sediment, (e) lipophobic (not likely to decompose to fats), and (f) not readily mineralized.

These lipophobic compounds are unlikely to adsorb on the sludge matrix if treated through activated sludge. Moreover, this mechanism limits the potential losses in the aqueous phase in the final effluent. Non-volatile organic compounds in sewage sludge are regarded as a potential risk to human health or the environment when sludge is used in agricultural soils (Langenkamp et al. 2001). Tenofovir, a nucleotide reverse transcriptase inhibitor, has been found to be largely and rapidly excreted unchanged in the urine. Al-Rajab et al. (2010) reported that biosolids or recycled wastewater could contain trace concentrations of tenofovir that are found to be persistent in soils and expected to limit availability for biodegradation. Research has also shown that oseltamivir is not degraded or removed during conventional wastewater treatment (Fick et al. 2007). It has been reported to be persistent in surface waters for a longer period of time. Its half-life in surface water has been reported to be 53 days (Accinelli et al. 2010a).

The pharmaceutical concentrations in WWTP in Switzerland receiving pharmaceutical formulation facilities discharge have been reported to range from less than 0.01 to 38 μg/L. Research has suggested that discharge from production units in Europe may result in increased antiviral drug concentrations in river water (Phillips et al. 2010). Recently, it has been found that the administration of the antiviral drug oseltamivir phosphate during a pandemic has posed a risk to drinking water safety and ecological health (Ghosh et al. 2010a). An active moiety of oseltamivir, used for treatment and prevention of pandemic influenza, has been found to be resistant to biological treatment and UV radiation treatment, and the active substance has

been released in wastewater leaving the treatment plant. The UV spectra of OC has absorbance in 295–700 nm range, and radiation with wavelengths less than 700 nm do not contain enough energy to break the bonds within the molecules (Fick et al. 2007). Efavirenz (another drug used in HIV treatment) has been reported in high production volume pharmaceuticals that have not been detected in the environment but are likely to be persistent and/or bioaccumulative (Howard and Muir 2011). Ritonavir was reported as the most commonly consumed (1,026 g/year) and found in high concentration in an effluent from a hospital in France (Jean et al. 2012). Ritonavir has gained substantial attention for bioaccumulation potential in the environment. Zanamivir is the second most prescribed drug in Japan used in the treatment of influenza A and influenza B viruses. It was also reported in high concentration (241.6 ng/L), more than the concentration of oseltamivir phosphate (87.6 ng/L) in STPs effluents (Takanami et al. 2012).

Kummerer (2009) suggested possible measures to reduce the load of pharmaceuticals in the environment. It included treatment of pharmaceuticals before their discharge into the environment using advanced oxidation processes (AOPs) or adsorption. The pharmaceutical industries should also publish data about the impacts of active pharmaceutical ingredients on the environmental components. Strict legislative measures are needed for the proper disposal of expired medications. Such unused drugs should not be disposed off down the drain but instead returned to pharmacy for which take-back system ought to be established.

Table 1 depicts the physicochemical properties of some antiviral drugs, their structure, and molecular weight. From the table, it can be observed that generally all antiviral drugs have high molecular weights (>200 g/mol) and are highly soluble in water. Molecular weight of antiviral drugs would be useful while selecting membrane-based treatment processes ranging from ultrafiltration to reverse osmosis, which depend on molecular weight cut-off. The carbonyl oxygen of ketone group capable of hydrogen bonding with water makes antiviral drugs highly soluble in water. Unsaturated cyclic rings are prominent in antiviral drugs. Presence of aromatic rings makes the compound toxic and resistant to conventional degradation methods. Solubility of the antiviral drug will determine the amount of drug remaining as suspended solid and the part which will go in dissolved solids. The soluble part will contribute to total organic carbon of waste streams. Acid dissociation constant, p*K*a, is a very useful parameter for understanding the behavior of antiviral drug molecules in water. For strong acids, the value of p*K*a is less than 2; for weak acids, p*K*a is between 2 and 7; for weak bases, p*K*a lies between 7 and 10, while for strong bases, the value of p*K*a is greater than 10. The wastewater from pharmaceutical industries has a wide range of pH, i.e., 2 to 9 (Yi-zhong et al. 2002). With the help of p*K*a values, appropriate selection of ion exchange adsorbents can be made. For example, for abacavir, having a p*K*a value of 16.71, representing its strongly basic nature, acidic adsorbents can be a viable option for its removal. Ionic species of drug molecules differ in chemical, physical, and biological properties, which can be used to predict the ionic form of the molecule that is present in aqueous solution. T_m, melting point, is an important physical property of the antiviral drugs, which determines the limiting temperature of the treatment process. Information about physicochemical properties of antiviral drugs is a useful tool to select appropriate treatment technology.

UV spectrophotometer and high performance liquid chromatography (HPLC) are the two most common techniques for the detection of antiviral drugs. Majority of the methods are based on the detection of antiviral drugs in biological samples like plasma, urine, and serum (Palacios et al. 2005; Pereira et al. 2000; Uslu et al. 2006). Jung et al. (2007) used a combination of liquid–liquid extraction and protein precipitation followed by LC/MS/MS with electrospray ionization for the analysis of 17 antiretroviral drugs in human plasma. Even though these methods are used for the analysis of antiviral drugs in biological samples, e.g., plasma and urine, they can provide useful hints for the method development for environmental matrices. For example, HPLC-UV is not suitable for the analysis of antiviral drugs in aqueous matrices considering the low concentrations (nanograms per liter range) of these drugs typically observed in the environment. A solid-phase extraction followed by HPLC method was used to quantify five human immunodeficiency virus protease inhibitors (PIs), namely, indinavir, amprenavir, saquinavir, ritonavir, and nelfinavir, and the non-nucleoside reverse transcriptase inhibitor, efavirenz in plasma (Marzolini et al. 2000). Antiviral drugs are not efficiently retained on common solid-phase extraction sorbent materials due to their high polarity, and the use of large sample volumes is necessary to achieve sufficient sensitivity. The same is

