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Diclofenac and its transformation products: Environmental occurrence and toxicity - A review

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Review article

Diclofenac and its transformation products: Environmental occurrence and toxicity - A review

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ABSTRACT

Diclofenac (DCF) is a prevalent anti-inflammatory drug used throughout the world. Intensive researches carried out in the past few decades have confirmed the global ubiquity of DCF in various environmental compartments. Its frequent occurrence in freshwater environments and its potential toxicity towards several organisms such as fish and mussels makes DCF an emerging environmental contaminant. At typical detected environmental concentrations, the drug does not exhibit toxic effects towards living organisms, albeit chronic exposure may lead to severe effects. For DCF, about 30–70% removal has been obtained through the conventional treatment system in wastewater treatment plant being the major primary sink. Thus, the untreated DCF will pass to surface water. DCF can interact with other inorganic contaminants in the environment particularly in wastewater treatment plant, such as metals, organic contaminants and even with DCF metabolites. This process may lead to the creation of another possible emerging contaminant. In the present context, environmental fate of DCF in different compartments such as soil and water has been addressed with an overview of current treatment methods. In addition, the toxicity concerns regarding DCF in aquatic as well as terrestrial environment along with an introduction to the metabolites of DCF through consumption as well as abiotic degradation routes are also discussed. Further studies are required to better assess the fate and toxicological effects of DCF and its metabolites and must consider the possible interaction of DCF with other contaminants to develop an effective treatment method for DCF and its traces.

Contents

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1. Introduction

Pharmaceutical industry has emerged as one of the largest and prominent industry worldwide. Large amount of pharmaceuticals of different categories are being used to cure and care human and animal health. In general, pharmaceuticals comprise compounds which include materials extensively used in medicine, agriculture and biotechnology, such as drugs, antibiotics and hormones. The worldwide average per capita consumption of pharmaceuticals per year is estimated to be about 15 g. In industrialized countries, the usage is even as high as 50 to 150 g (Alder et al., 2006). Pharmaceutically active compounds (PhACs) are one of the conspicuous classes of pharmaceuticals which by one route or another, enter the environment as the parent compound or as pharmacologically active metabolites (Halling-Sørensen et al., 1998). It is estimated that worldwide consumption of active compounds amounts to 100,000 tons or more per annum (Kummerer, 2004). Usually, drugs are developed with an intention of having a beneficial biological effect on the organism to which they are administered, though many such compounds will often pass into the environment where they may exert an unwanted biological effect (Halling-Sørensen et al., 1998). The global occurrences of pharmaceuticals and PhACs in aquatic environment have been arising as a problem with unknown consequences. PhACs have been reported to be present in different environmental compartments and often the short-term as well as long-term effects are obscure (Kunkel and Radke, 2012; Langford et al., 2011) Hence, it has been relatively recently that PhACs have become a subject of interest to environmentalists worldwide (Hao et al., 2007).

Among PhACs, non-steroidal anti-inflammatory drugs (NSAIDs) are widely used throughout the world and detected in different environmental compartments at concentrations ranging from ng L^{-1} to low mg L^{-1} (Halling-Sørensen et al., 1998; Khetan and Collins, 2007). Moreover, NSAIDs are over-the-counter (OTC) drugs in most of the countries and this in turn increases the chances for consumption and hence, their presence in the environment. DCF is often recognized as the 'world's most popular pain killer' and is also the most commonly used NSAID, with a market share close to that of the next three most popular drugs combined (ibuprofen, mefenamic acid, naproxen) (McGettigan and Henry, 2013). The name diclofenac is derived from its chemical name: 2-(2,6-dichloranilino)phenylacetic acid. Diclofenac was discovered by Ciba-Geigy, a Swiss pharmaceutical company in 1973 (now merged to Novartis). Diclofenac is commonly used to reduce inflammation and to relieve pain in diseased conditions, such as arthritis or acute injury. It also works as antiuricosurics and analgesic. DCF can be applied to skin or it can be administered orally. DCF is supplied as or contained in medications under a variety of trade names. In Canada, DCF is sold as Voltaren Emulgel and some other common names are Votalin (China), Diclofenaco Normon (Spain), Volini (India), Diclofenac-Asteria (USA and Korea), Diclo-Denk (Germany) Voltaren (Argentina, Australia, Belgium, Egypt, France, Germany, Israel, New Zealand, Norway, Portugal, Russia, South Africa, Sweden, Turkey) (sources - www.drugs.com/diclofenac, www.drugbank.ca, www.scbt.com).

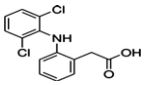
Pharmacological and physico-chemical properties of DCF are listed in Table 1. Often DCF is not completely removed from wastewater treatment plants (WWTP) due to its poor degradation and higher consumption rates (Fatta-Kassinos et al., 2011; Zorita et al., 2009). Hence, DCF is frequently detected in rivers, sediments and sludges (Kunkel and Radke, 2012; Langford et al., 2011). Relatively recently, DCF has drawn much more attention due to its frequent occurrence in drinking water sources (Gros et al., 2010) and its potential harmful effects on many organisms at significant concentration (Cleuvers, 2004; Oaks et al., 2004).

Diclofenac is normally used as salt of sodium or potassium for improved solubility and absorption. Until date, no literature is exclusively available on the environmental perspectives and concerns regarding the drug, DCF. Most of the previously published reviews have discussed the fate of diclofenac in WWTPs (Vieno and Sillanpää, 2014; Zhang et al., 2008). The objective of this review is to briefly summarize the current status of diclofenac in the environment, review the available information about its consumption, occurrence, toxicity, resistance, persistence and metabolites. In addition this review addresses the hypothetical possibility of potential interactions of DCF with other organic and inorganic contaminants, emerging contaminants along with its own metabolites. Major research gap in the current knowledge and future research need in diclofenac fate and transport in environment have also been highlighted.

2. Global consumption

It is fairly impossible to calculate the exact global consumption of diclofenac because of various reasons, such as use of different trade names for DCF, use for human and veterinary purposes and that the drug is an over the counter drug. Nevertheless, Zhang et al. (2008) estimated that about 940 tons of diclofenac is consumed globally on an annual basis from Intercontinental Marketing Services (IMS) health data (Zhang et al., 2008). About 877 tons of diclofenac were sold in 2007 in 76 major countries which are believed to account for about 96% of the global diclofenac pharmaceutical market (Zhang et al., 2008). In a 2012 report from "Fierce Pharma", diclofenac was listed as the 12th bestselling generic molecule globally. The total sales of diclofenac in

Table 1
Physico-chemical and pharmacological properties of diclofenac (in unionized form).

