Pamukkale Univ Muh Bilim Derg, 28(6), 937-952, 2022

Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi

Pamukkale University Journal of Engineering Sciences

A review on diclofenac degradation, transformation products and their fate in the environment

Diklofenak'ın bozunması, dönüşüm ürünleri ve çevresel akıbeti üzerine inceleme

Serenay Ceren TÜZÜN[1], *Ilgi KARAPINAR*[1*]

[1]Department of Environmental Engineering, Engineering of Faculty, Dokuz Eylul University, İzmir, Turkey.
serenay.tuzun@deu.edu.tr, ilgi.karapinar@deu.edu.tr

Received/Geliş Tarihi: 15.11.2021 Revision/Düzeltme Tarihi: 02.04.2022 doi: 10.5505/pajes.2022.71363
Accepted/Kabul Tarihi: 18.05.2022 Review Article/Derleme Makalesi

Abstract

Diklofenak, insan ve veterinerlik amaçlı yaygın kullanılan ilaçlardan biri olmuştur. Diklofenak'ın çevre ve organizmalar üzerindeki olumsuz etkilerinin ortaya çıkmasıyla, araştırma çalışmaları atıksudan uzaklaştırılmasına yönelmiştir. Atıksu ya da yüzeysel suda mikrogram düzeyindeki derişimlerine rağmen, endokrin sistemini bozabilir, bu da üst düzey organizmaların metabolizmasında dolayısıyla ekosistemde sorunlara yol açabilir. Bu nedenle diklofenakın tüketiminden başlayarak etkin diklofenak arıtımına, deşarj standartlarının belirlenmesine ve su yaşamının üst düzeyde korunmasına kadar uzanan bir yelpazede diklofenak kontrolü için acil önlemler alınmalıdır. Diklofenak ile ilgili diğer bir problem ise, fotoliz ve biyolojik reaksiyonları ile oluşan bozunma ürünlerinin atıksu arıtma tesislerinin çıkışında bulunmasıdır. Bu dönüşüm ürünleri organizmalar için daha fazla tehdit oluşturmaktadır. Dolayısıyla, bu derleme makalesi diklofenakın çevre üzerindeki olumsuz etkilerini, birincil diklofenak kaynaklarını, hayvanlar üzerindeki endokrin bozucu etkilerini, insan vücudundaki metabolizmasını, arıtma yaklaşımlarını, biyolojik ve kimyasal bozunma metabolitlerinin türünü veya dönüşüm ürünlerini vurgulamayı amaçlamaktadır.

Anahtar kelimeler: Diklofenak, Endokrin bozucu kimyasal, Farmasötikler, Dönüşüm ürünleri, Arıtma.

Öz

Diclofenac has been one of the widely consumed pharmaceuticals for human and veterinary purposes. The research studies are devoted to investigating its removal from wastewater since its adverse effect on the environment and the organisms have been revealed. Despite its microgram level concentrations in the wastewater or water bodies, it could disrupt the endocrine system, which leads to problems in the metabolism of the higher-level organisms and, thereby, the ecosystem. Therefore, urgent measures should be taken to control diclofenac starting from its consumption and extending to the efficient diclofenac treatment, determination of discharge standards, or the high-level protection of aquatic life. Another problem with diclofenac is the degradation end-products that form through photolysis, or biological reactions remain in the wastewater treatment plants' effluent. These transformation products create further threats for the organisms. This review paper aims to emphasize the adverse effects of diclofenac on the environment, the primary diclofenac sources, its endocrine disrupting effects on the animals, metabolism in the human body, treatment approaches, type of biological and chemical degradation metabolites, or transformation products.

Keywords: Diclofenac, Endocrine disrupting chemicals, Pharmaceuticals, Transformation products, Treatment.

1 Introduction

Water pollutants have been known as heavy metals, nitrogen, phosphorus, or organic ones named chemical oxygen demand (COD) and biological oxygen demand (BOD). The wastewater treatment plants were designed and constructed for the removal of these well-known conventional pollutants. However, the need to protect the environment and organisms from pollutants has changed recently. The term "pollutant" is now knowns as "conventional pollutants" and "specific pollutants." The advanced scientific background knowledge and engineering experiences are available about conventional pollutant control, though economical and more efficient treatment approaches are still under development. The discharge standards are well established for them, and existing wastewater treatment plants have been designed and constructed accordingly. The specific pollutants, which are recognized as micropollutants, have become the primary concern in the environment due to their unknown effects on organisms, including humans. Their carcinogenic effect was presumable, but surprisingly, they realized that they have the endocrine-disrupting effect, which has received worldwide research attention. Industrial chemicals, pesticides, pharmaceuticals, and personal-care products are now classified as endocrine-disrupting compounds.

Pharmaceuticals include antibiotics, anti-inflammatory drugs, pain killers, etc. Diclofenac (DCF) is a non-steroidal anti-inflammatory drug (NSAID) that is used widely throughout the world, with the estimation of 1443 tons consumption globally in 2015 [1]. The risk of DCF is not only the amount consumed by the human, but it is also a pharmaceutical used for veterinary purposes. Human consumption residues can be somehow managed or controlled before they reach the natural water bodies through treatment processes. However, it is hard to contain veterinary residues, which might be the most significant risk for the deposition of DCF in soil and natural waters, especially in groundwater. A study conducted by de Voogt et al. [2] about the development of a common priority list of pharmaceuticals relevant for the water cycle indicated that DCF is within Class I with ten high priority pharmaceuticals based on its resistance to treatment, high occurrence in the water, high consumption, physical properties, toxicity and

*Corresponding author/Yazışılan Yazar

persistence. It means that it has got a high potential to reach surface waters through wastewater treatment plants (WWTPs) discharges in the form of the parent compound and its transformation products (TPs) if a proper treatment technology or strict discharges standards are not applied in the countries. Therefore, the possible adverse effects of DCF and its TPs on aquatic organisms have been a significant concern in environmental research subjects. Numerous scientific studies regarding its toxicity and health effects on aquatic animals were conducted [3]-[6]. Through extensive research, it was discovered that DCF has endocrine-disrupting properties even at an environmentally relevant concentration around µg/L and even at ng/L [7],[8]. The toxicity studies encouraged the community to do more occurrence and fate studies of DCF to investigate the concentration levels in WWTPs [9]-[11] and surface water [12]-[14]. DCF removal studies in WWTPs revealed a wide range of efficiencies between less than 50% to higher than 90% [15],[16].

The DCF metabolites in wastewaters or WWTPs effluents is another problem affecting WWTP design and water quality. DCF is metabolized in the human body, and then different types of metabolites at varying concentrations are released through urine and bile [17],[18]. Therefore, the primary need is to understand the fate of DCF and its TPs in the environment after chemical oxidation, photolysis, aerobic, anaerobic, or anoxic treatment approaches. The primary degradation mechanism could be natural sunlight or photolysis due to the photosensitive nature of DCF [19],[20]. The end products regarding photolysis degradation were carbazole derivatives, which were determined as a possible reason for phototoxicity of DCF TPs [19],[21]-[23]. Aerobic bacterial degradation of DCF is limited, but high removal can be achieved through sludge adaptation [24]. The degradation can be enhanced by using special bacterial stains [25],[26] and white-rot fungi [27],[28]. But, partial degradation and hydroxylated end-product formation are observed [25],[26],[29].

It is evident that DCF received considerable research interests. These studies' results lightened the further studies that helped eliminate DCF from the environment to provide a sustainable world. This review paper was designed to do a detailed analysis of DCF, including its chemistry, which affects its removal, global consumption, its toxicity or adverse effect on the organisms, the main DCF contamination routes, the observed DCF concentrations in surface water and wastewater, the applied treatment technologies and their effectiveness on the DCF removal. The review mainly concentrates on the TPs generated after applied treatment technologies to emphasize their potential risks and devote the research interest to complete removal of DCF from wastewater without leaving its dangerous end products.

2 Physical and chemical properties of diclofenac

The physical and chemical properties of a pollutant determine its fate and transport in the environment. These properties specify the persistency, biodegradability, treatability, toxicity, and bioaccumulation in the organisms. The physical and chemical properties of DCF is given in Table 1 and its chemical structure is shown in Figure 1. The IUPAC name of DCF is 2-[2-(2,6-dichloroanilino)phenyl]acetic acid.

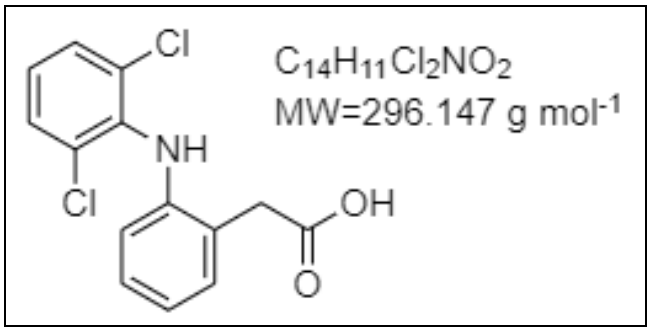


Figure 1. Chemical Structure of DCF (2-[2-(2,6-dichloroanilino)phenyl]acetic acid).

DCF's low air-water distribution coefficient (log Daw=-12.21) and low octanol-water distribution coefficient (log Dow=1.79) ensure that it stays in the dissolved phase. The pKa of 4.15 value of DCF also indicates that it will present as its dissociated form. Moreover, volatilization and sorption to solid-phase are considered insignificant processes [30]. Buser et al. showed that light enhances degradation of DCF, chemical and biological degradation in the dark is negligible [31], and no adsorption on sediment due to its low sorption ability [31].

Table 1. Chemical and physical properties of DCF.

Properties		Reference
Water Solubility	2.37 mg/L	[32]
Vapor pressure	6.14 x 10^{-8} mm Hg (25 0C)	www.pubchem.ncbi.nlm.nih.gov
Henry's Law Constant	4.79 x 10^{-7} Pa m^3/mol (25 ℃)	[33]
Melting point	283-285 ℃at 760 mm Hg (predicted)	[32]
Boiling point	412 ℃at 760 mm Hg	[32]
pKa	4.15	[34]
logKow	4.51	[34]
logKoc	2.20-3.42	[35]
Half-life in the body	~2 h	[33],[36]
Half-life in the atmosphere	0.8 h	[37]
Half-life in water (photolysis)	8 d-21 d	[30],[38],[39]
Half-life in soil	<5 d	[40]

The octanol-water partition coefficient value of DCF is logPow of 0.7, which means high water solubility. DCF can accumulate in the organs such as the liver, kidney, gills. Bioconcentration factors have been determined inversely proportional to the exposure concentration, which indicates a complete saturation of tissues by DCF in the highest concentration group [6]. Histopathological results revealed significant concentrations in the gills and kidneys responsible for the excretion of DCF. However, the highest concentration was found to be in the liver, where the cytochrome P450-dependent metabolism of DCF occurs. The exposure of 1 µg/L DCF caused an activated hepatic metabolism in the rainbow trout's liver [6].

The half-life of DCF in the body is around 2 hours [33],[36]. On the other hand, the half-life in the water phase when exposed to sunlight is about 8 days [38]. A longer half-life up to 23 days in the field experiment in springtime, has been reported [39]. The temperature and irradiation variations depending on the season, regional climate, and latitude are the main reasons for

the wide range of half-life through photolysis [30],[39]. Controlling the temperature decreased half-life to the range of 0.2-1.7 h [41]. The half-life of DCF in the soil is less than five days, as shown in Table 1. Xu et al. [42] reported that DCF has high mobility in the soil, in turn relatively low adsorption affinity. The microbial community in the earth is the significant parameter that determines the half-life of DCF. Although most of the studies concluded that the primary DCF degradation mechanism is photolysis in the natural waters, Radke et al. [43] claimed that photolysis is not important for even easily photodegradable compounds like DCF in real conditions, which indicates that DCF needs an efficient treatment before it is discharged into the water body.