Table 1 Physicochemical properties of some antiviral drugs

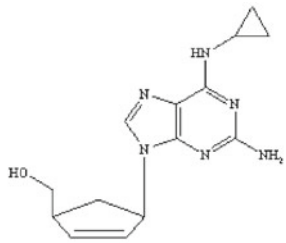
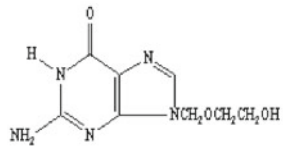
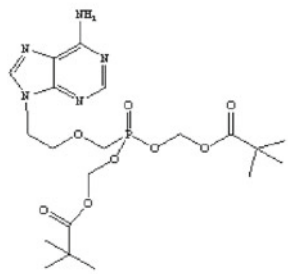
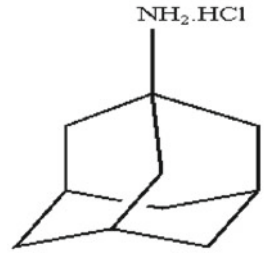
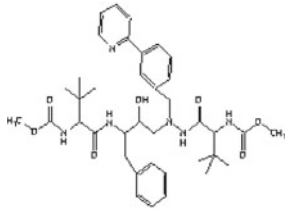
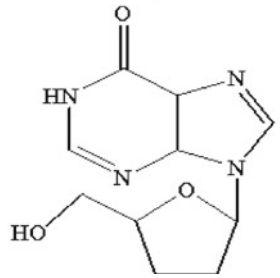
CAS. No.	Name of Salt	Structure	Molecular Formula and Weight (g/mol)	Solubility in water	pKa	T_m
136470-78-5	Abacavir		$C_{14}H_{18}N_6O$ and 286.33	77 mg/mL (Sulfate salt)	16.71	165°C
59277-89-3	Acyclovir		$C_8H_{11}N_5O_3$ and 225.20	1.3 mg/mL	2.27 and 9.25	257°C
142340-99-6	Adefovir dipivoxil		$C_{20}H_{32}N_5O_8P$ and 501.47	19 mg/mL at pH 2.0 and 0.4 mg/mL at pH 7.2.	-	-
31377-23-8	Amantadine		$C_{10}H_{17}N.HCl$ and 187.7	6290 mg/L	10.6 (Base)	300°C
198904-31-3	Atazanavir		$C_{38}H_{52}N_6O_7$ and 704.85	4-5 mg/mL, (Sulfate salt)	13.07	-
69655-05-6	Didanosine		$C_{10}H_{12}N_4O_3$ and 236.22	15.8 mg/mL	14.67	160-163°C

Table 1 (continued)

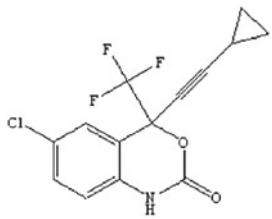
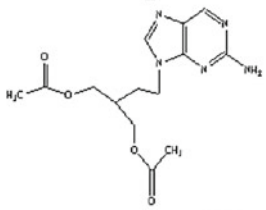
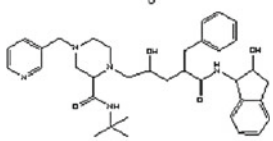
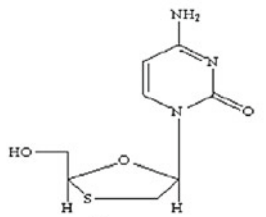
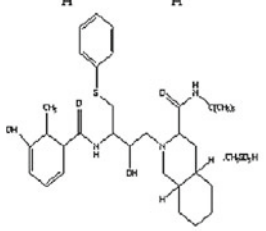
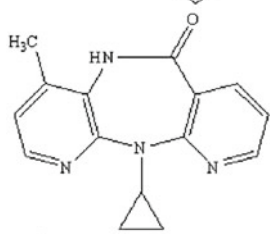
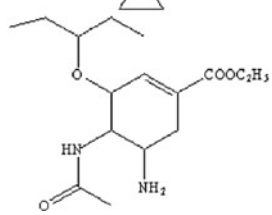
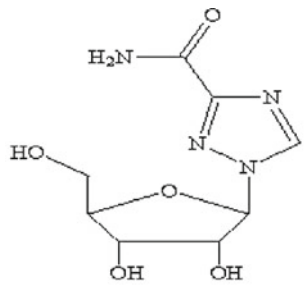
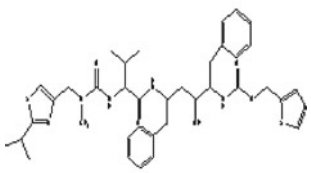
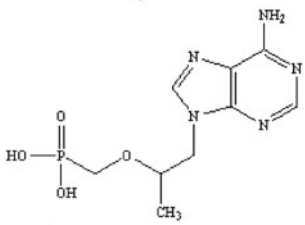
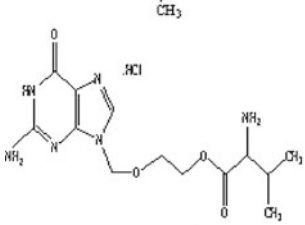
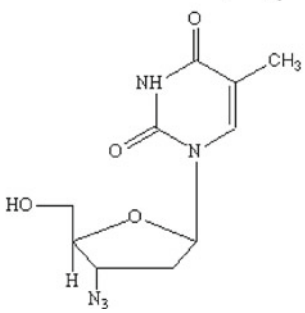
CAS. No.	Name of Salt	Structure	Molecular Formula and Weight (g/mol)	Solubility in water	pKa	T_m
154598-52-4	Efavirenz		$C_{14}H_9ClF_3NO_2$ and 315.68	Practically insoluble in water	-	139-141°C
104227-87-4	Famciclovir		$C_{14}H_{19}N_5O_4$ and 321.33	Soluble in water (25°C)>25% w/v	-	102-104°C
150378-17-9	Indinavir		$C_{36}H_{47}N_5O_4$ and 613.78	0.015 mg/mL	14.21	167.5-168°C
134678-17-4	Lamivudine		$C_8H_{11}N_3O_3S$ and 229.25	70 mg/mL	-	160-162°C
159989-64-7	Nelfinavir		$C_{32}H_{45}N_3O_4S$ and 567.78	Slightly soluble	14.13	349.8°C
129618-40-2	Nevirapine		$C_{15}H_{14}N_4O$ and 266.29	0.7046 mg/L	-	196.1°C
204255-11-8	Oseltamivir		$C_{16}H_{28}N_2O_4$ and 284.35 (OC)	Soluble in water > 500 mg/L	3.6 (acid), 8.9 (base)	192-196°C

