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Non-Steroidal Anti-Inflammatory Drugs in Water

Emerging Contaminants and Ecological Impact

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Volume 96

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Non-Steroidal Anti-Inflammatory Drugs in Water

Emerging Contaminants and Ecological Impact

Volume Editor: Leobardo Manuel Gómez-Oliván

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Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last four decades, as reflected in the more than 150 volumes of *The Handbook of Environmental Chemistry*, there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry*, the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of

"pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via www.springerlink.com/content/110354/. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Series Editors

Preface

Pharmaceuticals are designed to persist and perform their therapeutic action and, consequently, once they enter the aquatic environment, they persist in it, damaging the health of organisms living in these ecosystems and even human health. Therefore, these products are currently of worldwide environmental concern and have been called "emerging contaminants". The latter term includes non-steroidal anti-inflammatory drugs (NSAIDs), a heterogeneous group of pharmaceuticals with anti-inflammatory, analgesic and antipyretic properties, which act as selective inhibitors of the enzyme cyclooxygenase (COX), inhibiting cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) which are responsible for the production of prostaglandins, prostacyclins and thromboxanes.

Some NSAIDs do not degrade in the environment, others are degradable but at a very slow pace and although some others are not persistent in the environment, they can be transformed through natural processes. The continuous introduction of this type of products from various sources (municipal, hospital and industrial effluents) prolongs and maintains its presence in the waters increasing any possible impact on aquatic life.

The appearance of NSAIDs in water represents a high risk to the environment for many reasons. The main one is that they contain active ingredients that were designed to induce specific pharmacological effects in humans but, when they dissolve in water, can reach non-target populations such as fish, amphipods, amphibians, among others, which produces toxicological effects. Diverse studies have reported NSAID-induced toxicity in aquatic organisms since these organisms are more susceptible to toxic effects due to their continued exposure to wastewater discharges throughout the life cycle.

In the chapters included in this book are indicated data about fate, occurrence, toxicological findings identified by the presence of NSAIDs in various aquatic organisms of economic and ecological interest. Also are included avant-garde technologies for the removal of NSAIDs and the regulatory framework for the presence of these drugs in the world.

The authors are well-known researchers from Mexico, Spain, Portugal, Italy, Australia and India and make exhaustive reviews and show the findings identified in their investigations related to NSAIDs occurrence, toxicity characterization using different biomarkers, as well as showing some technologies for the removal of NSAIDs and legal framework of the NSAIDs around the world. This compilation of research in world countries allows us to have a very specific vision of the specific water problem by NSAIDs and consider solution proposals.

The authors and I hope that our book complies with the diverse and generalized expectations and needs for information about the contamination problem in the world by NSAIDs.

I thank all the authors of this book for their professional expertise and thoroughness in writing up their chapters; the Universidad Autónoma del Estado de México for the unending support it has shown as my employing entity; my research group; and my family, most especially my mother, Aida Oliván, and friends for the enthusiasm and support they have always shown.

Toluca, Mexico Leobardo Manuel Gómez-Oliván

Contents

Introduction and Historical Findings That Focused Nonsteroidal Anti-Inflammatory Drugs as Emerging Pollutant

Gustavo Axel Elizalde-Velázquez and Leobardo Manuel Gómez-Oliván

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Abstract Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most consumed pharmaceuticals worldwide due to their significant anti-inflammatory and antipyretic properties. These drugs are mainly excreted from the body in their metabolized form and may enter into the environment through different pathways. In wastewater treatment plants (WWTPs), these contaminants are mainly removed by biological treatment processes. However, even after these treatments, high concentrations of these drugs have been found in WWTPs effluents, surface water, and

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drinking water. NSAIDs are likely to bioaccumulate in aquatic organisms such as *Mytilus galloprovincialis*. Furthermore, toxic effects such as oxidative stress, developmental abnormalities, hepatotoxicity, immunosuppressive effects, and hematological alterations have been found in several freshwater species exposed to these pollutants. Therefore, NSAIDs are a threat to the human being as well as to our environment. This review comprehensively discusses the worldwide consumption of NSAIDs, their occurrence in the aquatic environments, and the toxic effects produced by these drugs in nontarget organisms. This is to raise awareness of the negative consequences of their occurrence in freshwater ecosystems and promote the creation of new alternatives for their removal from water.

Keywords Fate, NSAIDs, Occurrence, Toxic effects

1 Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are an important heterogeneous group of drugs prescribed to treat inflammation, pain, and fever [1]. Their therapeutic effects are mainly mediated by the inhibition of cyclooxygenase isoforms, COX-1 and COX-2, involved in the synthesis of different prostaglandins from arachidonic acid [2]. Although, several studies have informed about the high toxicity and side effects of these drugs in humans, NSAIDs are among the most consumed drugs worldwide. Furthermore, it is expected their consumption steadily increases in the forthcoming years, as the prevalence of painful conditions, such as osteoarthritis and inflammatory diseases, will also likely to increase [3].

The growing consumption of NSAIDs reflects the ubiquitous occurrence of these pharmaceuticals in the aquatic environment. Numerous studies have reported the presence of these pollutants in several water bodies at concentrations ranging from ng/L to μg/L [4, 5]. This is a problem of global concern as NSAIDs tend to accumulate and produce oxidative stress in aquatic species [6–8].

Since NSAIDs are often found at high concentrations in the aquatic environment and can exhibit different toxicities in nontarget organisms, a timely review seems appropriate. The aim of this work was to comprehensively investigate the occurrence of NSAIDs in the aquatic environment and discuss the toxic effects of NSAIDs toward different aquatic organisms.

2 Pathways to the Environment: Life Cycle of NSAIDs

The production of NSAIDs in the pharmaceutical industries may lead to their direct discharge into liquid or solid waste systems. The manufactured NSAIDs are transported and distributed to hospital pharmacies, which are prescribed for treating musculoskeletal disorders, headaches, dysmenorrhea, and postoperative pain. Table 1 summarized the NSAIDs consumption data of some countries in terms of prescriptions issued. Acetylsalicylic acid (ASA) and paracetamol (PCT) lead the list of the most prescribed NSAIDs. This could be explained due to their significant benefits in a variety of indications. For instances, current evidence suggests ASA is helpful for primary prevention of cardiovascular disease, primary prevention of preeclampsia, and secondary prevention of colorectal adenomas [17, 18]. On the other hand, PCT is the drug of choice in patients that cannot be treated with other NSAIDs, such as people with bronchial asthma, peptic ulcer disease, hemophilia, and salicylate-sensitized people. Furthermore, it is recommended as a first-line treatment of pain associated with osteoarthritis [19].

Once administered NSAIDs are mainly excreted from the body in their metabolized form. Table 2 summarized the main metabolites of the NSAIDs found in human urine. Since the elimination of NSAIDs depends largely on hepatic biotransformation, less than five percent of the dose is renal excreted in unchanged form. Finally, this waste material is collected in wastewater treatment plants (WWTPs) and either directly discharged untreated into the environment or passed through one or more treatment steps before being discharged as effluent into the natural aquatic environment.

3 Occurrence of NSAIDs

The occurrence of NSAIDs in the aquatic environment was firstly reported in 1985, when Richardson and Bowron found ASA in multiple wastewater effluents of England. Thirteen years later, Ternes [32] reported the occurrence of several NSAIDs in multiple German WWTPs and rivers. Table 2 summarizes the data collected from literature regarding the occurrence of NSAIDs about their presence in the aquatic environment. Data collected have dates between 1985 and 2017 and will be discussed in the subsequent sections (Table 3).

3.1 Wastewater

Metcalfe et al. [33] analyzed influent and effluent samples from 18 WWTPs of Canada. Their results demonstrated ASA, naproxen (NPX), and ibuprofen (IBF)

Table 1 Prescriptions of NSAIDs in 2017

NSAIDs	Country	Prescriptions per year	Source
Acetylsalicylic acid	USA	[a]19,753,190	MEPS [9]
	England	20,152,891	Prescribing and Medicines Team Health and Social Care Information Centre [10]
	North Ireland	886,747	Mulholland [11]
	Wales	1,814,859	National Statistics Ystadegau Gwladol [12]
	Scotland	2,014,623	Information Services Division National Services Scotland [13]
	Netherlands	7,408,200	Zorginstituut Nederland [14]
	Sweden	2,951,829	Socialstyrelsen [15]
Paracetamol	USA	[a]29,325,845	MEPS [9]
	England	20,152,891	Prescribing and Medicines Team Health and Social Care Information Centre [10]
	North Ireland	640,274	Mulholland [11]
	Wales	1,302,422	National Statistics Ystadegau Gwladol [12]
	Scotland	2,507,530	Information Services Division National Services Scotland [13]
	Denmark	142,346	Sundhedsdata-Styrelsen [16]
	Netherlands	1,800,700	Zorginstituut Nederland [14]
	Sweden	5,030,429	Socialstyrelsen [15]
Ibuprofen	USA	[a]21,329,751	MEPS [9]
	North Ireland	162,647	Mulholland [11]
	Scotland	530,917	Information Services Division National Services Scotland [13]
	Denmark	55,790	Sundhedsdata-Styrelsen [16]
	Netherlands	756,420	Zorginstituut Nederland [14]
	Sweden	316,633	Socialstyrelsen [15]
Naproxen	USA	[a]11,470,076	MEPS [9]
	North Ireland	377,669	Mulholland [11]
	Scotland	854,887	Information Services Division National Services Scotland [13]
	Denmark	4,773	Sundhedsdata-Styrelsen [16]
	Netherlands	1,232,500	Zorginstituut Nederland [14]
	Sweden	668,604	Socialstyrelsen [15]
Diclofenac	USA	[a]9,907,530	MEPS [9]
	North Ireland	123,195	Mulholland [11]
	Scotland	196,215	Information Services Division National Services Scotland [13]
	Denmark	39,987	Sundhedsdata-Styrelsen [16]

(continued)

Table 1 (continued)

NSAIDs	Country	Prescriptions per year	Source
	Netherlands	1,961,000	Zorginstituut Nederland [14]
	Sweden	439,793	Socialstyrelsen [15]
Ketoprofen	North Ireland	29,692	Mulholland [11]
	Scotland	1,111	Information Services Division National Services Scotland [13]
	Sweden	140,353	Socialstyrelsen [15]
Mefenamic acid	North Ireland	19,138	Mulholland [11]
	Scotland	31,941	Information Services Division National Services Scotland [13]
Indomethacin	Scotland	12,837	Information Services Division National Services Scotland [13]
	Sweden	861	Socialstyrelsen [15]

[a]Prescriptions in 2016

which were the most consumed NSAIDs, reaching maximum concentrations of up 874 μg/L in the influents and 59.6 μg/L in the effluents.

Other countries with high concentrations of NSAIDs in WWTPs influents and effluents are the USA, Korea, China, and Spain. In the USA, [34] quantified multiple NSAIDs in four different removal steps of three WWTPs. According to their results, NPX and IBF were the most dominant pharmaceuticals in the influents samples. Furthermore, they also demonstrated that the majority influent load of these pharmaceuticals was removed during secondary treatments. On the other hand, in Korea, Sim et al. [35] measured multiple pharmaceuticals in ten municipal WWTPs, one hospital WWTP, and five rivers. Their results agreed with those found by Thomas and Foster and concluded that the most efficient mechanism for the removal of NSAIDs is the biological treatment processes. This is because PTC, ASA, IBF, and NPX showed relatively high removal rates during these processes.

Finally, Huang et al. [56] investigated the behavior and fate of five NSAIDs in two WWTPs located in South China. In their results, biodegradation was also the main elimination mechanism for the removal of NSAIDs. Furthermore, they also demonstrated diclofenac (DCF) which was mainly degraded anaerobically, whereas ASA, NPX, IBF, and indomethacin (IDM) were largely eliminated under aerobic conditions.

3.2 Surface Water

Most of the countries have reported low concentrations of NSAIDs in WWTPs effluents. However, high concentrations of these pharmaceuticals have been found in

Table 2 Main metabolites of NSAIDs

Drug	Metabolites	Source
Phenazone	4-Hydroxyantipyrine Norantipyrine 3-Hydroxyantipyrine	Eichelbaum et al. [20]
Paracetamol	Paracetamol glucuronide Paracetamol sulfate 3-Methoxy paracetamol N-Acetyl-p-benzo quinoneimine Cysteine paracetamol Mercapturate paracetamol	Forrest et al. [21]
Acetylsalicylic acid	Salicyluric acid Salicyl phenolic glucuronide Salicylic acid Salicyl acyl glucuronide Gentisic acid	Reidl [22]
Fenoprofen	4′-Hydroxyfenoprofen Fenoprofen acyl glucuronide	Volland et al. [23]
Naproxen	6-O-desmethylnaproxen 6-O-desmethylnaproxen acyl glucuronide Naproxen acyl glucuronide	Davies and Anderson [24]
Diclofenac	3′-Hydroxydiclofenac 3′-Hydroxy-4′-methoxydiclofenac 4′-Hydroxydiclofenac 4′,5-Dihydroxydiclofenac 5-Hydroxydiclofenac Diclofenac acyl glucuronide p-Benzoquinone imine of 5-hydroxydiclofenac	Davies and Anderson [25], Tang [26]
Ibuprofen	1-Hydoxyibuprofen 2-Hydroxyibuprofen 3-Hydroxyibuprofen Carboxyibuprofen Ibuprofen acyl glucuronide	Davies [27]
Indomethacin	O-Desmethylindomethacin N-Deschlorobenzoylindomethacin O-Desmethyl-N-deschlorobenzoylindomethacin	Nakajima et al. [28]
Nimesulide	2-(4′-hydroxyphenoxy)-4-nitro-methanesulfonanilide 2-(4′-hydroxyphenoxy)-4-N-acetylamino-methanesulfonanilide 2-Phenoxy-4-amino-methanesulfonanilide 2-(4′-hydroxyphenoxy)-4-amino-methanesulfonanilide 2-Phenoxy-4-N-acetylamino-methanesulfonanilide	Bernareggi [29]
Mefenamic acid	Mefenamyl-S-acyl-glutathione thioester Mefenamyl-1-β-O-acyl glucuronide Mefenamyl-S-acyl-CoA thioester	Grillo et al. [30]

(continued)

Table 2 (continued)

Drug	Metabolites	Source
Ketoprofen	2-[3-(4-hydroxybenzoyl)-phenyl]-propanoic acid 2-[(3-hydroxy(phenyl)methyl)-phenyl]-propanoic acid 2-[3-hydroxy(phenyl)methyl)-phenyl]-propanoic acid Ketoprofen acyl glucuronide	Skordi et al. [31]

surface waters. Heath et al. [45] investigated and compared the influence of different filter materials on the analysis of multiple NSAIDs samples from a Spain river water. Although the main scope of this study was to determine possible sources of variation, researchers reported IBF reached concentrations of up 11.89 μg/L in that river.

Other NSAIDs, such as NPX and ASA, have been also found in concentrations of environmental relevance. For instance, Selke et al. [5] quantified NPX in eight different surface water samples of Pakistan. According to their results, NPX reached maximum concentrations of 32 μg/L in one major river. Researchers explained this was due to eight different pharmaceutical manufacturers which did not treat their effluents before entering the river.

In Canada, Brun et al. [4] investigated the occurrence of multiple pharmaceuticals in WWTP effluents and receiving waters of the four Atlantic Canadian Provinces. Their results demonstrated ASA persisted in samples as far as 17 km downstream from the WWTPs, with concentrations ranging from 0.057 to 17 μg/L.

3.3 Drinking Water and Groundwater

Groundwater constitutes 97% of the global freshwater and is the single most important supply for the production of drinking water. Therefore, it is vital that the quality of groundwater must be protected. However, compared with the thoroughly research undertaken to assess the occurrence of NSAIDs in surface water, there is a lack of interest in evaluating their presence in groundwater.

Spain, France, Iran, Germany, and Norway are the only countries that have reported the occurrence of NSAIDs in drinking water. The highest concentrations of these drugs in this vital resource were found in Spain, when Heath et al. [45] demonstrated NPX, DCF, IBF, and ketoprofen (KTP) which were present in concentrations of over 0.500 μg/L.

Table 3 Worldwide occurrence of NSAIDs

Concentrations in µg/L								
NSAIDs	Country	WWTP influent (min.–max.)	WWTP effluent (min.–max.)	Surface water (min.–max.)	Drinking water (min.–max.)	Sludge (µg/g) (min.–max.)	Groundwater (min.–max.)	Source
Naproxen	Germany	n.a	ND–0.52	ND–0.39	n.a	n.a	n.a	Ternes [32]
	Brazil	n.a	n.a	0.02–0.05	n.a	n.a	n.a	Stumpf et al. [36]
	Canada	n.a	0.019–1.048	0.037–0.551	n.a	n.a	n.a	Metcalfe et al. [37]
	Canada	13–611	7.2–33.9	n.a	n.a	n.a	n.a	Metcalfe et al. [33]
	Switzerland	n.a	ND–3.3	n.a	n.a	n.a	n.a	Tixier et al. [38]
	Germany	ND–0.732	ND–0.261	n.a	n.a	n.a	n.a	Quintana and Reemtsma [39]
	Finland	0.007–0.008	n.a	n.a	n.a	n.a	n.a	[40]
	USA	10.3–12.8	0.023–0.031	n.a	n.a	n.a	n.a	Thomas and Foster [34]
	Slovenia	n.a	n.a	ND–0.080	n.a	n.a	n.a	Kosjek et al. [41]
	Canada	n.a	0.220–14	0.036–4.5	n.a	n.a	n.a	Brun et al. [4]
	France	n.a	n.a	< 0.002–0.275	n.a	n.a	n.a	Togola and Budzinski [42]
	Spain	n.a	0.018–2.140	0.053–2.446	n.a	n.a	n.a	Farré et al. [43]
	France	n.a	0.042–0.289	ND–0.091	ND–2×10^{-4}	n.a	n.a	Togola and Budzinski [44]
	Spain	n.a	0.317–1.030	0.609–2.993	0.026–1.014	n.a	n.a	Heath et al. [45]
	Pakistan	n.a	n.a	11.4–32	n.a	n.a	n.a	Selke et al. [5]
	Taiwan	n.a	n.a	0.035–0.270	n.a	n.a	n.a	Lin et al. [46]

	South Korea	n.a	n.a	0.005–0.100	n.a	n.a	n.a	Yoon et al. [47]
	Greece	0.219–0.302	0.021–0.253	n.a	n.a	n.a	n.a	Samaras et al. [48]
	Sweden	n.a	n.a	n.a	n.a	0.136–0.140	n.a	Sagristà et al. [49]
	Hungary	n.a	n.a	0.005–0.062	n.a	n.a	n.a	Helenkar et al. [50]
	Singapore	n.a	n.a	0.013–0.030	n.a	n.a	n.a	Wu et al. [51]
	Korea	0.278–1.21	0.048–0.254	0.012–0.013	n.a	n.a	n.a	Sim et al. [35]
	Spain	2.01–2.23	1.34–1.52	n.a	n.a	n.a	n.a	Villar Navarro et al. [52]
	Singapore	n.a	n.a	0.008–0.108	n.a	n.a	n.a	Xu et al. [53]
	Poland	n.a	n.a	ND–0.753	n.a	n.a	n.a	Baranowska and Kowalski [54]
	Spain	n.a	n.a	ND–0.109	n.a	n.a	n.a	Da Silva et al. [55]
	China	n.a	n.a	ND–0.074	n.a	n.a	n.a	Huang et al. [56]
	Spain	n.a	n.a	ND–0.006	n.a	n.a	n.a	Gros et al. [57]
	USA	n.a	n.a	ND–0.026	n.a	n.a	n.a	Vidal-Dorsch et al. [58]
	Spain	ND–0.75	ND–0.109	n.a	n.a	n.a	n.a	Rodil et al. [59]
	Sweden	n.a	n.a	ND–0.447	n.a	n.a	n.a	Daneshvar et al. [60]
	Japan	n.a	n.a	ND–0.240	n.a	n.a	n.a	Komori et al. [61]
	Turkey	n.a	n.a	0.002–12.3	n.a	n.a	n.a	Aydin and Talinli [62]
	Italy	n.a	n.a	0.200–0.264	n.a	n.a	n.a	Patrolecco et al. [63]

(continued)

Table 3 (continued)

Concentrations in µg/L								
NSAIDs	Country	WWTP influent (min.–max.)	WWTP effluent (min.–max.)	Surface water (min.–max.)	Drinking water (min.–max.)	Sludge (µg/g) (min.–max.)	Groundwater (min.–max.)	Source
	India	n.a	n.a	ND–0.028	n.a	n.a	n.a	Shanmugam et al. [64]
	Portugal	n.a	n.a	ND–0.178	n.a	n.a	n.a	Lolić et al. [65]
	Iran	0.088–0.430	0.033–0.054	0.029–0.041	0.037–0.039	n.a	n.a	Eslami et al. [66]
	South Africa	n.a	n.a	0.193–1.631	n.a	n.a	n.a	Archer et al. [67]
	Czech Republic	n.a	n.a	2.2×10^{-3}–1.689	n.a	n.a	n.a	Marsik et al. [68]
Diclofenac	Switzerland	0.470–1.920	0.310–0.930	$<1 \times 10^{-3}$–0.370	n.a	n.a	n.a	Buser et al. [69]
	Germany	n.a	ND–2.1	ND–1.20	n.a	n.a	n.a	Ternes [32]
	Brazil	n.a	n.a	0.02–0.06	n.a	n.a	n.a	Stumpf et al. [36]
	Canada	n.a	0.004–0.061	0.017–0.194	n.a	n.a	n.a	Metcalfe et al. [37]
	Canada	ND–1.3	n.a	n.a	n.a	n.a	n.a	Metcalfe et al. [33]
	Germany	ND–2.333	ND–1.561	ND–0.272	n.a	n.a	n.a	Quintana and Reemtsma [39]
	Germany	n.a	n.a	0.015–0.025	<0.005–0.035	n.a	n.a	Heberer et al. [70]
	USA	0.33–0.49	n.a	n.a	n.a	n.a	n.a	Thomas and Foster [34]
	Slovenia	n.a	n.a	ND–0.282	n.a	n.a	n.a	Kosjek et al. [41]

	France	n.a	n.a	0.071–0.172	n.a	n.a	n.a	Togola and Budzinski [42]
	UK	n.a	n.a	ND–< 1.2×10^{-4}	n.a	n.a	n.a	Nebot et al. [71]
	Japan	n.a	n.a	n.a	n.a	ND–0.035	n.a	Kimura et al. [72]
	USA	n.a	n.a	n.a	n.a	0.010–0.023	n.a	Chenxi et al. [73]
	Spain	n.a	0.092–3.343	0.129–0.767	n.a	n.a	n.a	Farré et al. [43]
	France	n.a	0.210–0.486	0.001–0.033	ND–0.002	n.a	n.a	Togola and Budzinski [44]
	Pakistan	n.a	n.a	0.700–4.4	n.a	n.a	n.a	Scheurell et al. [74]
	Spain	n.a	n.a	n.a	n.a	bql – 0.424	n.a	Radjenović et al. [75]
	Korea	0.002–0.042	0.002–0.046	n.a	n.a	n.a	n.a	Sim et al. [35]
	Singapore	n.a	n.a	0.004–0.038	n.a	n.a	n.a	Wu et al. [51]
	Taiwan	n.a	n.a	ND–0.056	n.a	n.a	n.a	Lin et al. [46]
	Greece	bql–0.117	bql–0.084	n.a	n.a	n.a	n.a	Samaras et al. [48]
	Sweden	n.a	n.a	n.a	n.a	0.034–0.044	n.a	Sagristà et al. [49]
	Spain	n.a	0.059–1.444	0.300–4.715	0.010–0.515	n.a	n.a	Heath et al. [45]
	Hungary	n.a	n.a	0.024–0.930	n.a	n.a	n.a	Helenkar et al. [50]
	Germany	n.a	n.a	ND–0.045	n.a	n.a	n.a	Meyer et al. [76]
	Spain	n.a	n.a	ND–0.148	n.a	n.a	n.a	Da Silva et al. [55]

(continued)

Table 3 (continued)