3 Global diclofenac consumption

The consumption can vary depending on the economics, medical and cultural differences of the countries. It is not possible to find accurate data about its consumption in all countries. Therefore, Zhang et al. [33] proposed a simple calculation method based on the average dose per capita of countries where this data is avaliable. Then they constituted the Equation (1) and

$$\frac{Average\ DPC\ of\ developed\ countries\ \times (world\ population\ \times 0.2)}{0.8} \quad (1)$$

estimated the annual global consumption for the year 2007. It was reported that the possible global DCF utilization all over the world is around 940 tons. The estimated consumption was parallel with the available data from Intercontinental Marketing Statistics (IMS) Health company. Altman et al. [36] were able to find data regarding DCF consumption in the USA. It was stated that the prescription of DCF exceeded 10 million in 2012. Among those prescriptions, DCF sodium as topical gel accounted for 3% of the total annual prescriptions for NSAIDs. Lonappan et al. [32] compiled the information in the review article, and it was concluded that the estimated numbers could be higher due to unavailable data about veterinary consumption. According to DCF consumption records in 2001 in the European Union, Germany is the biggest consumer with 86 tons/ year [44]. The annual consumption in England and France were 26 tons and 16 tons, respectively [45],[46]. Another estimation done in 2015 showed that the consumption increased to 1443 tons globally [32] since the amount reported by Zhang et al. in the year 2007 [33].

In conclusion, there is limited information in the literature regarding the consumption of DCF for individual countries or global consumption. Moreover, the data that can be found in the literature do not include veterinary usage. Nevertheless, the DCF utilization is increasing day by day, which will end up in the natural waters. The cautions should be taken to protect the water resources against DCF contamination.

4 Diclofenac contamination routes

The main routes of DCF contamination in the environment are through wastewater discharges and solid wastes. The drug disposal could be the most dangerous DCF contamination route in the surface, groundwater, and soil. Percolation from landfills to groundwater and surface water poses a significant risk [32],[47]. It was shown that among 400 households in the UK, 63.2% of people threw away their unfinished drugs as waste, and 11.5% threw them away through a sink or toilet [47]. The pharmaceutical industry and hospital effluents are the other possible entry routes for DCF to find its way into surface waters [48]. The biosolids from wastewater treatment plants could be used as a soil conditioner, which creates another risk for surface water and groundwater contamination [49]. The source from veterinary or DCF treated animals could be a significant concern for water and soil contamination. Finally, the dermal application is another source of DCF in the aquatic environment. It has a low absorption rate to the skin, around 6% of applied DCF, and the rest (94%) is washed away to the sewage system. Therefore, the dermal treatment gels end up in the form of the parent compound in the domestic effluents rather than its metabolites [18],[50].

Table 2 depicts some of the reported DCF concentrations in the influents and effluents with removal efficiencies of the WWTPs around the world. The large amount of DCF in domestic wastewaters is encountered due to the heavy consumption of DCF by the human in some regions. The influent concentrations for Europe, Asia, and America do not differ from each other. The reported DCF concentration is less than the minimum quantification limit (MQL) and could reach up to 5300 ng/L. An exceptional concentration between 6200-53000 ng/L was encountered in South Africa. The removal efficiency of DCF is usually lower than 50%, but it could be as high as 97% as it was reported in Sweden [16].

Table 2. The observed DCF removal efficiencies, influent and effluent of concentrations in different countries.

Location	WWTP Influent (ng/L)	WWTP Effluent (ng/L)	Removal Efficiency (%)	Ref.
USA	140-280	<10	51-80	[51]
Canada	50-2450	70-250	-	[52]
Mexico	2325-2470	1865-2180	<46	[53]
China	13-445	2-72	<0->90	[54]
	14.8-71.8	17.7-69.2	<0-25	[55]
South Korea	59-243	13-49	81.4	[56]
India	3100-5300	1100-2300	56-64	[57]
Singapore	318-390	271-394	<0-28.5	[58]
South Africa	22300	19000	14	[12]
	6200-53000	2600-15000	4-88	[9]
Spain	414-1080	189-1150	<0-54	[59]
	400-1500	ND-900	<24-60	[15]
	1660	430	74	[11]
	400-1500	-	-	[11]
Greece	ND-4869	ND-2668	45	[11]
	77.8-5164	ND-382.5	<0-65	[60]
Turkey	295-1376	119-1012	12-65	[61]
United Kingdom	26-1161	6-496	<0	[62]
Austria	905-4114	780-1680	0-70	[63]
Poland	2251-4477	1597-5630	<0-40	[64]
Sweden	1550-2250	40-36	~97	[16]
Finland	250-750	1000-2250	<0	[65]
	250-500	200-400	9-60	[66]

Similar to DCF concentration in wastewater, the observed concentrations in the countries' surface waters vary substantially (Table 3).

Table 3. The observed DCF concentrations in surface waters around the world.

Location	Concentration (ng/L)	Type of surface water	Ref.
Mexico	988-1398	River	[53]
France	35	Lake	[67]
Brazil	136-2625.7	Paraopeba River	[14]
	19000-193000	River	[68]
South Africa	600-8170	River	[12]
Finland	20-475	Lake	[65]
Greece and Turkey	<LOQ (2.0)-9.7	Aegean Sea	[69]
Turkey	<28-1300	Sea of Marmara	[70]
Poland	<LOD (0.25)-92.6	Baltic Sea	[71]
Saudi Arabia	<LOD (1.6)->3000	Red Sea	[13]
China	<LOD (0.058)-843	Yangtze River Estuary	[51]
Antarctica	<LOD (4.3)-7761	Northern Antarctic Peninsula	[67]
Finland	2-35	River	[66]
South Africa	900-5300	River	[9]
Pakistan	100-4400	River	[72]
Malaysia	ND-15.49	River	[73]

LOQ: Limit of Quantification; LOD: Limit of Detection; ND: Not detected.

The concentration range in surface waters for Asia and South America could be less than 0.058 ng/L and could reach up to 3000 ng/L as observed in the Red Sea and Paraopeba River. In Europe, the concentrations are in the range of <0.25-475 ng/L. Implementation of EU Water Framework Directive and Environmental Quality Standards for controlling hazardous and specific pollutants, including DCF, in EU surface waters could be the main reason for the low concentration of DCF in European surface waters. The highest surface water concentrations were observed in South Africa due to, probably, a high level of DCF discharge of 8000 ng/L from the WWTP (Table 2). Another very high concentration of 15087 ng/L in wastewater discharge point was observed in Antarctica [74]. The surface water or streamline in Seymour/Marambio Island receives direct liquid effluent discharges. The concentration measured in the surface water was 7761 ng/L. The high DCF concentration was reasoned by the population increase in the summer months due to touristic or military activities.

The discovery of decline of the vulture population in Asia due to DCF has enlightened the science community about the bioaccumulation in the food chain considering the utilization of DCF in veterinary application has been limited to cattle, pigs, and horses [75]. Since then, the utilization of DCF for veterinary purposes, was banned in India, Nepal, Pakistan, Bangladesh, Spain, Italy, and Slovenia. Instead, Dolofenac and Diclovet, which contain DCF-sodium, were approved for swine, cattle, and horse treatment [76].

It is evident that DCF has multiple routes that contaminate the environment. Its concentration in the different environmental phases depends on the amount of consumption, the effectiveness of wastewater treatment plants, and the control measures taken or established discharge standards of the countries. Besides, the reported studies consider only the DCF itself. The metabolites and TPs that form during human metabolism, photodegradation or biodegradation are not taken into account. The DCF treatment efficiency in WWTPs is available only for the parent compound. The metabolites and TPs could pose more health risks. In other words, the awareness about the adverse effect of DCF and its fate in the environment could be the main starting point to control DCF contamination.

5 The adverse effects of diclofenac on organisms

Global consumption of DCF indicates that its utilization is already very high even though veterinary purpose is not included. Its adverse effects on aquatic animals is a problem from an environmental point of view. However, there are shreds of evidence of its impact on human health. For example, DCF treatment was associated with the development of acute kidney injury (AKI). A meta-analysis study showed that individuals treated with DCF appeared to have a 77% increased risk of developing AKI. DCF treatment during pregnancy adversely affects the renal function of the newborn. Briefly, it can cause toxicity in kidneys in adults while children and older people can have renal complications due to DCF [77].

The adverse effect of DCF on animals is more concerning due to its endocrine disrupting compound (EDC) potential, which may cause a population shift from male to female. The change in the population may result in the permanent destruction of the ecosystem. Some of the studies on animals revealed its effects on organs, its bioaccumulative, and EDC features. The survey done by McRae et al. [4] focused on the impact of environmentally relevant (0.17 µg/L) and high (763 µg/L) concentrations on galaxiid fish, inanga (*Galaxias maculatus*), which is an essential fish species for Southern Hemisphere. Inanga was exposed to DCF for 96-h. Bioaccumulation in the inanga exposed to 763 µg/L was significantly higher (1811 µg/kg) than the inanga exposed to 0.17 µg/L (14.9 µg/kg). The calculated bioconcentration factor (BCF) of 2.1±1.2 from 763 µg/L was significantly lower than the BCF value of 87±55 from 0.17 µg/L. The results showed that inanga's human consumption exposed to the environmentally relevant concentration of 0.17 µg/L would lead to 4.25 µg of DCF per serving. Schwaiger et al. [6] stated that four weeks of exposure to environmentally relevant concentrations of DCF caused histopathological changes in the kidney and the gills of rainbow trout. The concentration which causes renal lesions and alterations of the gills has been determined as 5 µg/L. It was concluded that 1 µg/L was no observed effect concentration (NOEC).

Eades and Waring [78] focused on the effects of substantially low concentration of DCF (10 ng/L and 100 ng/L) at two salinity levels (35 and 17.5 psu) to the green shore crab *Carcinus maenas*. The study was about the effect of DCF on the osmoregulatory capacity (OC), which means regulation to balance the fluid and the concentration of electrolyte in the metabolism of the organisms. It maintains the homeostasis of the organism's water content, and a disruption in the OC could cause a detrimental effect on the organisms. Therefore, it is used as an indicator of early warning of any potential environmental problems that are not immediately apparent [79]. The crabs exposed to both 10 ng/L and 100 ng/L of DCF showed an increase in the OC at both salinities. The results indicated that DCF significantly impairs the OC balance of carbs in marine and estuarine environments. This result also shows

that the effect of DCF on the organisms could be observed even at ng/L concentrations [78].

The adverse effects of DCF can be observed through the food chain, which could cause further threats for the endangered species. The case was observed in Pakistan and India where three vulture populations, namely; *Gyps bengalensis*, *Gyps indicus*, and *Gyps tenuirostris* unexpectedly, but severely declined (95%) the last decade. The investigations indicated that DCF poisoning of wild vultures is thought to occur when they feed on carcasses treated with DCF [3]. A similar study was conducted by Oaks et al. in the year 2004. They investigated the correlation between exposure to DCF and death from renal failure in vulture species. The experiment included 20 vultures fed with DCF-treated animals and six control vultures fed with untreated animals. 13 vultures out of 20 (65%) exposed group died due to renal failure. In contrast, none of the vultures in the control group died. A significant correlation between renal failure and DCF exposure is apparent [5]. These findings emphasize the risk of DCF through the food chain.