Properties		Reference
Structure		www.pubchem.ncbi.nlm.nih.gov, www.chemspider.com
Molecular formula and molecular weight	$C_{14}H_{11}Cl_2NO_2$, 296.16 g mol^{-1}	www.drugbank.ca, www.pubchem.ncbi.nlm.nih.gov
CAS no.	15307-86-5 15307-79-6 (disodium salt)	www.drugbank.ca, www.pubchem.ncbi.nlm.nih.gov
Water solubility	2.37 mg L^{-1} (25 °C)	www.drugbank.ca, www.chemspider.com
Henry's law constant	4.79×10^{-7} Pa m^3 mol^{-1} (25 °C)	www.scbt.com, www.pubchem.ncbi.nlm.nih.gov
Melting and boiling points	283–285 °C and 412 °C at 760 mm Hg (predicted) respectively	www.drugbank.ca, www.chemspider.com
p*K*a	4.15	www.drugbank.ca, www.chemspider.com
Log K_{ow} (logarithm of octanol-water partition coefficient)	4.51	www.scbt.com, www.pubchem.ncbi.nlm.nih.gov

2011 were estimated to be $1.61 billion dollars with a sales charge of 15.5% annually (Palmer, 2012). While considering the sales change information along with previously carried-out consumption estimation, there is high probability that the annual consumption of DCF might have crossed 1000 tons. Moreover, recent reports suggests that along with conventional and developed markets, such as United States, emerging markets such as India, China and Brazil also consume >60 tons of DCF on annual basis (Acuña et al., 2015). In addition, the consumption estimation of DCF does not cover the veterinary consumption due to non-availability of data and hence the consumption can be even higher while considering the veterinary usages.

Diclofenac is included in the emergency medical list (EML) of 74 countries. The exact annual consumption of diclofenac in North America is not available, even though in North America, the market shares of DCF in the drug market are on a continuous rise. In US, DCF contributes to about 5–6% of the total NSAID market while in Canada, 17% of NSAID consumed is DCF (Henry, 2013). According to the current trend, consumption of DCF will keep on increasing in North America since lifestyle diseases, such as arthritis and heart diseases are now becoming common and the also the ageing population will require medicines, such as pain killers.

The annual consumption or prescription data for diclofenac is available for some countries. According to consumption estimation models, in Australia, it was estimated that 4 tons of DCF was used annually (Khan and Ongerth, 2004). In Europe, the largest user of DCF is Germany with 86 tons of usage in the year 2001 (Huschek et al., 2004). For rest of the European countries; the consumption comprises for England 26.13 tons per year (Jones et al., 2002), Austria 6.14 tons per year (Strenn et al., 2004) and France 16 tons per year (Ferrari et al., 2003). The total consumption of DCF in the entire European continent was estimated to be 179.8 tons per year (Ferrari et al., 2003). For most of the Asian and African countries, data on consumption of DCF is not available due to the lack of studies on consumption and also due to the absence of inventory of sales. With the frequent reports on toxicological effects observed in these countries on vultures, it can be assumed that the consumption can be colossal.

Recent studies based on IMS health data (which serves 82% of the global population) from 86 countries estimated that at present on an average 1443 ± 58 tons of DCF is consumed globally (Acuña et al., 2015). In this study authors also indicated that 39.5% of DCF was consumed in Asia and 28.7% Europe. However, this is only an indication on the consumption DCF for human health related applications and does not include the consumption of DCF for veterinary uses. At present it is impossible to calculate the total consumption of DCF since the data for veterinary consumption is not available.

3. Legislation

DCF in environment has been lately acknowledged to constitute a health risk to terrestrial organisms. DCF is extremely toxic to vultures (even though they do not consume DCF directly) and its use on cattle has wiped out and threatened vulture populations in India, Pakistan and Nepal since the vultures consume cattle carcasses. India was the first country to bring in regulations on the consumption of DCF. In 2006, the manufacture and veterinary use of DCF was banned in India (The Drug controller General, 2006). Further in 2008, India placed additional restrictions on diclofenac for animal use, with contravention punishable with imprisonment. Further, the same year, Nepal and Pakistan banned the drug for veterinary use followed by Bangladesh in 2010 (Venkateshwarlu, 2011).

For the veterinary use, diclofenac does not have a central marketing approval from the European Medicines Agency (EMA) and it is authorized independently in each member state. Further, EMA has set a Maximum Residue Limit for DCF in bovine and porcine species. Since 2013, the commercial production of DCF started in Spain and Italy and is being exported to other European Union countries. A coalition of famous organizations including the Vulture Conservation Foundation, The Royal Society for the Protection of Birds, Bird Life Europe and the IUCN Vulture Specialist Group are campaigning for an EU-imposed, continent-wide ban on veterinary diclofenac following the lessons from India (BirdLife, 2013; Tavares, 2014a; Tavares, 2014b). Diclofenac has been recently added to Environmental Quality Standards (EQS) of Europe. According to the European Community document (COM(2011)876), the annual average value of EQS (evaluation of quality standards) for DCF was 0.1 $\mu g\ L^{-1}$. However, this document has been amended and DCF is put on watch list until next review (Johnson et al., 2013). In 2013, DCF has been selected for inclusion in the watch list of "EU Water Framework Directive" in order to collect sufficient monitoring data for the determination of risk reduction measures. According to this proposed EQS document , the maximimum alloable concentrations are 0.1 $\mu g\ L^{-1}$ in fresh waters and 0.01 $\mu g\ L^{-1}$ in marine waters. Another European coutry, United Kingdom (UK) has placed DCF in 'the list of priority substances' which forced the water industries to search technologies to remove DCF from wastewater.

Regulatory measures for the use of DCF has been imposed by few countries. Apart from very few regional/country wise regulations in global scale scenario, strict legislation or directives does not exist to regulate the production and consumption of DCF to control environmental presence of diclofenac. Since DCF is emerging as a contaminant of concern and has been acknowledged to pose health risks towards terrestrial organisms, such as vultures, recent efforts have focussed on survey on contamination of diclofenac in the environment and drafting the rules for the regulation of the drug. However, there is a need to control the consumption of drug and thereby reduce the environmental presence. Besides, it is important to set appropriate directives, such as setting a maximum concentration limit in the environment.

4. Environmental fate

Even though the general purpose of the pharmaceuticals is to positively affect human or animal health in a unique manner, they often have some adverse effects on the environment. When these pharmaceuticals enter the environment, they may affect the same pathways in animals having identical or similar target organs, tissues, cells or biomolecules (Fent et al., 2006). Studies have demonstrated the potential adverse effects of diclofenac in the environment (Cleuvers, 2004; Gros et al., 2010; Kunkel and Radke, 2012; Oaks et al., 2004). Hence, it is important to understand the origin and fate of pharmaceuticals and diclofenac in particular in the environment for designing appropriate pollution remedial measures.