Table 1 (continued)

CAS. No.	Name of Salt	Structure	Molecular Formula and Weight (g/mol)	Solubility in water	pKa	T_m
36791-04-5	Ribavirin		$C_8H_{12}N_4O_5$ and 244.2	142 mg/mL at 25°C	-	166-176°C
155213-67-5	Ritonavir		$C_{37}H_{48}N_6O_5S_2$ and 720.94	Practically insoluble in water	14.23	-
147127-20-6	Tenofovir		$C_9H_{14}N_5O_4P$ and 287.21	13.4 mg/mL in distilled water at 25°C	7.91	276-280°C
124832-27-5	Valacyclovir hydrochloride		$C_{13}H_{20}N_6O_4.HCl$ and 360.80	170 mg/mL	-	170-172°C
30516-87-1	Zidovudine		$C_{10}H_{13}N_5O_4$ and 267.24	Sparingly soluble in water, soluble in ethanol	9.96	106-112°C

true for reversed phase HPLC columns, which have limitations with respect to chromatographic resolution. In recent years, hydrophilic interaction liquid chromatography has been successfully employed for the analysis of polar substances in biological matrices. Contrary to reversed-phase liquid chromatography, polar compounds, viz. acyclovir, are well retained on hydrophilic interaction liquid chromatography columns, due to their interaction with the water layer formed at the surface of the stationary phase (Prasse et al. 2010).

Survey of the literature reveals that limited methods are available for determination of antiviral drugs in

aqueous solutions like reversed phase HPLC, HPLC–tandem mass spectrometry, and UV–spectrophotometric methods (Basavaiah and Anil Kumar 2007; Djurdjevic et al. 2004; Ghoshal and Soldin 2003; Kapoor et al. 2006). The concentration of antiviral drugs in the wastewater discharge from production units may range in milligrams per liter (Mascolo et al. 2010a), and as such there are very few methods of detection in aqueous sample at such high concentrations documented in literature. Presently available methods suffer with detection limits confined to low concentrations only, tedious experimental conditions, low sensitivity, and sometimes complex procedures for the preparation of samples or standard solutions. Considering limited methods for detection of antiviral drugs in aqueous solution, there is an urgent need to develop other reliable detection methods. However, the analysis of antiviral drugs in aqueous medium is a challenging task due to their different structure and wide range of p*K*a values.

3 Antiviral Drug Resistance and Health Problems

A change in a viral genome after prolonged exposure makes the virus resistant towards that particular drug which is referred to as antiviral drug resistance. The incomplete removal of antiviral drugs from effluent of STPs results in their increased concentration in receiving waters, which may lead to the development of microbial or viral resistance with adverse health effects on humans and harmful effects on environment (Kummerer 2008).

Presence of a wide range of pharmaceuticals in water bodies may pose significant danger to aquatic life and wild birds (Bound and Voulvoulis 2005; Jarhult 2012; Singer et al. 2011). Examples include development of oseltamivir-resistant virus in animals like wild fowl and dabbling ducks, in which their bowel contains replicating virus as well as oseltamivir (Singer et al. 2007; Söderström et al. 2009). Waterfowls, which live close to the treated wastewater effluent stream, were found to be resistant towards Tamiflu® during pandemic influenza (Ghosh et al. 2010a). The increased concentration of antiviral drugs in natural waters due to their extensive use during influenza outbreak may aggravate the risk of development of drug resistance in human beings (Bartels and von Tümpling Jr. 2008). These antiviral drugs have been found even in drinking water (Ghosh 2009; Ghosh et al. 2010b). If wastewater containing resistant bacteria and antibiotics are used for irrigation, and sewage sludge as a fertilizer, the resistant bacteria can enter the food chain also (Kummerer 2008). Same behavior is expected for viruses and antiviral drugs.

Table 2 shows a list of some FDA-approved antiviral drugs, class, their mechanism of action, and the most frequent adverse events. Zidovudine, an antiretroviral drug, is excreted as metabolite and parent compound via urine and is reported to show hematological toxicity and also found to be carcinogenic in rodents (Vanková 2010). As far as unmetabolized part is concerned, indinavir, a PI, has been reported to be associated with certain side effects such as urinary complications, nephrolithiasis and crystalluria, renal atrophy, tubulointerstitial nephritis, and hypertension (De Araujo and Seguro 2002). Prolonged exposure of tenofovir has also been reported to lead to reduced bone mineral density (Fontana 2009). All other drugs used for treatment of other viral infections such as herpes simplex virus (HSV), varicella zoster virus (VZV), cytomegalovirus (CMV), hepatitis B virus (HBV), human papillomavirus, chronic viral hepatitis, and others fall under the category of general antiviral drugs. General antiviral drugs (non-antiretroviral) include nucleoside analogs, nucleotide analogs, antisense drugs, and all other antiviral drugs. Acyclovir, famciclovir, ganciclovir, etc. are categorized under nucleoside analog groups which inhibit viral DNA polymerase and used in the treatment of HSV, VZV, and CMV. Nucleotide analogs include cidofovir and adefovir used in the treatment of HBV and CMV infections. Nucleoside analogs and other antiviral drugs are associated with kidney failure, neuropsychiatric side effects, encephalopathy, delirium, tremors, etc.