The adverse effect of DCF is not limited to the failure of organs and then death. Another problem with DCF is its endocrine-disrupting effect, which could destroy the ecosystem irreversibly and increase species extinction risk. As it is well known, EDCs constitute a significant concern regarding both environmental and human health. Shortly, EDCs can be natural or synthetic compounds that can interfere with the hormonal system; they can mimic or block hormones, alter the pattern of hormone synthesis or metabolism and modify the hormone receptor levels [80]. They can create deviations in the population from male to female by affecting the aquatic organisms' reproducibility. A good indicator of the endocrine disruption for a chemical in the organism is the level of vitellogenin (VTG). It is a naturally synthesized egg protein in females, and it is inactive in males. Increased alkali-labile phosphate (ALP) concentration produced by VTG alkali hydrolysis can be used as an indicator of the endocrine-disrupting (ED) effect of a chemical. Gonzalez-Rey and Bebianno [7] exposed female and male mussels *M. galloprovincialis* gonads to 250 ng/L of DCF for 15 days. The result was a 3-fold increase in females' ALP concentrations while males showed consistent ALP concentrations during 15 days of experiments. This study showed that 250 ng/L of DCF for 15 days affected female mussels more than male mussels. The increased vitellogenin gene expression in *Oreochromis niloticus* was observed at 1µg/L DCF [81]. In another study, the effects of DCF and its major metabolite 4'-hydroxydiclofenac (4'-OH-DCF) were investigated [82]. The results showed that both DCF and 4'-OH-DCF have anti-estrogenic and anti-androgenic effects, meaning that they block estrogen and androgen binding receptors. On the other hand, 4'-OH-DCF itself can mimic the estrogens and androgens in the receptors, and it has weak estrogenic and androgenic activity. Hong et al. [8] showed that WWTP effluent and surface water samples contain over 1000 ng/L DCF concentration, which could induce an increase in vitellogenin production in fish, and thereby, it could cause deviation in the population.

It is now known that DCF has endocrine-disrupting properties, and its metabolites and TPs could pose the same risk. DCF has been put watch-list of EU in 2015 based on toxicity results of DCF [83]. Therefore, it is vital to determine the effects of environmentally relevant concentrations of DCF.

6 Diclofenac treatment and transformation products

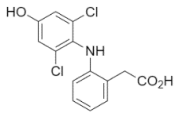
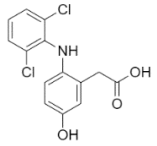
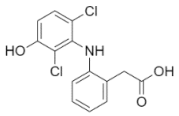
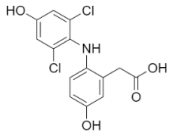
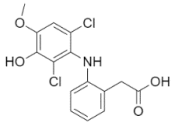
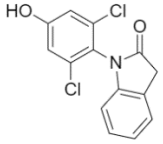
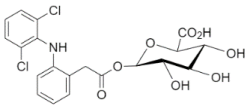
DCF is either partially degraded through natural physical and chemical reactions or degraded biologically in wastewater treatment plants and metabolized in humans/animals' body. So, DCF may not be observed as itself in the water, but its degradation products may still exist in the environment. The metabolites and TPs that occur as a result of these reactions may cause a detrimental impact on the organism. Therefore, the studies on the removal of DCF and its toxicity should include TPs, and metabolites.

Micropollutants have received extensive research attention for the past two decades since their effects on the organisms have been revealed. DCF is one of the most studied micropollutants in the literature. There are many studies regarding its fate in WWTPs and the aquatic environment. The recent studies confirmed the presence of TPs of micropollutants in WWTPs devoted attention to occurrence and fate studies of DCF together with its TPs. The studies without considering TPs could result in a false narrative that may give the impression of good removal when, in reality, they could be transforming into their TPs, which later could deconjugate into the parent compounds again. Therefore, fate studies should include monitoring TPs as well as parent micropollutants. In brief, any wastewater treatment process used for DCF removal should consider both DCF and its transformation products to ensure non-toxic water discharges.

6.1 Metabolites of diclofenac in human

DCF is subjected to aromatic hydroxylation and conjugation through hepatic metabolism in humans and animals [84]. The identified metabolites of DCF in the human body are shown in Table 4. These metabolites are found in humans/animals' excreta and blood at varying percentages. 4'-hydroxydiclofenac (4'-OH-DCF), 5-hydroxydiclofenac (5-OH-DCF), 4',5-dihydroxydiclofenac (4',5-diOH-DCF), 3'-hydroxydiclofenac (3'-OH-DCF) and 3'-hydroxy-4'-methoxydiclofenac (3'-OH-4'-OCH_3-DCF) were discovered in human urine, plasma, and in bile [17]. Stülten et al. [85] identified a new minor metabolite in human urine called lactam-dehydrate of 4'-OH-DCF. Stierlin and Faigle [86] identified 1-O-acyl-glucuronide-DCF (DCF-AG), which can form through direct conjugation of DCF or hydroxylation followed by conjugation. DCF-AG is found to be unstable in bile and possibly hydrolyzes in the gastrointestinal tract to form DCF which, then enters the circulation again. A study about DCF-AG showed that after 50 mg DCF administration, the mean C_{max} of DCF and DCF-AG in plasma were comparable and C_{max} ratio of DCF-AG/DCF was 0.62 [87]. The enzymes responsible for specific metabolites were studied to investigate the DCF metabolism in detail. Bort et al. [17] incubated DCF in human hepatocytes, human liver microsomes and genetically engineered cytochrome P450 (CYP)-expressing cells. The results suggested that 4'-OH-DCF is entirely formed by CYP2C9 enzyme (>99.5%) while CYP2C9 had >97% participation in forming 5-OH-DCF in the human liver. The isoforms; CYP2C8, CYP2C19, CYP2C18, and CYP2B6 were also suggested in the formation of 5-OH-DCF. CYP2C9 was the only one to initiate the formation of 3'-OH-DCF.

Table 4. Identified human metabolites of DCF.

Metabolite	Molecular Structure	Ref.
4'-OH-DCF		[86],[88]
5-OH-DCF		[86],[88]
3'-OH-DCF		[86],[88]
4',5-DiOH-DCF		[86],[88]
3'-OH-4'-OCH3-DCF		[84],[86]
4'-OH-DCF-dehydrate		[85]
DCF-AG		[86]

The amount of metabolite and the types of metabolite depend on the DCF application. The metabolites in the urine for DCF administered orally were 4'-OH-DCF (16.0%), 5-OH-DCF (6.1%), 3'-OH-DCF (2.0%), and 3'-OH-4'-OCH_3-DCF (<0.01%). Approximately 6.2% of DCF remains unchanged. However, if there is declined renal function, 3'-OH-DCF, 5-OH-DCF, 3'-OH-4'-OCH_3-DCF, and DCF constitute less than 2% of the dose. The amount of 4',5-DiOH-DCF in urine was 9.4% of the dose [18]. Oral administration of DCF resulted in 6.5% DCF, 18.1% 4'-OH-DCF, 8.2% 5-OH-DCF, 1.4% 3'-OH-DCF, and 15.4% 4',5-DiOH-DCF of urine samples. DCF and its metabolites in human bile were mainly 10-20% 4'-OH-DCF, 5-10% 5-OH-DCF and less than 5% unchanged DCF [85].

6.2 Photodegradation and transformation products

Photodegradation of organic pollutants in the aquatic environment occurs by two mechanisms: direct absorption of solar radiation and reaction with photosensitized species. Direct photodegradation occurs via non-reversible bond cleavage or re-arrangement of organic molecules with the energy coming from sunlight. Indirect photodegradation is the degradation of molecules by chromophoric compounds which are brought to a higher level of energy by solar radiation. In both mechanisms, energy gained from solar radiation breaks covalent bonds and transforms organic pollutants into more biodegradable and hydrolyzable compounds. There are multiple factors affecting photodegradation, such as the mechanism of degradation (direct or indirect), the chemical structure of pollutant, temperature, pH, depth of surface water, cloud coverage, altitude, latitude, and time of day [20],[89].

The photodegradation of DCF is related to the concentration of DCF in the wastewater. Zhang et al. [90] stated that the degradation rate is affected by the concentration due to competition in the absorption of a limited number of photons by DCF. Dissolved oxygen plays a significant role in the photooxidation of DCF. The mechanism of indirect DCF photooxidation in the presence of dissolved oxygen in the water has two stages. First, direct photodegradation occurs through exciting DCF by solar radiation. Later, dissolved oxygen can absorb the energy of excited DCF, resulting in reactive oxygen species, which induces photooxidation of DCF. On the other hand, direct photodegradation, which is known as the primary mechanism for DCF, is inhibited in the presence of dissolved oxygen due to suppression of excited DCF.

The presence of other pollutants could adversely affect photodegradation. The rate will be more affected by increasing the concentration of these pollutants. The organic matter in the water could enhance the target compound's oxidation through the free radical formation of existing other organic ones. However, in the case of DCF, the rate could decrease due to competition in receiving photons from UV irradiation [23]. Nitrogen in the form of NO_3, NO_2, and ammonia are the common pollutants in domestic wastewater. Therefore, their effects on photodegradation must be considered. Zhang et al. [90] reported that the inhibition effect of NO_2 is more potent than that of NO_3, but ammonia has no effect. The absorption wavelength of NO_2 and DCF are almost the same, which sets a competition to receive the photon that caused decreasing in the degradation rate of DCF. On the other hand, Sokól et al. [91] claimed that NO_3 and NO_2 ions positively affected the DCF decomposition rate due to photosensitizing properties of these ions.

Sokól et al. [91] investigated the influence of pH, humic substances, and complex matrixes on the photodegradation of DCF. It was observed that acidic conditions (pH=2 and 4) enhances the degradation rate implying that H^+ ions could be the catalyst for the photochemical reactions. Similarly, Zhang et al. [92] claimed that the reaction rate increased when pH was between 3 and 5 but decreased when the pH was around 5-8. Further increase in the reaction for the alkaline range (pH=8-12) was observed. Sokól et al. [91] concluded that the humic acids have photosensitizing properties that lead to the increased rate. Zhang et al. [92], reported an opposed conclusion to Sokól et al. [91]. His experience was hindering in DCF photodegradation rate in the presence of humic acid. Three mechanisms were suggested to reveal the effect of humic substance on DCF degradation. The first one was the competition between DCF and humic acid to absorb photons in the emission spectrum resulting in decreasing in the rate. The second one provides an increase in DCF degradation under UV irradiation by forming reactive oxygen species through exited humic acid. However, UV irradiation was not enough under simulated sunlight to support this mechanism. The third mechanism suggests that humic acid can be a scavenger towards DCF [92].

Buser et al. [31] investigated the elimination pathway of DCF in the lake. The results showed that adsorption onto lake sediment was negligible while there was no evidence of chemical and biological degradation. However, sunlight-exposed DCF resulted in a rapid degradation in 4 days. A high concentration (36 mg/L) of DCF was exposed to sunlight to discover photodegradation TPs. Three TPs of photolysis called PH1, PH2, and PH3 were observed. PH1 was the most abundant, and it was identified as an unchlorinated product of DCF. PH2 was suggested to be chloro analog of PH1. Both of them were observed as the initial TPs of photolysis. PH1 was identified as methyl esters of carbazole-1-acetic acid, and PH2 was 8-chloro derivative of PH1. The chemical structure of PH3 was not identified. Scheurell et al. [72] studied DCF and its several TPs in two river waters. The TPs detected in the samples were reported as new in the literature in the year 2009 namely 8-chlorocarbazole-1-yl-ethanoic acid at 0.08-0.3 µg/L concentration. The others were the product of photolysis as 3'-OH-DCF, 4'-OH-DCF, 5-OH-DCF and 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indole-2-one with the concentrations of, 0.03-0.4 µg/L, 0.4-1.8 µg/L, 0.01-0.3 µg/L and 0.02-0.2 µg/L, respectively.