Fig. 1 demonstrates the possible entry routes of DCF to the environment. Being an anthropogenic pollutant, the source of diclofenac is the drug industry, and it is used for both human and veterinary purposes. Through both human and veterinary routes, DCF ends up in wastewater treatment plants or in landfills as DCF or its metabolites. Also, there are fewer but considerable probabilities for the drug to reach wastewater treatment plants directly from pharmaceutical industrial residues. Likewise, the conventional treatment process of DCF in wastewater treatment plants are ineffective (Fatta-Kassinos et al., 2011; Zorita et al., 2009) and hence DCF can end up in the surface water and the possibility for the percolation of DCF to the drinking water sources cannot be overruled. In addition, percolation probabilities for DCF from landfills to surface water are fairly higher. At present, none of the studies reported DCF causing major risks to aquatic life at an environmentally relevant concentration. The potential detrimental effect of DCF in the aquatic environment has been revealed from many studies (Cleuvers, 2003; Fent et al., 2006; Jones et al., 2002; Lee et al., 2011). The no effect concentration (NEC) of DCF was calculated to be 100 $\mu g\ L^{-1}$ for freshwater cladocerans and Japanese medaka (Lee et al., 2011). Another study on rainbow trout and zebra fish, two typical examples for freshwater organisms no observed effect concentration (NOEC) was observed to be 320 $\mu g\ L^{-1}$ (Memmert et al., 2013).

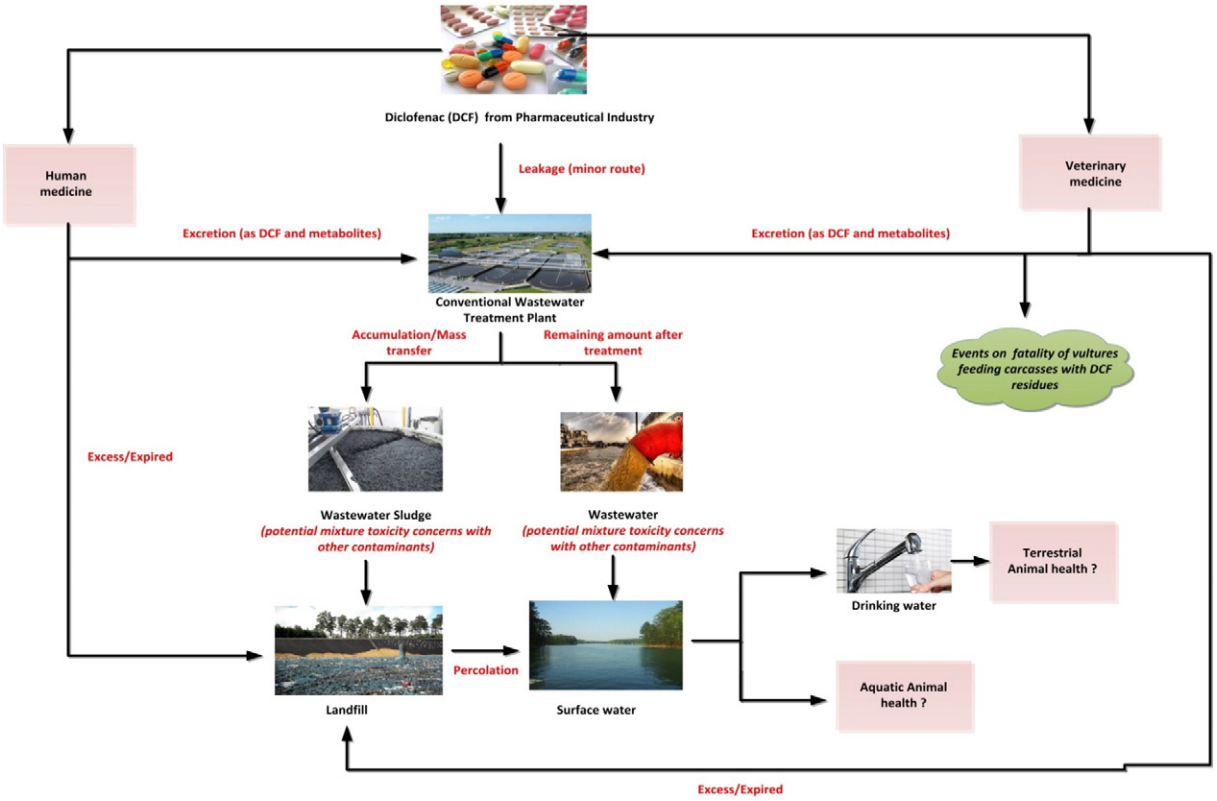


Fig. 1. Entry routes of diclofenac to the environment.

4.1. Removal processes

The conventional treatment system including wastewater treatment plants exhibited a moderate to higher degradation efficiency of DCF. DCF is moderately persistent in the environment. In the reviewed studies, the maximum removal obtained was about 93% by adsorption on activated carbon followed by ozonation (Beltran et al., 2009). Primary treatment was also efficient with coagulating and flocculating agents, such as $FeCl_3$, $Al_2(SO_4)_3$ but did not remove the drug completely from wastewater (Carballa et al., 2005). Conventional activated sludge processes showed better efficiency when compared to the MBR. On an average, 30–70% removal can be obtained by the existing removal methods (Vieno and Sillanpää, 2014; Zhang et al., 2008). An overview of the treatment methods is given in Table 2. The removal efficiency mostly depends upon the treatment methods. For instance, the conventional activated sludge process exhibited about 75% removal (Kimura et al., 2005; Lonappan et al., 2016), however mostly this removal was achieved through sorption to the sludge. This happened in conventional wastewater treatment plant where a certain percent of DCF removal occurred through the accumulation of the drug in wastewater sludge (Lonappan et al., 2016). Hence, the effective removal or degradation of the drug is minimal in conventional wastewater treatment system. Several other treatment options which are based on sorption process have been suggested by various researchers recently (Sotelo et al., 2014; Sotelo et al., 2012; Suriyanon et al., 2013). Even though various natural and synthetic adsorbent materials, such as activated carbon, biochar, silica based polymer based adsorbents exhibited excellent removal efficiency towards DCF, as a whole, these processes cannot be considered as sustainable methods; as they do not completely remove DCF from the environment. Instead, water/wastewater bound DCF is accumulated on the adsorbent. Another most widely used method for the treatment of DCF is based on advanced oxidation processes, such as ozonation (Beltran et al., 2009). However these methods also do have fallouts. These processes may create unwanted and toxic by-products. Nevertheless, few recent developments are promising and sustainable. Studies demonstrated the effective use of enzymes for the complete degradation of DCF from water (Marco-Urrea et al., 2010) which will create no harmful byproducts.