Antiviral agents in certain combinations compose highly active antiretroviral therapy (HAART). Generally, HAART combines three or more different drugs such as two nucleoside reverse transcriptase inhibitors (NRTIs) and a PI, two NRTIs and a non-nucleoside reverse transcriptase inhibitor (NNRTI), or other such combinations. The HAART therapy has proved its efficacy and reduced the activity of the target viruses (Tyring 2004). These drugs in certain combinations when released into aqueous bodies after partial metabolism can show potential adverse effects to contact organisms and may have fatal consequences. Gradual release and prolonged exposure of antiviral drugs and combination with other drugs through various channels can affect the human body to a severe extent.

Table 2 Some FDA-approved antiviral drugs, class, their mechanism of action, and adverse effects

S. No.	Antiviral drugs	Class	Mechanism of action	Adverse effects
1	Zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, emtricitabine	Nucleoside reverse transcriptase inhibitors (NRTIs)	Reverse transcriptase inhibitors	Lyell's syndrome, pancreatitis, peripheral neurotoxicity, hypersensitivity reaction, hyperlactatemia, nausea, diarrhea, and lactic acidosis
2	Tenofovir disoproxil	Nucleotide reverse transcriptase inhibitors (NtRTIs)	Acyclic nucleoside phosphonates	Renal failure, proximal tubular dysfunction, nephrogenic diabetes, nephrotoxicity
3	Nevirapine, delavirdine, efavirenz	Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Reverse transcriptase inhibitors	Cardiovascular complications, hepatic toxicity, hypersensitivity reactions, central nervous system side effects including dizziness, insomnia, impaired concentration, somnolence, and abnormal dreams
4	Saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir	Protease inhibitors (PIs)	Viral protease inhibitors	Hyperglycemia, urinary complications such as nephrolithiasis and crystalluria, tubulointerstitial nephritis, hypertension, and renal atrophy
5	Enfuvirtide, pentafuside (T-20)	Fusion inhibitors (FIs)	–	Insulin resistance and hypercholesterolemia
6	Ribavirin	Nucleoside analogs	IMP dehydrogenase inhibitors	Hyperlactatemia, mitochondrial toxic effects
7	Amantadine, rimantadine	Ion channel function inhibitors of M2 proteins and neuraminidase inhibitors	Viral uncoating process	Neurotoxicity, central nervous system side effects, insomnia, nervousness, anorexia, and nausea
	Zanamivir, oseltamivir		Viral neuraminidase inhibitors	Adverse effects related to the upper respiratory tract (nausea), gastrointestinal tract (abdominalgia and diarrhea), central nervous system (CNS; headache, vertigo, somnolence, insomnia, numbness, and behavioral excitement)
8	Adefovir dipivoxil	Nucleotide analogs	Acyclic nucleoside phosphonates	Renal tubular toxicity
9	Acyclovir and its oral prodrug valacyclovir, penciclovir and its oral prodrug famciclovir, ganciclovir and its oral prodrug valganciclovir, Foscarnet	Nucleoside analogs and other antiviral drugs	Viral DNA polymerase inhibitors	Renal failure, neuropsychiatric side effects, encephalopathy, delirium, tremors, headache, diarrhea, nephrotoxicity, hepatotoxicity, neutropenia, leucopenia, anemia, thrombocytopenia, nausea, vomiting, headache, fatigue, and rash

4 Risks Associated with Antiviral Drugs in Wastewater

Among pharmaceuticals, antibiotic and antiviral drugs are of emerging concern due to their growing role in antibiotic and antiviral drugs resistance among pathogenic bacteria and influenza viruses, respectively. These compounds may also upset sensitive ecosystems, as they are highly bioactive. Antiviral drugs may have both qualitative and quantitative effects upon the resident microbial population of sediments (Kummerer 2008). These drugs

vary widely in their molecular weight and chemical structure, and therefore, there is a possibility that they would show different nature to wastewater treatment in terms of recalcitrance and environmental behavior. Release of antiviral drugs like OC to the environment or water bodies may pose risks, which include drinking water safety, ecological health risk, the development of antiviral resistance, destabilization of microbial biofilms or flock or sensitive microbial community, and affecting the performance and function of STPs (Ghosh 2009). This may often lead to generation of metabolites or degradation-by-products, which may be more harmful than their parent compound and more difficult to remove from wastewater. OC and peramivir inhibited biofilm formation on microbial community like *Pseudomonas aeruginosa*, a typical bacterium found in soil, water, and skin flora (Soong et al. 2006). This may lead to hindrance in biological treatment of wastewater. Large amount of bioactive pharmaceuticals during an influenza pandemic poses significant ecotoxicological challenge and stops the growth of microbial consortia due to antiviral contamination of receiving water bodies, thereby causing concern for freshwater and marine organisms (Singer et al. 2011; Slater et al. 2011). With growing use of antivirals and antibiotics, 80 % to 100 % WWTPs will experience inhibition of microbial growth in plant operations (Reynolds 2011).