Concentrations in µg/L								
NSAIDs	Country	WWTP influent (min.–max.)	WWTP effluent (min.–max.)	Surface water (min.–max.)	Drinking water (min.–max.)	Sludge (µg/g) (min.–max.)	Groundwater (min.–max.)	Source
	China	ND–0.119	n.a	ND–0.094	n.a	n.a	n.a	Huang et al. [56]
	Poland	n.a	n.a	ND–0.429	n.a	n.a	n.a	Baranowska and Kowalski [54]
	Sweden	n.a	n.a	ND–0.286	n.a	n.a	n.a	Daneshvar et al. [60]
	Taiwan	n.a	n.a	<0.002–0.053	n.a	n.a	n.a	Fang et al. [77]
	Spain	n.a	n.a	ND–0.004	n.a	n.a	n.a	Gros et al. [57]
	Italy	n.a	n.a	ND–0.120	n.a	n.a	n.a	Patrolecco et al. [63]
	Japan	n.a	n.a	ND–0.140	n.a	n.a	n.a	Komori et al. [61]
	Turkey	n.a	n.a	bql–0.045	n.a	n.a	n.a	Aydin and Talinli [62]
	India	n.a	n.a	ND–0.103	n.a	n.a	n.a	Shanmugam et al. [64]
	Portugal	n.a	n.a	ND–0.241	n.a	n.a	n.a	Lolić et al. [65]
	Iran	0.230–0.044	0.022–0.033	ND–0.025	0.022–0.024	n.a	n.a	Eslami et al. [66]
	Czech Republic	n.a	n.a	5.9×10^{-4}–1.247	n.a	n.a	n.a	Marsik et al. [68]
	South Africa	n.a	n.a	0.291–1.970	n.a	n.a	n.a	Archer et al. [67]
	Latvia	n.a	n.a	0.008–0.009	n.a	n.a	n.a	[78]
	Norway	n.a	n.a	0.001–0.002	n.a	n.a	n.a	Reinholds et al. [78]

Ibuprofen	Germany	n.a	ND–3.4	ND–0.53	n.a	n.a	n.a	Ternes [32]
	Switzerland	0.990–3.300	0.013–0.081	0.001–0.007	n.a	n.a	n.a	Buser et al. [79]
	USA	n.a	n.a	ND–1.0	n.a	n.a	n.a	Kolpin et al. [80]
	Canada	n.a	0.077–2.051	0.007–0.790	n.a	n.a	n.a	Metcalfe et al. [37]
	Canada	14.2–75.8	0.3–24.6	n.a	n.a	n.a	n.a	Metcalfe et al. [33]
	Germany	ND–5.533	n.a	n.a	n.a	n.a	n.a	Quintana and Reemtsma [39]
	Finland	0.008–0.017	bql–0.008	n.a	n.a	n.a	n.a	Vieno et al. [40]
	USA	9.5–14.7	0.010–0.022	n.a	n.a	n.a	n.a	Thomas and Foster [34]
	Canada	n.a	0.037–22	0.075–6.4	n.a	n.a	n.a	Brun et al. [4]
	Romania	n.a	n.a	ND–0.115	n.a	n.a	n.a	Moldovan [81]
	France	n.a	n.a	<0.002–0.610	n.a	n.a	n.a	Togola and Budzinski [42]
	UK	n.a	n.a	ND–< 0.052	n.a	n.a	n.a	Nebot et al. [71]
	France	n.a	0.017–0.219	ND–0.004	ND–6×10^{-4}	n.a	n.a	Togola and Budzinski [44]
	Spain	n.a	0.233–2.951	0.022–1.067	n.a	n.a	n.a	Farré et al. [43]
	South Korea	n.a	n.a	ND–0.414	n.a	n.a	n.a	Kim et al. [82]
	Korea	0.213–1.50	ND–0.238	0.029–0.051	n.a	n.a	n.a	Sim et al. [35]
	Singapore	n.a	n.a	0.041–0.121	n.a	n.a	n.a	Wu et al. [51]
	Hungary	n.a	n.a	0.003–0.050	n.a	n.a	n.a	Helenkar et al. [50]
	Spain	n.a	0.433–2.633	2.358–11.891	0.029–0.571	n.a	n.a	Heath et al. [45]
	Taiwan	n.a	n.a	ND–4.350	n.a	n.a	n.a	Lin et al. [46]

(continued)

Table 3 (continued)

Concentrations in µg/L								
NSAIDs	Country	WWTP influent (min.–max.)	WWTP effluent (min.–max.)	Surface water (min.–max.)	Drinking water (min.–max.)	Sludge (µg/g) (min.–max.)	Groundwater (min.–max.)	Source
	Sweden	n.a	n.a	n.a	n.a	0.115–0.129	n.a	Sagristà et al. [49]
	Greece	0.096–0.403	5×10^{-3}–0.262	n.a	n.a	n.a	n.a	Samaras et al. [48]
	Singapore	n.a	n.a	0.002–0.076	n.a	n.a	n.a	Xu et al. [53]
	Germany	n.a	n.a	ND–2.383	n.a	n.a	n.a	Meyer et al. [76]
	China	0.264–0.997	n.a	0.079–0.609	n.a	n.a	n.a	Huang et al. [56]
	Sweden	n.a	n.a	ND–0.818	n.a	n.a	n.a	Daneshvar et al. [60]
	Kenya	n.a	n.a	10–30	n.a	n.a	n.a	K'oreje et al. [83]
	Spain	ND–7.5	ND–0.264	n.a	n.a	n.a	n.a	Rodil et al. [59]
	Spain	n.a	n.a	ND–0.016	n.a	n.a	n.a	Gros et al. [57]
	Taiwan	n.a	n.a	<0.002–0.057	n.a	n.a	n.a	Fang et al. [77]
	Italy	n.a	n.a	0.095–0.210	n.a	n.a	n.a	Patrolecco et al. [63]
	Italy	n.a	n.a	$<4.9 \times 10^{-5}$–0.001	n.a	n.a	n.a	Loos et al. [84]
	Turkey	n.a	n.a	bql–0.263	n.a	n.a	n.a	Aydin and Talinli [62]
	India	n.a	n.a	ND–0.200	n.a	n.a	n.a	Shanmugam et al. [64]
	Portugal	n.a	n.a	ND–0.222	n.a	n.a	n.a	Lolić et al. [65]

	Iran	0.233–1.051	0.031–0.045	0.022–0.037	0.021–0.047	n.a	n.a	Eslami et al. [66]
	Czech Republic	n.a	n.a	6.16×10^{-3}–3.394	n.a	n.a	n.a	Marsik et al. [68]
	Latvia	n.a	n.a	0.004–0.018	n.a	n.a	n.a	Reinholds et al. [78]
	Norway	n.a	n.a	0.001–0.005	0.001–0.009	n.a	n.a	Reinholds et al. [78]
	South Africa	n.a	n.a	0.107–0.516	n.a	n.a	n.a	Archer et al. [67]
Ketoprofen	Germany	n.a	ND–0.38	ND–0.12	n.a	n.a	n.a	Ternes [32]
	Canada	n.a	0.012–0.014	0.017–0.047	n.a	n.a	n.a	Metcalfe et al. [37]
	Canada	ND–5.7	n.a	n.a	n.a	n.a	n.a	Metcalfe et al. [33]
	Germany	ND–0.321	ND–0.146	ND–0.329	n.a	n.a	n.a	Quintana and Reemtsma [39]
	Finland	0.007–0.011	bql–0.008	n.a	n.a	n.a	n.a	Vieno et al. [40]
	USA	0.41–0.52	0.023–0.080	n.a	n.a	n.a	n.a	Thomas and Foster [34]
	Canada	n.a	0.052–0.310	ND–0.079	n.a	n.a	n.a	Brun et al. [4]
	France	n.a	n.a	<0.002–0.033	n.a	n.a	n.a	Togola and Budzinski [42]
	France	n.a	0.021–1.08	ND–0.014	ND–0.003	n.a	n.a	Togola and Budzinski [44]
	Spain	n.a	0.025–1.2	0.062–0.538	n.a	n.a	n.a	Farré et al. [43]
	Greece	0.036–0.097	0.039–0.083	n.a	n.a	n.a	n.a	Samaras et al. [48]
	Sweden	n.a	n.a	n.a	n.a	0.020–0.038	n.a	Sagristà et al. [49]
	Spain	n.a	0.107–1.705	0.069–1.389	0.030–0.854	n.a	n.a	Heath et al. [45]
	Taiwan	n.a	n.a	ND–0.045	n.a	n.a	n.a	Lin et al. [46]

(continued)

Table 3 (continued)

Concentrations in µg/L								
NSAIDs	Country	WWTP influent (min.–max.)	WWTP effluent (min.–max.)	Surface water (min.–max.)	Drinking water (min.–max.)	Sludge (µg/g) (min.–max.)	Groundwater (min.–max.)	Source
	Hungary	n.a	n.a	bql–0.077	n.a	n.a	n.a	Helenkar et al. [50]
	Korea	0.018–0.226	0.006–0.041	n.a	n.a	n.a	n.a	Sim et al. [35]
	Poland	n.a	n.a	ND–0.258	n.a	n.a	n.a	Baranowska and Kowalski [54]
	Spain	n.a	n.a	ND–1.060	n.a	n.a	n.a	Da Silva et al. [55]
	Portugal	n.a	n.a	ND–0.011	n.a	n.a	n.a	Sousa et al. [85]
	Sweden	n.a	n.a	ND–0.364	n.a	n.a	n.a	Daneshvar et al. [60]
	Spain	n.a	n.a	ND–<0.008	n.a	n.a	n.a	Gros et al. [57]
	Taiwan	n.a	n.a	<0.001–0.006	n.a	n.a	n.a	Fang et al. [77]
	Italy	n.a	n.a	ND–0.150	n.a	n.a	n.a	Patrolecco et al. [63]
	Japan	n.a	n.a	ND–0.063	n.a	n.a	n.a	Komori et al. [61]
	India	n.a	n.a	ND–0.100	n.a	n.a	n.a	Shanmugam et al. [64]
	Portugal	n.a	n.a	0.010–0.089	n.a	n.a	n.a	Lolić et al. [65]
	Czech Republic	n.a	n.a	8.4×10^{-4}–1.228	n.a	n.a	n.a	Marsik et al. [68]
	South Africa	n.a	n.a	0.011–0.649	n.a	n.a	n.a	Archer et al. [67]

Acetylsalicylic acid	England	n.a	ND–1	n.a	n.a	n.a	n.a	Richardson and Bowron [86]
	Germany	n.a	ND–1.5	ND–0.34	n.a	n.a	n.a	Ternes [32]
	Canada	29–874	0.3–59.6	n.a	n.a	n.a	n.a	Metcalfe et al. [33]
	Germany	ND–0.861	ND–0.092	ND–1.098	n.a	n.a	n.a	Quintana and Reemtsma [39]
	Romania	n.a	n.a	ND–0.037	n.a	n.a	n.a	Moldovan [81]
	Canada	n.a	0.053–35	0.057–17	n.a	n.a	n.a	Brun et al. [4]
	France	n.a	0.023–0.051	n.a	n.a	n.a	n.a	Togola and Budzinski [44]
	Korea	2.61–11.1	0.006–0.078	0.015–0.148	n.a	n.a	n.a	Sim et al. [35]
	Spain	2.66–3.08	1.81–2.15	n.a	n.a	n.a	n.a	Villar Navarro et al. [52]
	Poland	n.a	n.a	ND–4.7×10^{-4}	n.a	n.a	n.a	Baranowska and Kowalski [54]
	China	9.6–18.6	0.021–0.128	0.1–65.1	n.a	n.a	n.a	Huang et al. [56]
	Spain	ND–2.4	n.a	n.a	n.a	n.a	n.a	Rodil et al. [59]
	India	n.a	n.a	6×10^{-4}–0.660	n.a	n.a	n.a	Shanmugam et al. [64]
	Portugal	n.a	n.a	ND–5.34×10^{-3}	n.a	n.a	n.a	Lolić et al. [65]
Paracetamol	Germany	n.a	ND–6.0	n.a	n.a	n.a	n.a	Ternes [32]
	USA	n.a	n.a	ND–10	n.a	n.a	n.a	Kolpin et al. [80]
	France	n.a	0.108–1.13	0.010–0.072	ND–0.210	n.a	n.a	Togola and Budzinski [44]
	Korea	3.13–41.9	ND–6.76	0.018–0.076	n.a	n.a	n.a	Sim et al. [35]
	Spain	n.a	n.a	ND–0.023	n.a	n.a	n.a	Gros et al. [57]

(continued)

Table 3 (continued)

Concentrations in µg/L								
NSAIDs	Country	WWTP influent (min.–max.)	WWTP effluent (min.–max.)	Surface water (min.–max.)	Drinking water (min.–max.)	Sludge (µg/g) (min.–max.)	Groundwater (min.–max.)	Source
	Portugal	n.a	n.a	0.051–0.584	n.a	n.a	n.a	Lolić et al. [65]
	South Africa	n.a	n.a	0.016–0.139	n.a	n.a	n.a	Archer et al. [67]
Indomethacin	Germany	n.a	ND–0.60	ND–0.20	n.a	n.a	n.a	Ternes [32]
	Canada	n.a	0.009–0.393	0.004–0.019	n.a	n.a	n.a	Metcalfe et al. [37]
	Canada	n.a	0.035–0.310	ND–0.150	n.a	n.a	n.a	Brun et al. [4]
	China	n.a	n.a	ND–0.094	n.a	n.a	n.a	Huang et al. [56]
	Spain	n.a	n.a	ND–0.003	n.a	n.a	n.a	Gros et al. [57]
	Iran	0.039–0.110	0.028–0.057	0.025–0.041	0.021–0.037	n.a	n.a	Eslami et al. [66]
	Czech Republic	n.a	n.a	9.1×10^{-4}–0.0787	n.a	n.a	n.a	Marsik et al. [68]
Fenoprofen	Canada	n.a	0.058–0.443	0.064–0.150	n.a	n.a	n.a	Metcalfe et al. [37]
	Canada	ND–9.7	n.a	n.a	n.a	n.a	n.a	Metcalfe et al. [33]
	Canada	n.a	0.015–0.190	n.a	n.a	n.a	n.a	Brun et al. [4]
Phenazone	Germany	n.a	ND–0.41	ND–0.95	n.a	n.a	n.a	Ternes [32]
	Spain	n.a	n.a	ND–0.002	n.a	n.a	n.a	Gros et al. [57]
Mefenamic acid	UK	n.a	n.a	ND–< 4×10^{-5}	n.a	n.a	n.a	Nebot et al. [71]
Nimesulide	Portugal	n.a	n.a	ND–7.33×10^{-3}	n.a	n.a	n.a	Lolić et al. [65]

n.a not available, *bql* below quantification limit, *ND* non-detected

3.4 Sludge

In extend to our knowledge to date, only Japan, the USA, Spain, and Sweden have been the only countries that have reported the presence of NSAIDs on sludge. In Sweden [49] determinated the occurrence of some NSAIDs in dried sludge. NPX, DCF, IBF, and KTP were found in concentrations of up 0.140 μg/L.

Due to the high frequency of detection and the different distribution tendencies of NSAIDs in the aquatic environment, it is recommended that the usage pattern of these pharmaceuticals on every country must be reported annually.

Although KTP and FNP are not among the most consumed NSAIDs, they also have been found in concentrations of environmental relevance. Therefore, it is important to further monitor and assess their discharges in order to reduce the loading of these compounds to sensitive water bodies.

Overall, there is a huge knowledge gap regarding the occurrence of PCT in the aquatic environments. Since PCT consumption throughout the world has increased, it is important to further investigate the occurrence and fate of this pharmaceutical in the aquatic environments.

Several studies have reported biological treatment processes are the most efficient mechanisms for the removal of NSAIDs from wastewater. However, the high concentrations of these drugs in surface waters indicate biodegradation is insufficient for their completely removal from WWTPs. Therefore, the development of new treatment techniques for the removal of NSAIDs from aquatic environments should be addressed promptly.

Finally, little information is known about the occurrence of NSAIDs in drinking water. Future works should stimulate research to understand the potential risk of these contaminants in the human health.

4 Toxic Effects

Many studies have been conducted in aquatic organisms to evaluate the toxicity of NSIADs in freshwater environments. The following section describes the toxic concerns over NSAIDs in aquatic species (Table 4).

4.1 Diclofenac

Some studies have found DCF may lead to the alteration of hematological parameters in aquatic species. Hoeger et al. [87] demonstrated hematocrit and leucocrit levels were significantly reduced in brown trout adults exposed to DCF. On the other hand, Ribas et al. [88] found DCF increased red blood cells count and reduced hemoglobin levels in trahira fed with *Astyanax* sp., previously inoculated with the

Table 4 Toxic effects produced by NSAIDs

NSAIDs	Species	Concentrations	Time	Results	Source
Diclofenac	*Salmo trutta f. fario*	0.5, 5, 50 µg/L	7 d 14 d 21 d	Ht, serum lysozyme activity ↓ Ht, Lt ↓ Serum lysozyme activity ↑ Histopathology: monocyte infiltration in the liver; telangiectasis in the gills; intersticial hyaline droplets, interstitial proteinaceous fluid, and mild tubular necrosis in the trunk kidney Immunohistology: granulocyte in gill filaments ↑; MHC II expression in the kidney ↑	Hoeger et al. [87]
	Oryzias latipes	1 mg/L	9 d	Time to eat the midge larvae ↑ Medaka did not eat the midge larvae at all	Nassef et al. [93]
	Danio rerio	1.48, 2.95, 5.9, 11.9, 23.7	72 h	From 1.5 mg/L and upward effects were yolk sac and tail deformation From 2.9 mg/L and upward effects were growth and hatching retardation NOEC = 1.5 mg/L EC_{50} = 5.3 mg/L	Van den Brandhof and Montforts [91]
	Oncorhynchus mykiss	0, 0.5, 1, 5, 25 µg/L	21 d	Concentrations of diclofenac in the bile = 315−13,364 ng/mL BAF = 509–657 COX1 mRNA, COX2 mRNA expression ↓ CYP1a1 mRNA expression in the liver and gills ↑ CYP1a1 mRNA expression in the kidney ↓ Histopathology: tubular necrosis in the kidney and hyperplasia and fusion of the villi in the intestine	Mehinto et al. [89]
	Dreissena polymorpha	95, 318, 637 ng/L	96 h	Negligible cytotoxicity and genotoxicity on zebra mussel hemocytes Negligible effect on the activities of antioxidant and detoxifying enzymes	Parolini et al. [94]

	Danio rerio	10, 32, 100, 320, 1,000, 3,200 μg/L	34 d	NOEC (hatching rate, egg development) ≥ 1,134 μg/L NOEC (survival and growth) > 320 < 1,000 μg/L	Memmert et al. [90]
	Oncorhynchus mykiss	3.2, 10, 32, 100, 320, 1,000 μg/L	95 d	BCF < 10 NOEC (hatching rate, egg development, survival and growth) ≥ 1,084 μg/L Histopathology: minimal lesions in kidney (hyaline inclusions and single cell necrosis), in the liver (inflammatory cell foci and enhanced basophilia) and gills (increasing incidences of focal proliferation)	Memmert et al. [90]
	Cyprinus carpio	0.015, 0.03, 1, 3 mg/L	30 d	GST ↑ GR, TBARS ↓ NOEC = 0.015 mg/L LOEC: 0.03 mg/L	Stepanova et al. [95]
	Danio rerio	1.01, 3.38, 10.13, 15.20 μM	4 d	Developmental abnormalities (shorter body length, smaller eye, pericardial and body edema, lack of liver and intestine, muscle degeneration, and abnormal pigmentation)	Chen et al. [92]
	Danio rerio	5, 15, 30, 60 mg/L	28 d	LOEC = 15 mg/L NOEC = 5 mg/L No histopathological changes were observed	Praskova et al. [96]
	Hoplias malabaricus	[a]0, 0.2, 2.0, 20 μg/kg	6 w	HIS ↓ Testosterone levels ↓ Liver: SOD ↑; LPO and GST ↓	Guiloski et al. [97]
	Cyprinus carpio	7.098 mg/L	4 d 24 d	HPC in blood ↓; HPC in the muscle, gills, and liver ↑ LPO in brain ↓; LPO in the gills and liver ↑ PCC in blood ↓; PCC in the gills, brain and liver ↑ SOD in blood ↓; SOD in the gills and brain ↑ CAT in the blood and liver ↓; CAT in the muscle, gills, and brain ↑ GPx in the gills and brain ↓; GPx in the blood and liver ↑	Saucedo-Vence et al. [98]

(continued)

Table 4 (continued)

NSAIDs	Species	Concentrations	Time	Results	Source
				HPC in the blood, muscle, gills, and liver ↑ LPO in the brain, gills, and liver ↑ PCC in the blood ↓; PCC in the gills, brain, and liver ↑ SOD in the blood, muscle, and brain ↓; SOD in the gills ↑ CAT in the blood and liver ↓; CAT in the muscle, gills, and brain ↑ GPx in the muscle and brain ↓; GPx in the blood, liver, and gills ↑	
	Hoplias malabaricus	[a]0, 0.2, 2.0, 20 μg/kg	6 w	Red blood cell count and hematocrit ↑ Hb concentration and polymorphonuclear leukocytes count ↓ Lipopolysaccharide-induced nitric oxide production inhibited	Ribas et al. [88]
	Mytilus galloprovincialis	25 μg/L	14 d	Tissue concentrations = 7.01–22.79 ng/g Accumulation of lipofuscin in tertiary lysosomes DNA strand breaks and micronuclei frequency ↑ LMS ↓	Mezzelani et al. [7]
Paracetamol	*Danio rerio*	1, 5, 10, 50, 100 mg/L	7 d	Developmental abnormalities (the rate of hatching decreased, shorter body length, lack of pigmentation, deformity in tail and tail fin)	David and Pancharatna [99]
	Hyalella azteca	7.7 mg/kg	12 h 24 h 48 h 72 h	LPO ↑ SOD, CAT, GPx, PCC ↓ LPO, PCC ↑ SOD, CAT, GPx ↓ LPO, PCC ↑ SOD, CAT, GPx ↓ LPO, GPx, PCC ↑ SOD, CAT ↓	Gómez-Oliván et al. [100]

	Venerupis decussata	0.05, 0.5, 5 mg/L	96 h	GST ↓ GR, TBARS ↑	Antunes et al. [101]
	Venerupis philippinarum	0.05, 0.5, 5 mg/L	96 h	GST ↑	Antunes et al. [101]
	Oncorhynchus mykiss	0.05, 0.50, 5 mg/L 12.5, 25, 50 mg/L	96 h 28 d	CAT, GPx, GR, GST, TBARS ↑ CAT, GPx, GST, TBARS ↑	Ramos et al. [102]
	Anguilla anguilla	5, 25, 125, 625, 3,125 μg/L	96 h	In the liver: GST ↑ In the gills: GST ↓; TBARS ↑	Nunes et al. [103]
	Mytilus galloprovincialis	25 μg/L	14 d	Accumulation of lipofuscin in tertiary lysosomes Micronuclei frequency ↑ LMS ↓	Mezzelani et al. [7]
	Rhamdia quelen	0.25, 2.5 μg/L	21 d	Hb, Ht, leukocytes, and thrombocytes count, E2, 5-HT, DA, PCC, SOD ↑ T, GST, EROD ↓	Guiloski et al. [104]
	Ruditapes philippinarum	0.25, 2.50, 25 μg/L	96 h	SOD, GPx, GST, LPO, GR, GLY ↑ ChE, ETS ↓	Nunes et al. [105]
	Phalloceros harpagos	8, 80, 800, 8,000, 80,000 μg/L 5, 10, 20, 40, 80 μg/L	96 h 28 d	GST, ChE ↑ No significant changes were observed	Pereira et al. [106]
Ibuprofen	*Planorbis carinatus*	0.1, 1, 10, 100 mg/L 0.32, 1, 3.2, 10 mg/L	72 h 21 d	LC_{50} = 17.1 mg/L Survival: LOEC = >5.36 mg/L; NOEC = 5.36 mg/L Hatching success: LOEC = 5.36 mg/L; NOEC = 2.43 mg/L Growth: LOEC = 2.43 mg/L; NOEC = 1.02 mg/L	Pounds et al. [107]