4.2. Presence in aquatic environment

The potential detrimental effect of DCF in the aquatic environment has been revealed from many studies (Cleuvers, 2003; Fent et al., 2006; Jones et al., 2002; Lee et al., 2011). However, all these studies were conducted at laboratory scale. In surface water bodies, DCF has been detected in ng L^{-1}whereas in wastewater, the concentration has been as high as micrograms per liter. The concentration decreased by natural processes, such as soil retention, biodegradation and photo-transformation and also by physico-chemical processes in wastewater treatment plants. Table 3 presents the recent occurrence of diclofenac in the aquatic environment in different countries over a time period of 15 years. In surface waters, DCF contamination occurred in rivers, estuaries and lakes (Buser et al., 1998; Kim et al., 2007; Metcalfe et al., 2003; Öllers et al., 2001). Also, there are a few reports of detection in groundwater and drinking water (Benotti et al., 2008; Rabiet et al., 2006). Most of the detection has been from EU countries, but this does not indicate that DCF was only present in European countries. In Asia, the available data was insufficient to predict the environmental concentrations as there was no systematic annual data. When compared to Europe, the consumption of DCF in North America was lower. However, there are a few reported cases of DCF detection in the environment (Metcalfe et al., 2003; Sosiak and Hebben, 2005).

In reviewed data, the highest concentration detected was in rivers and was in Pakistan as 4900 ng L^{-1} (Scheurell et al., 2009) and one of the potential reason could be due to the absence of advanced WWTPs in Asian countries. Meanwhile, recent reports from Canada showed that WWTP effluents contain very high concentration of DCF such as 16 μg L^{-1} (Lonappan et al., 2016). German water bodies have also

Table 2
Removal of diclofenac in wastewater treatment plants.

Major treatment method	Process conditions and other treatment methods	Removal %	Comments	Reference
Submerged MBR	Membrane flux 0.4 m^3/ml/d HRT 9 h MLSS conc-10,000 mg L^{-1}	40%	–	Kimura et al. (2005)
Conventional activated sludge	HRT 13 h MLSS conc-1700 mg L^{-1}	75%	–	Kimura et al. (2005)
Adsorption on activated carbon followed by ozonation	–	>93%	Activated carbon reduced toxicity	Beltran et al. (2009)
Conventional biological wastewater treatment	–	65%	Average result of study on 5 biological wastewater treatment plants in Spain	Gomez et al. (2007)
Primary treatment (coagulation and flotation)	Coagulation-$FeCl_3$,$Al_2(SO_4)_3$ PAX	40–70%	–	Carballa et al. (2005)
	Flotation-12 °C–25 °C	20–40%		
Conventional active sludge (CAS) treatment	Anoxic pre-denitrification and phosphate precipitation with ferric chloride as tertiary treatment	65%	About 15% in 65% of total removal was through adsorption on to sludge	Larsson et al. (2013)
Conventional biological wastewater treatment	Bio-filtration type wastewater treatment and consists of 30 bio-filters	75%	Some percent of removal was occurred through adsorption onto sludge	Lonappan et al. (2016)
Activated sludge process	–	25%	–	Martín et al. (2012)
MBR	SRT(d)-10–55 HRT(d)-0.5–4	<50%	–	Clara et al. (2005)
Conventional biological wastewater treatment	WWTP: 1 SRT-8 days HRT-9 h	75%	Study on 2 WWTPs. Accumulation on sludge was of minor	Samaras et al. (2013)
	WWTP: 2 SRT-18 days HRT-23 h	39%		
Conventional activated sludge with chemical phosphorous removal	HRT (h) 15–16	22%	–	Bendz et al. (2005)
Conventional activated sludge with UV treatment as tertiary treatment	–	81.4%	–	Behera et al. (2011)
Conventional activated sludge with maturation pond	WWTP: 1 SRT-10 days HRT-6 h			
Conventional activated sludge with chlorination	WWTP: 2 SRT-6 days HRT-6 h	≈70% in all the 3 WWTPs	Study of 3 WWTPs.	Anumol et al. (2016)
Aerated lagoon with filtration and with chlorine addition	WWTP: 2 SRT-3.1 days HRT-74 h			
Activated sludge	–	45.6%	Average result for 15 different WWTPs	Pereira et al. (2015)

Abbreviations - MBR: membrane bioreactor, HRT: hydraulic retention time, SRT: sludge retention time, MLSS: mixed liquor suspended solids, WWTP: wastewater treatment plant.

been heavily polluted with DCF and maximum concentration detected was 1030 ng L^{-1} in river water (Heberer, 2002a). In Spain, DCF residues were detected in river Delta (Lopez-Serna et al., 2013). DCF residues were detected in almost all European Union countries (Hernando et al., 2006; Loos et al., 2010). In US and Germany, DCF was even detected in drinking water which warrants attention (Benotti et al., 2008; Heberer, 2002b).

DCF has been detected mostly in fresh water bodies all over the world. Water containing DCF is discharged to surface water from WWTPs after treatment. The concentration ranges from few hundreds of ng L^{-1} to thousands of ng L^{-1}. In Asian countries, there are no strict measures to monitor the concentration of DCF in aquatic environment. From the few available studies in rivers, it is clear that DCF is discharged to surface water without proper treatment. Therefore, in this region, the load of DCF into the aquatic environment could be reduced by proper treatment of wastewater. Mostly, these wastewater treatment plants must be equipped with train of primary, secondary and tertiary treatment for an effective removal.

4.3. Presence in soil

There are a few reported cases of detection in DCF in soil. Diclofenac could potentially reach agricultural lands through the application of municipal sewage sludge as a source of nutrients in soil or through wastewater and it has been detected in the Canadian province of Ontario (Al-Rajab et al., 2010). Studies on the sorption coefficient of diclofenac proved that sorption even in sandy sediment was relevant and therefore diclofenac was less mobile in groundwater (Scheytt et al., 2005). On the other hand, studies from Israel reported that DCF showed slower mobility in organic rich agricultural soils and higher mobility in fresh water column and which caused its leaching to the ground water and ultimately to drinking water after rain events (Chefetz et al., 2008; Drillia et al., 2005). Few other studies also pointed to the same possibility (Chefetz et al., 2008; Xu et al., 2009).

The toxicity of DCF in soil towards plants and soil organisms/microorganisms has been poorly understood. More investigations must be carried out in this environmental compartment. The only information that is available is that DCF has been readily degraded in soil and was highly adsorbed by organic rich soil (Al-Rajab et al., 2010; Xu et al., 2009). DCF is less toxic towards leguminous plants when compared to other pharmaceuticals, such as sulfamethazine (Ziółkowska et al., 2014) and DCF does not show any harmful effect towards plant growth (Carter et al., 2014b). Also, DCF does not show any toxic effects (behavior, weight change, mortality) towards soil organisms, such as earthworms (Carter et al., 2014a). However, recent reports on soil application of wastewater sludge containing DCF suggested medium risks towards soil microbes (Verlicchi and Zambello, 2015).

Table 3
Recent occurrences of diclofenac in aquatic environment.