Acute aquatic ecotoxicity data of famciclovir has been briefly cited in literature (Cunningham et al. 2006). Ritonavir has also been reported to exhibit a high ecotoxicity potential (Escher et al. 2011; Lienert et al. 2011). Zidovudine, an antiretroviral drug, has carcinogenic potential and has been classified in Group 2B which constitutes possible human cancerogens (Bottoni et al. 2010). Populations exposed to abacavir may have adverse drug reactions and hypersensitivity (Bonnefoi et al. 2010). Furthermore, drug residues at high concentrations can have toxic effects on aquatic organisms, e.g., on *Daphnia magma*, algae, bacteria, fish, and then to humans (Kummerer 2008).

Atazanavir, adefovir, acyclovir, valacyclovir, tenofovir, and other antiviral drugs have very limited experimental fate or toxicity data available. One such study about the presence of carboxy-acyclovir, a metabolite of acyclovir, in drinking water reported to be of major concern was because of the neuropsychiatric side effects in patients. Penciclovir (PCV), another antiviral drug used in treating herpes infections, and its transformation product (TP) formed after biological treatment are likely to be of ecotoxicological relevance. For example, a transformation product of penciclovir TP251, R,β-unsaturated aldehydes, results in inactivation of enzymes and mutagenesis in human body (Prasse et al. 2011). Valacyclovir is among the top 25 drugs found in WWTPs by mass (5,352 mg/day/1,000 persons) exhibiting high effluent concentration and has potential to induce ecotoxicity, but it has been neglected in prior research studies related to the environmental fate, transport, and occurrence (Ottmar et al. 2010). Risk assessment studies of oseltamivir (Tamiflu®) used in normal or pandemic influenza conditions during sewage treatment and in aquatic systems concluded that it is present in effluent from WWTP. Oseltamivir was found to induce a significant ecotoxicological risk in waterways and reported to be recalcitrant in sewage effluent (Goncalves et al. 2011; Singer et al. 2007; Straub 2009). OC was found not to be completely removed by conventional wastewater treatment processes (Fick et al. 2007), which results in adverse effects on aquatic ecosystems (Accinelli et al. 2007; Sacca et al. 2009; Singh et al. 2008). Activated sludge bacteria showed no growth on oseltamivir (Tamiflu®), and presence of lamivudine in WWTPs results in decrease in overall efficiency (Slater et al. 2011; Vanková 2010).

Reynolds (2011) developed a mathematical model to predict the effects of antibiotics and antiviral drugs on wastewater treatment ecosystems. It was observed that during mild pandemic, a slight increase in use of antiviral drugs has negligible effect on microbial community. While large increase in antiviral drugs during severe outbreak of influenza has completely inhibited the growth of microorganisms.

At present, potential health risks associated with antiviral drugs other than oseltamivir and its metabolite OC are far less reported in literature. Therefore, occurrence and inhibitory effects of other antiviral drugs on microbial community in the environment needs further attention. Increased use of antiviral medications for the treatment of influenza can potentially affect the ecosystem and WWTP operations, but to what degree and extent are currently unidentified and need to be further explored.

5 Removal of Antiviral Drugs from Wastewater

As far as removal of antiviral drugs from wastewater is concerned, majority of the studies are reported on removal of oseltamivir. The reported literature contains

only removal of some antiviral drugs through biological treatment or a combination of biological treatment with some other process.

Biodegradation effectiveness of three drugs (acyclovir, naproxen, and nalidixic acid) from pharmaceutical industrial wastewaters was investigated using Zahn–Wellens test (Mascolo et al. 2010a). Organic parent compounds and metabolites were reported to be recalcitrant to biodegradation. Out of the three main compounds which were detected in the acyclovir wastewater samples, one compound was reported to be quite persistent and accumulated during biodegradation (Mascolo et al. 2010a). Mascolo et al. (2010b) reported the removal of acyclovir up to 99.99 % in an integrated system consisting of membrane bioreactor followed by ozonation.

Biological treatment of synthetic wastewater of three antiretroviral drugs (used in treatment of HIV), lamivudine, nevirapine, and zidovudine, were carried out in a closed bottle system (Vanková 2010). These drugs were found to be non-biodegradable, toxic, and inhibitory to activated sludge bacteria and potentially referred as refractory in the environment. All anti-HIV drugs have been reported to be potential environmental pollutants. Long half-life of nevirapine and also photostability makes it toxic to larger organisms like rat. Nevirapine has been reported to persist for years in the environment due to its bioactive nature. Al-Rajab et al. (2010) showed less than 10 % mineralization of tenofovir when sorbed with dewatered biosolids in a 2-month incubation period during sewage treatment. This is reported as relatively persistent in soils but biodegraded by aerobic microorganisms.

Oseltamivir has been reported to be persistent in aquatic bodies and non-biodegradable in WWTPs or STPs (Accinelli et al. 2010b; Söderström et al. 2009). Biological removal of oseltamivir (Tamiflu®) and three different antibiotics (erythromycin, sulfamethoxazole, and ciprofloxacin) from Bologna WWTP was studied using white rot fungus, *Phanerochaete chrysosporium*. All three antibiotics have been significantly removed, but oseltamivir was found to be most persistent among four reported active substances (Accinelli et al. 2010b). Fick et al. (2007), through some experiments, showed that the active moiety of Tamiflu®, OC, was not found to be removed in normal sewage water treatments and also was not degraded by UV light radiation substantially. Bartels and von Tümpling Jr. (2008) demonstrated that direct photolysis does not affect degradation of OC. On the other hand, the combination of biological and indirect photolysis treatment resulted in decomposition of OC. Goncalves et al. (2011) conducted the photodegradation of OC and oseltamivir ester (OE) and identified degradation products. These degraded products were found to be more persistent than parent drugs, and showed very low sorption to sediments resulting in high hydrophilicity and low affinity to the particulate matter and limited mineralization which was found to be less than 20 % as CO_2 in a 28-day test by aerobic microorganisms. According to Bartels and von Tümpling Jr. (2008), OC can be removed by a combination of microbial metabolism and indirect photodegradation. Furthermore, addition of 5 % river sediments reported to result in rapid OC degradation (Sacca et al. 2009).