The depth of surface water can affect the photochemical reaction. The deeper the water, the slower the reaction. Bartels and Tümpling Jr. [22] examined the effects of surface depth by determining the relative fluence rate (photosynthetically active radiation [PAR]). The results showed decreased relative fluence rate with increasing depth.

The identification of end products through photolysis of DCF was studied under direct UV light. The reported end products of DCF through photodegradation could vary depending on the reaction condition. Buser et al. [31] stated that DCF showed affinity toward direct photolysis. This behavior was explained as the UV spectrum of DCF shows high-intensity adsorption at 275 nm to >290 nm. Roscher et al. [20] observed that although DCF disappears in 3 minutes, eleven different possible TPs formed similar to DCF in structure. The complete mineralization of DCF to CO2 and water through UV irradiation wasn't achieved. Dechlorination and oxygenation were presented as the dominant mechanisms in TP formation. Aguera et al. [21] provided extensive research on the possible photodecomposition pathway and TP formation reactions. The most important pathway to initiate the phototransformation of DCF is the photocyclization of DCF to carbazole derivatives (C-1), which starts the other reactions that affect the alkyl-chain. In the first route reported by Aguera et al. [21], chlorine atom was the most common product. The replacement of chlorine atom in the hydroxyl-group can occur, resulting in C-6 formation, namely 8-hydroxy-9,9a-dihydro-4 aH-carbazol-1-yl-acetic acid, which is highly reactive and the precursor of dimer formation. This route has been the focus of the research since carbazole formation is linked to the phototoxicity of DCF. Route 2 is initiated with decarboxylation of DCF (C-8) and oxidation of the alkyl-chain (C-9 to C-13). The compounds in this pathway showed persistence under photolysis.

Salgado et al. [23] reported four different TPs which were more polar than DCF and two new products formed through dechlorination, hydroxylation, oxygenation, decarboxylation reactions. Carbazol formation was observed in some end products. However, extending the UV irradiation up to 20 min provided the disappearance of all products apart from the end product (E)-6-[2,6-dichlorophenyl)-imino]-3-oxocyclohexa-1,4-dienecarbaldehyde (D2). It was stated that the transformation of DCF to D2 was the main degradation mechanism, and TPs other than D2 were transformed into other unknown compounds. Iovino et al. [19] carried out DCF photodegradation experiments under UV-light at 254 nm wavelength. The concentration of DCF was 20 mg/L, and over 98% removal of DCF was observed after 5 min UV treatment. A very stable and colored dimer carbazole formed at the end of the reaction. This product was named carbazole-1-acetic acid. However, further photolysis of dimer carbazole showed that this TP was persistent under UV treatment. Semi-laboratory and field experiment carried out for the photodegradation of [22] resulted in three new UV-decomposition products, namely 2-chloroaniline, 2,6-dichlorophenol, and 2,6-dichloroaniline beside to other four products 2-Chloro- and 2,6-Dichlorodiphenylamine derivatives, and 8-Hydroxy- and 8-Chlorocarbazole derivatives [21],[93].

6.3 Biological degradation and transformation products

6.3.1 Biodegradation of diclofenac by isolated and mixed cultures

Biodegradation of DCF is generally low (<50%) in conventional wastewater treatment systems due to mainly resistance of DCF to biological degradation and also limited survival of the microbial community in the DCF containing water [26],[94]. Therefore, the isolation of bacterial strains that are capable of biodegrading DCF ultimately is a real challenge. A bacterial strain *Raoultella sp* KDF8 isolated from contaminated soil was able to degrade 91% of DCF. This removal efficiency was achieved within 72 h at 28 ℃, pH 7, and 1 g/L DCF concentration. The end product identified after degradation was 4'-OH-DCF [26]. *Labrys portucalensis* F11 is another isolated bacterial strain used for DCF degradation. The results showed that the removal of 70% of DCF was achievable when DCF was the sole carbon source. On the other hand, if co-substrate such as acetate was supplied to the isolate, DCF was removed entirely within six days and 25 days of batch cultivation for DCF concentrations 1.7 and 34 µM, respectively. Two main metabolites, namely 4'-OH-DCF and 5-OH-DCF produced nine other intermediate metabolites formed through hydroxylation, methylation, decarboxylation, and oxidation reactions. The metabolites accumulated when there was no co-substrate. In the case of acetate addition, the metabolite generation was faster, but they were further degraded without accumulating in the media [25]. Nitratireductor, Asticcacaulis, and Pseudoxanthomonas were observed in fed-batch operated activated sludge exposed to 50-5000 µg/L DCF [94]. The removal of DCF by abiotic reactions was defined as hydrolysis, volatilization, photolysis, and adsorption with the removal efficiencies of 2.3 %, 2.5%, 3.2 %, 6.5%, respectively, while biodegradation was about 21.3%.

The study with the mixed aerobic bacterial culture was conducted by Jewell et al. [29] for DCF degradation. DCF-lactam, 4'-OH-DCF, 5-OH-DCF, and DCF-benzoic acid were the main metabolites. The secondary metabolites were formed from these main ones. For example, 4'-OH-DCF had six TPs that disappeared during treatment, meaning that they were either entirely oxidized to CO_2 or transformed to other tertiary products. On the other hand, 5-OH-DCF produced nine identified TPs in which some of them were intermediates leading to unidentified other TPs. DCF-lactam was transformed into 4'-OH-DCF-quinone imine and DCF-benzoic acid. Another aerobic DCF degradation study by mixed culture was carried out by Kosjek et al. [95]. The study showed seven TPs that

formed from DCF. Only four of the TPs were properly identified. DP1 ($C_{14}H_{11}NO_2Cl_2$) was found to be the product of only biological degradation since there was no DP1 in the abiotic control. The relationship of DCF and DP1 was inversely proportional, indicating that DP1 was an intermediate TP of DCF directly. DP3, identified as $C_{14}H_{10}NOCl_2$, was reported as the other biodegradation TP, although it may form through thermal decomposition of DCF or due to free OH radicals. Other identified TPs were DP4 and DP7 formed as a result of dehydrogenation. They had the same molecular weight, and it was reported as isomers with the proposed chemical structure of $C_{13}H_{10}NCl_2$. The TPs DP2, DP5, and DP6 were not identified due to their low concentration and weak fragmentation patterns [95]. Poirier-Larabie et al. [96] studied with the mixed culture from an aerobic wastewater treatment plant. The biodegradation rate was slow, and no degradation was observed for 24 hours. After 14 days, three TPs were identified as TP311 ($C_{14}H_{11}Cl_2NO_3$), TP265 ($C_{13}H_9Cl_2NO$), and TP324, which is nitroso-DCF. All three TPs stayed in the system for 57 days indicating that they are resistant to biodegradation.

Barbieri et al. [97] focused on the especially anoxic condition or denitrification in groundwater to emphasize the danger of this pollutant and its TPs in the natural water resources. The batch anoxic degradation test resulted in DCF removal, initially, but then the recovery of DCF to its initial concentration at the end of the reaction. In comparison to the most common TP, nitro-DCF was the main TP under anoxic conditions. The DCF disappearance and TP formation were directly related to the presence of nitrate. The conversion of nitrite to nitrogen resulted in the recovery of DCF concentration.

There are a limited number of studies about the anaerobic degradation of DCF and its TPs. Photodegradation is the initial step of DCF degradation. Anaerobic conditions are light-free media, and therefore, the initial degradation can not be achieved, which makes the anaerobic removal of DCF more difficult. On the other hand, DCF sorption onto the sludge is possible and anaerobic digestion (AD) of treatment sludges is one of the most widely used sludge stabilization methods. Therefore, the fate of DCF under anaerobic conditions is a research subject. Carballa et al. [35] studied the removal of DCF in AD and discovered that DCF could be removed (80%) after the sludge adaptation period, and there is no influence of sludge retention time (SRT) and temperature on the removal of DCF. Lahti and Oikari [98] investigated the removal of DCF from sewage treatment plant sludge under anaerobic conditions. The reduction in DCF was only 26% after 161 days for the initial concentration of 100 µg/L, and the degradation mechanism was reported as mainly abiotic but not anaerobic. Poirier-Larabie et al. [96] identified only TP324 (nitroso-DCF) under anaerobic degradation. This TP was the common one encountered under aerobic degradation. However, the concentration of TP324 was lower under anaerobic degradation due to low DCF biodegradation efficiency by anaerobic microorganisms.

The other microorganism with its well-known ability in the degradation of recalcitrant substances is white-rot fungi (WRF). They have been widely used for the degradation of textile dyestuff [99]-[101], PAH [102]-[104], and the other several pollutants such as BPA, 17-β-estradiol, ethinylestradiol [105],[106]. White-rot fungi can produce high redox potential peroxidases (lignin peroxidase, manganese peroxidase, and versatile peroxidase) and laccase, which are capable of oxidizing a wide variety of pollutants. They have an intracellular cytochrome P450 system, which takes place in the oxidation of these recalcitrant substances [28]. The use of WRF for the removal of pharmaceuticals from polluted water is rather a new concept. It has been shown that they can degrade ibuprofen, ketoprofen, carbamazepine, atenolol, etc. [107]-[110]. DCF degradation by white-rot fungus *Phanerochaete sordida* YK-624 was studied by Hata et al. [27] and 90% of DCF was removed within three days, and then complete degradation was achieved in 6 days. Three different metabolites, namely, 4'-OH-DCF, 5'-OH-DCF and, 4',5-diOH-DCF were observed, and 4'-OH-DCF was the major one. Another well-known white-rot fungi, *Trametes versicolor*, was used for degradation of DCF by Marco-Urrea et al. [28]. The metabolites 4'-OH-DCF and 5-OH-DCF formed disappeared after 24 hr [28].

White-rot fungi could be very talented in the degradation of recalcitrant substances. However, the organism's behavior in real wastewater, in the presence of other microorganisms with different optimal growth conditions and growth requirements, could be a limitation in the degradation ability of WFR. Yang et al. [106] investigated the use of bacteria and WRF *T. versicolor* ATCC 7731 to degrade DCF in a membrane bioreactor (MBR). It was observed that the removal efficiency in the presence of bacteria is lower than that of *T. versicolor* alone. The low removal in the co-culture was explained as the bacterial damage on fungal mycelium or enzymes, inactivation of ligninolytic enzymes, and competition between bacteria and fungi for organic substance resulting in inhibition on the fungal degradation. The TPs of DCF from various degradation processes were summarized in Table 5 and the chemical structures of metabolites and transformation products are given in Table S1.

6.3.2 The effects of wastewater treatment plant operation conditions in diclofenac removal and metabolite formation

Biological WWTPs are mainly designed to remove conventional organic pollutants and nutrients. However, it has been realized that the micropollutants end up in biological wastewater treatment plants where they have to be removed. Many researchers are investigating the removal of DCF and its degradation products since these pollutants have become an emerging concern for the environment. The reported studies showed that the removal of DCF varies widely from no reduction (0%) to 75% as given in Table 2. The wide range of treatment efficiency can be explained by the operating conditions employed in the process. DCF removal under anaerobic-aerobic or anoxic conditions, the relationship between SRT or hydraulic retention time (HRT) and environmental conditions such as temperature can affect the DCF removal. Therefore, it is crucial to define the role of process conditions in the biodegradation of DCF and its TP.