Environmental medium	Concentration (ng L^{-1})	Country	Reference
River	2–3	Finland	Lindqvist et al. (2005)
River	21–90	Canada	Sosiak and Hebben (2005)
River	18–50	Canada	Metcalfe et al. (2003)
Estuary	195	UK	Thomas and Hilton (2004)
River	6.2	Germany	Weigel et al. (2002)
River	1030	Germany	Heberer, (2002a)
Ground water/wells	2	Mediterranean region	Rabiet et al. (2006)
Wells	380	Germany	Heberer et al. (1998)
Drinking water tap	10	Germany	Heberer (2002b)
River	100–200	Germany	Letzel et al. (2009)
River	100–4900	Pakistan	Scheurell et al. (2009)
Lake	370	Switzerland	Buser et al. (1998)
River	5–40	UK	Kasprzyk-Hordern et al. (2008)
River	26–72	Spain, Belgium, Germany, Slovenia	Hernando et al. (2006)
River	20–91	UK	Hilton and Thomas (2003)
Rivers and lakes	1.1–6.8	South Korea	Kim et al. (2007)
River	0.7	France	Rabiet et al. (2006)
Well	0.9	France	Rabiet et al. (2006)
River	9–282	Slovenia	Kosjek et al. (2005)
River	20–150	Switzerland	Öllers et al. (2001)
Well	4.9–24	European Union (23 countries)	Loos et al. (2010)
Aquifer	1.7	Spain	Lopez-Serna et al. (2013)
Well	3.1	Spain	Lopez-Serna et al. (2013)
River delta	29.5–380	Spain	Lopez-Serna et al. (2013)
Drinking water	1.2	US	Benotti et al. (2008)
River	15.8–35.5	Austria	Ahrer et al. (2001)
River	7.8–64.8	China	Dai et al. (2015)
Harbor lagoon	100	Pakistan	Scheurell et al. (2009)
Well	590 (max. observed)	Germany	Sacher et al. (2001)
River	260 (max. observed)	Spain	López-Serna et al. (2012)
River	15	South Korea	Yoon et al. (2010)
River	49	Spain	Carmona et al. (2014)
Tap water	18	Spain	Carmona et al. (2014)
River	34–145	Argentina	Valdés et al. (2014)
Seawater (subtropical coastal zone)	19.4	Brazil	Pereira et al. (2016)
River	230 (max. observed)	China	Ma et al. (2016)

From the little available information on the fate and toxicity of DCF in soil, DCF exhibits lower toxicity and moderate persistence. However, in soils with large amount of organic matter, DCF gets adsorbed to the soil and exhibited resistivity towards aerobic/anaerobic degradation and may leach out to the groundwater causing accumulated toxic effects. Thus, future studies should focus on the fate of DCF in agricultural lands with further monitoring of fate through groundwater aquifers.

5. Toxicity

Earlier, most of the studies on the toxic effects of DCF were focused on its adverse effects on the aquatic animals. The toxic concerns regarding DCF in freshwater environment have been studied in the laboratories with the help of model organism for toxicological studies. The first widely noted case of pharmaceutical causing major ecological damage was the sudden collapse of vultures due to the consumption of carcasses containing residues of DCF. After these consecutive incidents in the first decade of 20th century, DCF has got much worldwide attention. The following section describes the toxic concerns over DCF in aquatic environment; particularly in freshwater environment and the events of DCF toxicity towards terrestrial animals.

5.1. Aquatic organisms

Many toxicity studies were conducted worldwide to evaluate the toxicity of DCF in aquatic organisms. One of the widely used and highly standardized methods for measuring toxicity was the acute immobilization tests. Ferrari et al. (2003) conducted one of the first studies on the toxic effects of DCF. This study was carried out on bacteria, algae, microcrustaceans and fish, and showed relatively less toxic effects even at environmental concentrations. On the contrary, later studies revealed the potential impacts of diclofenac on the environment. According to risk assessment studies, the potential ecological risk of diclofenac in surface waters was higher (Hernando et al., 2006). Cleuvers (2004) conducted ecotoxicity studies using acute *Daphnia* and algal tests and revealed that DCF was potentially harmful to aquatic organisms. In the same study, Cleuvers (2004) also reflected that under field or environmental concentrations, the adverse effects were either less or negligible and a mixture of pharmaceuticals can be considerably toxic even at lower concentrations. In crustacea (*Daphnia magna* sp.), at acute concentrations, such as mg L^{-1}, DCF induced high mortality rate. From different studies for 48 h exposure, the presence of DCF produced higher mortality and the EC_{50} values were reported to be 22.4 mg L^{-1} and 39.9 mg L^{-1} (Ferrari et al., 2003; Haap et al., 2008). Also, for *Ceriodaphnia dubia* sp., mortality was observed and the EC_{50} was 22.7 mg L^{-1} (Ferrari et al., 2003). On the contrary, Lee et al. (2011) reported 3 times higher values for 48 h EC_{50} tests for the same species. Surprisingly, studies from Canada reported that DCF was a major risk even at predicted environmental concentrations (10–100 ng L^{-1}) (Lawrence et al., 2007). These studies in river biofilm communities revealed the significant impacts of DCF on community structure and function at even lower concentrations of 100 ng L^{-1}.

DCF was known to exert deadly effects by damaging renal and gastrointestinal tissue in several vertebrates, such as fishes. In an exposure assessment study, Letzel et al. (2009) found that at environmentally relevant concentrations, such as nanograms per liter, DCF may cause chronic adverse effects on fish populations. In fish, Japanese medaka (*Oryzias latipes*), DCF adversely affected the growth in egg phase and resulted in significant reduction of hatchability and delay in hatching (Lee et al., 2011). In a study on zebra fish, Hallare et al. (2004) observed the same results. Hatching was delayed when the embryos were exposed to 2000 μg L^{-1}. In brown trout, DCF was not completely excreted through

first pass metabolism, but a significant part of the DCF entered enterohepatic circulation. The resulting prolonged availability of DCF in the organism possibly promoted accumulation of DCF (Hoeger et al., 2008). For the same species, a heavy damage of gill, liver and kidney was observed at 50 μg L^{-1} (Hoeger et al., 2005). For rainbow trout, even at environmentally observed concentrations, DCF interfered with the biochemical functions and lead to tissue damage (Mehinto et al., 2010; Schwaiger et al., 2004). DCF may accumulate in liver kidney, gills and muscle tissues of rainbow trout and can cause cytological alterations even at 1 μg L^{-1} (Schwaiger et al., 2004; Triebskorn et al., 2004).

Mussels got affected by DCF at concentrations that are prevalent in the environment. At nanograms per liter level concentrations, DCF significantly induced lipid peroxidation (LPO) in mussels indicating tissue damage (Schmidt et al., 2011). A relatively recent study (Gonzalez-Rey and Bebianno, 2014) proved that at 250 ng L^{-1}, which came very close to the concentrations in certain German rivers, DCF induced tissue specific biomarker responses leading to the tissue damage. DCF was also adversely affecting the metabolism and growth of the blue mussels which are common in Baltic Sea (Ericson et al., 2010).