The conventional treatment of antiviral drugs may result in intermediates which are poorly biodegradable. These intermediates are hard to biodegrade and/or hinder biological treatment system since these residual compounds have been reported to cause change in genome of viral or microbial cells (Sponza and Demirden 2007). The presence of antiviral drugs in a WWTP inhibits the growth of microorganisms and thus affecting the removal of the remaining organic matter content (Dantas et al. 2008). Longer retention time, usually in days to oxidize antiviral drug, is one of the drawbacks in biological oxidation systems. Incineration was applied to treat a variety of antiviral drugs resulting in the release of toxic fumes of NO_x, SO_x, NH_3, F^-, Cl^-, and PO_x (Sheahan 2008). However, this process requires high temperature incinerators, and moreover, there is a risk of diffusion of gases in the environment in case of any mishap.

Table 3 gives an overview of the recent work undertaken for the removal of antiviral drugs from wastewater. From the data of Table 3, several observations can be made as follows:

1. As far as treatment efficiency is concerned, biological treatment is capable of removing some antiviral drugs but not necessarily accompanied by total mineralization. In several cases, degradation by-products and transformation products are found to be more persistent or recalcitrant than the original compound, thus implying that post-treatment is required. For example, in case of acyclovir and penciclovir, the transformation products carboxy-acyclovir was found to be persistent under aerobic conditions with R,β-unsaturated aldehydes (e.g., penciclovir transformation product PCV TP251) lead to major changes in natural metabolism of living beings (Prasse et al. 2011).

Table 3 Removal of antiviral drugs from wastewater reported in literature

S. No.	Antiviral drugs	Waste type and % composition found in wastewater	Treatment process/study	Main findings	Reference
1	Abacavir, abacavir/lamivudine, acyclovir, atazanavir, efavirenz, efavirenz + emtricitabine + tenofovir, emtricitabine + tenofovir, indinavir, lamivudine, lamivudine/stavudine, lamivudine/stavudine + efavirenz, lamivudine/stavudine + nevirapine, lamivudine/zidovudine, lamivudine/zidovudine + abacavir, lamivudine/zidovudine + efavirenz, lamivudine/zidovudine + nevirapine, lopinavir + ritonavir, nelfinavir, nevirapine, ribavirin, ritonavir, saquinavir, stavudine, tenofovir, tenofovir disoproxil fumarate, tenofovir/emtricitabine	Pharmaceutical	Incineration	Toxic fumes upon incineration (NO_x, SO_x, NH_3, F^-, Cl^-, PO_x)	Sheahan (2008)
2	Zidovudine	Hazardous	Incineration	Toxic fumes of NO_x upon incineration	Sheahan (2008)
3	Amantadine (AMT), oseltamivir carboxylate (OC)	STP, 538 ng/L (AMT), 140 to 460 ng/L (OC)	Primary, secondary, extended aeration-based conventional activated sludge treatment and ozonation as tertiary treatment	Primary treatment resulted in 7–17 % and 2–9 % removal of AMT and OC, respectively. Removal of 20 % to 37 % of OC through anoxic–oxic and anaerobic–anoxic–oxic treatment. Less than 20 % removal in extended aeration-based conventional activated sludge treatment. More than 90 % removal when combined with ozonation (tertiary treatment)	Ghosh et al. (2010a)
4	Amantadine	Simulated solution of amantadine	Fenton process	100 % removal of amantadine and degradation of all intermediates	Zeng et al. (2008)
5	Acyclovir	3 different pharmaceutical wastewater (240, 170, and 2,580 mg/L)	Aerobic biological treatment (activated sludge)	One metabolite reported to be not completely biodegradable	Mascolo et al. (2010a)

Table 3 (continued)

S. No.	Antiviral drugs	Waste type and % composition found in wastewater	Treatment process/study	Main findings	Reference
6	Acyclovir	Pharmaceutical, 154 mg/L	Integrated MBR-ozonation system	99.99 % removal	Mascolo et al. (2010b)
7	Acyclovir, abacavir, lamivudine, nevirapine, oseltamivir, penciclovir,ribavirin, stavudine, zidovudine, and one active metabolite oseltamivir carboxylate	Raw and treated wastewaters (acyclovir, 1,800 ng/L; lamivudine, 720 ng/L; zidovudine, 380 ng/L; and abacavir, 220 ng/L)	German conventional WWTPs	Almost complete removal of abacavir, acyclovir, lamivudine, penciclovir, and stavudine ranging from 87 % to less than 99 % except zidovudine	Prasse et al. (2010)
8	Acyclovir (ACV) and penciclovir (PCV)	ACV (1,990, 1,800±300 ng/L), PCV (<50 ng/L)	Activated sludge treatment (biotransformation)	Carboxy-acyclovir, as transformation product (TP) from acyclovir degradation and 8 TPs from penciclovir biodegradation	Prasse et al. (2011)
9	Acyclovir and its transformation product, carboxy-acyclovir	Wastewater treatment plant (WWTP) effluent (dissolved organic carbon (DOC) 12 mg/L, pH 7.7)	Ozonation	*N*-(4-carbamoyl-2-imino-5-oxoimidazolidin)-formamido-*N*-methoxyacetic acid (COFA) as oxidation product of ACV	Prasse et al. (2012)
10	Amantadine hydrochloride	Pharmaceutical wastewater and 1,000 m^3	Adsorption using 5 adsorbents, namely, activated carbon, diatomite, zeolite, slag, and steel slag	Maximum adsorption reported from activated carbon followed by diatomite, zeolite, steel slag, and slag	Fu and Jiang (2010)
11	Amantadine (AMT)	AMT wastewater	Crystallization	95 % removal	Zou et al. (2009)
12	Lamivudine	Lamivudine wastewater, (13,600 mg/L of COD and 3,000 times of colority)	Iron–carbon micro-electrolysis process	COD lower down to 56 % and colority decreased by 90 %, respectively	Wang et al. (2010)
13	Lamivudine	–	Photocatalytic degradation	Complete mineralization	An et al. (2011)
14	Ribavirin	Ribavirin medicine wastewater (COD concentration, 7,000 mg/L)	Universal broadcast filter anaerobic reactor (UBF)	72.8 % COD removal	Ni and Li (2009)
15	Zidovudine	Pharmaceutical wastewater	Catalyzed iron electrolysis method	More than 60 % COD removal	Hu et al. (2006)
16	Zidovudine	Pharmaceutical wastewater	Ultrasonic and iron–carbon micro-electrolysis technology	85 % COD removal	Li et al. (2011)
17	Oseltamivir ester (OE) and oseltamivir carboxylate (OC)	River basin	Photodegradation (photolysis, artificial and natural solar irradiation)	Degradation products were reported less than 25 % of the parent compound in case of OE and 7 % in the case of OC	Goncalves et al. (2011)
18	Oseltamivir carboxylate	Irrigation canal	Microbial degradation	35 % degradation after 36 days of incubation and the drug was reported to be persistent	Accinelli et al. (2007)