(continued)

Table 4 (continued)

NSAIDs	Species	Concentrations	Time	Results	Source
	Daphnia magna	1.23, 3.7, 11.1, 33.3, 100 mg/L	21 d	$NOEC_{survival}$ = 33.3 mg/L $LOEC_{reprocution}$ = 1.23 mg/L PGR ↓	Han et al. [108]
	Moina macrocopa	3.13, 6.25, 12.5, 25, 50 mg/L	7 d	$NOEC_{survival}$ = >50 mg/L $NOEC_{reprocution}$ = 25 mg/L	Han et al. [108]
	Oryzias latipes	0.01, 0.1, 1, 10, 100, 1,000 μg/L	144 d	Males: vitellogenin in plasma ↑ Females: HSI, GSI ↑ Number of eggs per brood ↑ Survival ↓ Hatching was delayed	Han et al. [108]
	Pimephales promelas	250 μg/L	28 d	Gill, liver, and muscle tissue uptake ↑ BCF: 0.7–1	Nallani et al. [109]
	Ictalurus punctatus	250 μg/L	1 w	Liver, kidney, and plasma uptake ↑ BCF: 0.08–1.4	Nallani et al. [109]
	Mytilus galloprovincialis	250 μg/L	2 w	SOD, LPO ↑ CAT, GR, GST ↓	Gonzalez-Rey and Bebianno [110]
	Ruditapes philippinarum	100, 500, 1,000 μg/L	7 d	Hemocyte proliferation, LDH ↑ THC, NR uptake ↓	Matozzo et al. [111]
	Cirrhinus mrigala	14.2 ppm	35 d	Hb, Ht, WBC, MCV, MCH, plasma glucose, AST, ALT ↑ RBC, MCHC, plasma protein ↓	Saravanan et al. [112]
	Danio rerio	0.0001, 0.05, 1, 8, 25 mg/L	28 d	GPx, GST ↑ MDA ↓	Bartoskova et al. [113]
	Cyprinus carpio	17.7 mg/L	96 h	Blood, liver, brain, gill uptake LPO, CAT, SOD, GPx ↑	Islas-Flores et al. [6]
	Mytilus galloprovincialis	25 μg/L	14 d	Tissue concentrations = 0.63–2.63 ng/g Accumulation of lipofuscin in tertiary lysosomes DNA strand breaks and micronuclei frequency ↑ LMS, GST ↓	Mezzelani et al. [7]
	Tinca tinca	60 μg/L	35 d	GPx, GST ↓	Stancova et al. [8]

	Clarias gariepinus	0.28, 0.33, 0.38, 0.43, 0.48 mg/L	96 h	LC_{50} = 0.38 mg/L Abnormal behavior: regurgitation of food, hyperactive and jerky movements, loss of equilibrium and change of skin coloration from shining to dull ash, irregular fin movements increased mucus secretions and erosion of fins RBC, Hb, PCV ↑ MCV, MCH, MCHC ↓	Ogueji et al. [114]
	Rhamdia quelen	0.1, 1, 10 μg/L	14 d	GST, GPx, AChE, plasma magnesium ↑ WBC, CA ↓	Mathias et al. [115]
Acetylsalicylic acid	*Danio rerio*	550, 560, 570, 580, 590, 600 mg/L 100, 200, 300, 400, 500, 600, 700 mg/L	96 h 96 h	LC_{50} = 567.7 mg/L LC_{50} = 274.6 mg/L GST ↑	Prášková et al. [116]
		0.004, 0.04, 40, 120, 250 mg/L	28 d	GST, GR, CAT, GPx ↑ TBARS ↓	Zivna et al. [117]
	Salmo trutta fario	25, 50, 100 μg/L	28 d	In the liver: GR, GPx ↑ Histopathology: in the kidney (aneurism, epithelial cell proliferation of the primary lamellae, epithelial lifting, necrosis of the secondary lamellae, and lamellar tip fusion); in the liver (sinusoidal capillaries dilatation, hemorrhagic, inflammatory cell infiltration, hepatocellular vacuolization, and focal areas of necrosis)	Nunes et al. [118]
	Cyprinus carpio	0.004, 0.04, 0.4, 4, 20 mg/L	34 d	Developmental abnormalities (retarded development, hyperpigmentation, lordosis, kyphosis, scoliosis, intestinal damage, body weight lower) Histopathology: alterations to the dermo-epidermal junction, increased mucous cell numbers TBARS ↑	Zivna et al. [119]

(continued)

Table 4 (continued)

NSAIDs	Species	Concentrations	Time	Results	Source
				GPx, GR, CAT, ↓ LOEC = 0.004 mg/L	
Naproxen	*Danio rerio*	1, 100 µg/L	14 d	CAT mRNA, GST mRNA ↑ Ucp-2 mRNA ↓	Stancová et al. [120]
	Danio rerio	0.001, 0.1, 5 mg/L	14 d	GPx, GR, GST, CAT ↑ Histopathology: in the gills (hyperemia, widening of a leaflet's apex, desquamation of leaflet's epithelium), in the liver (hepatocytic trabeculae, hyperemia in liver parenchyme)	Stancova et al. [121]
	Danio rerio	10, 20, 50, 75, 100, 125, 150, 175, 200, 240 mg/L 10, 20, 50, 75, 100, 125, 150, 175 mg/L	120 h 120 h	LC_{50} = 115.2 mg/L Developmental abnormalities (delayed hatching, pericardial edema, yolk sac edema, hemagglutination, weak pigmentation, hemorrhage, yolk condensation, tail not detached, axial malformation, tail twisting) Heart rates ↓ LC_{50} = 147.6 mg/L Developmental abnormalities (pericardial edema, yolk sac edema, axial malformation) Histopathology: swelling of hepatic cells , cell borders became obscure, nuclei pyknosis occurred	Li et al. [122]
	Cyprinus carpio	10, 50, 100, 200 µg/L	32 d	Developmental abnormalities (delayed hatching, hyperpigmentation) Histopathology: skin mucous cells and gill lamellas deformations Body weight and length, GST ↑ GR, TBARS ↓ LOEC = 10 µg/L	Sehonova et al. [123]
Ketoprofen	*Danio rerio*	1, 3, 6, 9, 12 mg/L 550, 600, 650, 700, 750 mg/L	144 h 96 h	LC_{50} = 6.44 ± 2.22 mg/L LC_{50} = 632.30 ± 10.10 mg/L	Praskova et al. [124]
	Cyprinus carpio	0.003, 2.1, 6.3, 21 mg/L	30 d	LOEC = 0.003 mg/L Delayed hatching Body weight and length ↑	Prášková et al. [125]

	Mytilus galloprovincialis	25 μg/L	14 d	Accumulation of lipofuscin in tertiary lysosomes Micronuclei frequency ↑ LMS, CAT ↓	Mezzelani et al. [7]
	Danio rerio	1, 10, 100 μg/mL 1, 10, 100 μg/L	96 h 42 d	Developmental abnormalities (pericardial edema, delayed hatching, scoliosis, elongation of heart, yolk sac edema, pericardial edema) Heart rate ↓ AST, ALT, LDH ↑ SOD, CAT, GSH, GPx, LPO, Na^+/K^+-ATPase ↓ Histopathology: In liver (nuclear degeneration, necrosis, nuclear degeneration, cytoplasmatic degeneration, cytoplasmatic vacuolation, sinusoid and pyknotic nuclei)	Rangasamy et al. [126]
Mefenamic acid	*Daphnia magna*	0.39, 1.56, 6.5, 25, 100 mg/L 1×10^{-5}, 0.0001, 0.001, 0.01, 0.1, 1 mg/L	48 h 21 d	$EC_{50} = 17.16$ mg/L Population growth rate ↓	Collard et al. [127]
	Moina macrocopa	0.39, 1.56, 6.5, 25, 100 mg/L 0.063, 0.125, 0.250, 0.500, 1 mg/L	48 h 7 d	$EC_{50} = 2.93$ mg/L Population growth rate ↓	Collard et al. [127]
	Danio rerio	0.001, 0.01, 0.1, 1 mg/L	14 d	GnRH2 mRNA, GnRHR2 mRNA, VTG1 mRNA, CYP19B mRNA ↑ HMGRA mRNA, HMGRB mRNA, CYP11A mRNA ↓	Collard et al. [127]

(continued)

Table 4 (continued)

NSAIDs	Species	Concentrations	Time	Results	Source
Nimesulide	*Mytilus galloprovincialis*	25 μg/L	14 d	Tissue concentrations = 16.72–43.72 ng/g Accumulation of lipofuscin in tertiary lysosomes Micronuclei frequency ↑ LMS, CAT ↓	Mezzelani et al. (2016)

HIS hepatosomatic index, *SOD* superoxide dismutase, *LPO* lipid peroxidation, *GST* glutathione S-transferase, *Hb* hemoglobin, *HPC* hydroperoxide content, *PCC* protein carbonyl content, *CAT* catalase, *GPx* glutathione peroxidase, *GR* glutathione reductase, *TBARS* thiobarbituric acid-reactive substances, *BAF* bioaccumulation factor, *COX1* cyclooxygenase 1, *COX2* cyclooxygenase 2, *Ht* hematocrit, *Lt* leucocrit, *MHCII* major histocompatibility complex class II, *LMS* lysosomal membrane stability, *ChE* cholinesterase, *T* testosterone, *E2* estradiol, *5-HT* serotonin, *DA* dopamine, *EROD* ethoxyresorufin-O-deethylase, *EROD* ethoxyresorufin-O-deethylase, *ETS* electron transport system, *GLY* glycogen, *LMS* lysosomal membrane stability, *THC* total hemocyte count, *NR* Neutral Red, *LDH* lactate dehydrogenase, *RBC* erythrocytes, *PCV* pack cell volume, *WBC* leukocytes, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *BCF* bioconcentration factor, *AST* aspartate aminotransaminase, *ALT* alanine transaminase, *CA* carbonic anhydrase, *AChE* acetylcholinesterase, *PGR* population growth rate, *HIS* hepatosomatic index, *GSI* gonadosomatic index, *MDA* malondialdehyde, *Ucp-2* uncoupling protein 2, *GSH* glutathione, *TOSC* total oxyradical scavenging capacity, *GnRH1* gonadotropin-releasing hormone 1, *GnRH2* gonadotropin-releasing hormone 2, *VTG1* vitellogenin 1, *CYP19B* cytochrome 19B, *CYP11A* cytochrome 11A, *HMGRA* hydroxymethylglutaryl CoA reductase A, *HMGRA* hydroxymethylglutaryl CoA reductase B

[a]Fish were fed twice every week with *Astyanax* sp. submitted to intraperitoneal inoculation with diclofenac

drug. Furthermore, trophic exposure to DCF also inhibited lipopolysaccharide-induced nitric oxide production, suggesting a possible immunosuppressive effect.

DCF is known to produce deadly damaging effects in renal and gastrointestinal tissue of several fishes. Hoeger et al. [87] showed DCF exposure over 21 days resulted in the telangiectasia of the gills, increased monocyte infiltration in the liver, and the common histopathological effects produced in the trunk kidney. Moreover, in rainbow trout, it has been demonstrated this drug produced hyaline inclusions and cell necrosis in the kidney, as well as inflammatory cell foci and increased basophils in the liver, after a chronic exposure [89, 90].

Two studies conducted in zebrafish evaluated the developmental abnormalities induced by DCF. Van den Brandhof and Montforts [91] exposed *Danio rerio* embryos to this pollutant for 72 h and found yolk sac and tail deformation in concentrations of over 1.5 mg/L, whereas Chen et al. [92] observed several tail malformations, pericardial edema, muscle degeneration, several trunk curvature, shorter body length, lack of the liver, and abnormal pigmentation in concentrations of 3.78 μM.

4.2 Paracetamol

The high toxicity of PCT is mainly produced by an oxidative stress mechanism. Gómez-Oliván et al. [100] demonstrated PCT induces significantly lipid peroxidation and decreases the activity of the antioxidant enzymes on amphipods exposed to 7.7 mg/kg of this pollutant. Furthermore, studies conducted in rainbow trout, common eel, and two edible clams have also showed significant alterations in all oxidative stress biomarkers [101, 102, 105, 118]. This set of data thoroughly evidence the bioactivation of PCT into a harmful prooxidant substance.

Data regarding the toxic effects of PCT on embryonic development are limited. David and Pancharatna [99] exposed zebrafish embryos to different doses of this pharmaceutical for seven consecutive days. Their results demonstrated PCT interferes with the normal embryonic development, growth, behavior, and survival of zebrafish larvae.

Paracetamol is a potential endocrine disruptor and can cause hepatotoxicity in male fish. Guiloski et al. [104] exposed *Rhamdia quelen* fish to environmental concentrations of PCT for 21 days. According to their results, testosterone levels were significantly reduced, whereas estradiol, serotonin, and dopamine levels increased. Furthermore, hepatic tissues of exposed fish showed blood congestion and leucocytes infiltration. Their findings evidence PCT which requires further attention relative to its potential endocrine disruptor effect.

4.3 Ibuprofen

Nallani et al. [109] investigated the uptake and depuration of IBF in fathead minnow and channel catfish exposed to 250 μg/L of this drug. Their results demonstrated IBF is poorly bioconcentrated in both species. However, in a more recent study, Mezzelani et al. [7] demonstrated mussels exposed to environmental concentrations of IBF revealed a significant bioaccumulation of the drug.

Like PCT, IBF has been associated with the fluctuation of several oxidative stress biomarkers in multiple species. Gonzalez-Rey and Bebianno [110] demonstrated the breakdown of the redox defense system and the prooxidant activity of IBF in mussels exposed to environmental concentrations of this pollutant. Their results agree with those reported by Bartoskova et al. [113], Islas-Flores et al. [6], and Stancova et al. [8], who also demonstrated this pharmaceutical-induced oxidative stress in zebrafish, common carp, and tench, respectively.

The reproductive damage of IBF at environmental relevant concentrations was investigated by Han et al. [108]. They exposed fertilized eggs of Japanese medaka to several concentrations of this drug for 144 days. In their results, IBF induced the production of vitellogenin in male fish and increased the number of eggs per brood.

Information regarding IBF toxicity effects on behavior and hematological parameters in fish is scarce. Ogueji et al. [114] observed the behavioral responses of *Clarias gariepinus* fish exposed to several concentrations of IBF to 96 h. Fishes exposed to the drug exhibited abnormal behavior characterized by regurgitation of food, jerky movements, and loss of equilibrium. Furthermore, the acute exposure of the African catfish to IBF also resulted in the alteration of several hematological parameters, such as the increase of red blood count, hemoglobin, pack cell volume, and leukocytes.

4.4 Acetylsalicylic Acid

ASA may cause a negative impact on some biomarkers connected with the production of oxidative stress in aquatic organisms. Zivna et al. [117] exposed zebrafish larvae to several concentrations of this pollutant for 28 days. After the exposure, larvae demonstrated the activity of multiple antioxidant enzymes increased, whereas lipid peroxidation depleted. Two years later, Zivna et al. [119] also demonstrated ASA altered the activity of lipid peroxidation and antioxidant enzymes in common carp embryos exposed to this NSAID. However, in this case, the antioxidant activity diminished and lipid peroxidation increased.

In addition to the oxidative stress study, Zivna et al. [119] assessed the toxic effects of ASA on the growth and development of common carp embryos. Developmental abnormalities, such as hyperpigmentation, lordosis, kyphosis, scoliosis, intestinal damage, and lower body weight, were found in larvae exposed to ASA.

The histological alterations produced by ASA in freshwater fish were studied by Nunes et al. [103]. They chronically exposed brown trout juveniles to this drug, in order to assess its histological effects in the liver and gills. After 28 days of exposure, both tissues showed several degenerative alterations such as aneurisms, hemorrhagic signals, vacuolization, inflammation, and necrosis.

4.5 Naproxen

Studies in zebrafish and common carp have demonstrated NPX may alter the embryonic development of freshwater fish. Li et al. [122] exposed *Danio rerio* embryos and larvae to multiple concentrations of this pollutant. According to their results, embryos exposed to NPX showed more sensitivity than larvae. Furthermore, several developmental abnormalities were found in both larvae and embryos, after 120 h of exposure. On the other hand, Sehonova et al. [123] confirmed subchronic exposure to NPX had harmful effects on the development and growth of common carp.

In order to assess the histopathological effects induced by NPX in fish, Stancova et al. [121] exposed zebrafish adults to concentrations of environmental relevance. After 2 weeks of exposure, the gills and liver showed structural alterations, such as hyperemia, desquamation of epithelium, edema, and steatosis.

4.6 Ketoprofen

Unlike IBF, KTP did not show a significant bioaccumulation in in *Mytilus galloprovincialis* mussels exposed to 25 μg/L of this NSAID. However, after 14 days of exposure, KTP induced the alteration of some immunological parameters and inhibited the activity of the antioxidant enzyme, catalase, in this marine organisms [7].

In an effort to assess the ecotoxicity of KTP, Rangasamy et al. [126] exposed embryos and adults of zebrafish to different concentrations of this pollutant. After 96 h of exposure, developmental abnormalities, such as pericardial edema, delayed hatching, and scoliosis, were observed in zebrafish embryos. On the other hand, in zebrafish adults, KTP decreased significantly the levels of several enzymatic and nonenzymatic antioxidants. Furthermore, structural alterations in liver tissue were found at concentrations of environmental relevance.

4.7 Mefenamic Acid

Collard et al. [127] conducted a study to assess the endocrine disrupting effects and the chronic toxicity effects of mefenamic acid (MFA) in three different freshwater species. For this purpose, two crustaceans, *D. magna* and *M. macrocopa*, and one fish, *D. rerio*, were exposed to several concentrations of this NSAID. After chronic exposure, in both crustaceans, the population growth was considerably reduced, whereas, in zebrafish, vitellogenin gene expression and several hypothalamus-pituitary-gonad transcription genes were significantly affected.

4.8 Nimesulide

NIM exhibited a significant bioaccumulation in *Mytilus galloprovincialis* mussels, reaching tissue concentrations of up 43.72 ng/g. Furthermore, immunological parameters and oxidative stress biomarkers were affected in this bivalves species, after they were exposed to 25 μg/L of this NSAID.

In general, oxidative stress, embryotoxicity, and histopathological studies have been thoroughly investigated in NSAIDs. However, information regarding the effects of these drugs on the sex hormone balance of nontargeted organisms have been poorly investigated. Future works should examine the endocrine disruption potential of NSAIDs in a multigeneration exposure.

Since NSAIDs are mainly bioactivated to prooxidant substances, Nunes et al. [105] demonstrated oxidative stress may impair the activity of cholinesterase. It is recommended to study in extent the neurotoxicological effects of these pollutants in aquatic species.

Unlike the wide information found regarding toxic effects induced by DCF, PCT, IBF, and ASA in aquatic organisms, NSAIDs such as NIM, MFA, NPX, and KTP have received less importance. Further evaluation of the toxicity of these pharmaceuticals for nontarget aquatic organisms is necessary.

5 Conclusions

Several studies have confirmed the high and ubiquitous occurrence of NSAIDs in the freshwater. However, no information have been reported on the occurrence of this pollutants in the marine environments. Moreover, there is a lack of interest and necessity for monitoring the occurrence of NSAIDs in ground and drinking water. It is recommended to assess in depth the occurrence, behavior, and fate of these pollutants in these water bodies, as their consumption is likely to increase.

Removal of NSAIDs during primary treatment has been shown to be minimal, whereas in secondary treatment, they are highly biodegradable in both aerobic and

anaerobic conditions. However, several studies have found concentrations of environmental relevance in surface waters. Therefore, there is a need to investigate new alternative post-treatment techniques for their removal from waste water.

As numerous studies have shown NSAIDs-induced harmful toxic effects on nontargeted organisms, we conclude these drugs are a potential threat to the environment. However, further research is needed to better understand the neurotoxicity and endocrine disruption effects of these pollutants. Furthermore, as these drugs share similar mode of action is likely they coexist in the aquatic environments. Therefore, it is of paramount importance to assess the effects of these drugs on a complex mixture. Finally, future works must focus on only to investigate the toxic effects produced at environmentally relevant concentrations.

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Overview of Non-steroidal Anti-inflammatory Drugs as Emerging Contaminants

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Contents

Abstract Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most used pharmaceuticals in the human and veterinary medicine, and it has been demonstrated that their widespread consumption all over the world has led to their ubiquitous occurrence in water environment. Nowadays, there exist strong evidence about the presence of different NSAIDs, such as diclofenac, naproxen, ketorolac, ibuprofen, ketoprofen, and salicylic acid, among others, which are found in concentrations in the range of ng/L to mg/L on different water bodies. Besides, the toxicological effects that NSAIDs cause in aquatic organisms have been evaluated by working groups all over the world. Thus, the aim of this review is to provide a detailed overview about the presence of NSAIDs in aquatic environmental, in particular to summarizing the main toxicological effects on living organisms and occurrence in water bodies that has been documented.

Keywords Apoptosis, Bioaccumulation, Biomagnification, Genotoxicity ecotoxicological, Trophic chains

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1 Introduction

The emerging contaminants (EC), such as antibiotics, pharmaceuticals, personal care products, hormones, and artificial sweeteners, among others, are recognized as new classes of water contaminants due to their proven or potential adverse effects on aquatic ecosystems and human health. Besides, its ubiquitous detection in the aquatic environment around the world raises a great public concern [1, 2].

According to data reported in the literature [2], the emerging pollutants can be classified into various families, among which are:

1. Antibiotics
2. Antifungal/antimicrobial agents
3. Non-steroidal anti-inflammatory drugs (NSAIDs)
4. Anticonvulsants/antidepressants
5. Artificial sweeteners
6. β-adrenergic blocking agents
7. Plasticizers
8. Steroidal estrogens (EDCs; endocrine disruptor compounds)
9. X-ray contrast medium
10. UV filters

It has been reported that the most important anthropogenic compounds are pharmaceuticals (diclofenac, ibuprofen, naproxen, ofloxacin, acetaminophen, progesterone, ranitidine, and testosterone), which nowadays are recognized as a threat for aquatic ecosystems, agricultural products or pesticides (atrazine, carbendazim, fipronil), narcotics and illegal drugs (amphetamines, cocaine, and benzoylecgonine), food industry derivatives (bisphenol A and caffeine), and personal care products (triclosan and other related surfactants) [3, 4].

Non-steroidal anti-inflammatory drugs (NSAIDs) belong to most used pharmaceuticals in the human and veterinary medicine, and their widespread consumption all over the world has led to their ubiquitous occurrence in water environment including large river systems [5]. NSAIDs are world widely reported as one of the most dominant and frequently detected groups in environmental matrices including wastewater, surface water, suspended solids, sediments, groundwater, and even drinking water. Among the emerging contaminants, the top 5 most frequently studied NSAIDs included ibuprofen, diclofenac, naproxen, acetaminophen, and ketoprofen [6, 7].

Recently, these compounds have been recognized to constitute a health risk for aquatic ecosystems, affecting not only fish tissues (kidney, brain, liver, gill, muscle), but have several effects at different levels, for instance, bioaccumulation, trophic chains, and biomagnification that can be the cause of cellular toxicity, apoptosis, genotoxicity, and alterations in sex ratios in human beings [3, 8].

Non-steroidal anti-inflammatory drugs remaining in the environment are a kind of priority hazard substances, due, among others reported cases, to a notable incident in which diclofenac residues caused the loss of more than 99% of vultures across the

Indian subcontinent [9]; for this reason, the aim of this review is to provide a detailed overview about the presence of NSAIDs in aquatic environmental, in particular to summarizing the main toxicological effects on living organisms and occurrence in water bodies that has been documented.

2 Occurrence of NSAIDs in Water Bodies

Emergent contaminants from different sectors such as industrial, agricultural, and pharmaceutical are found in water bodies with considerable endocrine disruptor potency and can damage the biotic components of the environment, and their presence in different environmental matrices is a serious and unresolved concern and has been related to land use patterns and various human activities [1, 3].