SRT is one of the factors that affect the treatment performance of a suspended growth wastewater treatment systems. The effect of SRT on DCF removal is not apparent. Jelic et al. [15] stated that SRT has no influence on removing DCF in plants with only secondary treatment. Instead, WWTP with tertiary treatment including microfiltration and chlorination resulted in 60% DCF removal. Anumol et al. [57] drew the same conclusion as Jelic et al. [15] that chlorination after secondary treatment significantly improved the reduction. It was also observed that aerated lagoon before secondary treatment could help the degradation [57].

Table 5. DCF metabolites and TPs from different degradation processes.

Process	Metabolites and TPs	Ref.
Human	4'-hydroxydiclofenac, 5-hydroxydiclofenac, 4',5-dihydroxydiclofenac, 3'-hydroxydiclofenac, 3'-hydroxy-4'-methoxydiclofenac	[18],[84], [86],[88]
Photodegradation	2-(8-hydroxy-3-oxo-3H-carbazol-1-yl)acetic acid (E)-6-[2,6-dichlorophenyl)-imino]-3-oxocyclohexa-1,4-dienecarbaldehyde) 2-(8-hydroxy-3-oxo-9,9a-dihydro-3H-carbazol-1-yl)acetic acid (E)-2-[3-(2,6-dichloro-?-hydroxyphenylimino)-6-oxocyclohexa-1,4-dienyl]acetic acid 2-[2-(phenylamino]phenyl)acetic acid 2-(8-chloro-9H-carbazol-1-yl)acetic acid	[23]
	PH1: methyl esters of carbazole-1-acetic acid PH2: 8-chloro derivative of carbazole-1-acetic acid PH3: unknown	[31]
	carbazole-1-acetic acid.	[19]
	2-chloroaniline 2,6-dichloroaniline 2,6-dichlorophenol 1-(2,6-dichlorophenyl)indolin-2-one 2-(2-chlorophenylamino)benzaldehyde 2,6-dichloro-N-o-tolybenzenamine 9H-carbazole-1-carbaldehyde 8-chloro-9H-carbazole-1-carbaldehyde 2-(2,6-dichlorophenylamino)benzaldehyde	[22]
	8-chlorocarbazole-1-yl-ethanoic acid 3'-hydroxydiclofenac, 4'-hydroxydiclofenac, 5-hydroxydiclofenac 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indole-2-one	[72]
Biodegradation *Raoultella sp* KDF8	4'-hydroxydiclofenac	[26]
Labrys portucalensis F11	4'-hydroxydiclofenac 5-hydroxydiclofenac	[25]
Mixed culture-Aerobic	DCF-lactam, 4'-hydroxydiclofenac 5-hydroxydiclofenac DCF-benzoic acid	[29]
	C14H11NO2Cl2 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one Contains structural fragment "2,6-dichloro-N-(phenyl)aniline"	[95]
Phanerochaete sordida YK-624	4'-hydroxydiclofenac 5-hydroxydiclofenac 4',5-dihydroxydiclofenac	[27]
Trametes versicolor	4'-hydroxydiclofenac 5-hydroxydiclofenac	[28]
WWTPs	4'-hydroxydiclofenac 5-hydroxydiclofenac carboxydiclofenac (C-DCF)	[16]
	4'-hydroxydiclofenac DCF-lactam	[111]
Anoxic	Nitro-Diclofenac (NO2-DCF)	[97]

Tran and Gin [58] emphasized that higher biomass concentration, longer SRT, and better adsorption ability of sludge could be the reason for better removal of DCF in high biomass holding process such as membrane bioreactor (MBR) compared to conventional wastewater treatment systems. It was also concluded that one of the other reasons for low DCF removal is the absence of electron-donating groups in DCF. Clara et al. [63] investigated the effect of SRT on DCF removal efficiency in three different conventional activated sludge wastewater treatment plants. A pilot-scale membrane reactor was installed in one of the WWTPs. No DCF removal was observed in two conventional WWTPs operated at SRT=2 days and SRT=46 days. MBR reactor had a positive effect on DCF removal in the system. Complete removal of DCF was achieved in MBR at SRT=10 days and HRT=0.5 days. The contribution of conventional WWTP removed about 50% of DCF. Increasing SRT to 27 days resulted in 50% DCF removal in MBR and 20% in WWTP with a total removal efficiency of 70%. Extending SRT and HRT in MBR to 55 days and four days, respectively, did not provide further improvement in DCF for the total of the system. The mechanism of DCF removal in this two-stage combined system was not clearly explained. But, the operation temperature was almost ideal (16.8- 22.1 ℃) for the biological activity when 50% and 70% removal efficiency was obtained. Besides, long SRT combined with high temperature was explained as the reason for improved removal efficiency [63].

Suarez et al. [24] studied the removal of DCF in a full-scale WWTP working under sequential denitrifying and nitrifying conditions. DCF removal at the denitrification unit was only 2%, suggesting resistance to biodegradation under anoxic conditions. The nitrification unit provided an increase in the removal efficiency from 0% to 25%, which was explained as the initial adaptation (~170 d) with the washout of heterotrophic organisms. Further increase in the removal efficiency to 74% was explained as the full adaptation of microorganisms in relation to SRT and temperature. In another study conducted by Suarez et al. [112] in the single sludge denitrification and nitrification process, the removal of DCF was below 20%, which was explained by the long half-life of DCF. The biological degradation of DCF was determined as pseudo-first-order with degradation constant below 0.1 L.g/SS.d. The half-life of DCF in a plant with a biomass concentration of 2-4 g/L was calculated as 2-3.5 d, from which it was concluded that only WWTPs with high HRT (in the order of days) could achieve biodegradation of DCF to some extend.

It is evident that DCF is biotransformed to other products that may not be degraded further in WWTP, and then they are directly discharged into surface waters. The biotransformation of DCF is initiated by cytochrome P450 (CYP2C9) oxidation to hydroxylated metabolites, mainly 4'-OH-DCF, 5-OH-DCF and DCF-lactam [28],[29],[113]. Some researchers have investigated the metabolite types and their concentrations in existing full-scale wastewater treatment plants. Jewell et al. [29] monitored a domestic wastewater treatment plant in a three-stage process as denitrification (anoxic), aerobic, and nitrification with high aeration and hybrid moving bed bioreactor (MBBR) to evaluate DCF removal and degradation products. DCF-lactam was the most dominant metabolite encountered in the process. The human metabolites such as 4'-OH-DCF and 5-OH-DCF were detected in WWTP influent, but not in the effluent due to probably fast transformation. No evidence for the formation of these TPs during the treatment process was observed. DCF-BA concentration was 23±8 ng/L in WWTP effluent, although it was not detected in the influent, showing that it formed at a low fraction. The major reactions in DCF removal were explained as hydroxylation, decarboxylation, oxidation amide formation, ring-opening, and reductive dechlorination. The DCF removal was mainly

achieved in MBBR [29]. Schmidt et al. [111] monitored the concentrations of DCF and its metabolites 4'-OH-DCF and DCF-lactam in water samples taken from two Berlin WWTPs and a canal receiving wastewater. No DCF removal was observed in one of the WWTPs while it was around 50% in the other. A slight increase in the metabolite concentration of both WWTP effluent occurred. DCF-lactam concentration was below LOD while it was 1 ng/L in WWTP effluents, although there was no DCF removal in one of them. Interestingly, lower DCF and metabolite concentrations were detected in the effluent receiving water.

Another study was conducted by Kolecka et al. [64] in a WWTP with nitrification and denitrification units. The effluent DCF concentration was either higher or lower than the influent one depending on the sampling time and wastewater characteristics. On the other hand, the most common metabolites 5-OH-DCF and 4'-OH-DCF were removed in the WWTP. The maximum efficiencies were 40.6% for DCF and between 58.6-78.6% for the metabolites.

Larsson et al. [16] monitored DCF with its TPs, 4'-OH-DCF, 5-OH-DCF, and carboxydiclofenac (C-DCF) in a full-scale WWTP included primary treatment, conventional activated sludge, pre-denitrification, and phosphate precipitation with ferric chloride. The influent wastewater contained DCF itself as well as human metabolites as 4'-OH-DCF, 5-OH-DCF, and C-DCF. Primary treatment provided only 8-20% DCF and metabolite removal with only 1% adsorption on primary sludge. Secondary treatment resulted in 40% to 90% removal of DCF and its metabolites with 5 to 10 % adsorption on biological sludge. The tertiary treatment employed for phosphorus removal did not reduce the concentrations further. It was concluded that the secondary treatment is efficient enough for DCF and metabolites removal. The low amounts of metabolites detected in the effluent suggested that they are quickly transformed even if they are formed in biological treatment [16].

Lindholm-Lehto et al. [65] touched upon a subject that might answer why there are negative removals of DCF in the literature. It was stated that DCF is excreted as conjugates, which can deconjugate to the parent compound later in the treatment system, causing higher DCF concentration in the effluent than in the influent. This phenomenon occurs in the summertime, so no removal is obtained. In the wintertime, DCF consumption increases, but the deconjugation reduces due to low temperature. Lower effluent DCF concentration can be observed with limited removal efficiency. Moreover, photolysis is an important elimination mechanism of DCF. Therefore, the decreased UV light in winter could influence the removal as well [65]. Finally, Lindqvist et al. [66] explained that industrial wastewater entering the system could have toxic effects on the biomass resulting in lower efficiencies.

6.4 Diclofenac in soil and wastewater treatment sludges

Wastewater treatment sludge can be used for energy production, fertilizer, or soil conditioner. It is declared that 53% of sludge produced in the European Union is used in agriculture directly or after composting [114]. However, heavy metals and other organic pollutants such as pharmaceuticals on sludge make its application to soil very dangerous due to its possible toxic effects on biota. Rastetter and Gerhardt [49] studied the toxicity of non-dewatered bio-P sludge (S1) and dewatered sludges (S2 and S3). The results showed that S1 was more toxic than S2 and S3 due to the high concentrations of benzotriazole (6122 ng/g TS), carbamazepine (2106 ng/g TS), DCF (1935 ng/g TS), and heavy metal iron (18.9 mg/g TS). The dewatered sludge was unlikely to create an acute toxicity response, while S1 could harm soil invertebrates and freshwater organisms (plants and crustacean). Kummerova et al. [115] reported the impact of DCF on a duckweed plant, Lemna minor. The results indicated that the environmentally relevant concentrations of DCF could affect the plant's biochemical process by producing reactive oxygen species (ROS) and reactive nitrogen species. The production of these species created different effects such as increased lipid peroxidation, loss of plasma membrane integrity, etc.

7 Conclusions

The studies on DCF reveals that DCF threatens ecological balance. Photolysis through direct and indirect UV initiates the DCF destruction, and biodegradation depends on the microbial cultures, environmental conditions, and WWTPs operation. Photolysis or biological DCF degradation mechanisms end up with the TPs, which need further treatment. The wastewater treatment plants are insufficient for the complete removal of DCF. The degradation of DCF by pure culture could give an idea about the TPs, degradation rates, or environmental conditions required. Nevertheless, the real wastewater conditions could be different due to inhibitors, readily biodegradable substances, or other toxic non-biodegradable ones in the water. Therefore, monitoring studies in WWTP should be conducted to understand the nature of degradation or transformation mechanisms in real conditions. The formation of various TPs and the lack of information about their ecotoxicological effects are the problems. Therefore, the transformation of DCF in a full-scale wastewater treatment plant should be questioned in detail to protect the natural water bodies.