Table 3 (continued)

S. No.	Antiviral drugs	Waste type and % composition found in wastewater	Treatment process/study	Main findings	Reference
19	Oseltamivir carboxylate	Synthetic influent wastewater	Microbial diversity and nutrient removal performance in a simulated activated sludge system	41 % removal of OC	Slater et al. (2011)
20	Oseltamivir carboxylate	River catchment areas (26–32 μg/L)	Photolysis (daylight experiment)	Direct photolysis resulted in non-degradation of OC	Bartels and von Tümpling Jr. (2008)
21	Oseltamivir carboxylate	River water and lake water	Biological treatment (activated sludge)	Persistence of oseltamivir with half-life of 53 days in surface waters. Presence of sediments (5 %) reported to affect oseltamivir degradation	Accinelli et al. (2010a)
22	Oseltamivir carboxylate, oseltamivir phosphate	–	Bioremediation (granular bioplastic formulation entrapping propagules of fungi *Phanerochaete chrysosporium*)	Oseltamivir was found to be the most persistent among four active substances (erythromycin, sulfamethoxazole, ciprofloxacin, and oseltamivir)	Accinelli et al. (2010b)
23	Oseltamivir carboxylate	Influent wastewater	WWTP (activated sludge)	20 % removal efficiency	Matsuo et al. (2011)
24	Oseltamivir carboxylate	STP discharges (293.3 ng/L)	STP with tertiary treatment (ozonation) and activated sludge process as secondary treatment)	OC (>85 % from secondary effluent)	Ghosh et al. (2010b)
25	Oseltamivir, oseltamivir carboxylate (OC), ritonavir	Hospital wastewater, oseltamivir, 0.125 μg/L; OC, 0.151 μg/L; ritonavir, 0.108 μg/L	Membrane bioreactor	Minor or no elimination of oseltamivir and OC and 78 % ritonavir removal	Kovalova et al. (2012)
26	Oseltamivir phosphate (OP)	–	UV, UV/H_2O_2, and UV/H_2O_2/Fe^{II}	Hydroxylated OP derivative (3S,4R,5S)-ethyl 4-acetamido-5-amino-2-hydroxy-3-(pentan-3-yloxy) cyclohexane carboxylate as photoproduct, overall no ecotoxicity was reported	Tong et al. (2011)
27	Oseltamivir acid (OA, the active metabolite of Tamiflu®)	Secondary wastewater effluent	Ozonation and AOPs	Efficiently removed	Mestankova et al. (2012)

2. Ozonation and AOPs can be considered to be effective methods for removal of antiviral drugs since AOPs are based on the intermediacy of hydroxyl and other radicals to oxidize recalcitrant/persistent, toxic, and non-biodegradable compounds to various by-products resulting in the formation of inert end products. However, majority of the ozonation experiments are conducted on acyclovir and oseltamivir only. These advanced treatment processes can be employed for removal of other antiviral drugs also.
3. Majority of the work has been carried out on removal of antiviral drugs on laboratory scale using biological treatment. Therefore, further studies are required to be made in terms of the design strategies to scale up the treatment process.

Highly bioactive in nature, partially resistant towards biological degradation, and often ending up in generation of recalcitrant or persistent by-products are some of the characteristics of these emerging contaminants. As conventional wastewater and other wastewater treatment processes are unable to act as a reliable barrier towards some of recalcitrant antiviral drugs, it is necessary to work upon some of the additional advanced treatment technologies. It has been assumed that antiviral drugs would behave in the same way as that of antibiotics. Hence, the removal processes which have been employed for antibiotics can be effectively used for antiviral drugs also. Various techniques are available in literature for the removal of antibiotics from wastewater (Adams et al. 2002; Bolong et al. 2009; Heberer 2002). It includes adsorption, oxidation using chlorination, H_2O_2-UV, ozonation, anaerobic biological processes, and membrane separation techniques. Electrochemical methods can also be considered as viable options for treatment of polar antiviral compounds.

Removal or degradation rates depend on the treatment used and duration, concentration, and physical properties of the antiviral drugs in the influent. Kummerer (2009) proposed and investigated some risk management strategies to eliminate or remove pharmaceuticals from effluent of STPs or wastewater. Considering the research work done so far on removal of antiviral drugs, it is desirable to study the other well-established methods of removal of pharmaceuticals from wastewater.