The occurrence of pharmaceuticals used as non-steroidal anti-inflammatory drugs (NSAIDs) in the aquatic environment is a threat to humans and aquatic species at large [10]. Thousands of tons of pharmaceuticals are introduced into the aqueous environment due to their incomplete elimination during treatment process in wastewater treatment plants (WWTPs) and water treatment plants (WTPs) [11]. It has been reported that the principal pathways of EC to enter the aquatic environment are excretion of human waste such as urine and feces and discharge of effluents through sewage treatment plants [8, 10], but these compounds have been found in different environmental matrices, such as water reservoirs for human consumption, WWTPs, drinking water treatment plants (DWTPs), groundwaters, surface waters, rivers, and seas, which demonstrate their free movement within the environment in an uncontrolled manner [3]; in particular, the WWTPs have been identified as a major route for release of pharmaceuticals in aquatic bodies where concentrations ranging from ng/L to μg/L are ubiquitously detected [4].

According to the literature, the profile of NSAIDs was dominated by acetaminophen in wastewater influents and effluents, and ibuprofen was the most abundant NSAID in surface water, and majority of NSAIDs were detected in solid matrices at below 1 μg/g except for ketoprofen, diclofenac, and ibuprofen [7].

Patrolecco et al. [12] provided data on the occurrence of selected human pharmaceuticals, including NSAIDs such as ibuprofen, ketoprofen, and naproxen, which were found in influents/effluents to/from the four principal wastewater treatment plants serving the city of Rome (Italy), in a range from 5 to 2,230 ng/L in influents and from 5 to 1,424 ng/L in effluents, indicating that after the treatment processes, most of pharmaceuticals were not completely eliminated, as average removal efficiencies were in the 14–100% [12].

The transformation products of diclofenac were identified by Scheurell et al. [13] in Malir River and Lyari River water as well as in effluent samples from Karachi, Pakistan, at μg/L concentrations: 3′-hydroxydiclofenac (0.08–0.3 μg/L), 8-chlorocarbazole-1-yl-ethanoic acid (0.03–0.4 μg/L), and 4′- and 5-hydroxydiclofenac as well as 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one were detected in the samples at concentrations between 0.4–1.8, 0.01–0.3, and

0.02–0.2 μg/L, respectively [13]. Besides, Khan et al. [14] in Mardan City, Pakistan, showed that four NSAIDs (paracetamol, diclofenac, ibuprofen, and codeine) were found in sewage and surface water of River Kabul and River Indus, at different concentrations; in particular, paracetamol was found at the higher end (32.4 μg/L) of the reported ranges in literature for other countries, and river samples showed that the target compounds were usually lower in concentration than the respective EC_{50} values for aquatic organisms [14].

In the first report on the occurrence of NSAIDs in Indian rivers, Shanmugam and co-workers, in 2014, determined the concentrations of diclofenac, ketoprofen, naproxen, ibuprofen, and acetylsalicylic acid in surface waters from 27 locations of the Kaveri, Vellar, and Tamiraparani rivers; all NSAIDs were found in concentration ranging from not detected to 200 ng/L, except for acetylsalicylic acid, which was found at all sites at considerably higher concentrations up to 660 ng/L, which represents risks of direct toxicity to aquatic wildlife [15]. Meanwhile, a study performed in Tehran, Iran, showed that NSAIDs were present in samples from surface, drinking, and wastewater. The highest concentrations of NSAIDs were found in the municipal WWTP influents where ibuprofen, naproxen, diclofenac, and indomethacin were found at 1.05, 0.43, 0.23, and 0.11 μg/L, respectively, while in tap water samples, their concentration was very low, and the maximum values were 47, 39, 24, and 37 ng/L, respectively, and, due to their low measured concentrations, no ecotoxicological effect is suspected to occur [16].

The analysis of influent and effluent wastewater and sludge samples from 3 conventional WWTPs in Catalonia (Spain) showed that non-steroidal anti-inflammatory drugs such as ketoprofen, naproxen, and diclofenac were present in 72 samples recollecting over a period of 2 years, in concentrations ranging from low ng/L to a few μg/L, and were present in the highest percentage (35–40%) in effluents water samples, in comparison with other 43 pharmaceutical compounds analyzed [17], while acetaminophen, ketoprofen, and the metabolites hydroxyibuprofen and carboxyibuprofen were detected in seawater samples from Portuguese coast at concentrations of 584, 89.7, 287, and 1,227 ng/L, respectively. On the other hand, the environmental risk in seawaters toward different trophic levels (fish, daphnids, and algae) was also assessed, and only diclofenac showed hazard quotients above 1 for fish, representing a potential risk for aquatic organisms [18].

Madikizela and Chimuka [19] showed that naproxen, ibuprofen, and diclofenac were present in water samples from Mbokodweni River and WWTPs located around the city of Durban in KwaZulu-Natal Province of South Africa. The NSAIDs were found at maximum concentrations of 6.84, 19.2, and 9.69 μg/L, respectively [19]. Besides, the drugs analyzed by Agunbiade and Moodley [20] in wastewater, surface water, and sediment samples from the Msunduzi River in the province of KwaZulu-Natal, South Africa, showed that aspirin was the most abundant pharmaceutical observed (118 ± 0.82 μg/L) in wastewater influent and that the downstream distribution patterns for both water and sediment indicate discharge contributions from wastewater, agricultural activities, domestic waste disposal, and possible sewer system leakages [20].

NSAIDs such as ibuprofen, diclofenac, naproxen, ketoprofen, and indomethacin were determined in the watercourses of the river Elbe basin in Czech Republic. In the study performed by Marsik et al. [5], ibuprofen was found to be the most abundant drug with maximum concentration of 3,210 ng/L, followed by naproxen, diclofenac, and ketoprofen (1,423.8 ng/L, 1,080 ng/L, and 929.8 ng/L, respectively). Indomethacin was found only at some sampling sites (maximum concentration of 69.3 ng/L) [5]. Kot-Wasik et al. [11] showed that the most often detected pharmaceuticals in water samples include ibuprofen (98% of samples), concluding that they may be considered as pollution indicators of the aqueous environment in tested area and that drugs concentrations were much higher in winter season, especially for NSAIDs, probably due to the inhibited degradation related to lower temperatures and limited sunlight [11].

The use of new technologies, with greater sensitivity and resolution, have allowed the determination of pharmaceutical compounds in different water samples, for instance, using a solid-phase extraction method based on multi-walled carbon nanotubes, Reinholds and collaborators, in 2017, reported the presence of diclofenac (1.7–8.4 ng/L) and ibuprofen (ranged between 1.0 and 9.2 ng/L) in surface water samples from Latvia and Norway [21]. Also, Ruixue and co-workers demonstrated for the first time the enantiospecific occurrence of NSAIDs in surface water in Beijing, China, in the monitoring of 34 sites along rivers showed that ibuprofen was the most abundant, with mean concentration of 114.9 ng/L and detection frequency of 91%, naproxen was also detectable at all sites for maximal concentration of 43.2 ng/L, both presenting an excess of the S-(+)-enantiomer [22].

3 Toxic Effects of NSAIDs in Aquatic Organisms

Pharmaceuticals are becoming widely distributed in waters and wastewaters and pose a serious threat to public health. There are a vast number of studies published regarding their input, presence, effects, and risks in ecosystems [16, 23]. Since medicine principles are designed to be effective at very low concentrations, they have the potential to interfere with biochemical and physiological processes of aquatic species over their entire life cycle. Besides, there is definitive evidence for the adverse impacts of NSAID residues on scavenging birds and aquatic species, for instance, ketoprofen, a widely used NSAID with comparable or even higher global consumption than diclofenac, in the environment has been shown to present a potential risk to nontarget terrestrial and aquatic species [4, 6, 9].

Non-steroidal anti-inflammatory drugs have been detected in the aquatic environment, but little is known about either their impact or mode of action in aquatic organisms [24]. NSAIDs such as ibuprofen, diclofenac, and paracetamol are causing increasing environmental concern due to their incomplete removal in wastewater treatment plant and potential toxicity on endocrine, kidney, and reproduction in teleost fish. Xia et al. [25] demonstrated that ibuprofen and diclofenac significantly affected embryo locomotivity and were potentially neurotoxic, thus posing threats to

zebrafish development by exposing embryos to the target chemicals at 5, 50, and 500 μg/L starting from 6 h postfertilization (hpf). Exposure to high concentration of ibuprofen significantly decreased the spontaneous movement by 25% and reduced the free-swimming distance, duration, and speed under dark condition by 41%, 29%, and 30%, respectively [25]. Besides, all three NSAIDs showed remarkable time-dependent and concentration-dependent effects on *Daphnia magna*, with diclofenac the highest and paracetamol the lowest toxic. Survival, growth, and reproduction data of *D. magna* from all bioassays were used to determine the LC_{10} and LC_{50} as well as the EC_{10} and EC_{50}, which were mainly in the low ppm-range, of which reproduction was the most sensitive one, indicating that nontarget organisms might be adversely affected by relevant ambient low-level concentrations [26].

One of the most widely NSAIDs evaluated is diclofenac. There exist a vast number of articles regarding its toxicological effects on several aquatic organisms. McRae and co-workers, in 2018, determined that diclofenac at environmentally relevant (0.17 μg/L) and elevated (763 μg/L) concentrations caused lipid peroxidation in the liver but, in the kidney and gill, was decreased after diclofenac exposure in *inanga fish* [27]. Conversely, juvenile *Rhamdia quelen* fish species were exposed to diclofenac for 96 h at concentrations of 0.2, 2, and 20 μg/L, and no oxidative stress was observed in the liver but in the kidney the superoxide dismutase activity was increased in all concentrations, suggesting an alteration in the hydrogen peroxide production, and DNA damage and lipid peroxidation were not detected; besides, diclofenac exposure increased the red blood cells number (0.2 and 2 μg/L) and monocytes and neutrophils (2 and 20 μg/L) [28].

Oreochromis niloticus, exposed to diclofenac for 80 days post-hatch to a 0.1 and 1 μg/L, showed altered biomarkers associated with reproduction indicating the potential to affect sexual differentiation and gametogenesis by acting as an estrogenic endocrine-disrupting compound; also, vitellogenin gene expression was significantly induced at 1 μg/L [29]. In 2017, Pandey and colleagues evaluated the DNA damage, hematological changes, and activities of oxidative stress enzymes in Nile tilapia, *Oreochromis niloticus*, in response to diclofenac and found that at 0.17, 0.34, and 0.68 mg/L, diclofenac caused a reduction in hemoglobin and red blood cell counts and an elevation on the indices of hepatic oxidative stress biomarkers, including lipid peroxidation and carbonyl protein [30].

High concentrations of diclofenac, present in effluents and water bodies of different countries, including Mexico, caused toxicity on aquatic organisms. Cardoso-Vera et al. [31] demonstrated that the exposition of oocytes in mid-blastula transition of *Xenopus laevis* and *L. catesbeianus* to diclofenac at 1, 4, 8, 16, 32, and 62.5 mg/L induced diverse malformations in both species, the most frequent of these being axial malformations in the tail and notochord, edema, and stunted growth, using FETAX assay [31]. Also, it was demonstrated that the exposition of *Gasterosteus aculeatus* to 0, 4.6, 22, 82, and 271 μg/L of diclofenac in flow-through systems for 28 days caused histological changes in the proportion of renal hematopoietic tissue (renal hematopoietic hyperplasia), but no histological changes were observed in the liver at low μg/L concentrations; moreover, an

increment in the relative hepatic mRNA levels of c7 (complement component 7), a gene involved in the innate immune system, was found (at 22 μg/L) [32].

The fish *Rhamdia quelen* was exposed to diclofenac at concentrations ranging from 0 to 20 μg/L, and, as shown by Guiloski et al. [33], diclofenac reduced the catalase and ethoxyresorufin-O-deethylase activities in fish exposed to 2 μg/L, in the liver, and superoxide dismutase in all exposed groups; besides, the levels of reduced glutathione and glutathione S-transferase activity increased at all tested concentrations, and lipid peroxidation was reduced (0.2 and 20 μg/L), but there was no protein oxidation [33]. Diclofenac caused immune responses in gastropod species *Lymnaea stagnalis* at environmental realistic (1–10 μg/L) and therapeutic (100–1,000 μg/L) concentrations; the immune parameters of individual snails were measured: hemocyte density and viability, hemocyte phagocytosis capacity, and hemocyte-related oxidative activities (basal and NADPH oxidase stimulated with zymosan particles) [34]. The toxic effects of diclofenac were evaluated on *Clarias gariepinus* by acute and chronic static renewable bioassay carried out by Ajima et al. [35]. Exposure to acute toxicity resulted in abnormal behavior and mortality of some fish, but compared with the control, chronic exposure to 1.57, 3.14, and 6.28 mg/L showed hematological alterations, including significantly higher mean corpuscular hemoglobin concentration, mean corpuscular volume, and white blood cell, with significantly lower hemoglobin, hematocrit, red blood cell, and mean corpuscular hemoglobin with increase in the concentration of the drug [35].

Cyprinus carpio is one of the most frequently bioindicators used to assess the toxicological effects of NSAIDs on aquatic organisms. A study carried out by Saucedo-Vence's group showed that the exposure of *Cyprinus carpio* to median lethal concentration of diclofenac caused alterations on the oxidative stress status in the blood, muscle, gills, brain, and liver [36]. Islas-Flores et al. [37] evaluated the toxicity induced by diclofenac, ibuprofen, and their mixture on *Cyprinus carpio*; the results showed that diclofenac, ibuprofen, and a mixture of these pharmaceuticals induced free radical production, oxidative stress, and cytogenotoxicity in tissues of *C. carpio*, but a greater effect was elicited by the mixture than by either pharmaceutical alone in some biomarkers evaluated, particularly in the gill [37].

In order to assess the sub-chronic toxicity of naproxen, *Cyprinus carpio* was exposed to 10, 50, 100, and 200 μg/L, and the results showed a strong effect on the early life stages of the common carp. Besides, naproxen caused effects on hatching, developmental rate, morphology, and histopathology [38]. Studies conducted to evaluate the genotoxicity and cytotoxicity induced in the common carp using the effluent emanating from a non-steroidal anti-inflammatory drug (NSAID)-manufacturing plant, in Mexico, showed that carps exposed to the lowest observed adverse effect level (LOAEL, 0.1173%) for 12, 24, 48, 72, and 96 h present a significant positive correlations between NSAID concentrations and biomarkers of geno- and cytotoxicity [39]. Besides, it was demonstrated that salicylic acid has effects on the growth and development of common carp early life stages with respect to antioxidant defense enzymes; in particular hatching, early ontogeny, and both morphometric and condition characteristics were significantly influenced by sub-chronic exposure to salicylic acid [40].

The experimental groups of Galar-Martínez et al. [41] and Pérez-Coyotl [42], using *C. carpio* as bioindicator, evaluated the oxidative stress and genotoxicity induced by sub-lethal concentrations of ketorolac (1 and 60 μg/L) in the liver, brain, and blood as well as the genotoxicity and cytotoxicity induced in the blood, liver, and gill of *C. carpio* by the pollutants present in a water reservoir. Ketorolac induced oxidative damage (increased lipid peroxidation, hydroperoxide content, and protein carbonyl content) and changes in antioxidant status (superoxide dismutase, catalase, and glutathione peroxidase activity) in the liver and brain of carp, and in the blood, ketorolac increased the frequency of micronuclei and is therefore genotoxic for the test species. On the other hand, the water reservoir caused significant increases in all biomarkers in all tissues evaluated (DNA damage, frequency of micronuclei, apoptosis, and caspase-3 activity) [41, 42].

Another bioindicator using frequently for evaluation of toxic effects of NSAIDs is *Danio rerio.* Exposure of adult zebrafish (*Danio rerio*) to naproxen caused moderate effects on the expression of antioxidant genes in the intestine rather than in the liver, including *Ucp-2* at 1 μg/L, and an increased expression of *GST p2* at 100 μg/L, demonstrating that the intestine is more sensitive than the liver [43]. van den Brandhof and Montforts [44], using a fish embryo toxicity (FET) test, evaluated the effects of diclofenac and metoprolol on *Danio rerio*, finding specific effects on hatching, yolk sac, and tail deformation above 1.5 mg/L for diclofenac and on scoliosis and growth retardation above 12.6 mg/L for metoprolol [44]. On the other hand, Li et al. [45] obtained the values of 96-h LC_{50} of 115.2 mg/L for embryos and 147.6 mg/L for larvae indicating that zebrafish embryos were more sensitive than larvae to naproxen exposure and naproxen-treated zebrafish larvae exhibited histopathological liver damage, including swollen hepatocytes, vacuolar degeneration, and nuclei pyknosis, indicating that naproxen is a potential threat to aquatic organisms [45].

The exposition of juvenile zebrafish to salicylic acid at concentrations of 0.004, 0.04, 0.4, 4, and 40 mg/L caused no effects on histological changes, specific growth rate glutathione reductase, and lipid peroxidation but increased the catalytic activity of GPx (at 0.04 mg/L) catalase (at 0.04 and 4 mg/L) and glutathione-S-transferase (at 0.004 and 0.04 mg/L) compared to controls [46]. Moreover, *Danio rerio* was exposed to naproxen (0.1, 1, 10, and 100 μg/L) and its thyroid-disrupting effects were evaluated. Xu et al. [47] showed that naproxen caused a decrease of cytochrome P450 gene expression and enzyme activity might inhibit its metabolism which might resulted in the significant bioconcentration; besides, both triiodothyronine and thyroxine levels were substantially decreased; thus, thyroid disruption should be considered when assessing the aquatic risk of long-term exposure to environmentally relevant concentrations [47]. Also, adult zebrafish, of both sexes, were exposed to NSAIDs such as atenolol, ketoprofen, and diclofenac, and their UV photolysis products resulted more toxic than the parental compounds, causing an increase in GST, MDA, and CAT levels [48].

Amphibians represent particularly vulnerable organisms, and many populations around the world are currently at risk of extinction. *Limnodynastes peronii* were exposed to a mixture of the common pharmaceutical contaminants including

diclofenac and naproxen at 0.1, 1, 10, 100, and 1,000 μg/L throughout the developmental period. Morphological endpoints were associated with significantly altered levels of hepatic triglycerides, which in turn were correlated with increased peroxidase activity at the highest concentration (1,000 μg/L) [49]. On the other hand, Veldhoen et al. [50] assessed the ability of sub-lethal and environmentally relevant concentrations (13.7 μg/L) of ibuprofen to function as a disruptor of endocrine-mediated post-embryonic development of the *Rana catesbeiana tadpoles*; the results indicated that the exposure caused subsequent disruption of thyroid hormone-mediated reprogramming in the liver transcriptome affecting constituents of several metabolic, developmental, and signaling pathways and raised the possibility that ibuprofen may alter the post-embryonic development of anuran species in freshwater environs [50].

Studies about the metabolism of ibuprofen were carried out by Jones and collaborators, in 2012, through the exposition of zebrafish larvae to 100 μg/L of ibuprofen; the results provide strong evidence that zebrafish larvae can metabolize and excrete ibuprofen in a manner known to be cytochrome P450-dependent in mammals [51]. Exposure of trout fry to a range of salicylate or ibuprofen concentrations (1, 10, 100, or 1,000 μ/L) for 4 d caused an increment on heat-shock protein 70 (mRNA and protein levels), in the liver; also, liver glucose levels and the activities of hexokinase, pyruvate kinase, and lactate dehydrogenase were elevated by NSAIDs suggesting enhanced tissue glycolytic capacity [24].

Due to the fact that paracetamol induces oxidative stress in mammals, Bebianno et al. [52] evaluated if similar effects were observed in oysters *Crassostrea gigas*, through the exposition for 1, 4, and 7 days to two different sub-lethal concentrations (0.1 and 100 μg/L). The results showed that no changes in cell viability and DNA damage were observed in oysters exposed to both concentrations. Similarly, no significant changes were detected in the major antioxidant enzymes (except for glutathione reductase) in oyster gills, suggesting that changes in glutathione reductase activity are enough to counteract a potential oxidative stress in *C. gigas* gills under these experimental conditions [52]. On the other hand, the ecotoxicity of naproxen in aquatic organisms is limited primarily to acute lethal effects. In 2018, Kwak and colleagues demonstrated that the chronic no observed effect concentrations (NOECs) of naproxen for reproduction were determined to be 10 mg/L in *Daphnia magna* and 0.3 mg/L in *Moina macrocopa*. At concentrations of 0.5 mg/L, the survival of juvenile medaka fish was significantly decreased and transcription of *erβ2* gene was significantly increased [53].

Finally, the evaluation of a drug mix composed of different medication classes (antibiotic, NSAIDs, antidepressant, anxiolytic, analgesic, and antacid drugs), at environmentally relevant concentrations, was carried out by do Amaral et al. [54] in *Lithobates casteibeianus tadpoles*, finding that drug mix promoted changes in mandibular sheath pigmentation, dentition, and swimming activity, as well as atypical behavior in the social aggregation test; besides, the mutagenic analysis revealed higher frequency of nuclear abnormalities in the erythrocytes of tadpoles that were exposed [54].

4 Conclusions

In this review, we provide data regarding the importance of non-steroidal anti-inflammatory drugs (NSAIDs), focusing in data concerning its occurrence studies on several water bodies and its toxicological effects on different aquatic organisms.

As shown above, there exist vast information about the presence of different NSAIDs in the environment, such as diclofenac, naproxen, ketorolac, ibuprofen, ketoprofen, and salicylic acid among others, which have been found in concentrations in the range of ng /L to mg/L and in particular cases in concentrations even higher than the limits allowed by regulatory agencies, suggesting severe problems not only for the health of aquatic organisms that live in different bodies of water where they are detected but also pose a risk to human health.

Also, in this review we present the main results obtained by different working groups around the world in which the toxic effects produced by NSAIDs have been evaluated, focusing mainly on aquatic organisms highlighting the use of bioindicators as *Cyprinus carpio* and *Danio rerio*, among others. Among the main effects of the exposure to NSAIDs, the DNA damage, alterations on the oxidative stress enzymes, effects on cyto- and genotoxicity, hematological alteration, effects on behavior, and changes on expression of several genes involved in defense and metabolism, among others, were described.

Finally, with the information presented here, we can conclude that it is necessary to carry out further investigation of both the occurrence of NSAIDs in the environment and their toxic effects, since the information in the literature is still limited, and these efforts could lead to the execution of ecopharmacovigilance programs with a view to the creation of adequate legislation on the production, uses, and wastes of these compounds in the environment.

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Worldwide Occurrence, Detection, and Fate of Nonsteroidal Anti-inflammatory Drugs in Water

Lucila I. Castro-Pastrana, Erika Palacios-Rosas, M. Luisa Toledo-Wall, and Mónica Cerro-López

Contents

Abstract Finding over-the-counter drugs such as NSAIDs in wastewater is somewhat expected and understandable; however, finding them on surface or groundwater is more worrisome as it demonstrates, in part, the inefficiency of the methods used today to treat wastewater as well as practices of inadequate use and indiscriminate disposal of these emerging contaminants.

The objective of this chapter is to provide a systematic review of the worldwide occurrence of NSAIDs in three environmental reservoirs of water (saline and fresh and groundwater) and in drinking water.

Our results showed that the worldwide distribution of studies on the subject is practically concentrated in Asia and Europe. Acetaminophen, diclofenac, ibuprofen, indomethacin, ketoprofen, naproxen, mefenamic acid, and salicylates are the NSAIDs investigated and most frequently detected in all studies.

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Since 2010, research on the occurrence of NSAIDs in environmental water reservoirs has been continuous and consistent. However, the analysis of river waters is much more abundant than those of seas, groundwater, or drinking water. Fortunately in recent years, research has focused on the development of more sensitive but at the same time simpler methods for the detection of NSAIDs in water bodies. Moreover, during the article screening process, we found that in recent years, publications related to toxicity studies in diverse model organisms have increased.

Concentrating the information available to date on the quantities of NSAIDs found in different aquatic ecosystems, through different methods of extraction and analysis, is very useful to direct future research, to design more efficient strategies to minimize the ecological impact of these water pollutants, and to develop evidence-based regulation.