Except for mussels, environmentally relevant concentrations appeared to be less toxic for aquatic animals. Most of the studies suggested that continuous exposure to DCF even at very low concentrations may lead to some adverse effects in aquatic animals. It was estimated that the no effect concentration of DCF was 0.1 mg L^{-1} (Lee et al., 2011), which is very high when compared to those observed in aquatic systems/real environmental conditions (Table 3). Moreover, DCF and its metabolites were observed in fish bile (Kallio et al., 2010). Several photo-transformation products can be more toxic than DCF at the concentration that may come close to the environmental concentrations (Schmitt-Jansen et al., 2007). However, the toxicity of metabolites of DCF was poorly understood in the environment. The future studies may include the toxicity studies of photo-transformation products of DCF and mixture toxicity studies. Also, the future studies should concentrate on the bioaccumulation of DCF in the food web and chronic exposure studies at lower but environmentally relevant concentrations since the DCF residue is continuously introduced and dynamically increasing in the environment.

5.2. Terrestrial organisms

The first widely noted case of pharmaceutical causing major ecological damage was the sudden collapse of vultures due to the consumption of carcasses containing residues of DCF, and this threatened several vulture species to extinction (Oaks et al., 2004; Taggart et al., 2007b). DCF was the major cause of collapse of population of three *Gyps* vulture species (*Gyps bengalensis, Gyps indicus, Gyps tenuirostris*) which were severely affected, reduced by 98% in the Indian sub-continent and was included in "critically endangered" species list of IUCN (Das et al., 2010).

In 2003, studies reported the catastrophic collapse of Indian white-backed *Gyps bengalensis* and long-billed *Gyps indicus* vulture populations due to some unknown reason or epidemics (Ferrari et al., 2004; Prakash et al., 2003). However, the exact reason for this sudden collapse was discovered by Oaks et al. (2004), and it was found to be the renal portal vasoconstriction caused by DCF. On the contrary, another study reported the cause of death as decreased uric acid excretion (Naidoo and Swan, 2009). Most of the investigations led to the probability of renal failure due to the consumption of DCF (Sharma et al., 2014; Swan et al., 2006; Taggart et al., 2007a). The major food source of vultures was the livestock from cows and goats. These animals were treated with DCF and although DCF was short lived in these animals, the prevalence in carcasses available to vultures may still be very high (Taggart et al., 2007a) and *Gyps* vultures were extremely susceptible to very lower doses of DCF (Swan et al., 2006). These events even affected the ecosystem. DCF did not only affect the population of vultures but also the community structure of the ecosystem. Vultures are keystone species and their decline has a range of socio-economic as well as culture and biodiversity impacts. As an example, the rabies causing dogs and vultures were having same food source. The decrease in the number of vultures increased the availability of food for dogs by reducing competition over food (Markandya et al., 2008). Hence, the decline in vultures had a biological and social effect in the specific region of concern.

The veterinary application of DCF is also threatening the vulture species in Africa (Naidoo et al., 2009; Virani et al., 2011). There are reported cases of major decline in the abundance of vultures and other scavenging raptors from Kenya and the reason was suspected to be DCF (Virani et al., 2011). Moreover, relatively recently, DCF has been reported to be fatal for eagles which widens the diversity of raptors threatened by DCF (Sharma et al., 2014).

Recent studies reported that the ban of DCF for veterinary use in south Asian countries was an effective measure and the vulture population is on rise now (Khadka and Mandal, 2013; Prakash et al., 2012). Hence, legislative measures succeeded for DCF in South Asia through its ban for veterinary use. Nevertheless, there are DCF residues already present in the environment, especially those that pass through the soil into groundwater which need to be investigated to avoid any future chronic toxicity effects on organisms. However, there are other terrestrial animals which feed on the carcasses of cattle and which need to be studied as they are the intermediate transporters of DCF during its veterinary use.

6. Metabolites

In animals, after consumption, DCF is mostly degraded into hydroxyl derivatives. DCF is easily degraded to its transformation products in the environment. The major natural process of degradation is photo-transformation by sunlight. DCF is one of the best investigated pharmaceutical residues in the environment (Vieno and Sillanpää, 2014; Zhang et al., 2008). However, studies on the occurrence and toxicity of its metabolites in the environment are not well understood.

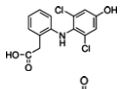
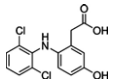
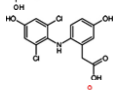
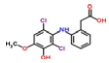
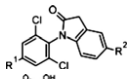
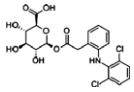
6.1. Via consumption routes

In human body, DCF derivative is found in urine and plasma. The hydroxylated and methoxylated derivatives of DCF are present in their free forms well as glucuronide-conjugated forms. In an earlier study, Stierlin et al. identified the metabolites of DCF in human body and the main metabolite was identified to be 4′-hydroxydiclofenac (2-[2,6-dichloro-4 hydroxyphenylamino] phenylethanoic acid) (30%) and other major metabolites are 5′-hydroxydiclofenac (2-[2,6-dichlorophenylamino]-5-hydroxyphenylethanoic acid) (10%), 3′-hydroxydiclofenac (2-[2,6-dichloro-3-hydroxyphenylamino] phenylethanoic acid) and 4′,5-dihydroxydiclofenac (2-[2,6-dichloro-4-hydroxyphenylamino]-5-hydroxyphenylethanoic acid) (15%) (Boettcher et al., 1991; Stierlin et al., 1979). Recently, some minor metabolites were also identified in human body (Stulten et al., 2008a). Along with hydroxyl derivatives of DCF, acyl glucuronide and hydroxyl acyl glucuronide were also found in mouse and fish (Kallio et al., 2010; Naisbitt et al., 2007). The identified human metabolites of DCF are depicted in Table 4. The wide occurrence of human metabolites of diclofenac in water and its structural similarity towards diclofenac is a matter of concern on toxicity grounds and needs to be investigated further.

6.2. Via abiotic degradation route

DCF was readily degraded in the sunlight. The half-life of DCF is estimated to be 3.3 h (Schmitt-Jansen et al., 2007). DCF followed first order kinetics during photodegradation and was detected in water cycle (Qin and Yang, 2012). Photodegradation was identified as the main removal process for the degradation of DCF in lakes and it was estimated that about 90% of DCF was eliminated by this process (Buser et al., 1998). Many phototransformation products of DCF were identified by various

Table 4
Human metabolites of diclofenac.

Metabolite	Molecular structure	Reference
4′-Hydroxydiclofenac		Boettcher et al. (1991), Stierlin et al. (1979), Stulten et al. (2008a))
5′-Hydroxydiclofenac		Boettcher et al. (1991), Stierlin et al. (1979), Stulten et al. (2008a)
3′-Hydroxydiclofenac		Boettcher et al. (1991), Stierlin et al. (1979)
4′,5-Dihydroxydiclofenac		Boettcher et al. (1991), Stierlin et al. (1979)
3′-Hydroxy-4′-methoxy diclofenac		Boettcher et al. (1991)
4′-Hydroxy diclofenac dehydrate		Stulten et al. (2008a)
1-*O*-acyl glucuronide (DCF-gluc)		Willis et al. (1979)

researchers (Agüera et al., 2005; Moore et al., 1990; Qin and Yang, 2012). Most significant process of DCF phototransformation was identified as the photocyclisation to the corresponding monohalogenated carbazole (Eriksson et al., 2010). Most of the photochemical decomposition products occurred as two main sub-structures: 2-chloro- and 2,6-dichlorodiphenylamine derivatives and also 8-hydroxy- and 8-chlorocarbazole derivatives (Agüera et al., 2005; Moore et al., 1990).