6 Current and Future Research Needs

Based on a literature review and an overview of occurrence, ecotoxic effects, and detection methods of antiviral drugs in aquatic bodies and environment, this paper proposes a few recommendations in research of antiviral drugs in wastewater by highlighting the potential harmful effects of antiviral drugs on organisms.

To date, limited number of studies has indicated occurrence of antiviral drugs in the low nanograms per liter or milligrams per liter range in STPs or effluents from pharmaceutical industries. Antiviral drugs have also been found to develop resistance in dabbling ducks. It is recommended to identify potential hazards associated with antiviral drugs when encountered with other animals through waste streams.

Limited literature data is available on toxicological effects of antiviral drugs on aquatic organisms. Therefore, more studies are needed to investigate the toxic nature and degradation mechanisms of these antiviral drugs and their metabolites in the environment.

Biodegradation of antiviral drugs is a cost effective method, but degradation products are found to be more persistent than parent compounds. More work is needed to establish the degradation of antiviral drugs from wastewaters. Results show that there is a pronounced lack of data in removal of antiviral drugs; majority of the work has been carried out only on removal of oseltamivir. There are many other antiviral drugs like abacavir, indinavir, ritonavir, and emtricitabine and certain combination of antiviral agents, HAART having the potential to contaminate water bodies, which are found in significant quantities in water bodies and STPs. The removal method of such antiviral drugs is not well established. Adsorption is one of the most efficient methods for the removal of contaminants from wastewater producing high quality treated effluent. This process may provide a remarkable alternative in a sense that it does not require any additional pre-treatment step for the treatment of wastewater containing antiviral drugs. Adsorption has been found to be a better option compared to other techniques in terms of ease of operation, flexibility, initial cost and design, and inertness to toxic pollutants. Adsorption also does not generate any harmful by-product (Ahmaruzzaman 2011).

AOPs are efficient methods for the treatment of pharmaceutical wastewaters containing recalcitrant and toxic compounds. AOPs work in two steps: (1)

the formation of strong oxidants and (2) the reaction of these oxidants with organic contaminants in water. AOPs include heterogeneous processes, e.g., photocatalysis based on titanium dioxide (TiO_2/hν), ozonation, and homogeneous processes like γ-radiolysis, sonolysis, electrolysis, near ultraviolet (UV) or solar visible irradiation, Fenton and photo-Fenton processes, and wet air oxidation, while less conventional but evolving processes consist of ionizing radiation, microwaves, pulsed plasma, and the treatment with ferrate reagent (Klavioriti et al. 2009; Ikehata et al. 2006).

Membrane separation processes, viz. reverse osmosis, ultrafiltration, and electrodialysis, are gaining considerable attention in many industrial applications. Low pressure membrane filtration, such as microfiltration (MF) and ultrafiltration (UF), can be feasible options for addressing the present removal needs. Antiviral drugs can be recovered and commercially utilized without any chemical modification using suitable membranes. The resulting water would be relatively clean and can often be directly reused with no further treatment (Strathmann 1976).

Electrochemical methods like electrocoagulation, electrooxidation, electroreduction, and electroflotation offer various advantages over other treatment methods. High efficiency, ease of operation, and compact facilities are some of the key features of these methods. As some of the drugs are charged molecules carrying negative, positive, or zwitterions in their ionic forms, these methods can potentially be used to effectively remove antiviral drugs from wastewater.

Depending on the properties of the antiviral drug to be treated, above-mentioned treatment processes can be employed either alone or may be used in combination with other physicochemical and/or biological processes. Combining two or more processes can be advantageous leading to improved treatment efficiencies. For instance, adsorption may be employed to remove recalcitrant compounds to a great extent followed by biological treatment. It would minimize the possibility of formation of transformation products. Therefore, it is recommended that the removal processes or treatment methods should be done or tried on other antiviral drugs also. In addition, the lack of appropriate detection methods of antiviral drugs and their metabolites in aqueous solution is also a problem.

As people buy and take more and more antiviral drugs to treat their ailments/diseases in the event of pandemic outbreak, the concentration of drugs increases in the wastewater, and also sometimes, unused or expired medications can accumulate. A problem arises, however, when unused medications are disposed off as such in trash or untreated in drains. Globally, majority of the population dispose their pharmaceuticals, whether analgesics, antiviral drugs, or antibiotics, in the household garbage or by flushing in sink or toilet. Therefore, there is also a need to properly educate and set guidelines for proper disposal of unused and expired pharmaceuticals.

7 Conclusions

A wide range of antiviral drugs has been detected in the water bodies and environment worldwide. Many of the compounds are chemically stable in water, lipophobic, and often not be effectively removed by conventional biological treatment processes. Many of these compounds are also toxic and relatively persistent in water bodies. Persistent presence of antiviral drugs in the environment is a cause of concern due to several reasons. One of them is the resistance developed in the aquatic organisms if drug and virus both are simultaneously present in water bodies. There is a threat of resistance development in humans also from antiviral drugs present in water. In order to examine the hazards presented by antiviral drugs on usage and persistence in the environment, robust and sensitive analytical methods are required. Considering limited methods for detection of antiviral drugs in aqueous solution, there is a need to work upon more reliable detection methods. Presently, available methods suffer with detection limits confined to low concentrations in biological samples only. Majority of the literature are on occurrence and removal of oseltamivir, used in treatment of H5N1 and H1N1 influenza. With the advent of time, use of antiviral drugs is increasing resulting in increased concentration of these drugs in the environment. Hence, research is needed to remove other antiviral drugs from wastewater efficiently. So, it is desirable to study other well-established methods on removal of pharmaceuticals from wastewater which have not been tried so far on other antiviral drugs. However, for metabolites of antiviral drugs, their transformation products, this information can be sparse, and their fate and transport data in the water bodies require substantial attention. There is still an urgent need to fill the gaps in our knowledge.

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