Keywords Anti-inflammatory agents, Drinking water, Fresh water, Groundwater, Nonsteroidal, Saline waters, Water pollutants

1 Introduction

The Earth's surface is 70% covered with water, of which 97.5% is considered salt water and the remaining 2.5% as fresh water. Frozen water represents 69.7% of fresh water, groundwater represents 30%, and in rivers and lakes, we only find 0.3% of fresh water [1].

Among the most prescribed pharmaceutical products used by both humans and veterinary medicine are nonsteroidal anti-inflammatory drugs (NSAIDs).

Although it is well-known that pharmaceutical products have been present in the aquatic environment for 30 years [2, 3], it was not until the last half of the 1990s that their presence began to arouse widespread concern in the scientific community.

The consumption of NSAIDs is increasing, and with it the danger of environmental pollution. Its widespread consumption has caused us to find these drugs in the environment, especially in aquatic compartments, including large and small river systems such as rivers, lakes, and lagoons, which has generated a growing international concern [4–6].

The migration of these pharmaceutical products is promoted by riverbank filtration, artificial groundwater recharge, or natural groundwater flow, among other processes [7, 8]. In addition, these emerging pollutants can end up in the aquatic ecosystems due to an incomplete process of elimination during wastewater treatment [9].

Traditionally, water quality control has focused on the elimination of conventional priority pollutants, especially those considered as persistent, toxic, or bioaccumulative, but in recent years interest in the appearance of pharmaceutical

pollutants in water, their environmental fate, and their potential ecotoxic effects has increased [10–12].

For example, based on a Scopus literature review (2017–2018), López-Pacheco et al. [13] informed summarized concentration ranges of more than 100 pharmaceuticals in different water sources. For effluents of wastewater treatment plants (WWTPs), there is evidence of nine studies reporting levels of various pharmaceuticals between 0.103 and 1,673,000 ng/L; for river and surface waters, the literature (15 studies) revealed a concentration range of 0.11–276,000 ng/L; and for groundwater, the range is 0.33–339 ng/L according to two articles reviewed. Moreover, ocean/seawaters were found to contain pharmaceutical levels between 0.0038 and 1,219 ng/L in five studies, and for drinking water only, one study reported a concentration of 10.3 ng/L for the drug carbamazepine. NSAIDs with more available data among all these studies were acetaminophen, diclofenac, ibuprofen, ketoprofen, naproxen, and salicylic acid.

Diclofenac was the only NSAID conforming the first Watch List for emerging pollutants from the European Water Framework Directive (WFD; Directive 2000/60/EC) which covers both fresh waters and transitional waters (the estuarine and coastal area up to one nautical mile, or 1.85 km, from the shore) [14]. However, since the median surface water concentration of diclofenac was already established between 0.027 and 0.047 μg/L and a lower Predicted No Effect Concentration (PNEC) of 0.05 μg/L has been updated, the European Commission's Joint Research Centre (JCR) determined that there is no need to collect additional monitoring data for this substance and decided in 2018 to remove it from the Watch List [15]. To date no other NSAID has been selected as a new Watch List substance for inclusion. While the countries of the European continent have responded satisfactorily with sufficient and relevant evidence to the prioritization of diclofenac as a substance to be monitored in bodies of water, the rest of the world still has the pending task for prioritizing and monitoring this and many other pharmaceuticals.

Finding drugs and other emerging pollutants in wastewater is somewhat expected and understandable; however, finding them on surface or groundwater is more worrisome as it demonstrates, in part, the inefficiency of the methods used today to treat wastewater.

That is why concentrating the information available to date of the levels found of different NSAIDs in various types of aquatic compartments, with diverse methods of extraction and analysis, is useful to direct future research, guidelines, and policies that facilitate the understanding of the problem of NSAIDs in the environment, the design of efficient strategies to minimize their presence and the development of evidence-based regulation.

Thus, the objective of this chapter is to provide a systematic review of the worldwide occurrence of NSAIDs in three environmental reservoirs of water (saline, fresh, and groundwater) and in drinking water by means of identifying and analyzing the available literature to date that communicates about the detection and quantification of these drugs in natural water samples.

The reported presence of one or more NSAIDs in the four different types of water was investigated through a qualitative systematic review assembled in accordance

Table 1 Search statements used for identifying the available evidence on the presence of NSAIDs in different types of water

Water type	Pubmed search strategy
Fresh water (lakes, ponds, rivers)	("Fresh Water"[MeSH]) AND (("Anti-Inflammatory Agents, Non-Steroidal"[MeSH]) OR "Anti-Inflammatory Agents, Non-Steroidal" [Pharmacological Action])
Saline waters (oceans and seas)	((("Saline Waters"[MeSH]) OR "Seawater"[MeSH])) AND ((("Anti-Inflammatory Agents, Non-Steroidal"[MeSH]) OR "Anti-Inflammatory Agents, Non-Steroidal" [Pharmacological Action]))
Groundwater	("Groundwater"[MeSH]) AND (("Anti-Inflammatory Agents, Non-Steroidal"[MeSH]) OR "Anti-Inflammatory Agents, Non-Steroidal" [Pharmacological Action])
Drinking water	("Drinking Water"[MeSH]) AND ("Anti-Inflammatory Agents, Non-Steroidal"[MeSH] OR "Anti-Inflammatory Agents, Non-Steroidal" [Pharmacological Action])

with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [16].

Systematic search was performed for abstracts of all articles published up to October 2019. We used MEDLINE/PubMed for the identification of studies. Four search strategies were designed using MeSH terms as shown in Table 1.

The criteria for considering studies for the different sections of this chapter were at least one NSAID was detected and quantified (levels or concentrations are reported); at least one of the water samples in which the analyzed NSAIDs were determined corresponds to the types of water of interest; and the sample in which the NSAID or NSAIDs were determined was natural (i.e., not artificially created for the experiment). Reviews and systematic reviews were excluded. Ecotoxicity assays in model organisms as well as studies where methods for NSAID degradation, extraction, and/or analysis were developed but did not quantify at least one NSAID in real (natural) water samples were also excluded. Only articles in English and Spanish were included, but no limitations on the year of publication were applied. For each type of water, Figs. 1, 2, 3, and 4 show the complete and reproducible search and data management strategies applied by the authors.

Articles were retrieved and reviewed for relevance. Data were collected using a data extraction form developed specifically to evaluate the records of each type of water. Full text articles were obtained for the screening phase in order to assess which studies met the inclusion criteria. Data extraction and analysis were completed independently by pairs. Discrepancies in classification were resolved through discussion among all authors.

A summary of the NSAIDs and their concentrations found in different investigations conducted around the world is presented below for each type of water.

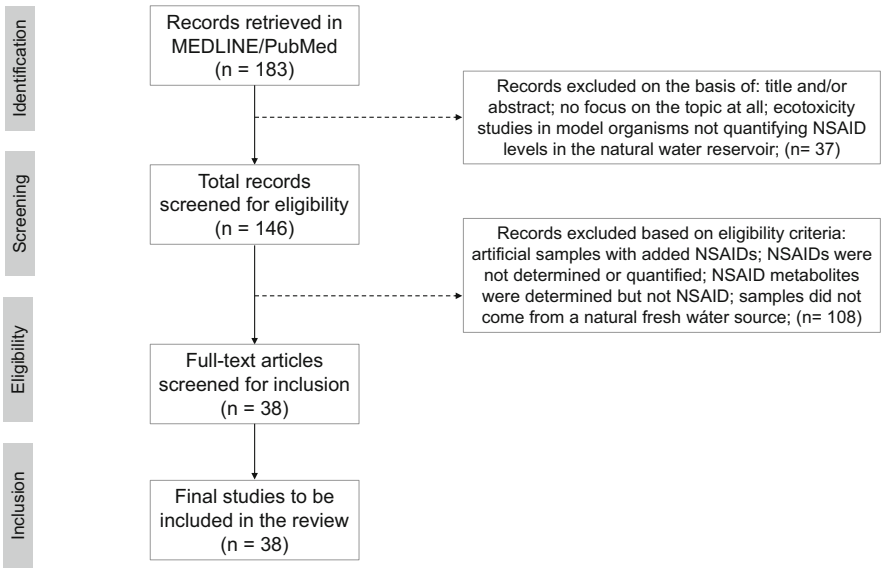

Fig. 1 PRISMA flow chart of studies identified in the systematic review of the occurrence of NSAIDS in freshwater samples

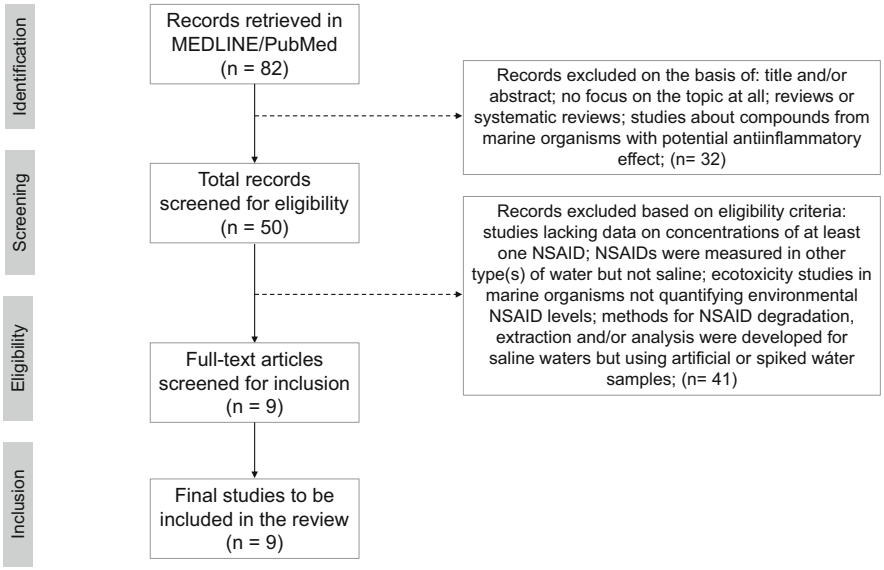

Fig. 2 Selection of studies retrieved for the systematic review of the presence of NSAIDS in saline waters

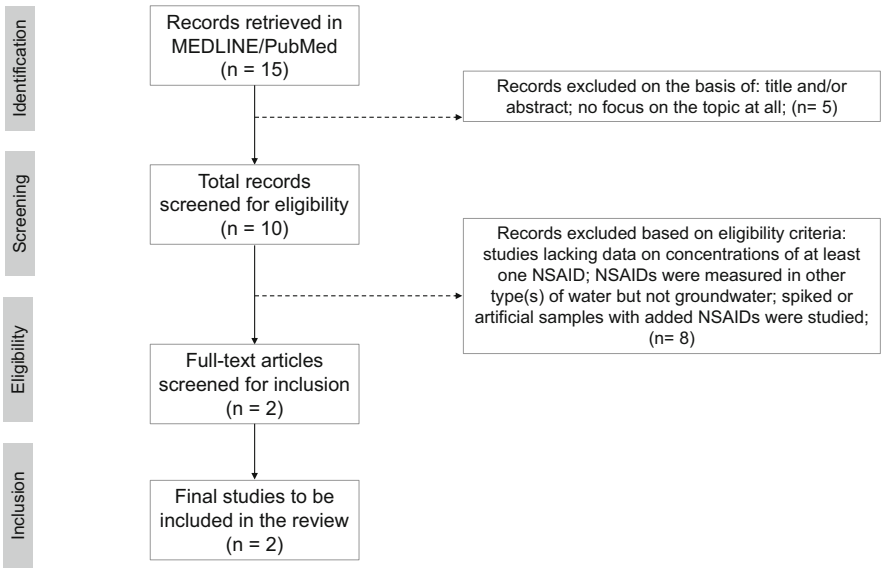

Fig. 3 Flow chart of included studies in the systematic review of the occurrence of NSAIDS in groundwater samples

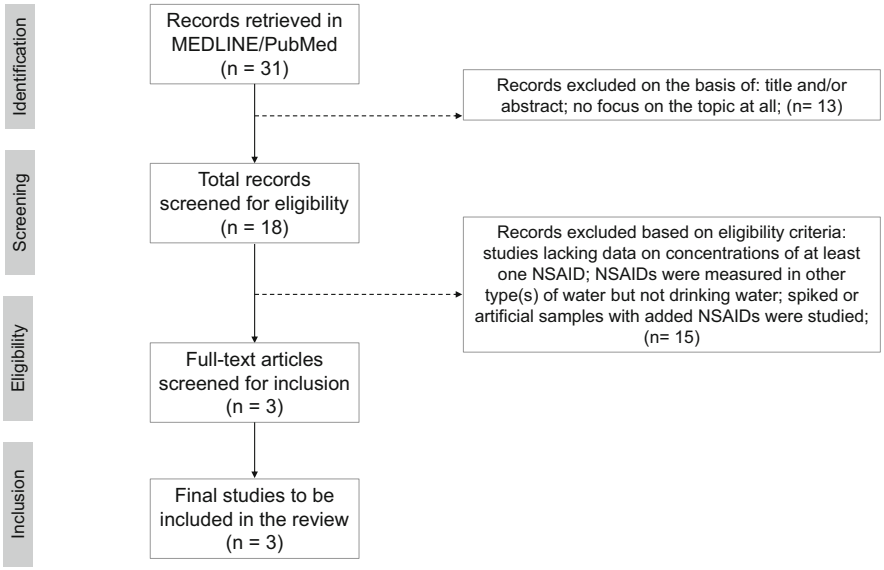

Fig. 4 Selection of studies retrieved for the systematic review of the presence of NSAIDS in samples of drinking water

2 Fresh Water

After analysis and selection of 183 retrieved records, the search strategy for fresh water ends up with 38 studies for inclusion in this section of the review. From the total, only 7 articles were published in the period from 1976 to 2000, but as of 2001 the investigations on the subject began to rise. Particularly since year 2010, research about the occurrence of NSAIDs in freshwater reservoirs has been continuous and consistent. In fact, in recent years, research has basically focused on the development of more sensitive but at the same time simpler methods for the detection of NSAIDs both in river and lake water and in sediments. Novel and more effective sample preparation methods have also been under development.

Magnetic adsorption, enantiomeric analysis, immunosensors, and nanomaterials stand out among the most explored techniques to improve NSAID detection. Regarding degradation methods, photodegradation, and biodegradation of NSAIDs using algae and aquatic plants have been the most published techniques in recent years.

The main feature of the articles excluded from this review was that the analyzed water samples were artificial, since even when the matrix samples were taken directly from a river or lake, spiked NSAID standards of known concentrations were added in the laboratory to evaluate the performance of an analytical method or of a degradation procedure developed for these drugs.

Other articles were excluded because the measurement of the presence of NSAIDs in water samples was made indirectly, for example, by measuring the changes detected in the pH of the analyzed water, or the concentration of their metabolites or photodegradation products. Also, some studies were excluded because the water studied did not come from a natural source (rivers, lakes, lagoons) but from aquariums. Figure 1 shows the process and eligibility criteria used to rule out and include studies for this section of the chapter.

Tables 2, 3, and 4 present the summary of the included studies reporting the prevalence of NSAIDs in fresh surface waters in two different concentration ranges, micrograms per liter (μg/L) and nanograms per liter (ng/L), and for river sediments in terms of dry weight (μg/kg and ng/kg).

Most of the articles included in this review section presented two variables in common: they studied samples from river waters and used liquid chromatography coupled to mass spectrometry (LC-MS/MS) to determine the concentration of NSAIDs.

An Italian study carried out by Zuccato et al. [42] detected through LC-MS/MS the presence of ibuprofen in the Lambro River at concentrations of 20 ng/L and in the Po River at a maximum concentration of 17.4 ng/L. The authors mentioned that the quantities reached by pharmaceutical products in surface waters are affected by effluents from treatment plants but also by their degradation susceptibility. Unfortunately, the degradation rates of several drugs in the environment are not known, and to a limited extent they are estimated from degradation data under laboratory conditions.

Table 2 Maximum concentrations of NSAIDs in surface waters of rivers reported in concentrations within the range of micrograms per liter (μg/L)

NSAID measured	Maximum level reported (μg/L)	River and site	References
Acetaminophen	32.4	Kabul and Indo River, Pakistan	[17]
Acetylsalicylic acid	91.3	Msunduzi River, South Africa	[18]
	14.74	Shijing River, China	[19]
	6.7	Pearl River, China	[20]
	1.17	Yeongsan River, South Korea	[21]
	0.52	Rivers of the Doñana National Park, Spain	[22]
Diclofenac	243	Danube River, Europe	[7]
	100.8	Msunduzi River, South Africa	[18]
	8.5	Karachi River, Pakistan	[23]
	2.19	Yamuna River, India	[24]
	0.16	Kabul and Indo River, Pakistan	[17]
	0.09	Rivers of the Doñana National Park, Spain	[22]
Ibuprofen	165	Danube River, Europe	[7]
	92.8	Msunduzi River, South Africa	[18]
	11.4	Mbokodweni, South Africa	[25]
	1.21	Rivers of the Doñana National Park, Spain	[22]
	1.09	Yamuna River, India	[24]
	0.8	Furong Lage, China	[26]
	0.21	Kabul and Indo River, Pakistan	[17]
Ketoprofen	192	Danube River, Europe	[7]
	102.7	Msunduzi River, South Africa	[18]
	0.20	Rivers of the Doñana National Park, Spain	[22]
Mefenamic acid	0.94	Furong Lake, China	[26]
Naproxen	166	Danube River, Europe	[7]
	0.68	Mbokodweni, South Africa	[25]
	0.64	Rivers of the Doñana National Park, Spain	[22]
	0.53	Yeongsan River, South Korea	[21]

Similarly, concentrations of ibuprofen between 54.4 and 62.3 ng/L were found in the Qiantang River in southeastern China by means of LC-MS/MS [41].

Na et al. [21] using LC-MS examined the distribution of various drugs in surface waters collected from 7 main streams and 11 tributaries of the Yeongsan River in South Korea, finding average concentrations of naproxen of 0.0516 and 0.0447 μg/L of acetylsalicylic acid.

In Western Ukraine, also using the LC-MS technique, a study was carried out in waters of neighboring European Union transboundary rivers, finding diclofenac

Table 3 Maximum concentrations of NSAIDs in surface waters of rivers reported in concentrations within the range of nanograms per liter (ng/L)

NSAID measured	Maximum level reported (ng/L)	River and site	References
Acetaminophen	264	Thames River, England	[27]
Acetylsalicylic acid	0.5	Edo River, Japan	[28]
Diclofenac	3,560	Rivers of Western Ukraine	[29]
	1,080	Elbe River, Czech Republic	[30]
	900	Rhine River, Europe	[31]
	432	Aisonas, Greece	[32]
	370	Elbe River, Germany	[5]
	166	Danube River, Romania	[33]
	150	Shijing River, China	[19]
	65	Vantaa River, Finland	[34]
	35	Päijänne Lake, Finland	[34]
	17	Tiber and Aniene Rivers, Italy	[35]
	10	Klang River, Malaysia	[36]
	2	Edo River, Japan	[28]
	0.025	Rivers of Tehran, Iran	[37]
Etodolac	0.3	Naruo-shin River, Japan	[38]
Felbinac	10	Edo River, Japan	[28]
Ibuprofen	3,210	Elbe River, Czech Republic	[30]
	783	Thames River, England	[27]
	723	Lima River, Portugal	[39]
	685	Shijing River, China	[19]
	288	Pearl River, China	[20]
	114.9	Rivers of the North Channel Basin, Beijing China	[40]
	120	Rhine River, Europe	[31]
	62.3	Qiantang River, China	[41]
	58	Danube River, Romania	[33]
	33	Vantaa River, Finland	[34]
	31	Päijänne Lake, Finland	[34]
	22	Aisonas, Greece	[32]
	20	Tiber and Aniene Rivers, Italy	[35]
	17.4	Lambro River, Italy	[42]
	1	Edo River, Japan	[28]
	0.31	Rivers of Tehran, Iran	[37]
Indomethacin	210	Rhine River, Europe	[31]
	7.7	Danube River, Romania	[33]
	0.7	Naruo-Shin River, Japan	[38]
	0.04	Rivers of Tehran, Iran	[37]
Ketoprofen	929.8	Elbe River, Czech Republic	[30]
	66	Aisonas, Greece	[32]

(continued)

Table 3 (continued)

NSAID measured	Maximum level reported (ng/L)	River and site	References
	31	Vantaa River, Finland	[34]
	27	Päijänne Lake, Finland	[34]
	25	Rhine River, Europe	[31]
	20	Tiber and Aniene Rivers, Italy	[35]
	1	Edo River, Japan	[28]
Mefenamic acid	24.6	Shijing River, China	[19]
	2	Edo River, Japan	[28]
	0.4	Naruo-shin River, Japan	[38]
Naproxen	1,423.8	Elbe River, Czech Republic	[30]
	146	Aisonas, Greece	[32]
	125	Shijing River, China	[19]
	43.2	Rivers of the North Channel Basin, Beijing China	[40]
	32	Päijänne Lake, Finland	[34]
	22	Danube River, Romania	[33]
	17	Tiber and Aniene Rivers, Italy	[35]
	6.4	Vantaa River, Finland	[34]
	2	Edo River, Japan	[28]
	0.04	Rivers of Tehran, Iran	[37]
Piroxicam	32	Danube River, Romania	[33]

Table 4 Maximum reported concentrations of NSAIDs in river sediments (dry weight)

NSAID measured	Maximum level reported	River and site	References
Diclofenac	144 μg/kg	Danube River, Europe	[7]
	13.88 μg/g	Klang River, Malaysia	[43]
	0.10 μg/g	Novo mesto nearby rivers, Slovenia	[12]
	12.9 ng/g	River in Shanghai, China	[44]
Ibuprofen	31 μg/kg	Danube River, Europe	[7]
	0.21 μg/g	Novo mesto nearby rivers, Slovenia	[12]
Ketoprofen	99 μg/kg	Danube River, Europe	[7]
	0.25 μg/g	Novo mesto nearby rivers, Slovenia	[12]
Naproxen	57 μg/kg	Danube River, Europe	[7]
	0.15 μg/g	Novo mesto nearby rivers, Slovenia	[12]

levels of 4 ng/L in upstream, 98 ng/L in waters of the center of a large Ukrainian city (Kharkiv city), and 3,560 ng/L downstream. Thus, the authors suggest that cross-border water management is required, including preventive pollution measures, since without this it would be impossible to achieve the water quality standards established by the European Union [29].

In surface waters of the Kan, Darband, Farahzadi, and Karaj rivers in Tehran (Iran), the presence of ibuprofen (IBU), naproxen (NPX), diclofenac (DIC), and indomethacin (IDM) was determined by solid-phase extraction followed by LC-MS/MS detecting the presence of these NSAIDs in low concentrations; the maximum detected were IBU 0.31 ng/L, NPX 0.041 ng/L, DIC 0.025 ng/L, and IDM 0.041 ng/L (see Table 3). Except for DIC that was found only in one river, the other anti-inflammatories were detected in all the rivers. Thus, the authors conclude that the NSAIDs studied are ubiquitously present in the analyzed aquatic environment [37].

In another investigation, diclofenac concentrations were analyzed by LC-MS/MS in the Elbe, Saale, and Leine rivers in Northern Germany, detecting levels of 370, 160, and 153 ng/L, respectively. The highest concentrations were found downstream compared to the concentrations measured upstream [5].

In the Edo river basin in Japan, Nishi et al. [28] determined the concentrations of seven NSAIDs finding values of 0.5 ng/L for salicylic acid, ibuprofen 1 ng/L, felbinac 10 ng/L, naproxen 2 ng/L, mefenamic acid 2 ng/L, ketoprofen 1 ng/L, and diclofenac 2 ng/L, while the presence of meclofenamic acid in the studied samples was undetectable (see Table 3). The authors suggested that COX inhibitory activity by NSAIDs may be potentially toxic to aquatic organisms. Therefore, COX inhibition assays may be useful for assessing the ecotoxicity of COX inhibitors.

The detection of four nonsteroidal anti-inflammatory drugs was carried out in Lake Päijänne and Vantaa River in Finland. The samples were analyzed by LC-MS/MS. The concentrations of diclofenac, ibuprofen, ketoprofen, and naproxen in Lake Päijänne ranged between 15–35, 13–31, 16–27, and 3.3–32 ng/L, respectively. Similarly, the results found in the Vantaa River samples ranged from 15–65, 13–33, 16–31, to 3.3–6.4 ng/L. Lindholm-Lehto et al. [34] concluded that possibly the environmental conditions and dilution of pharmaceutical products vary due to different loads and along different types of water bodies.