Not only diclofenac, but also its metabolites are globally entering the aqueous environment. There are reports that some of the DCF metabolites are even more toxic than DCF. Some of the phototransformation products exhibited a six fold increase in toxicity in algal reproduction tests (Schmitt-Jansen et al., 2007; Schulze et al., 2010). In view of the toxic effects of diclofenac on several water organisms, it appeared that the metabolites also initiated objectionable reactions in other organisms and needed strict surveillance during drug toxicological and environmental monitoring experiments (Stulten et al., 2008b). Therefore, future studies need to investigate the potential toxicity of DCF metabolites. However, the analysis of DCF transformation products is often laden with challenges. For example, unavailability of metabolite standards obstructs the exact quantification of DCF transformation products. Another analytical challenge is the low level (often nanograms per liter) occurrence of the drug and its transformation products. Hence, MS/MS instruments with high sensitivity and appropriate methods are needed to quantify this "micro-pollutant".

7. Interactions with other pollutants-proposed approach

For diclofenac, WWTPs being one of the major sink; interaction with other contaminants is a possibility that has to be studied. A hypothetical representation of a typical secondary treatment system in wastewater treatment plant depicting interaction of diclofenac with other pollutants is shown in Fig. 2. Municipal wastewater treatment plants are complex systems that receive contaminants from a variety of sources. These include contaminants, such as suspended solids, biodegradable organics such as proteins, carbohydrates and fats (particularly in hospital wastewater), nutrients, such as nitrogen, phosphorus and carbon - (from various origin), refractory organics, such as pesticides, phenols, surfactants, heavy metals, dissolved organics, and pathogens of various category (bacteria, viruses, protozoa, etc.). In addition, they also receive various pharmaceuticals and personal care products (PPCPs) termed as emerging contaminants. Often emerging contaminants are detected in minute concentrations, such as ng L^{-1} or μg L^{-1}. These lower concentrations are even undetectable without specific sensitive methods. Moreover, for pharmaceuticals, corresponding metabolites are formed during in vivo human/veterinary metabolism and they could become a new category of potential emerging contaminants.

Micro-pollutants, such as pharmaceuticals are present in 'micro' concentrations. However, even at this micro concentration, they can cause adverse impacts in the environment. In addition, these contaminants can interact/combine/aggregate with other pollutants of the same or different class. For example, for pharmaceuticals, including DCF, it is proven that in a mixture of pharmaceuticals, considerable combination effects could also occur if some or even all substances were applied in concentrations below their NOEC (no observed effect concentration) (Cleuvers, 2004; Cleuvers, 2008).

As described in Section 5, many studies already evaluated the toxic concerns regarding DCF in various environmental compartments. However, very few studies have examined the mixture toxicity concerns regarding DCF along with other pharmaceuticals. A recent study suggested antagonistic interactions between nutrients and emerging contaminants including DCF in stream biofilms (Aristi et al., 2016); however long-term real exposure experiments are required to bring out actual mixture toxicity effects. Hence, to interpose realism into future risk assessment studies, mixture toxicity studies must focus on: (i) whether ecotoxicity of a pharmaceutical mixture is higher than that of the toxicity of each individual pharmaceutical; and (ii) if acting singly at lower concentrations what will be the toxicity of the individual drug, DCF (Backhaus, 2014). These studies will thus need efforts from environmental exposure assessment studies for toxicity evaluation. Furthermore, since the number of drugs and its varieties are on an increase along with hundreds of existing pharmaceuticals, the need for creating database for the mixture toxicity concerns of DCF along with other pharmaceuticals is vital. These interactions and mixture toxicity studies must consider toxicokinetic and toxicodynamic interactions in test organisms along with ecological interactions for real scale studies (Backhaus, 2014).

7.1. Proposed interactions of DCF with metals, other inorganics and organics

Conventionally, diclofenac treatment occurs in the wastewater treatment plant. WWTP receive wastewater from various sources, such as industries, hospitals, households and municipal wastewater, among others. Because of the differences in origin, the contaminants are also diverse. Metals and heavy metals, in particular is a major class of contaminants that are usually present (Barakat, 2011; da Silva Oliveira et al., 2007; Karvelas et al., 2003). Various physico-chemical conditions in a conventional wastewater treatment plant can catalyze this process. Previous studies already proved that DCF along with certain metals possess higher anti-inflammatory activity and antioxidant property than DCF alone (Kovala-Demertzi, 2000). Moreover, DCF has active groups such as amino, hydroxyl, carbonyl, and carboxyl groups in its structure. These groups can enhance the metal complexation/binding properties with most of the metals. Hence, organometallic complexation of drugs via chelation is a possibility that can happen in WWTP. DCF can act as ligand coordinated to the metal ions via the deprotonated functional groups present on it. Moreover, DCF complexes with Hg(II), Pb(II), and Sn(II) are already known to be antibacterial agents (Refat et al., 2014) while Cu(II) complex of DCF can cleave DNA (Theodorou et al., 1999). Hence, on reaction with metal complexes, the properties of DCF can change completely and become another potential pollutant possessing antibacterial and cell destruction capacity. Until date, most of the metal complexation studies of DCF commonly have been used to uncover its novel therapeutic values and underlying mechanisms. However, to the best of our knowledge, none of the studies focused on DCF metal complexes and its toxicity concerns in wastewater effluent. Hence, while considering DCF as an emerging