Further research about DCF can be addressed as follows;

- The toxicity of the DCF metabolites can be investigated,
- The degradation pathway can be studied in detail,
- Veterinary sources and the DCF concentrations can be monitored,
- DCF disposal through unused pills and its fate in solid waste disposal areas or landfills can be monitored.
- The fate and degradation of DCF in leachate can be studied.

8 Author contribution statements

In the scope of this study, Serenay Ceren TUZUN and Ilgi KARAPINAR, in the formation of the idea, literature review, writing and editing the article, were contributed.

9 Ethics committee approval and conflict of interest statement

There is no need to obtain permission from the ethics committee for the article prepared.

There is no conflict of interest with any person/institution in the article prepared.

10 References

[1] Acuña V, Ginebreda A, Mor JR, Petrovic M, Sabater S, Sumpter J, Barceló D. "Balancing the health benefits and environmental risks of pharmaceuticals: Diclofenac as an example". *Environment International*, 85(12), 327-333, 2015.

[2] de Voogt P, Janex-Habibi ML, Sacher F, Puijker L, Mons M. "Development of a common priority list of pharmaceuticals relevant for the water cycle". *Water Science and Technology*, 59(1), 39-46, 2009.

[3] Green RE, Newton IAN, Shultz S, Cunningham AA, Gilbert M, Pain DJ, Prakash V. "Diclofenac poisoning as a cause of vulture population declines across the Indian subcontinent". *Journal of Applied Ecology*, 41(5), 793-800, 2004.

[4] McRae NK, Glover CN, Burket SR, Brooks BW, Gaw S. "Acute exposure to an environmentally relevant concentration of diclofenac elicits oxidative stress in the culturally important galaxiid fish *Galaxias maculatus*". *Environmental Toxicology and Chemistry*, 37(1), 224-235, 2018.

[5] Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Shivaprasad HL, Ahmed S, Iqbal Chaudhry MJ, Arshad M, Mahmood S, Ali A, Ahmed Khan A. "Diclofenac residues as the cause of vulture population decline in Pakistan". *Nature*, 427(1), 630-633, 2004.

[6] Schwaiger J, Ferling H, Mallow U, Wintermayr H, Negele RD. "Toxic effects of the non-steroidal anti-inflammatory drug diclofenac. Part I: histopathological alterations and bioaccumulation in rainbow trout". *Aquatic Toxicology*, 68(2), 141-150, 2004.

[7] Gonzalez-Rey M, Bebianno MJ. "Effects of non-steroidal anti-inflammatory drug (NSAID) diclofenac exposure in mussel *Mytilus galloprovincialis*". *Aquatic Toxicology*, 148(3), 221-230, 2014.

[8] Hong HN, Kim HN, Park KS, Lee S-K, Gu MB. "Analysis of the effects diclofenac has on Japanese medaka (*Oryzias latipes*) using real-time PCR". *Chemosphere*, 67(11), 2115-2121, 2007.

[9] Madikizela LM, Chimuka L. "Occurrence of naproxen, ibuprofen, and diclofenac residues in wastewater and river water of KwaZulu-Natal Province in South Africa". *Environmental Monitoring and Assessment*, 189(7), 348-359, 2017.

[10] Tran NH, Reinhard M, Gin KY-H. "Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review". *Water Research*, 133(6), 182-207, 2018.

[11] Yang YY, Liu WR, Liu YS, Zhao JL, Zhang QQ, Zhang M, Zhang J-N, Jiang Y-X, Zhang L-J, Ying G-G. "Suitability of pharmaceuticals and personal care products (PPCPs) and artificial sweeteners (ASs) as wastewater indicators in the Pearl River Delta, South China". *Science of the Total Environment*, 590-591(14), 611-619, 2017.

[12] Agunbiade FO, Moodley B. "Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, Kwazulu-Natal, South Africa". *Environmental Toxicology and Chemistry*, 35(1), 36-46, 2016.

[13] Ali AM, Rønning HT, Alarif W, Kallenborn R, Al-Lihaibi SS. "Occurrence of pharmaceuticals and personal care products in effluent-dominated Saudi Arabian coastal waters of the Red Sea". *Chemosphere*, 175(10), 505-513, 2017.

[14] de Barros ALC, Schmidt FF, de Aquino SF, Afonso R. "Determination of nine pharmaceutical active compounds in surface waters from Paraopeba River Basin in Brazil by LTPE-HPLC-ESI-MS/MS". *Environmental Science Pollution Research*, 25(20), 19962-19974, 2018.

[15] Jelic A, Gros M, Ginebreda A, Cespedes-Sánchez R, Ventura F, Petrovic M, Barcelo D. "Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment". *Water Research*, 45(3), 1165-1176, 2011.

[16] Larsson E, Al-Hamimi S, Jonsson JA. "Behaviour of nonsteroidal anti-inflammatory drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid phase microextraction and liquid chromatography mass spectrometry". *Science of the Total Environment*, 485-486(13), 300-308, 2014.

[17] Bort R, Macé K, Boobis A, Gómez-Lechón Ma-J, Pfeifer A, Castell J. "Hepatic metabolism of diclofenac: role of human CYP in the minor oxidative pathways". *Biochemical Pharmacology*, 58(5), 787-796, 1999.

[18] Stülten D, Zühlke S, Lamshöft M, Spiteller M. "Occurrence of diclofenac and selected metabolites in sewage effluents". *Science of the Total Environment*, 405(1), 310-316, 2008.

[19] Iovino P, Chianese S, Canzano S, Prisciandaro M, Musmarra D. "Photodegradation of diclofenac in wastewaters". *Desalination and Water Treatment*, 61(3), 293-297, 2017.

[20] Roscher J, Vogel M, Karst U. "Identification of ultraviolet transformation products of diclofenac by means of liquid chromatography and mass spectrometry". *Journal of Chromatography A*, 1457(31), 59-65, 2016.

[21] Aguera A, Perez Estrada LA, Ferrer I, Thurman EM, Malato S, Fernandez-Alba AR. "Application of time-of-flight mass spectrometry to the analysis of phototransformation products of diclofenac in water under natural sunlight". *Journal of Mass Spectrometry*, 40(7), 908-915, 2005.

[22] Bartels P, von Tümpling W. "Solar radiation influence on the decomposition process of diclofenac in surface waters". *Science of the Total Environment*, 374(1), 143-155, 2007.

[23] Salgado R, Pereira VJ, Carvalho G, Soeiro R, Gaffney V, Almeida C, Vale Cardoso V, Ferreira E, Benoliel MJ, Ternes TA, Oehmen A, Reis MA, Noronha JP. "Photodegradation kinetics and transformation products of ketoprofen, diclofenac and atenolol in pure water and treated wastewater". *Journal of Hazardous Materials*, 244-245(1), 516-527, 2013.

[24] Suarez S, Lema JM, Omil F. "Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions". *Water Research*, 44(10), 3214-3224, 2010.

[25] Moreira IS, Bessa VS, Murgolo S, Piccirillo C, Mascolo G, Castro PML. "Biodegradation of Diclofenac by the bacterial strain *Labrys portucalensis* F11". *Ecotoxicology and Environment Safety*, 152(6), 104-113, 2018.

[26] Palyzova A, Zahradnik J, Maresova H, Sokolova L, Kyslikova E, Grulich M, Stepanek V, Rezanka T, Kyslik P. "Potential of the strain *Raoultella sp*. KDF8 for removal of analgesics". *Folia Microbiologica*, 63(3), 273-282, 2018.

[27] Hata T, Kawai S, Okamura H, Nishida T. "Removal of diclofenac and mefenamic acid by the white rot fungus *Phanerochaete sordida* YK-624 and identification of their metabolites after fungal transformation". *Biodegradation*, 21(5), 681-689, 2010.

[28] Marco-Urrea E, Perez-Trujillo M, Cruz-Morato C, Caminal G, Vicent T. "Degradation of the drug sodium diclofenac by *Trametes versicolor* pellets and identification of some intermediates by NMR". *Journal of Hazardous Materials*, 176(1-3), 836-842, 2010.

[29] Jewell KS, Falas P, Wick A, Joss A, Ternes TA. "Transformation of diclofenac in hybrid biofilm-activated sludge processes". *Water Research*, 105(18), 559-567, 2016.

[30] Zou H, Radke M, Kierkegaard A, MacLeod M, McLachlan MS. "Using chemical benchmarking to determine the persistence of chemicals in a swedish lake". *Environmental Science & Technology*, 49(3), 1646-1653, 2015.

[31] Buser H-R, Poiger T, Müller MD. "Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: rapid photodegradation in a lake". *Environmental Science & Technology*, 32(22), 3449-3456, 1998.

[32] Lonappan L, Brar SK, Das RK, Verma M, Surampalli RY. "Diclofenac and its transformation products: Environmental occurrence and toxicity-A review". *Environment International*, 96(9), 127-138, 2016.

[33] Zhang Y, Geissen SU, Gal C. "Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies". *Chemosphere*, 73(8), 1151-1161, 2008.

[34] Vieno N, Sillanpää M. "Fate of diclofenac in municipal wastewater treatment plant-a review". *Environment International*, 69(8), 28-39, 2014.

[35] Carballa M, Fink G, Omil F, Lema JM, Ternes T. "Determination of the solid–water distribution coefficient (Kd) for pharmaceuticals, estrogens and musk fragrances in digested sludge". *Water Research*, 42(1), 287-295, 2008.

[36] Altman R, Bosch B, Brune K, Patrignani P, Young C. "Advances in NSAID development: evolution of diclofenac products using pharmaceutical technology". *Drugs*, 75(8), 859-877, 2015.

[37] Meylan WM, Howard PH. "Computer estimation of the Atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone". *Chemosphere*, 26(12), 2293-2299, 1993.

[38] Tixier C, Singer HP, Oellers S, Muller SR. "Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface waters". *Environmental Science and Technology*, 37(6), 1061-1068, 2003.

[39] Zou H, Radke M, Kierkegaard A, McLachlan MS. "Temporal Variation of Chemical Persistence in a Swedish Lake Assessed by Benchmarking". *Environmental Science & Technology*, 49(16), 9881-9888, 2015.

[40] Al-Rajab AJ, Sabourin L, Lapen DR, Topp E. "The non-steroidal anti-inflammatory drug diclofenac is readily biodegradable in agricultural soils". *Science of the Total Environment*, 409(1), 78-82, 2010.

[41] Poiger T, Buser H-R, Müller MD. "Photodegradation of the pharmaceutical drug diclofenac in a lake: Pathway, field measurements, and mathematical modeling". *Environmental Toxicology and Chemistry*, 20(2), 256-263, 2001.

[42] Xu J, Wu L, Chang AC. "Degradation and adsorption of selected pharmaceuticals and personal care products (PPCPs) in agricultural soils". *Chemosphere*, 77(10), 1299-1305, 2009.

[43] Radke M, Ulrich H, Wurm C, Kunkel U. "Dynamics and Attenuation of Acidic Pharmaceuticals along a River Stretch". *Environmental Science & Technology*, 44(8), 2968-2974, 2010.

[44] Huschek G, Hansen PD, Maurer HH, Krengel D, Kayser A. "Environmental risk assessment of medicinal products for human use according to European Commission recommendations". *Environmental Toxicology*, 19(3), 226-240, 2004.

[45] Ferrari Bt, Paxéus N, Giudice RL, Pollio A, Garric J. "Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac". *Ecotoxicology and Environmental Safety*, 55(3), 359-370, 2003.

[46] Jones OAH, Voulvoulis N, Lester JN. "Aquatic environmental assessment of the top 25 English prescription pharmaceuticals". *Water Research*, 36(20), 5013-5022, 2002.