For a decade (years 1997–2007), a monitoring program at four sampling sites along the Rhine River in Europe showed concentrations with maximum levels of 900, 120, 210, and 25 ng/L of diclofenac, ibuprofen, indomethacin, and ketoprofen, respectively . The authors noted that the contamination of the river by the pharmaceutical residues described during the decade of study was almost constant, so the results of this research demonstrate that the control measures undertaken during this period of time to reduce the discharge of pharmaceutical residues to the aquatic environment did not result in a significant improvement in the quality of the receiving waters. Consequently, more effective measures are necessary if it is desired to significantly reduce contamination of the Rhine River with pharmaceutical waste in the coming years [31].

A study conducted in South Africa on the Msunduzi River by Agunbiade and Moodley [18] reported by means of LC-MS/MS the presence of NSAIDs in the surface water of this river in concentrations of 91.3, 92.8, 102.7, and 100.8 μg/L for ibuprofen, acetylsalicylic acid, ketoprofen, and diclofenac, respectively. Interestingly in this study, in addition to performing these NSAID determinations in surface water, they also measured their levels in wastewater, finding that the wastewater treatment process in the area did not significantly reduce these contaminants.

Through LC-MS/MS, Hoshina et al. [38] determined the presence of NSAIDs in Naruo-Shin River samples in Japan. The determination of NSAIDs in river water samples was carried out using a molecularly imprinted restricted access polymer (RAM-MIP) for flufenamic acid as a pretreatment column. The concentrations of mefenamic acid, indomethacin and etodolac in the river water samples were determined to be 0.4, 0.7 and 0.3 ng/L, respectively, while ketoprofen was below the limit of quantification.

In the city of New Delhi (India), a study was conducted to analyze the presence of NSAIDs in the surface waters of the Yamuna River. The collected water samples were quantified using a triple quadrupole tandem mass spectrometer coupled with ultrahigh-performance liquid chromatography system (UHPLC), finding ibuprofen concentrations of 1.09 ± 1.05 and 2.19 ± 4.40 μg/L of diclofenac. Additionally, they revealed that, apart from their therapeutic use, the main source of ecological exposure could be due to the elimination of expired pharmaceutical products in landfills [24]. Similarly, the presence of five NSAIDS (salicylic acid, ibuprofen, naproxen, indomethacin and diclofenac) was investigated in river waters of the urban section of the Pearl River in Guangzhou in southern China by liquid chromatography coupled with a triple quadrupole mass spectrometer with electrospray ionization in negative mode. All pharmaceutical products were detected at least once in the Pearl River. Salicylic acid had the highest maximum concentration (6.7 μg/L) and average concentration (109 ng/L). The second most abundant was ibuprofen with a maximum and average concentration of 288 and 78 ng/L respectively. The average of the other compounds ranged from undetectable to 13 ng/L [20]. In the Kabul and Indo rivers in Pakistan, the presence of three NSAIDs (paracetamol, diclofenac, ibuprofen) was investigated by liquid chromatography with triple quadrupole tandem mass spectrometry. Authors reported paracetamol in concentrations of 32.4 μg/L while ibuprofen and diclofenac at 0.21 and 0.16 μg/L, respectively. These three drugs are those reported with the highest use in Pakistan compared to other pharmacotherapeutic groups [17].

Using Q Exactive high-performance quadrupole-Orbitrap benchtop, 20 water samples from the Danube river basin in Romanian territory were examined by LC-MS/MS to identify and quantify NSAIDs in surface water, reporting the highest concentrations found in the samples for diclofenac, piroxicam, ibuprofen, indomethacin, and naproxen of 166, 32, 58, 7.7, and 22 ng/L, respectively. The study emphasizes that the Danube River crosses several countries in Europe, so it is the responsibility of each one to take care of the river's water quality, as well as to evaluate the levels of pollutants and to implement policies for their detection and elimination [33].

By means of high performance liquid chromatography equipped with a photodiode detector, Madikizela and Chimuka [25] monitored naproxen, ibuprofen, and diclofenac in the Mbokodweni River in South Africa. Ibuprofen was the most frequently detected NSAID, and the maximum detection was 0.68 and 11.4 μg/L for naproxen and ibuprofen, respectively. Diclofenac was found at concentrations below the limit of quantification in these aqueous samples. The authors conclude that

with this study they demonstrated the need to conduct research on the prevalence of NSAIDs in all bodies of water, including lakes and dams.

Patrolecco et al. [35] studied surface water samples from the Tiber and Aniene rivers in Italy using liquid chromatography coupled to tandem mass spectrometry, finding that diclofenac and ibuprofen were at high concentrations 17 and 20 ng/L, respectively, while other NSAIDs such as naproxen and ketoprofen were present in maximum concentrations of 15 and 9 ng/L. In their investigation, they found that diclofenac and ibuprofen were still the NSAIDs most present in the treated waters since after the treatment processes, most of the pharmaceutical products were not completely eliminated. In another investigation, diclofenac and five of its metabolites were identified in different water samples from rivers in Karachi, Pakistan; finding diclofenac in high concentrations of up to 8.5 μg/L and only in a sample of water was found in moderate concentrations of 0.1 μg/L. When studying the metabolites of diclofenac, the authors conclude that hydroxy derivatives appear to reflect human excretions through domestic wastewater, while the chlorocarbazole derivative would be related to the abiotic photolytic transformation of diclofenac [23].

Other studies used different techniques for the detection of NSAIDs. Li et al. [26] evaluated a new adsorbent for magnetic solid-phase extraction using samples from Furong Lake in Xiamen, China, finding ibuprofen levels of 0.80 and 0.94 μg/L of mefenamic acid. In some studies the concentration of NSAIDs in surface waters is determined by sampling in estuaries. Omar et al. [43] conducted a study of this type to determine concentrations of diclofenac in the estuary of the Klang river in Malaysia, which is a section of a river that has been invaded by the sea due to the influence of tides and the sinking of riversides in which large deposits of sludge accumulate, so this matrix of sediments is considered a sink for several pollutants. This study revealed the presence of diclofenac at 13.88 ng/g dry weight. The same authors in 2019 studied the presence of diclofenac in the same river, applying C18 polymer cartridges as extraction sorbent and measuring LC MS/MS technique, finding diclofenac levels equal to 10.8 ng/L. Although this evaluation revealed a negligible risk, its methods and results can be used to monitor changes in the future and to compare with other tropical aquatic ecosystems [36].

Antonic and Heath [12] selected the four most widely used NSAIDs in Slovenia and Central Europe (ibuprofen, naproxen, ketoprofen, and diclofenac) to determine their presence through sediment sample extraction and GC-MS. The analysis was carried out with two river samples from the vicinity of Novo mesto, the largest city in the southeastern part of Slovenia. Analysis of sediment samples showed maximum levels of 0.21, 0.15, 0.25, and 0.10 μg/g for ibuprofen, naproxen, ketoprofen, and diclofenac, respectively. Duan et al. [44] determined through LC-MS concentrations of NSAIDs (ng/g dry weight) in sediments of a river that receives wastewater in Shanghai, China. Ketoprofen, naproxen, and ibuprofen were not detected or were found within the limits of quantification. The maximum concentration of diclofenac was determined 1.6 km downstream (12.9 ± 5.69 ng/g). For their part, Dobor et al. [7] studied sediment samples collected in the urban area of the Danube River, between 1,642 and 1,622 km. The determination of the concentration of NSAIDs

was performed by gas chromatography and mass spectrometry both in liquid and solid phase. Their results show that ibuprofen, naproxen, ketoprofen, and diclofenac had maximum concentrations of 31, 57, 99, and 144, respectively, in solid phase (μg/kg) and 165, 166, 192, and 243 μg/L in liquid phase. As is evident, the highest concentrations of NSAIDs were found in the aqueous samples.

In four different points of the Aisonas River in northern Greece, water samples were taken to determine the presence of ibuprofen, ketoprofen, naproxen, and diclofenac by gas chromatography with mass spectrometry (GC-MS). The Aisonas River receives treated municipal wastewater. NSAIDs were detected mainly in the part of the river that receives water from the wastewater treatment plant. The highest average concentration detected was for diclofenac with 432 ng/L [32].

Ten NSAIDs were analyzed in the Pearl River system in China (i.e., the Liuxi, Zhujiang, and Shijing rivers). Zaho et al. [19] were able to detect five of them by gas chromatography and mass spectrometry under negative chemical ionization mode. NSAIDs salicylic acid, ibuprofen, diclofenac, mefenamic acid, and naproxen were measured with average concentrations between 11.2 and 102 ng/L in the mentioned rivers. Authors observed spatially considerable variations in concentrations for all pharmaceutical products in the three rivers. It was discovered that the water of the Shijing River is the main source of discharge from the Zhujiang River.

Bound and Voulvoulis [27] analyzed surface water samples from the southeast of England on the River Thames by GC-MS, detecting upstream concentrations of ibuprofen and paracetamol at 783 and 264 ng/L, respectively, while downstream the maximum concentrations of these drugs were 846 and 165 ng/L. In this investigation, samples from water treatment plants in West London were also studied, revealing that apparently these waters were not a path for the release of selected drugs into the environment.

In a study carried out by Paíga et al. [39], the concentration of ibuprofen was determined by solid-phase extraction of the analyte and subsequent determination with liquid chromatography coupled to fluorescence detection in surface waters of rivers in the northern area of Portugal, which is one of the most densely populated areas of the country. Their results indicated the highest concentration of ibuprofen in the Lima River, with concentrations of 723 ng/L.

The concentrations of the five most frequently used NSAIDs (ibuprofen, diclofenac, naproxen, ketoprofen, and indomethacin) were determined in the Elbe river basin in the Czech Republic. For quantification of NSAIDs, the methodology combined pentafluorobenzyl bromide (PFBBr) derivatization with highly sensitive two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS). This study determined that ibuprofen was the most abundant NSAID with a maximum concentration of 3,210 ng/L, followed by naproxen, diclofenac, and ketoprofen (1,423.8, 1,080, and 929.8 ng/L, respectively). Indomethacin was found at a maximum concentration of 69.3 ng/L [30].

Some authors have chosen to use other methods of detecting drugs in aqueous samples. Huebner et al. [54] determined the presence of diclofenac in the Isar River and Lake Wörthsee in the German Bavaria using a highly sensitive ELISA developed to detect antibodies against diclofenac. Although the concentrations in their

surface water samples were low (average of 0.031 μg/L), their study proposes a highly sensitive new technique for the detection of drugs in aqueous samples.

Tanwar et al. [55] used two different approaches to determine NSAIDs in water: stir bar sorptive extraction (SBSE) and passive sampling, followed by electrospray ionization liquid chromatography-tandem mass spectrometry. Unfortunately they only found undetectable levels of diclofenac, ketoprofen, mefenamic acid, naproxen, and ibuprofen in samples taken from the Arno river in Italy.

The photolysis of ibuprofen was studied by exposure to a solar simulator in solutions of fulvic acid isolated from Lake Pony in Antarctica: Suwannee River, GA, United States. High-pressure liquid chromatography using a UV-visible dual wavelength detector found ibuprofen concentrations of 7.20 mgC/L in the Suwannee River and 5.45 mgC/L in Lake Pony. The authors conclude that the photolytic fate of ibuprofen in sunlit waters is affected by its concentration and the source of dissolved organic matter present [56].

In the Doñana National Park in southern Spain, one of the most emblematic protected areas in Europe included in the UNESCO World Heritage List, a 1-year monitoring study was conducted to investigate the presence of NSAIDs in waters of rivers and streams that affect the Park. Using high-performance liquid chromatography with diode matrix and in-line fluorescence detectors, the presence of diclofenac was detected with a maximum average of 0.09 μg/L, ketoprofen 0.20 μg/L, naproxen 0.64 μg/L, and salicylic acid at 0.52 μg/L. Ibuprofen was the compound found at the highest concentration levels, with an average of 1.21 μg/L. The authors observed an increase in concentration levels in surface waters in the summer months due to the reduction in river flows [22].

For their part, Ma et al. [40] developed a study to determine, through a direct chiral analysis by means of LC-MS/MS, NSAIDs in the mainstream of the North Canal Basin and its main tributaries (Qinghe, Bahe, Tonghui, and Liangshui) in the most urbanized and industrialized zone in the northeast of Beijing (China). Their analyses revealed that ibuprofen was the most abundant NSAID, with an average concentration of its enantiomers of 114.9 ng/L, naproxen was also detectable at concentrations of 43.2 ng/L, both presenting an excess of the S enantiomer. Therefore, they argued that to better understand the ecological risk, chiral contaminants must be analyzed at enantiomeric levels. The authors indicate that this study is the first to outline the enantiospecific occurrence of NSAIDs in surface waters in Beijing.

Finally, another study that evaluated by means of LC-MS/MS the chiral fractions of ibuprofen, ketoprofen, and naproxen was the one conducted by Camacho-Muñoz and Kasprizyk-Hordern with surface water samples from a river in South West England. They found ibuprofen as the maximum contaminant in both chiral fractions (S 466 ng/L, R 1076 ng/L) followed by chiral fractions of naproxen (S 23.7 ng/L, R 25.9 ng/L) and finally those of ketoprofen (S 4.37 ng/L, R5.29 ng/L) [57].

This review has revealed the scarce research that still exists on the subject around the world. It is a pressing need to further develop techniques with satisfactory sensitivity to detect very low levels of NSAIDs and other pharmaceutical contaminants in natural water sources, in particular in the surface water of rivers, lakes, and

lagoons, both through the optimization of those approaches already useful for quantifying levels of these drugs in wastewater (pre- and posttreatment) as through the development of new and more efficient methods.

3 Saline Waters

Saline waters comprise environmental aquatic reservoirs represented mainly by oceans, seas, and coasts. Marine ecosystems are the final recipient of surface waters, but also urban sewage effluents, medicinal products used in marine aquaculture, animal husbandry and horticulture along rivers and in coastal areas, as well as leachates from coastal landfills and seafills constitute the main sources of arrival of pharmaceuticals as pollutants to marine waters [14]. Seawater presents important differences in physicochemical conditions like salinity, pH, and organic matter in comparison to fresh water which can significantly modify the environmental fate of pharmaceuticals. This makes this ecosystem very different from that of fresh water so widely investigated and justifies the urgency of qualitative and quantitative research on the presence of pharmaceuticals in marine waters.

Moreover, coastal areas are home to large megacities and thus continually impacted by anthropic activities. Together with many other emerging contaminants, medicinal products and their metabolites directly or indirectly end up reaching the marine environment, and to date, research on the ecotoxicological effects of those pollutants on aquatic organisms, especially on tropical species, is still very limited [58]. Fortunately, publications have been increasing in recent years dealing with toxicity studies in marine organisms such as the sea snail *Gibbula umbilicalis*; the marine crustaceans *Gammarus* spp., *Artemia* sp., and *Mysidopsis juniae*; the echinoderm *Echinometra lucunter*; the Manila clam *Ruditapes philippinarum*; mussels such as *Mytilus galloprovincialis* and *Mytilus edulis*; algae such as *Laminaria digitata* and *Fucus vesiculosus*; and the Pacific oyster *Crassostrea gigas*.

Recently, several NSAIDs have been detected specifically in seawater from different parts of the globe.

In the Saudi Arabian coastal waters of the Red Sea, Ali et al. reported diclofenac, ibuprofen, and acetaminophen maximum concentrations of 14,020, 508, and 2,363 ng/L, respectively [59]. Ibuprofen was also identified in different sites of the coastal and ocean waters from the Gulf of Cadiz (SW Spain) in concentrations up to 32.3 ng/L in oceanic water, where acetaminophen, diclofenac, mefenamic acid, and salicylic acid were also detected in concentrations up to 2.8, 2.5, 2.7, and 86.3 ng/L, respectively. Coastal water presented higher maximum concentrations for 10 out of a total of 11 NSAIDs investigated by Biel-Maeso et al.: acetaminophen (41.5 ng/L), diclofenac (31.9 ng/L), fenoprofen (7.5 ng/L), ibuprofen (1,219.70 ng/L), indomethacin (4.5 ng/L), ketoprofen (2.6 ng/L), mefenamic acid 4.5 ng/L), naproxen (95.8 ng/L), phenazone (309.8 ng/L), and salicylic acid (977.2 ng/L) [60].

For the purposes of this chapter and based on the systematic review conducted in MEDLINE/PubMed, we found 9 out of a total of 82 articles focused on and reporting

NSAID concentrations in saline waters. Figure 2 details the defined inclusion criteria used for study selection.

Table 5 shows a summary of the NSAIDs and their maximum concentrations found in different saline water sources worldwide.

Fontes et al. took water samples from six sites surrounding the submarine sewage outfall in Santos Bay in Sao Paulo, Brazil, to evaluate the occurrence of diclofenac. By means of LC-MS/MS and with a limit of detection of 0.81 ng/L and a limit of quantification of 3.0 ng/L, they were able to determine diclofenac concentrations up to 4.01 ng/L in surface samples and 4.78 ng/L in bottom samples. Authors explained the occurrence of diclofenac, especially in the bottom samples, by the lower sunlight in these samples, avoiding photodegradation. In this study, ecotoxicity assays were also conducted by assessing the effects of diclofenac containing water on the brown mussel *Perna perna*. Authors found cyto-genotoxicity in adult mussels exposed to ng/L levels as well as lipid peroxidation, lysosomal membrane destabilization, and COX inhibition [52].

Pusceddu et al. *used* liquid chromatography/electrospray ionization tandem mass spectrometry on a negative acquisition mode (LC-ESI-MS/MS) to determine ibuprofen from sediments of five sampling sites within the surroundings of the sewage outfall of Santos Bay. Limits of detection and quantification were 2.12 and 7.09 ng/g of sediment, respectively. They also evaluated the chronic effects of ibuprofen for three marine species. Ibuprofen affected the development of *Lytechinus variegatus* and *Perna perna* and caused a significant decrease in *Mytella charruana* lysosomal membrane stability at environmentally relevant concentrations (0.15 ng/g of sediment). Authors declared that these are the first data of sediment risk assessment of pharmaceuticals and personal care products of Latin America [53].

Several NSAIDs were evaluated by Lolić et al. in 14 bathing beaches of the North Portuguese coast. LC-MS was utilized with the following limits of detection in ng/L: acetaminophen 0.3, acetylsalicylic acid 0.10, carboxyibuprofen 8.18, diclofenac 0.02, hydroxyibuprofen 3.90, ibuprofen 0.08, ketoprofen 0.3, naproxen 0.02, and nimesulide 0.06. Authors highlighted that sampling locations were chosen taking into account the bathing water quality of the beaches (excellent, good, or sufficient, according to European regulation). Interestingly, and since the quality classification only takes into account microbiological parameters, hazard quotients higher than one were observed in seawaters classified as excellent bathing water. The highest concentrations for most of the pharmaceuticals found in seawaters were reported in a very densely populated area, namely, the Porto coastal area. Finally, the results showed that diclofenac was the only pharmaceutical that might be expected to pose an ecotoxicological risk to organisms [45].

Paíga et al. developed a UHPLC-ESI-MS/MS analytical method to study 13 NSAIDs and metabolites in three beaches located near Oporto in the Northern Portuguese coast. Limits of detection and of quantification ranged from 0.02 (naproxen) to 8.18 ng/L (carboxyibuprofen) and 0.06 (naproxen) to 24.8 ng/L (carboxy-ibuprofen), respectively. The concentrations detected for the different pharmaceuticals varied from 0.46 ng/L for nimesulide to 600.5 ng/L for carboxyibuprofen, being the highest concentrations reported for ibuprofen and its

Table 5 NSAIDs and some metabolites detected in marine aquatic environments around the globe

NSAID measured	Maximum level reported in ng/L	Saline water source	References
Acetaminophen	584	Atlantic Ocean, North Portuguese coast, 14 bathing beaches	[45]
	275	North Portuguese coast, 3 bathing beaches near to Oporto	[46]
	201	Baltic sea, Gdańsk Bay, Poland	[47]
	16.7	Coastal southwestern Taiwan	[48]
Acetylsalicylic acid	25	Pacific Northwest coasts and fjords	[49]
	5.34	Atlantic Ocean, North Portuguese coast, 14 bathing beaches	[45]
	5	North Portuguese coast, 3 bathing beaches near to Oporto	[46]
Carboxyibuprofen	1,227	Atlantic Ocean, North Portuguese coast, 14 bathing beaches	[45]
	600	North Portuguese coast, 3 bathing beaches near to Oporto	[46]
	7	Seawater from Tromsø-Sound, Norway	[50]
Diclofenac	241	Atlantic Ocean, North Portuguese coast, 14 bathing beaches	[45]
	102	Baltic sea, Gdańsk Bay, Poland	[47]
	33	North Portuguese coast, 3 bathing beaches near to Oporto	[46]
	11.6	Singapore's coastal waters from 8 sites	[51]
	4.01–4.78	Santos Bay, Sao Paulo, Brazil	[52]
Flurbiprofen	87	Baltic sea, Gdańsk Bay, Poland	[47]
Ketoprofen	616	Baltic sea, Gdańsk Bay, Poland	[47]
	89.7	Atlantic Ocean, North Portuguese coast, 14 bathing beaches	[45]
	23.3	Coastal southwestern Taiwan	[48]
	17	North Portuguese coast, 3 bathing beaches near to Oporto	[46]
Ibuprofen	222	Atlantic Ocean, North Portuguese coast, 14 bathing beaches	[45]
	110	North Portuguese coast, 3 bathing beaches near to Oporto	[46]
	48	Baltic sea, Gdańsk Bay, Poland	[47]
	12.1	Coastal southwestern Taiwan	[48]
	9.1	Singapore's coastal waters from 8 sites	[51]
	0.7	Seawater from Tromsø-Sound, Norway	[50]
	49 ng/g of sediment	Santos Bay, Sao Paulo, Brazil	[53]

(continued)

Table 5 (continued)

NSAID measured	Maximum level reported in ng/L	Saline water source	References
Hydroxyibuprofen	287	Atlantic Ocean, North Portuguese coast, 14 bathing beaches	[45]
	190	North Portuguese coast, 3 bathing beaches near to Oporto	[46]
	1.5	Seawater from Tromsø-Sound, Norway	[50]
Naproxen	178	Atlantic Ocean, North Portuguese coast, 14 bathing beaches	[45]
	171	Baltic sea, Gdańsk Bay, Poland	[47]
	59	North Portuguese coast, 3 bathing beaches near to Oporto	[46]
	7.3	Singapore's coastal waters from 8 sites	[51]
Nimesulide	7.33	Atlantic Ocean, North Portuguese coast, 14 bathing beaches	[45]
	0.46	North Portuguese coast, 3 bathing beaches near to Oporto	[46]

metabolites and acetaminophen, two pharmaceuticals with a high consumption rate among Portuguese population [46].

To be able to overcome the challenges of analyzing highly complex matrices and in order to detect and quantify NSAIDs in seawater (Baltic Sea, port of Gdynia, and the Gulf of Gdansk near the village of Mechelinki) and wastewater samples collected in Poland, Caban et al. developed a SPE-GC-MS (with selected ion monitoring modes) method based on the derivatization of NSAIDs by dimethyl(3,3,3-trifluoropropyl)silyldiethylamine. Authors provided a very useful new method for the determination of NSAIDs especially in complex matrices such as wastewaters [47].

Seven NSAIDs among many other organic contaminants were analyzed in coastal waters of southwestern Taiwan (Tainan coast, Kaohsiung coast, and Pingdong coast) by solid-phase extraction and liquid chromatography coupled to tandem mass spectrometry (SPE-LC-MS/MS). Method detection limits for NSAIDs ranged from 2 (acetaminophen) to 5.5 (naproxen) ng/L in seawater. Only, ketoprofen, ibuprofen, and acetaminophen were detected; the latter was detected in 100% of the samples analyzed. Authors emphasized the importance of further studies in coastal areas in order to investigate temporal pollutant variations, such as periods of reduced river discharge in dry season and stormwater discharges during wet season [48].