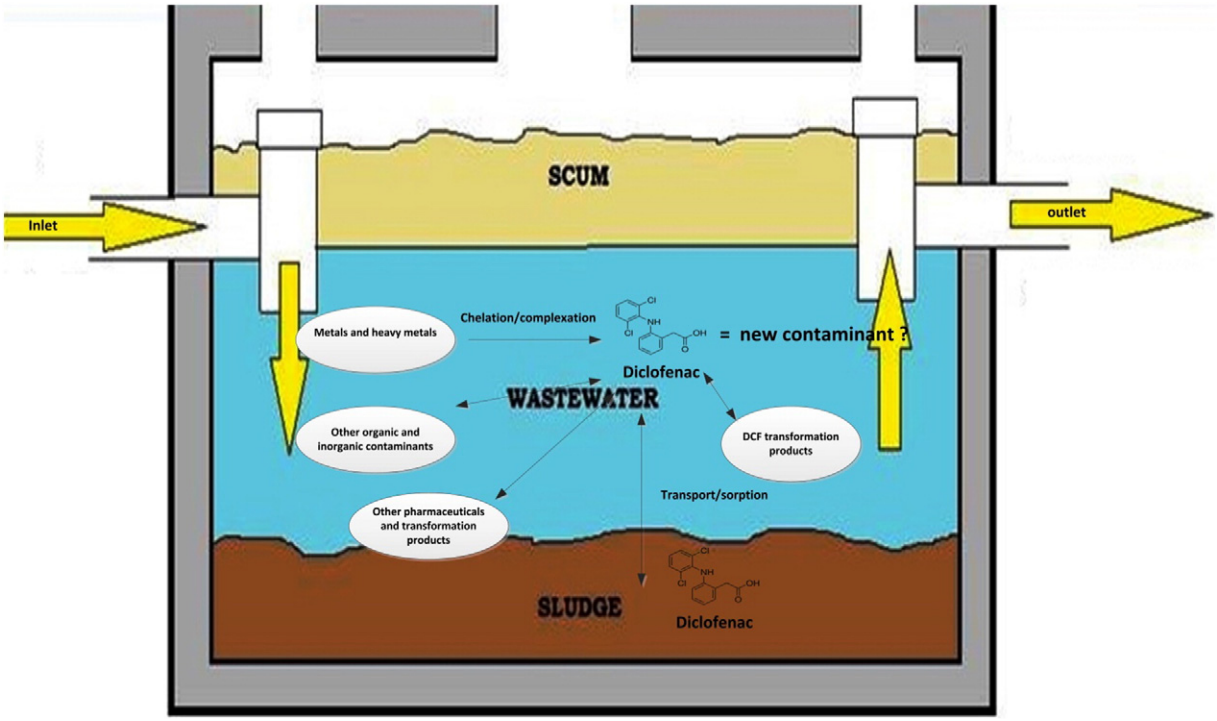


Fig. 2. Hypothetical representation of a typical secondary treatment system in wastewater treatment plant showing interaction of diclofenac with other pollutants.

contaminant having potential toxic concerns towards several organisms, the metal complexes of DCF must be considered as an emerging contaminant and must be treated with care while considering its antibacterial properties. The metal complexes of DCF add another chemical complexity raising toxicity concerns for several organisms.

Moreover, because of the structural properties, DCF can act as a ligand for other inorganic elements/groups. This property of DCF, particularly in WWTP is crucial from toxicological point of view. Unfortunately, none of the published studies investigated these aspects on DCF toxicity. Theoretically, the interactions with other inorganic pollutants, such as sulfates, nitrates chlorides are a certain possibility for DCF along with possible complexation/aggregation with other numerous organic pollutants in wastewater matrix.

7.2. Proposed interactions of DCF with other ECs and DCF metabolites

Although mixture toxicity concerns with other pharmaceuticals are already established and studied, the toxicity concerns over DCF metabolites are still valid. The major metabolites of DCF are hydroxy-metabolites (3′-hydroxydiclofenac, 4′-hydroxydiclofenac, 5′-hydroxydiclofenac). The mixture toxicity effects of these metabolites are still unknown. There is a possibility for the mixture toxic effect of DCF and these metabolites similar to one reported with other pharmaceuticals. In addition, various conditions existing in WWTP can catalyze the effective combination of these metabolites and DCF, and it may leads to the creation of another potential contaminant. In addition, about 65% percent of DCF is present as DCF metabolites (Boettcher et al., 1991) and this increases the availability for all metabolites and DCF.

The reported metabolites of DCF have –OH groups at various positions in the DCF structure. Hence, the basic molecular structure of DCF largely remains unaltered. The presence of hydroxyl groups on the structure can enhance the interactions with metals through π-π interactions. Yamada et al. (1990) applied this principle to DCF metabolites which can easily form metal complexes since the DCF metabolites are the hydroxyl derivatives of DCF. Moreover, due to the presence of active groups, such as amino, hydroxyl, carbonyl, and carboxyl groups in metabolites of DCF, there is a possibility of interaction between these molecules. Hence, these possibilities point towards creation of "new emerging contaminant" of unknown properties. Likewise, for DCF, it is possible to have interactions with other ECs. Several other ECs, such as pesticides, surfactants, PPCPs are present in wastewater (Petrović et al., 2003). Often these ECs are compounds having several active groups in their structure. Thus, it is possible to have multiple interactions with one or many other ECs and DCF along with its metabolites. These interactions could be with other ECs and/or its metabolites/transformation products during treatment process. Recently, the synergistic effect of DCF and its nitrogen transformation products were studied along with sulfamethoxazole and its transformation products (Osorio et al., 2016) suggesting that contribution of these compounds to overall toxicity of complex environmental samples, should not be dismissed.

Hence, experimental evidence is necessary for these interactions to perform toxicity studies and drafting adequate risk assessment for DCF. However, the existing ecotoxicological and environmental exposure data is not sufficient and realistic while considering WWTP as the major sink for DCF. Future studies, particularly toxicity focus on these mixtures of ECs, metabolites, metals and DCF that are either proven or likely to form new products. To further increase the realism of ecotoxicological studies, investigation based on these approaches is necessary with overwhelming number of emerging contaminants being added each day.

8. Conclusions

Diclofenac is one of the major PhACs which has a far flung usage throughout the world. The residues of diclofenac are found worldwide in surface, ground and drinking water. Even though diclofenac is removed by natural processes, such as photodegradation, the residue still remains in the environment as potential toxic metabolites and as diclofenac. Diclofenac in the environment is detected in lower concentrations, such as nanograms per liter to micro grams per liter and from the available ecotoxicology data, it is apparent that these lower concentrations can cause acute toxic effects to many organisms, such as mussels. At lower measured concentrations, there are fewer chances of acute toxicity. However, extended exposure to lower concentrations

may lead to chronic toxicological effects In the case of diclofenac, continuous entry into the environment due to the year-round use of medication is increasing the diclofenac residue in the environment. The fate of diclofenac in soil is poorly understood. In soils, with large amount of organic matter, diclofenac gets adsorbed to the soil and shows resistivity towards aerobic/anaerobic degradation and may leach out to the groundwater causing toxic effects. The toxicity of diclofenac metabolites is not well investigated and some studies suspected that few metabolites can be potentially more toxic than the parent compound. There still remains a lacuna for investigating the environmental impact of metabolites and also the toxicological effects if any, on the flora and fauna. Diclofenac can interact with other inorganic contaminants, such as metals, organic contaminants and even with diclofenac metabolites as they all are present in a complex wastewater matrix in wastewater treatment plant. This process may lead to the formation of another possible emerging contaminant. Further studies are required to better assess the fate and toxicological effects of diclofenac and its metabolites and must consider the possible interaction of diclofenac with other contaminants to develop an effective treatment method for diclofenac and transformation products. Moreover along with DCF, DCF metabolites must be considered as another emerging contaminant and treatment methods must emphasize metabolites as well. The tertiary treatment system must be equipped with more advanced treatment methods, such as advanced oxidation and environmental friendly enzymatic treatment methods which are known to be effective for several contaminants including DCF.

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