[47] Bound Jonathan P, Voulvoulis N. "Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom". *Environmental Health Perspectives*, 113(12), 1705-1711, 2005.

[48] Patel M, Kumar R, Kishor K, Mlsna T, Pittman CU, Mohan D. "Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods". *Chemical Reviews*, 119(6), 3510-3673, 2019.

[49] Rastetter N, Gerhardt A. "Toxic potential of different types of sewage sludge as fertiliser in agriculture: ecotoxicological effects on aquatic, sediment and soil indicator species". *Journal of Soils and Sediments*, 17(1), 106-121, 2017.

[50] Heberer T, Feldmann D. "Contribution of effluents from hospitals and private households to the total loads of diclofenac and carbamazepine in municipal sewage effluents--modeling versus measurements". Journal of *Hazardous Materials*, 122(3), 211-218, 2005.

[51] Yang Y, Fu J, Peng H, Hou L, Liu M, Zhou JL. "Occurrence and phase distribution of selected pharmaceuticals in the Yangtze Estuary and its coastal zone". *Journal of Hazardous Materials*, 190(1), 588-596, 2011.

[52] Lee H-B, Peart TE, Svoboda ML. "Determination of endocrine-disrupting phenols, acidic pharmaceuticals, and personal-care products in sewage by solid-phase extraction and gas chromatography–mass spectrometry". *Journal of Chromatography A*, 1094(1), 122-129, 2005.

[53] Rivera-Jaimes JA, Postigo C, Melgoza-Aleman RM, Acena J, Barcelo D, Lopez de Alda M. "Study of pharmaceuticals in surface and wastewater from Cuernavaca, Morelos, Mexico: Occurrence and environmental risk assessment". *Science of the Total Environment*, 613-614(3), 1263-1274, 2018.

[54] Yang Y, Ok YS, Kim K-H, Kwon EE, Tsang YF. "Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review". *Science of the Total Environment*, 596-597(18), 303-320, 2017.

[55] Sun Q, Li M, Ma C, Chen X, Xie X, Yu CP. "Seasonal and spatial variations of PPCP occurrence, removal and mass loading in three wastewater treatment plants located in different urbanization areas in Xiamen, China". *Environmental Pollution*, 208(1), 371-381, 2016.

[56] Behera SK, Kim HW, Oh JE, Park HS. "Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea". *Science of the Total Environment*, 409(20), 4351-4360, 2011.

[57] Anumol T, Vijayanandan A, Park M, Philip L, Snyder SA. "Occurrence and fate of emerging trace organic chemicals in wastewater plants in Chennai, India". *Environment International*, 92-93(6), 33-42, 2016.

[58] Tran NH, Gin KY. "Occurrence and removal of pharmaceuticals, hormones, personal care products, and endocrine disrupters in a full-scale water reclamation plant". *Science of the Total Environment*, 599-600(20), 1503-1516, 2017.

[59] Osorio V, Imbert-Bouchard M, Zonja B, Abad JL, Perez S, Barcelo D. "Simultaneous determination of diclofenac, its human metabolites and microbial nitration/nitrosation transformation products in wastewaters by liquid chromatography/quadrupole-linear ion trap mass spectrometry*". Journal of Chromatography A*, 1347(25), 63-71, 2014.

[60] Kosma CI, Lambropoulou DA, Albanis TA. "Investigation of PPCPs in wastewater treatment plants in Greece: Occurrence, removal and environmental risk assessment". *Science of the Total Environment*, 466-467(1), 421-438, 2014.

[61] Sari S, Ozdemir G, Yangin-Gomec C, Zengin GE, Topuz E, Aydin E, Pehlivanoglu-Mantas E, Okutman Tas D. "Seasonal variation of diclofenac concentration and its relation with wastewater characteristics at two municipal wastewater treatment plants in Turkey". *Journal of Hazardous Materials*, 272, 155-164, 2014.

[62] Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. "The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters". *Water Research*, 43(2), 363-380, 2009.

[63] Clara M, Strenn B, Gans O, Martinez E, Kreuzinger N, Kroiss H. "Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants". *Water Research*, 39(19), 4797-4807, 2005.

[64] Kołecka K, Gajewska M, Stepnowski P, Caban M. "Spatial distribution of pharmaceuticals in conventional wastewater treatment plant with Sludge Treatment Reed Beds technology". *Science of the Total Environment*, 647(2), 149-157, 2019.

[65] Lindholm-Lehto PC, Ahkola HS, Knuutinen JS, Herve SH. "Widespread occurrence and seasonal variation of pharmaceuticals in surface waters and municipal wastewater treatment plants in central Finland". *Environmental Science Pollution Research*, 23(8), 7985-7997, 2016.

[66] Lindqvist N, Tuhkanen T, Kronberg L. "Occurrence of acidic pharmaceuticals in raw and treated sewages and in receiving waters". *Water Research*, 39(11), 2219-2228, 2005.

[67] Vulliet E, Cren-Olivé C, Grenier-Loustalot M-F. "Occurrence of pharmaceuticals and hormones in drinking water treated from surface waters". *Environmental Chemistry Letters*, 9(1), 103-114, 2011.

[68] Veras TB, Luiz Ribeiro de Paiva A, Duarte MMMB, Napoleão DC, da Silva Pereira Cabral JJ. "Analysis of the presence of anti-inflammatories drugs in surface water: A case study in Beberibe river- PE, Brazil". *Chemosphere*, 222(9), 961-969, 2019.

[69] Nödler K, Voutsa D, Licha T. "Polar organic micropollutants in the coastal environment of different marine systems". *Marine Pollution Bulletin*, 85(1), 50-59, 2014.

[70] Korkmaz NE, Savun-Hekimoğlu B, Aksu A, Burak S, Caglar NB. "Occurrence, sources and environmental risk assessment of pharmaceuticals in the Sea of Marmara, Turkey". *Science of the Total Environment*, 819(18), 1-13, 2022.

[71] Borecka M, Siedlewicz G, Haliński ŁP, Sikora K, Pazdro K, Stepnowski P, Białk-Bielińska A. "Contamination of the southern Baltic Sea waters by the residues of selected pharmaceuticals: Method development and field studies". *Marine Pollution Bulletin*, 94(1), 62-71, 2015.

[72] Scheurell M, Franke S, Shah RM, Huhnerfuss H. "Occurrence of diclofenac and its metabolites in surface water and effluent samples from Karachi, Pakistan". *Chemosphere*, 77(6), 870-876, 2009.

[73] Praveena SM, Shaifuddin SNM, Sukiman S, Nasir FAM, Hanafi Z, Kamarudin N, Ismail THT, Aris AZ. "Pharmaceuticals residues in selected tropical surface water bodies from Selangor (Malaysia): Occurrence and potential risk assessments". *Science of the Total Environment*, 642(24), 230-240, 2018.

[74] González-Alonso S, Merino LM, Esteban S, López de Alda M, Barceló D, Durán JJ, López-Martínez J, Aceña J, Pérez S, Mastroianni N, Silva A, Catalá M, Valcárcel Y. "Occurrence of pharmaceutical, recreational and psychotropic drug residues in surface water on the northern Antarctic Peninsula region". *Environmental Pollution*, 229(11), 241-254, 2017.

[75] Abu-Seida AM. "Efficacy of diclofenac sodium, either alone or together with cefotaxime sodium, for control of postoperative pain, in dogs undergoing ovariohysterectomy". *Asian Journal of Animal and Veterinary Advances*, 7(2), 180-186, 2012.

[76] Camiña A, Aguilera J, Sarrazin F, Duriez O. "Potential exposure to diclofenac in Spain of European vultures". *Vulture News*, 75(2), 1-20, 2018.

[77] Borghi SM, Fattori V, Ruiz-Miyazawa KW, Bertozzi MM, Lourenco-Gonzalez Y, Tatakihara RI, Bussmann AJC, Mazzuco TL, Casagrande R, Verri WA, Jr. "Pyrrolidine dithiocarbamate inhibits mouse acute kidney injury induced by diclofenac by targeting oxidative damage, cytokines and NF-kappaB activity". *Life Sciences*, 208(17), 221-231, 2018.

[78] Eades C, Waring CP. "The effects of diclofenac on the physiology of the green shore crab *Carcinus maenas*". *Marine Environment Research*, 69 (Supplement 1), 46-48, 2010.

[79] Bamber SD, Depledge MH. "Responses of shore crabs to physiological challenges following exposure to selected environmental contaminants". *Aquatic Toxicology*, 40(1), 79-92, 1997.
[80] Sonnenschein C, Soto A. "An updated review of environmental estrogen and androgen mimics and antagonists". *The Journal of Steroid Biochemistry and Molecular Biology*, 65(1-6), 143-150, 1998.
[81] Gröner F, Höhne C, Kleiner W, Kloas W. "Chronic diclofenac exposure affects gill integrity and pituitary gene expression and displays estrogenic activity in nile tilapia (*Oreochromis niloticus*)". *Chemosphere*, 166(1), 473-481, 2017.
[82] Klopčič I, Markovič T, Mlinarič-Raščan I, Sollner Dolenc M. "Endocrine disrupting activities and immunomodulatory effects in lymphoblastoid cell lines of diclofenac, 4-hydroxydiclofenac and paracetamol". *Toxicology Letters*, 294(13), 95-104, 2018.
[83] European Commission. "Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council (notified under document C(2015) 1756) Text with EEA relevance". http://data.europa.eu/eli/dec_impl/2015/495/oj (08.03.2022).
[84] Stierlin H, Faigle JW, Sallmann A, Kung W, Richter WJ, Kriemler HP, Alt KO, Winkler T. "Biotransformation of diclofenac sodium (Voltaren®) in animals and in man". *Xenobiotica*, 9(10), 601-610, 1979.
[85] Stülten D, Lamshoft M, Zuhlke S, Spiteller M. "Isolation and characterization of a new human urinary metabolite of diclofenac applying LC-NMR-MS and high-resolution mass analyses". *Journal of Pharmaceutical and Biomedical Analysis*, 47(2), 371-376, 2008.
[86] Stierlin H, Faigle JW. "Biotransformation of diclofenac sodium (Voltaren) in animals and in man. II. Quantitative determination of the unchanged drug and principal phenolic metabolites, in urine and bile". *Xenobiotica*, 9(10), 611-621, 1979.
[87] Zhang Y, Han YH, Putluru SP, Matta MK, Kole P, Mandlekar S, Furlong MT, Liu T, Iyer RA, Marathe P, Yang Z, Lai Y, Rodrigues AD. "Diclofenac and Its Acyl Glucuronide: Determination of In Vivo Exposure in Human Subjects and Characterization as Human Drug Transporter Substrates In Vitro". *Drug Metabolism and Disposition*, 44(3), 320-328, 2016.
[88] Wiesenberg-Boettcher I, Pfeilschifter J, Schweizer A, Sallmann A, Wenk P. "Pharmacological properties of five diclofenac metabolites identified in human plasma". *Agents Actions*, 34(1-2), 135-137, 1991.
[89] Wilkinson J, Hooda PS, Barker J, Barton S, Swinden J. "Occurrence, fate and transformation of emerging contaminants in water: An overarching review of the field". *Environmental Pollution*, 231(1), 954-970, 2017.
[90] Zhang N, Liu G, Liu H, Wang Y, He Z, Wang G. "Diclofenac photodegradation under simulated sunlight: Effect of different forms of nitrogen and Kinetics". *Journal of Hazardous Materials*, 192(1), 411-418, 2011.
[91] Sokół A, Borowska K, Karpińska J. "Investigating the ınfluence of some environmental factors on the stability of paracetamol, naproxen, and diclofenac in simulated natural conditions". *Polish Journal of Environmental Studies*, 26(1), 293-302, 2017.
[92] Zhang N, Li JM, Liu GG, Chen XL, Jiang K. "Photodegradation of diclofenac in aqueous solution by simulated sunlight irradiation: kinetics, thermodynamics and pathways". *Water Science and Technology*, 75(9), 2163-2170, 2017.
[93] Moore DE, Roberts-Thomson S, Zhen D, Duke CC. "Photochemical studies on the antiinflammatory drug diclofenac". *Photochemistry and Photobiology*, 52(4), 685-690, 1990.
[94] Nguyen LN, Nghiem LD, Pramanik BK, Oh S. "Cometabolic biotransformation and impacts of the anti-inflammatory drug diclofenac on activated sludge microbial communities". *Science of the Total Environment*, 657(12), 739-745, 2019.
[95] Kosjek T, Heath E, Pérez S, Petrović M, Barceló D. "Metabolism studies of diclofenac and clofibric acid in activated sludge bioreactors using liquid chromatography with quadrupole-time-of-flight mass spectrometry". *Journal of Hydrology*, 372(1), 109-117, 2009.
[96] Poirier-Larabie S, Segura PA, Gagnon C. "Degradation of the pharmaceuticals diclofenac and sulfamethoxazole and their transformation products under controlled environmental conditions". *Science of the Total Environment*, 557-558(15), 257-267, 2016.
[97] Barbieri M, Carrera J, Ayora C, Sanchez-Vila X, Licha T, Nödler K, Osorio V, Pérez S, Köck-Schulmeyer M, López de Alda M, Barceló D. "Formation of diclofenac and sulfamethoxazole reversible transformation products in aquifer material under denitrifying conditions: Batch experiments". *Science of the Total Environment*, 426(11), 256-263, 2012.
[98] Lahti M, Oikari A. "Microbial transformation of pharmaceuticals naproxen, bisoprolol, and diclofenac in aerobic and anaerobic environments". *Archives of Environment Contamination and Toxicology*, 61(2), 202-210, 2011.
[99] Jayasinghe C, Imtiaj A, Lee GW, Im KH, Hur H, Lee MW, Yang HS, Lee TS. "Degradation of three aromatic dyes by white rot fungi and the production of ligninolytic enzymes". *Mycobiology*, 36(2), 114-120, 2008.
[100] McMullan G, Meehan C, Conneely A, Kirby N, Robinson T, Nigam P, Banat I, Marchant R, Smyth W. "Microbial decolourisation and degradation of textile dyes". *Applied Microbiology and Biotechnology*, 56(1), 81-87, 2001.
[101] Swamy J, Ramsay JA. "The evaluation of white rot fungi in the decoloration of textile dyes". *Enzyme and Microbial Technology*, 24(3), 130-137, 1999.
[102] Bhatt M, Cajthaml T, Šašek V. "Mycoremediation of PAH-contaminated soil". *Folia Microbiologica*, 47(3), 255-258, 2002.
[103] Field JA, de Jong E, Feijoo Costa G, de Bont JA. "Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white rot fungi". *Applied and Environmental Microbiology*, 58(7), 2219-2226, 1992.