Bayen et al. analyzed seawater from Singapore. Sampling occurred below surface (3 m depth) and during the relatively dry phase of Singapore's Southwest Monsoon Season, characterized by mean daily rainfall (<150 mm). LC-ESI-MS/MS analysis was performed, and naproxen, ibuprofen, and diclofenac were quantified. According to the authors of the study, their results indicate that concentrations of emerging

contaminants in this coastal marine system follow a trend where levels are highest at sites having the lowest flushing potential (i.e., high residence time) [51].

In 2011, Keil et al. evaluated the presence of 37 organic compounds in 66 locations of Barkley Sound (British Columbia, Canada) and Puget Sound (Washington State, USA), since marine organisms in the Pacific Northwest, particularly those in Puget Sound, were already showing signs of environmental stress. Those locations are proximal to large and growing urban populations. After GC-MS analysis, salicylic acid was the only NSAID ranking among the compounds with statistically higher concentrations in Puget Sound and higher frequencies of detection [49].

Finally in Norway, selected pharmaceuticals were determined in seawater from Tromsø-Sound, into which the sewage treatment plant effluents and non-treated sewage were discharged. Analyses were performed by means of LC-MS, and only ibuprofen and two of its metabolites were detected. By that time, this report represented the first scientific evidence on the presence of ibuprofen and its metabolites in a marine environment. Authors also concluded that despite the strong tidal current and the resulting dilution with presumably non-contaminated North Atlantic water, ibuprofen and/or its metabolites can be found in most seawater samples [50].

The review of these studies allowed us to observe that fortunately, the most recent research conducted in marine waters complements the quantitative analysis of pollutants with the ecotoxic evaluation of the waters sampled on marine model organisms.

Table 6 NSAIDs quantified in groundwater samples

	Study country [reference] Maximum concentration measured (ng/L)	
NSAID	India [24]	Portugal [61]
Diclofenac	Surface: 2.19; aquifer: 73.86	n.i.
Ibuprofen	Surface: 1.09; aquifer: 0.44	n.i.
Acetaminophen	n.a.	n.i.
Nimesulide	n.a.	9.24
Salicylates	n.a.	71
Ketoprofen	n.a.	n.i.
Detection method	LC-MS/MS	UHPLC-MS/MS system triple quadrupole mass spectrometer with an electrospray ionization source (ESI)

n.a. not analyzed in the study, *n.i.* not identified in any sample, *UHPL-LC-MS/MS* ultra-performance liquid chromatography coupled to tandem mass spectrometry, *LC-MS/MS* liquid chromatography coupled to tandem mass spectrometry

4 Groundwater

Figure 3 details the defined inclusion criteria used for study selection, and Table 6 presents the summary of the included studies reporting the prevalence of NSAIDs in groundwater.

Velpandian et al. analyzed many drugs in groundwater samples (aquifers) from 35 locations around Delhi and National Capital Region. Diclofenac was found at higher levels in aquifers as compared to surface waters (Yamuna River). The authors determined the Predicted Free Drug Levels (PFDL) in the aquifers based on the annual drug consumption data collection from the Central Government Health Scheme (CGHS) in India. Diclofenac usage was estimated to be 188.17 kg/million/year; thus PFDL was calculated as 1.88 kg/million/year. Based on this, the expected free drug would be 1.88 μg/L, but in aquifers (200 m from landfill) concentrations measured were 1,390 μg/L. The lack of correlation showed that several factors were involved in the occurrence of diclofenac and other drugs in the aquifers. Particularly, an unscientific landfill where the un-segregated garbage was being dumped and the active leach continuously drained into the water bodies was found to be responsible for the presence of drugs in groundwater. From these results, authors strongly recommended the implementation of the policy for the segregation and destruction of bioactive compounds in densely populated places to avoid their accumulation in the environment [24].

The other study included in our review was carried out by Paíga and Delerue-Matos in 2016 through an interesting approach by analyzing groundwater from five cemetery areas since those places may have serious environmental consequences, particularly on quality of adjacent groundwater. The study assessed 33 different pharmaceuticals, NSAIDs among them. Salicylic acid, ibuprofen, and ketoprofen were the NSAIDs present in all samples with 100% of detection frequency. Acetaminophen and nimesulide were also detected and quantified. Only salicylic acid was found in relatively high concentrations [61].

Even when literature is scarce, it is clear that in terms of public health, the continuous investigation and monitoring of groundwater and the substances polluting it is of paramount importance.

5 Drinking Water

For water potabilization from rivers, oceans, or groundwater sources, drinking water treatment plants (DWTPs) usually apply combinations of several processes such as preoxidation with chlorine, coagulation, flocculation, sedimentation, sand filtration, ultrafiltration, postchlorination, disinfection, desalination, ozonization, or granular-activated carbon treatments [62]. Raw water sources have demonstrated the presence of several pharmaceuticals, some of them in high concentrations, which makes them

Table 7 NSAIDs quantified in drinking water samples

	Study country [reference] Maximum concentration measured (ng/L) [LOQ (ng/L)]		
NSAID	Spain [62]	France [63]	Hungary [64]
Diclofenac	n.i.	<LOQ [0.025]	<LOQ [0.05]
Ibuprofen	12–17 [10]	n.a.	<LOQ [0.05]
Acetaminophen	n.i.	n.a.	<LOQ [0.05]
Naproxen	n.i.	n.a.	<LOQ [0.01]
Salicylates	n.i.	n.a.	<LOQ [0.1] Acetylsalicylic acid <LOQ [0.05] Salicylic acid
Ketoprofen	n.i.	n.a.	<LOQ [0.025]
Other NSAIDs	n.i. (fenoprofen, indomethacin, mefenamic acid)	n.a.	<LOQ [0.025] Fenoprofen <LOQ [0.05] Flurbiprofen <LOQ [0.005] Indomethacin
Detection method	UHPLC-MS/MS + HRMS (quadrupole-Orbitrap)	Electrochemical displacement immunosensor for diclofenac	MS/MS method optimization

LOQ limit of quantification, *n.i.* not identified in any sample, *n.a.* not analyzed in the study, *UHPLC-MS/MS* ultra-performance liquid chromatography coupled to tandem mass spectrometry, *HRMS* high-resolution mass spectrometry (Q Exactive quadrupole-Orbitrap), *MS/MS* tandem mass spectrometry

more prone to occur in drinking water, although the efficiencies of the treatments used in the DWTPs play a crucial role.

Therefore, it is of great interest to monitor the presence of drugs in drinking water and thereby control and regulate those that could be putting human health at risk.

Figure 4 shows the process and eligibility criteria used to rule out and include studies for this section of the chapter, and Table 7 shows a summary of the NSAIDs and their maximum concentrations found in different drinking water samples worldwide.

Boleda et al. analyzed the presence of 53 pharmaceuticals, 9 NSAIDs among them, in 50 samples of the drinking water provided to 12 million of Spaniards. Overall, the presence of the selected compounds in finished drinking water was very scarce. Practically, only ibuprofen was detected at low concentration levels in 12% of the samples. According to the authors, diclofenac, naproxen, acetaminophen, and indomethacin were not found at measurable levels in the studied samples probably because they undergo oxidation with chlorine, chlorine dioxide, and/or ozone and that leads to them being absent which was observed in similar samples from other studies [62].

Nguyen et al. developed an innovative electrochemical displacement immunosensor able to detect diclofenac from 0.1 pM to 0.1 nM (25 pg/L up to

25 ng/L). They suggested that biosensors constitute detection methods more easily to be transferred to continuous on in-the-field monitoring applications than high-performance liquid chromatography, mass spectrometry, or capillary electrophoresis. Authors used the sensor to quantify diclofenac in real samples of tap water [63].

Finally, an accurate and sensitive micro UHPLC-MS/MS method was developed and validated by Márta et al. for the simultaneous determination of ten nonsteroidal anti-inflammatory drugs (NSAIDs) from different environmental matrices, including drinking tap water. Limits of detection ranged from 0.001 ng/L for indomethacin to 0.05 ng/L for acetylsalicylic acid and limits of quantification from 0.005 ng/L for indomethacin to 0.1 ng/L for acetylsalicylic acid. NSAIDs were not detected in higher concentration than the LOD values. Since pharmaceutical compounds occur in very low concentrations in environmental matrices, the very low LOQ values achieved by the developed method make it very useful to quantify NSAIDs from real samples and for routine water analysis [64].

6 Discussion and Final Thoughts

In this chapter it was shown that there is a great amount of research related to the presence of NSAIDs in water bodies to date and that the approaches of such investigations are very diverse, useful, and innovative.

Our systematic review certainly has limitations that are important to highlight. On the one hand, the search through MeSH terms can lead to the inadvertent omission of several studies caused by the manual and human allocation methodology made of these terms. Also, by focusing our search solely on PubMed, other published studies may also have been omitted. The MeSH database is a very useful tool since it offers controlled vocabulary and well-identified concepts. However, the combination of MeSH terms with natural language terms, synonyms, and alternative terms may have optimized the search strategy and the information retrieval. However, we believe that our sample was sufficiently large to demonstrate the state of the art of world research on NSAIDs as water pollutants.

Acetaminophen, diclofenac, ibuprofen, indomethacin, ketoprofen, naproxen, mefenamic acid, and salicylates are the NSAIDs investigated and most frequently detected in all studies. On the contrary, etodolac, felbinac, piroxicam, nimesulide, and flurbiprofen are very little studied due to their patterns of use by the population in different countries. This evidence points to the need to prioritize the detection of specific NSAIDs in different types of water based on their consumption in each region of the world. In fact, just as the case of the occurrence of drugs of abuse in surface waters (e.g., environmental cocaine levels) has been considered as an approach with the unique potential ability to monitor local drug abuse trends in real time [65], also the detection of NSAIDs in the different types of water can bring us closer to their magnitude and trends of use as well as to the practices of inadequate and indiscriminate disposition that a population may be doing with these medicinal products.

The concentrations found for each NSAID constitute highly relevant information for the design of ecotoxicity studies in different species based on "environmentally relevant" levels. This in turn will favor the establishment of permissible limit values for specific NSAIDs and, on the other hand, will direct the development of water degradation, treatment, or purification methods capable of truly solving the problem.

The worldwide distribution of studies on the subject is practically concentrated in Asia and Europe, although research is still isolated and dispersed and is carried out by very specific research groups around the world. This does not mean that following the precautionary principle, the evidence already available should not be used to reinforce regulations and continuous monitoring by all environmental regulation agencies in different countries. Pollutants do not know about political borders, much less in the case of water. However, and despite all the evidence that has been published for more than a decade, regulation on pharmaceuticals discharges and environmental risk assessment remain largely unchanged.

Analytical methods have been optimized to become increasingly sensitive and capable of quantifying a large number of substances from the same sample even if it can be a complex mixture. However, the need to develop easy-to-apply methods, even portable, but also with good sensitivity, was evident so that they can be used directly at the sampling sites thereby optimizing routine monitoring.

The fate of pharmaceutical products and their metabolites in surface and groundwater is still incompletely understood, especially since the results and knowledge generated through laboratory experiments cannot be translated with complete reliability to the real world.

Moreover, at the end of this review, we also realized that comprehensive studies are needed to monitor the concentrations of the highest consumption NSAIDs from wastewater, through the treatment plants and following their course to rivers, lakes, groundwater, seas, or drinking water where they flow depending on each region. The foregoing would allow a better understanding of the conditions that favor or hinder the gradual disappearance of said compounds throughout their journey in the environment. In turn, this information would be very useful for monitoring the toxicity in sentinel organisms.

From a global perspective, the problem is undoubtedly very complex because different mixtures of pollutants coexist whose composition is dynamic because it depends on phenomena of biotic and abiotic degradation, water flows, pseudopersistence, predominance of certain stereoisomers to stay in the compartments or bioaccumulate, and, of course, the effects of climate change.

Finally, despite the fact that the published studies concentrate on very few countries and regions of the world, this review shows that the contamination of surface waters by NSAIDs is not an exclusive problem of industrialized countries and of those with developed markets, since the presence of these products was detected in various effluents around the world. Even when some countries have more developed policies and regulations, these efforts are diluted and lose impact when they share natural resources with other countries that control and monitor the pollution very poorly. Due to this, the objectives and actions undertaken to avoid the occurrence of medicinal products as water pollutants must be globally

orchestrated so that there are really favorable results for the environmental well-being and health of all living organisms that inhabit the planet.

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Quantification of Non-steroidal Anti-inflammatory Drug in Water

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Contents

Abstract This chapter discusses the main reasons for viewing non-steroidal anti-inflammatory drug (NSAIDs) as emerging contaminants. Their access routes into the environment are specified, in particular into natural and wastewater, and current evidence about their possible toxic effects is described. Mention is made of the most commonly used methods for routine determination from sampling to final quantification of NSAIDs in water samples of diverse origins. The most important aspects of sampling, extraction, and concentration, including microextraction methods, are

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detailed. The methods most commonly used in separation, identification, and quantification of NSAIDs in water are described. These methods include, in particular, gas and liquid chromatography systems and capillary electrophoresis that are coupled to different detectors. It was concluded that it is necessary to develop new methodologies that allow continuous monitoring at even lower costs than presently available.

Keywords Chromatographic methods, MS and MS/MS spectrometric detectors, Natural and wastewater, NSAIDs analysis

1 Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are a heterogeneous group of medications with different chemical structures but with similar effects. These drugs are recommended to relieve fever and pain associated with colds, influenza, and arthritis [1]. They constitute the first therapeutic step in the World Health Organization (WHO) analgesic scale [2], which is why they are the most used medications in veterinary and human medicine [3–5].

NSAIDs have become emerging pollutants in natural and wastewater [3–7] in very varied concentrations depending on the number of inhabitants of the localities and the activities carried out therein. In most cases, they are not completely eliminated in conventional treatment plants [4, 6, 8], so they accumulate in bodies of water at very low concentrations, but over time, they can accumulate in the environment and become harmful to human and animal health. Such concentrations require sensitive analytical methods with low detection limits. This chapter gives an overview of the most used analytical methods for NSAIDs detection today.

2 NSAIDs as Emerging Pollutants

Emerging pollutants (EP) are compounds whose presence in the environment is not considered significant in terms of concentration and distribution and therefore have gone unnoticed for many years [9]. Many of them are not subject to government regulations [10, 11] since there are not enough data available on their impact on the human and animal health and ecosystem status although it has been shown that they can potentially have an appreciable ecological impact and cause damage to health of humans and animals [9–12].

NSAIDs are the most widely used drugs worldwide in human and veterinary medicine and are self-medicated [3–5, 13, 14]. It is estimated that they are responsible for 5–10% of medications prescribed annually [15] and have been recognized as important emerging contaminants [3, 5, 12–16]. Waters from domestic waste

[5, 6, 12–14, 16], the pharmaceutical industry [12, 16–18], hospitals [7, 12–14, 19], veterinary waste [3, 12–14], and water treatment plants are the main contributors of these products to the water cycle and the soil [4, 6, 13, 18, 19].

Detectable NSAIDs levels in surface water sources [3, 5, 9, 12, 14, 18, 20, 21], underground water sources [9, 11, 12, 18, 20, 21], drinking water [3, 5, 9, 12, 18], and treatment plant effluents [5, 9, 12, 18, 21] range from ngL^{-1} to μgL^{-1} [3–5, 9, 14, 19, 21]. Although such concentrations are not harmful to health, these products accumulate in aquatic bodies [4, 6, 21] or are retained in the soil and sediments for long periods of time and finally produce changes that could affect the ecosystem and humans through the food chain [9, 14, 21]. The consequences of their presence in the environment are not yet completely understood [5] although it is known that at the usual doses, they can cause unwanted effects. For example, the decline in the population of vultures in Pakistan and India is attributed to diclofenac that was used in normal veterinary doses [22, 23].

2.1 NSAIDs Access Routes to the Environment

The physicochemical properties of NSAIDs, their metabolites, and degradation products determine their access routes to the environment [5, 6, 9, 11, 14, 18]. When conjugated to polar molecules, they can enter into dosmestic wastewater through the urine and feces of humans and animals, and from there, they travel to treatment plants and water sources [3, 5, 6, 9, 11–14, 21]. Improper disposal of unused or expired drugs, waste from the pharmaceutical industry, and filtering from cemeteries to groundwater of products administered in the final phase of life have also been considered access routes [5, 21], as well as sewers and septic tanks with leaks [11, 18]. In some cities such as Taiwan, hospitals discharge directly into rivers [24] with a consequent increase in pharmaceutical products, including NSAIDs, in the environment.

The characteristics of rural soils also influence the entry of NSAIDs into the environment. The application of human and animal waste to farmland, veterinary products that are administered to livestock and poultry, and sludge from sewage plants that are used as fertilizers pass to groundwater by filtration and leaching [11, 12, 14, 18, 20, 21, 25–27]. This process is coupled with the climatic factor, which also contributes to the entry of these products into bodies of water from the soil [18, 26–28]. Several studies have shown that the content of contaminants in bodies of water varies significantly at different times of the year [26–28].

Technological methods for the treatment of household waste are other essential factors in introducing NSAIDs into the environment. Treatment plants are considered to be primarily responsible for the introduction of NSAIDs to surface and groundwater [3, 5, 24, 26, 29]. Some authors point out that only 40–65% of them are eliminated from treatment plants [30], while others reported percentages of 30–99% [6]. Such proportions vary from one plant to another depending on the technology used and the characteristics of each product [6, 21, 26, 31]. NSAIDs have

a small tendency for removal by adsorption due to their pKa values [31]. However, the relatively high ibuprofen sorption coefficient [21, 28] allows for its elimination by this method and by biodegradation [26, 32]. Diclofenac is eliminated minimally by adsorption and biodegradation [26, 28] although under certain conditions it can be eliminated by photodegradation and biodegradation [21, 31].

NSAIDs that are not eliminated in treatment plants penetrate the environment in their original form or as degradation products and accumulate in water sources in a continuous process that can present a threat to humans and the ecosystem.

2.2 Toxic Effects of NSAIDs at Low Concentrations

There are few data on the toxic effects that low concentrations of NSAIDs produce on human health, aquatic organisms, and ecosystems [21, 31]. Some authors assume that their presence in the waters can have subtle effects of the normal biochemistry of human beings and aquatic species since the latter is exposed to these pollutants throughout their lives [12, 19, 21, 27, 31, 33, 34]. However, data concerning chronic toxic effects in aquatic organisms, except for aspirin, diclofenac, and naproxen, are still scarce. Diclofenac, for example, has been included in the list of products that require surveillance by the European Union [4, 35] due to the harmful effects it causes at low concentrations. Salicylic acid and naproxen affect reproduction of algae and planktonic crustaceans at concentrations of 1.8 mgL^{-1} and 330 μgL^{-1}, respectively [31, 36, 37]. Diclofenac affects the kidneys and gills of rainbow trout and salmonids at concentrations of 5 μgL^{-1} [38, 39]. One thousand times higher concentrations of diclofenac, naproxen, and ibuprofen have been found in fish exposed to effluents from a treatment plant compared to fish in their natural habitat [34]. The degradation products of naproxen and diclofenac are even more toxic than the original compounds [36, 40].

The concentrations of NSAIDs found in drinking water in various countries [14, 17, 18, 31, 33, 41] are of concern to researchers because effects that their continuous intake can have in the long-term are unknown. The situation is aggravated in countries with poor infrastructure for water treatment since the concentrations of these products may be higher than reported [27]. There are mathematical risk models that allow estimating or predicting the possible effects of these products on animals and aquatic plants [27, 31], but it is necessary to accumulate data on continuous exposure in different environments and times of the year in order to accurately deduce the real risks both in humans and other species.

3 Analytical Determination of NSAIDs in Waters of Different Origin

3.1 Main NSAIDs Found in Bodies of Water

The NSAIDs constitute a family of about 50 different products, but the most studied in different water bodies are shown in Table 1. To a lesser extent, pyroxican [3, 42, 55, 103, 104, 118], indoprofen [3], ketocorolac [7, 42] pyroxican [3, 42], diflunisal [43, 55, 104], tolmetin [56], nimesulide [57], meclofenamic acid [44, 45, 58, 59, 112], tolfenamic acid [44, 53, 59, 60], flufenamic acid [58, 60], meloxicam [61], niflumic acid [113], sulindac [3, 55, 103, 104], and metamizol [62]. Among veterinary NSAIDs, carprofen and flunixin have been evaluated [60].

The different types of water from which NSAIDs have been analyzed are shown in Table 2. The terms used by the authors were maintained, although it is important to note that there is great confusion in the literature regarding the terms used to catalog the bodies of water. As can be seen in the table, most of the work on natural waters has been carried out on surface waters, particularly in rivers, while the emphasis on wastewater is in the effluents of the treatment plants.

3.2 General Methodology

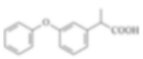
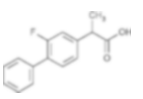
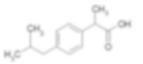
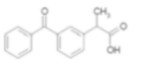
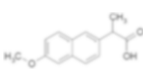
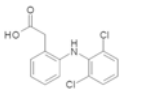
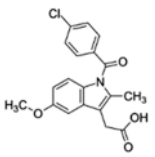
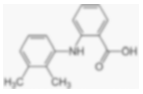
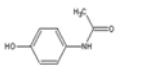
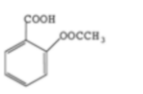
Different analytical methods are used for the determination of NSAIDs in water. Regardless of the selected method, researchers follow a general method that consists of three main stages:

1. Collection and preservation of the sample
2. Extraction and concentration of the analytes of interest
3. Separation, identification, and quantification of analyte

3.2.1 Stage 1: Collection and Preservation of the Sample

Sampling is an important step to ensure quality and reliability of analytical determinations and obtain significant results. The samples must be representative of water quality at the time and place of sampling and relevant depending on the objective of the analysis. There is abundant information on the proper sampling of various types of water. For example, the International Organization for Standardization (ISO) 5667-1 [120] provides guidance on sampling programs and techniques, as well as different types of samples depending on the purpose of the analysis. Different parts and updates of this standard complement the information on the sampling according to the type of water to be analyzed.