[104]Zhang H, Zhang S, He F, Qin X, Zhang X, Yang Y. "Characterization of a manganese peroxidase from white-rot fungus Trametes sp.48424 with strong ability of degrading different types of dyes and polycyclic aromatic hydrocarbons". *Journal of Hazardous Materials*, 320(20), 265-277, 2016.

[105]Shreve MJ, Brockman A, Hartleb M, Prebihalo S, Dorman FL, Brennan RA. "The white-rot fungus *Trametes versicolor* reduces the estrogenic activity of a mixture of emerging contaminants in wastewater treatment plant effluent". *International Biodeterioration & Biodegradation*, 109(4), 132-140, 2016.

[106]Yang S, Hai FI, Nghiem LD, Nguyen LN, Roddick F, Price WE. "Removal of bisphenol A and diclofenac by a novel fungal membrane bioreactor operated under non-sterile conditions". *International Biodeterioration & Biodegradation*, 85(10), 483-490, 2013.

[107]Asif MB, Hai FI, Singh L, Price WE, Nghiem LD. "Degradation of Pharmaceuticals and Personal Care Products by White-Rot Fungi-a Critical Review". *Current Pollution Reports*, 3(2), 88-103, 2017.

[108]Marco-Urrea E, Pérez-Trujillo M, Cruz-Morató C, Caminal G, Vicent T. "White-rot fungus-mediated degradation of the analgesic ketoprofen and identification of intermediates by HPLC-DAD–MS and NMR". *Chemosphere*, 78(4), 474-481, 2010.

[109]Marco-Urrea E, Pérez-Trujillo M, Vicent T, Caminal G. "Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by *Trametes versicolor*". *Chemosphere*, 74(6), 765-772, 2009.

[110]Rodarte-Morales AI, Feijoo G, Moreira MT, Lema JM. "Degradation of selected pharmaceutical and personal care products (PPCPs) by white-rot fungi". *World Journal of Microbiology and Biotechnology*, 27(8), 1839-1846, 2011.

[111]Schmidt S, Hoffmann H, Garbe LA, Schneider RJ. "Liquid chromatography-tandem mass spectrometry detection of diclofenac and related compounds in water samples". *Journal of Chromatography A*, 1538(8), 112-116, 2018.

[112]Suarez S, Reif R, Lema JM, Omil F. "Mass balance of pharmaceutical and personal care products in a pilot-scale single-sludge system: influence of T, SRT and recirculation ratio". *Chemosphere*, 89(2), 164-171, 2012.

[113]Bouju H, Nastold P, Beck B, Hollender J, Corvini PFX, Wintgens T. "Elucidation of biotransformation of diclofenac and 4'hydroxydiclofenac during biological wastewater treatment". *Journal of Hazardous Materials*, 301(1), 443-452, 2016.

[114]Zhou H, Liu J, Chen X, Ying Z, Zhang Z, Wang M. "Fate of pharmaceutically active compounds in sewage sludge during anaerobic digestions integrated with enzymes and physicochemical treatments". *Waste Management*, 78, 911-916, 2018.

[115]Kummerová M, Zezulka Š, Babula P, Tříska J. "Possible ecological risk of two pharmaceuticals diclofenac and paracetamol demonstrated on a model plant Lemna minor". *Journal of Hazardous Materials*, 302(2), 351-361, 2016.

Appendix A

Table S1. The chemical structures of metbolites and transformation products of Diclofenac

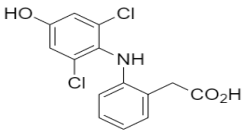
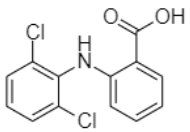
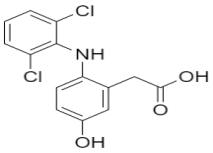
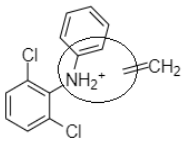
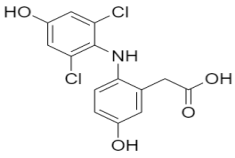
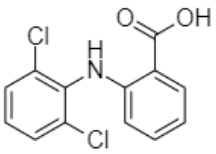
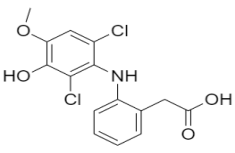
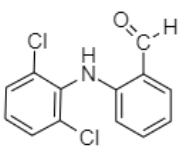
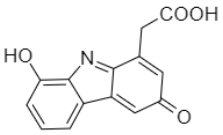
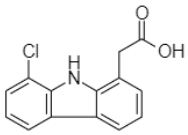
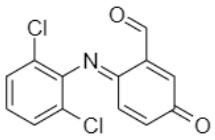
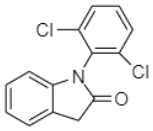
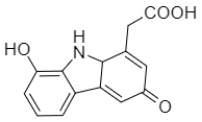
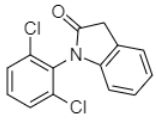
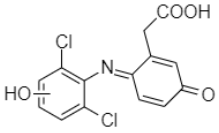
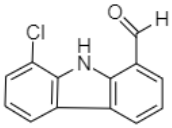
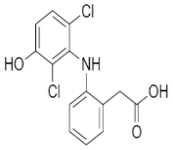
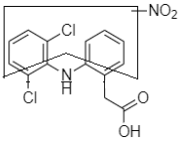
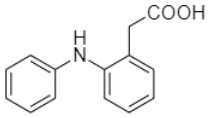
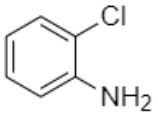
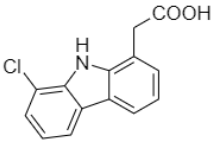
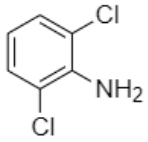
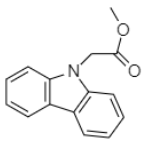
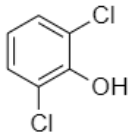
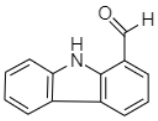
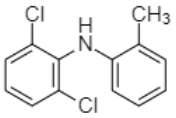
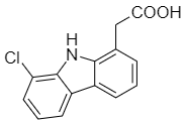
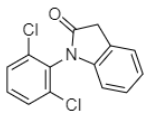
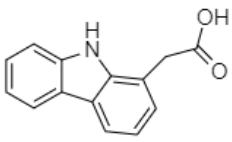
Transformation Products	Chemical Structure	Transformation Products	Chemical Structure
4'-hydroxydiclofenac (4'-OH-DCF)		DCF-benzoic acid	
5-hydroxydiclofenac (5-OH-DCF)		Contains structural fragment "2,6-dichloro-N-(phenyl)aniline Position of CH_2 group not defined	
4',5-dihydroxydiclofenac (4',5-diOH-DCF)		Carboxydiclofenac (C-DCF)	
3'-hydroxy-4'-methoxydiclofenac (3'-OH-4'-OCH_3-DCF)		2-(2,6-dichlorophenylamino)benzaldehyde	

Table S1. Continued.

Transformation Products	Chemical Structure	Transformation Products	Chemical Structure
2-(8-hydroxy-3-oxo-3H-carbazol-1-yl)acetic acid		8-chlorocarbazole-1-yl-ethanoic acid	
(E)-6-[2,6-dichlorophenyl)-imino]-3-oxocyclohexa-1,4-dienecarbaldehyde)		1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indole-2-one	
2-(8-hydroxy-3-oxo-9,9a-dihydro-3H-carbazol-1-yl)acetic acid		DCF-lactam	
(E)-2-[3-(2,6-dichloro-?-hydroxyphenylimino)-6-oxocyclohexa-1,4-dienyl]acetic acid		8-chloro-9H-carbazole-1-carbaldehyde	
3'-hydroxydiclofenac (3'-OH-DCF)		Nitro-Diclofenac (NO_2-DCF)	
2-[2-(phenylamino]phenyl)acetic acid		2-chloroaniline	
2-(8-chloro-9H-carbazol-1-yl)acetic acid		2,6-dichloroaniline	
methyl 2-(9H-carbazol-9-yl)acetate		2,6-dichlorophenol	
9H-carbazole-1-carbaldehyde		2,6-dichloro-N-o-tolybenzenamine	
2-(8-chloro-9H-carbazol-1-yl)acetic acid		1-(2,6-dichlorophenyl)indolin-2-one	
carbazole-1-acetic acid		2-(2-chlorophenylamino)benzaldehyde