Table 1 Main NSAIDs analyzed from different bodies of water

Functional chemical group	Product	Structural formula	M.W (g/mol)	References
Propionic acid derivatives	Fenoprofen		242.27	[3, 17, 42–52]
	Flurbiprofen		244.26	[3, 46, 47, 53, 54]
	Ibuprofen		206.28	[3–5, 7, 8, 16, 17, 19, 24, 26, 28, 30, 32, 33, 42–102]
	Ketoprofen		254.28	[3, 5, 8, 16, 17, 24, 26, 28, 32, 33, 44–57, 59–61, 64, 65, 70–72, 74–76, 78, 82–84, 89, 91, 93, 94, 97, 98, 100, 102–109]
	Naproxen		230.26	[3–5, 7, 8, 16, 17, 26, 28, 30, 32, 33, 42–45, 47, 49–61, 63–65, 67, 68, 70–72, 74, 76, 78, 79, 82, 83, 86, 91–98, 100, 101, 103–106, 110, 111]
Phenyl acetic acid derivatives	Diclofenac		296.15	[3–5, 7, 8, 16, 17, 19, 24, 26, 28, 32, 33, 42–45, 47, 48, 50–57, 59, 61–66, 68–77, 79, 82–84, 86, 91–93, 96, 98, 99, 101–109, 111–116]
Indole derivatives	Indomethacin		357.79	[3, 7, 42, 47, 48, 50–52, 54, 55, 72, 92, 101, 104, 108, 112]
N-acetylanthranilic acid derivatives	Mefenamic acid		241.28	[7, 19, 26, 49, 58, 60, 69, 72, 73, 75, 96, 98, 103, 108, 109]
p-amino phenol derivatives	Acetaminophen		151.17	[7, 19, 26, 33, 53, 57, 62, 63, 67, 69, 72, 73, 76, 87, 88, 90, 91, 106, 108].
Salicylates	Acetylsalicylic acid		180.16	[17, 26, 33, 44, 45, 48–50, 57, 63, 91, 96, 98, 99, 105, 106, 117]

In natural waters, particularly surface waters, simple samples are usually taken [3, 5, 16, 17, 24, 33, 53, 54, 57, 60, 61, 63–70, 105–107, 112], which can subsequently be integrated [28, 32, 46] or not. The samples from the treatment

Table 2 Types of water from which NSAIDs have been analyzed

Group	Subgroup	Type	Category	References
Natural waters	Surface	Rivers	Without another qualifier	[3, 44, 61, 64, 65, 96, 103, 109, 114, 115, 119]
			Where treatment plants discharged	[5, 8, 17, 24, 26, 28, 32, 33, 47, 48, 63, 67, 71, 73, 77, 83–85, 91, 105, 107, 112, 113]
			Where untreated residuals Discharged	[24, 70]
		Lakes	Without another qualifier	[17, 44, 65, 85, 89, 104]
			Where treatment plants discharged	[16, 32, 34, 113]
		Ponds and dams		[47, 54, 65]
		Sea		[16, 50, 57, 66, 96, 112]
		Without other specification		[33, 53, 54, 60, 69, 102, 105, 106, 114]
	Underground			[3, 33, 51, 53, 64, 70, 87, 114, 119].
	Common		From the tap	[3, 17, 43, 50, 54, 60, 61, 64, 69–71, 103, 109, 111].
			Drinking water	[16, 28, 53, 64, 91, 102, 103, 109–111, 114]
Sewage water	Hospital			[7, 19, 68, 89, 95]
	Urban and industrial		Treatment plants input	[8, 16, 28, 30, 42, 44, 45, 47–50, 55, 56, 59, 62, 63, 68, 71–75, 79, 80, 84–86, 92, 93, 98, 101–103, 106, 108, 110, 113]
	Treatment plants		Effluents	[4, 5, 8, 16, 17, 24, 28, 30, 32, 33, 42, 44–48, 50, 52, 55, 56, 59, 62, 63, 65, 66, 68, 69, 71–82, 84–86, 88, 91, 93, 95, 96, 98, 101–103, 106, 108, 110, 113, 117]

plants are almost always composed [28, 30, 32, 46–50, 62, 68, 71–81, 113, 117] with the objective of calculating average concentrations that allow the efficiency of the plant to be evaluated. Sampling can be manual or via automatic sequential samplers [48, 62, 66, 69, 73, 75, 80–82, 121, 122] that allow for increasing the sampling frequency. In the case of water-soluble organic pollutants, such as NSAIDs, there are specific samplers that facilitate obtaining the average analyte concentration over periods ranging from weeks to months [74, 82, 83, 121, 122].

In the rivers, samples are taken in the central area at a depth of 0.25–1 m [8, 24, 44, 53, 57, 64, 70]. It is recommended to account for the flow rate on the date the sample is taken [28, 75, 118]. Samples of lakes are taken at the deepest point [32]. The sample volume depends on the type of analysis to be performed. It usually ranges between 0.5 and 4 L [8, 16, 24, 28, 32, 47, 51, 53, 54, 56, 57, 60–62, 64, 70, 73–76, 80, 83–85, 113, 117] although it can be greater [47, 48, 107, 112] or less [45, 69, 72, 113]. To take samples of tap water, the water source is first allowed to run for 1–30 min and then the desired amount is collected [17, 61]. In treatment plants, the samples are taken at specific points according to the installed technology [30, 56, 68, 72, 79, 83] and the objective of the analysis. Water from the entry and exit points is essential [28, 47–49, 62, 66, 71, 74–77, 81, 86, 103, 106, 108, 110, 117].

The container into which the sample is collected is very important. There are various types of containers for collecting water samples. The most widely used is amber glass [7, 16, 50, 62, 63, 67, 69, 72–74, 76, 83, 87, 110, 112, 113] that is recommended for the analysis of organic compounds [118]. Clear plastic ones [45, 47, 53, 60, 106] are also used and are indicated for trace analysis [118]. Some authors collect samples in stainless steel containers [32, 48, 66, 82] and then transfer them to glass containers. Use of new containers is suggested, and reuse of those that have contained contaminated samples, concentrated solutions, or fuels should be avoided [118]. Glass containers are usually rinsed previously with acid [16, 117], organic solvents [16, 48, 52, 66, 69, 73, 85, 112, 113], or only ultrapure water [5, 7, 16, 17, 48, 52, 57, 83, 105, 112]. It is suggested to coat the container prior to sample collected, two to three times, with the water to be sampled [61, 118] and dry the vessels in an oven after washing in order to reduce subsequent biological degradation [16, 112].

In the case of natural water, it is useful to describe the location of the sampling points [8, 16, 28, 32, 33, 47, 57, 73, 81, 87, 88, 106, 107, 110, 113] and the dates or periods of sample collection [8, 17, 28, 33, 60, 75, 87, 88, 105, 107, 108, 110, 113] since changes in the meteorological conditions of the sampling site can induce marked variations in the results.

Care must be taken to prevent sample deterioration or contamination before running the analysis. Water samples are susceptible to changes due to physical, chemical, or biological reactions that may occur during the time between sampling and analysis [118]. The magnitude of these reactions depends on the chemical and biological nature of the sample, the temperature, light exposure, the nature of the container into which the sample is placed, the time between sampling and analysis, and the conditions to which the sample is subjected (agitation or rest during transport). Therefore, it is necessary to take precautions to minimize these reactions. Ideally, the analysis should be conducted in the first 72 h after the sample is collected [118]. The most common methods for preserving the sample are removal of suspended material, control of temperature and pH, and the addition of reagents that do not interfere with the subsequent analysis.

Removal of the suspended material allows exclusion of the particles that may interfere with the extraction stage, in particular if this stage is executed in the solid

phase. It is usually performed by filtration. For this purpose, different filters are used. The most commonly used filters are membranes with a pore diameter of 0.22 μm [8, 57, 86] or 0.45 μm [3, 50, 58, 63, 64, 103, 104, 109] and 2.7 μm glass microfiber filters [5, 107], 1.6 μm [16], 1.2 μm [57, 60, 66], 1 μm [71], 0.7 μm [7, 62, 74, 83, 87, 88, 112], and 0.45 μm [5, 16, 24, 52, 69, 71, 73, 89] that have been previously baked at 450°C for at least 2 h [118]. Sometimes, ultracentrifugation is used [113].

Refrigeration minimizes chemical changes caused by biological activity [121]. For this reason, once the sample is collected, it is transferred to the laboratory at 4°C [5, 8, 16, 17, 24, 32, 57, 71, 105, 108], packed on ice [76, 82, 110], or frozen [45].

Acidification prevents biological degradation [30] and prevents cation precipitation [118]. In order to acidify the sample, hydrochloric (HCl) [3, 24, 28, 33, 49, 57, 58, 63, 69–71, 73, 103, 104], phosphoric (H_3PO_4) [16], formic (CH_2O_2) [51], or sulfuric (H_2SO_4) acid is used [52, 66, 76, 82, 90, 110]. The volume that is added depends on its concentration. The pH is generally adjusted to between 2 and 3 [3, 16, 24, 28, 30, 32, 33, 45, 49, 51, 52, 57, 58, 63, 64, 67, 69–71, 73, 76, 79, 90, 104] although some authors adjust the pH to <2 [110, 113] and others adjust it to pH > 4 [66, 103]. Sodium azide has also been used to prevent biological degradation [112].

Once filtered and acidified, the sample is stored until the next stage of the analysis at 4°C [44, 50, 53, 62–66, 74–77, 80, 82–84, 86, 89, 91, 92, 104, 109] or frozen at temperatures between −20 and −4°C [17, 57, 60, 67, 70, 84, 103, 112].

3.2.2 Stage 2: Extraction and Concentration of the Analytes of Interest

The low concentrations of NSAIDs in water samples and the complexity of the matrices require that the sample undergo extraction and concentration stages before carrying out the final analytical determination. The most common route of extraction is to put the matrix in which the analytes are in contact with another phase, so they are transferred to the extraction phase. Ideally, the extraction should be exhaustive, but this depends on different factors, such as the nature of the extraction phase, the sample and extraction phase volumes, temperature, pH, salinity, among other parameters. The techniques used for this purpose are ion exchange (IE) [117], liquid-liquid extraction (LLE) [13, 60, 106], and solid phase extraction (SPE) [5, 7, 8, 16, 17, 24, 28, 30, 32, 33, 43, 48, 49, 51, 56, 62, 66, 67, 70–72, 74, 79–81, 84, 86, 88, 93, 105, 107, 108, 110, 114], which is the most used technique to analyze NSAIDs in water due to the high availability of sorbents, lower amounts of organic solvents, shorter extraction times, and the possibility of coupling it in line with the chromatographic methods used in the next stage. Combined with ultrasound (US), the SPE method is called SPE assisted by US [74, 121].

For SPE, reverse phase cartridges of different materials and formats are used. The Oasis HLB stand out [5, 7, 8, 16, 17, 24, 32, 48, 50, 62, 66, 70, 72, 79, 80, 82, 88, 108, 110, 112] and is composed of a copolymer of N-vinyl pyrrolidone and divinylbenzene [13] with different hydrophilic-lipophilic balance that serve to extract both polar and non-polar compounds [121, 123]. The MCX Oasis [28, 30,

33, 56, 80, 81, 84, 86, 105, 107] with mixed fill (ion exchange-reverse phase), and those of high-purity silica RPC-18 [46, 49, 51, 52, 70, 71, 74, 80, 89, 107] are effective for extracting non-polar or moderately polar compounds. Strata X [19, 43, 57, 64, 69, 73, 76, 77] consists of a polymeric sorbent and is used over a wide range pH values. The ENVI 18, designed for water samples, is less used [70, 75, 83]. Disk-shaped cartridges tolerate higher sample flows [53, 67, 121]. Both the cartridges and the disks can be coupled with detection equipment to perform the process automatically [51–54, 67, 123]. Molecularly printed polymer (MIP) cartridges have been used in recent years [65, 68].

The cartridges must be preconditioned according to the manufacturer's instructions. It is usual to wash them first with methanol [5, 7, 8, 17, 19, 24, 52, 56, 57, 62, 64, 66, 70–73, 75, 76, 82–84, 105, 106, 108, 112] followed by deionized water [5, 7, 8, 16, 17, 49, 62, 66, 70–73, 85, 110], which can be acidified to the pH of the sample [5, 7, 16, 19, 24, 28, 33, 43, 52, 56, 57, 64, 73, 75, 76, 83, 84, 86, 105, 108]. Ethyl acetate [16, 33], n-hexane [28, 49, 78], dichloromethane [49, 110], acetonitrile [86], tert-butyl methyl ether [110], and acetone [28, 43] are also used. Sometimes, before washing with methanol, cartridges are washed with ethyl acetate [8, 62, 72, 106], n-hexane [52, 84, 106], or acetone [52, 75, 84].

After conditioning, the extraction cartridge is loaded with the aqueous solution of the sample, and the next step depends on the selected analytical method. Some authors wash with deionized water [5, 19, 43, 50, 57, 62, 66, 71, 74, 75, 110], while others wash with a methanol-water mixture [8, 16, 17, 24, 53, 70, 76, 83, 86]. The cartridges are then dried under vacuum [5, 16, 24, 33, 52, 53, 57, 64, 70, 71, 74, 75, 83, 107] or with N_2 gas [7, 28, 43, 46, 50, 51, 56, 62, 66, 84, 105, 110]. The process can be manual or automated [46, 62, 108, 113].

Extraction of the analyte retained in the cartridge is carried out with an organic solvent. The most commonly used solvents are ethyl acetate [8, 62, 66, 74, 77, 79, 80, 107], acetone [28, 51, 56, 67, 83, 114], methanol [7, 17, 19, 24, 48–50, 52, 57, 64–67, 69, 71–73, 75, 80, 88, 91, 107, 110, 112, 114], and mixtures thereof [16, 33, 70, 77, 86, 88, 107, 110]. N-hexane [66] and dichloromethane [67] are used but to a lesser extent. The extracted analyte is evaporated to dryness and reconstituted for the final analysis step. Evaporation to dryness is almost always carried out under an atmosphere of N_2 [7, 8, 16, 17, 28, 33, 49, 59, 63, 67, 68, 71, 78–81, 83, 93, 94, 99, 102, 107] although some authors do so under a vacuum [19, 24, 52, 70]. The final reconstitution allows the sample to be concentrated several hundred times, depending on the subsequent analysis.

The need to increase the extraction efficiencies, use smaller volumes of samples and organic solvents, reduce extraction times, and simplify times and costs of the stages prompted the development of microextraction techniques. These techniques use very little or no organic solvent, isolate and concentrate analytes, have greater specificity and selectivity, are highly enriched, rapid, and allow automation [42, 45, 50, 55, 58, 59, 82, 89, 94–98, 103, 104, 118, 121, 122]. The microextraction of NSAIDs from water samples has been carried out both in solid and liquid phases (SPME and LPME, respectively).

In SPME, the extractant phase consists of a small volume of sorbent material (μL), which is a solid or semi-solid polymer in many cases. The analytes are extracted in the extractant phase and desorbed by temperature or organic solvent, depending on the subsequent analytical technique [59, 60, 104]. If gas chromatography (GC) is selected, the desorption is thermal, and if the technique is high-performance liquid chromatography (HPLC), the desorption is with an organic solvent. It can be carried out both in static and dynamic modes. The interaction with the aqueous matrix can occur via adsorption or absorption, according to the nature of the extractant material.

In static SPME, the fiber variant (IF-SPME) [59, 90, 97, 104], in stir bar (SBSE) [58, 96], with rotating disk (RDSE) [45, 98], dispersive (DSPME) [60, 115] methods, including magnetic particle dispersion (MSPM) [92], have been used. Dynamic SPME has been used in the tube-denominated variant (IT-SPME) [55, 95].

In the IF-SPME, a solid rod (1–2-cm-long) of very small diameter (fiber) is coated with the sorbent (~100 μL). The fiber is attached to a stainless steel piston covered by a protective needle that is adapted to a syringe. The plunger causes the fiber to come into contact with the sample. The analytes are transported from the aqueous matrix to the coating until equilibrium is reached [59, 90, 97, 104]. Therefore, extraction occurs in the outer phase of the fiber. Once the equillibrium is reached, the fiber is removed from the solution, and the analytes are desorbed in the detection equipment itself [59].

The SBSE uses magnetic glass-coated stir bars and is subsequently wrapped with a film (50–300 μL) of sorbent material [58, 96]. In the RDSE, a rotating Teflon or silica disk coated on one of its surfaces instead of a stir bar is used by the material and constitutes the extraction phase [45]. In some cases, the entire disk consists of the sorbent material [98]. The contact area is larger than in the case of SBSE; it can be stirred at higher speeds, and lower detection limits are achieved [45, 98]. In the RDSE, the disk does not touch the bottom of the container, which prevents cracking that occurs in the lining due to friction, which occurs in the SBSE. On the other hand, the extraction times are shorter than in the SBSE [98].

The DSPME, also known as DSPE, is based on the direct addition of the sorbent (insoluble) material in the aqueous solution containing the analytes followed by dispersion to favor their contact with the sorbent [60, 115, 124]. Upon completion of the sorption process, the sorbent containing the analytes retained on its surface is separated by filtration or centrifugation. Interferences are easily removed by elution with suitable solvents [60, 115, 124]. Sorbent particles can be chemically modified to improve selectivity for analytes of interest [99, 100]. If the sorbent material consists of inorganic magnetic particles coated with silicon dioxide (SiO_2), aluminum oxide (Al_2O_3), or polymeric materials, they can be separated with a magnet. The technique is then called magnetic solid phase microextraction (MSPE) [92] and is simpler and cheaper than the common DSPME [124]. The most attractive property of the DSPME is its small extraction time [115, 124].

In the IT-SPME the extractive phase is placed inside a very small hollow capillary tube consisting of molten silica [55, 95, 97] through which the sample moves. Sorption is performed in the internal phase of the tube. The extractive phase may

be the coating of the inner walls of the capillary, a filling of particles or packed fibers, or a monolithic bed [97]. The sample is aspirated and expelled from the capillary until equilibrium is reached. It has the advantage that the process of extraction and final determination can be automated. It is very convenient for HPLC [55, 97]. The extracted analytes are desorbed statically or dynamically [104]. The extraction efficiency depends on the nature and thickness of the sorbent and the length and internal diameter of the capillary [55].

There are large amounts of fibers and polymeric sorbents for the extraction and concentration of molecules by SPME. The most commonly used fiber is fused silica [90, 104]. The polymer coating includes polydimethylsiloxane (PDMS) [58, 59, 97] and polyacrylate (PA) [58, 59, 90, 97, 104], the first polymeric sorbents used in SPME. PDMS is less efficient for NSAID extraction than PA [90, 104]. Further development of combinations, such as PDMS-divinylbenzene (PDMS-DVB) [59, 90, 97, 104], carbowax-DVB (CW-DVB) [59, 90, 104], PA-PDMS [93], and carboxeno-PDMS (CAR-PDMS) [59, 97], have improved extraction efficiencies. Nanostructured materials, such as carbon nanotubes (CNT) [60], graphene oxide (GO) [95], iron oxide (Fe_3O_4) nanoparticles [92], molecularly printed polymers (MIP) with specific recognition sites for the target molecules [45, 98, 115], and others, have facilitated development of SPME sorbents. These materials are characterized by porous structures, high specific surface areas, and high thermal and mechanical stabilities. The selection of one or the other depends on the polarity, volatility, hydrophilicity, size of the analyte to be extracted, and the interference to be eliminated [98, 104].

Variants of static SPME can be executed in three modes of extraction: (1) by direct immersion of the sorbent in the solution (DI-SPME) [59, 90, 96–98, 104, 115, 124]; (2) in the free volume of the vial containing the sample (head space microextraction [HS-SPME]) [58]; and (3) with a protective membrane, in which a semipermeable membrane is placed around the fiber to avoid being damaged by compounds of high molecular weight that could be present in the matrix. It is used for heavily contaminated matrices [96]. The most commonly used variants of LPME for the concentration of NSAIDs in water samples are those with a hollow fiber liquid membrane (HF-LPME, which is used in dynamic mode called CHF-LPME) [82, 94] and the dispersive (DLLME) [61, 103, 119].

HF-LPME uses porous hollow fibers, composed of a hydrophobic polypropylene polymer whose pores are impregnated with a small volume of organic solvent (usually 1-octanol). After the pores are filled with the solvent, the hollow fiber is sealed and introduced into the aqueous solution containing the analyte, supported in the cannula of a syringe [42, 94, 95, 99], or in a Teflon microtube [82]. Extraction and preconcentration can be easily performed [42, 82, 94, 99]. If an aqueous phase is also introduced into the fiber in addition to the organic phase, it is then possible to re-extract previously extracted analytes. In this case, the technique is called HF-LLLME [42, 82, 99]. An interesting variant of HF-LPME, proposed by Rezafeizar et al. [95], reinforces the acceptor organic phase (1-octanol) with a nanocomposite of functionalized graphene oxide with hyperbranched polyglycerol (BPH). This modification increases the extraction efficiency even more. The

HF-LPME is simple, fast, and inexpensive allowing for the extraction and concentration of the analytes in a single step [99]. It allows for use of different configurations depending on the particular analysis that is chosen.

The DLLME consists of a very small acceptor organic phase, almost microscopic droplets, on the surface of the donor aqueous phase in order to achieve a large exchange surface. To achieve this, the organic phase is dispersed with a second solvent so that two organic solvents are used concurrently, one immiscible in the aqueous phase (acceptor) and the other miscible in the aqueous phase (dispersant). When the dispersant-acceptor mixture comes into contact with the aqueous phase, and the emulsion is formed, the analyte is then transferred from the sample to the extraction phase. It is then centrifuged in order to separate the two phases. The microvolume of the organic phase contains the extracted analytes, and the aqueous phase contains the impurities and the dispersing agent. The extraction is carried out quickly with high enrichment values [61, 103, 119]. As dispersing solvents, methanol [61, 103], acetone [61, 119], or acetonitrile [61, 103] are used. Instead of a third solvent, ultrasound is used to achieve dispersion; the technique is called ultrasound-assisted emulsification microextraction (USAEME) [54, 103].

3.2.3 Stage 3: Separation, Identification, and Quantification

The complexity of the water samples and the low concentrations of NSAIDs in them require that identification and quantification be carried out using very sensitive separation and detection methods, which allow multiple quantifications of analytes. The most commonly used for this purpose are chromatographic techniques, especially GC and HPLC. To a lesser extent, capillary electrophoresis (CE) [3, 100, 125–132], supercritical fluid chromatography (SFC) [111], and other techniques that are characterized by their speed and selectivity have been used, such as determination using biosensors [116]. For the final quantification of the products, different types of detectors are used.

Gas Chromatography Despite the advantages of GC for the determination of analytes in complex samples, the data provided is not sufficient for an unequivocal identification of the sample components. For its part, mass spectrometry (MS) identifies pure substances almost unequivocally but not the individual components of a mixture that have not been previously separated. The association of both techniques constitutes a powerful tool for the analysis of water samples. Both are compatible because they work in the gas phase and require a very small amount of sample. GC provides the successive elution of the analytes isolated from the mixture, which are then identified in the mass spectrometer (MS) based on their spectra. In this way, the MS acts as a chromatographic detector.

GC-MS It was the first technique used successfully for the determination of NSAIDs in water samples [123] and is still widely used [16, 30, 32, 44–49, 53, 54, 56, 58, 59, 62, 64, 66, 67, 70, 75, 76, 79, 85, 87, 88, 90, 98, 106, 113, 114, 123]. It detects concentrations in the order of $\mu g L^{-1}$ to $ng L^{-1}$ and less. It is fast,

simple, and less expensive than HPLC and has fewer problems associated with the matrix effect. However, due to the low volatility, high polarity, and thermal fragility of the NSAIDs, it requires previous derivatization of the compound, which lengthens the time of the analysis, although the microextraction techniques, which allows extraction and derivatization in one step shorten the analysis time. GC-MS/MS has been used less in aqueous samples [93, 123].

Derivatization is performed by methylation, acetylation, or silylation. Methylation uses diazomethane [32, 64, 70, 85, 94, 101, 113, 114, 117], pentafluorobenzyl bromide (PFBBr) [76], methyl chloroformate [47, 54, 106], dimethyl sulfate (DMS) [58], or tert-butyl ammonium sulfate (TBA-HSO4) [58, 70, 94]. Acetylation is carried out by the addition of acetic anhydride/triethanolamine [101, 114]. In silylation, different silyl reagents are used, such as N-(tert-butyldimethylsilyl)-N-methyl-trifluoroacetamide (MTBSTFA) [5, 16, 45, 59, 79, 93, 98], N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) [16, 33, 35, 64, 93], bis (trimethylsilyl)-trifluoroacetamide (BSTFA) [48, 67, 75, 90, 93], and dimethyl (3,3,3-trifluoropropyl)silyldiethylamine (DIMETRIS) [53].

The GC capillary columns for NSAIDs in water samples generally consist of a stationary phase composed of 5% diphenyl/95% dimethylpolysiloxane or equivalent [16, 43, 45, 47, 48, 54–56, 58, 67, 73, 75, 78, 79, 81, 85, 86, 93, 94, 96, 98, 106, 108, 109, 116, 119, 121] with different lengths, internal diameters, and film thicknesses. Preferred injectors are split/splitless and automatic/not, which are very suitable for trace determination [16, 34, 43, 45–47, 53, 56, 58, 59, 67, 73, 75, 76, 78, 85, 86, 90, 94, 98, 106, 116, 121]. As a carrier gas, high-purity helium (He) is commonly used, [16, 45, 46, 53, 54, 58, 59, 64, 66, 75, 78, 86, 90, 106, 116, 119, 121] although some authors prefer argon (Ar) [68]. The programmed temperature ranges between 50 and 300°C [16, 45–47, 53, 54, 59, 62, 64, 66, 67, 70, 75, 76, 90, 93, 106, 117, 123] and run times between 2 and 45 min [45, 64, 70, 75, 76, 123].

MS used in combination with the GC for the NSAIDs determinations in water are usually quadrupole, single, or triple analyzers [16, 26, 32, 48, 53, 54, 58, 62, 73–76, 90, 106, 108] or ion traps [30, 46, 59, 66, 70]. The ion sources consist of the electron impact ionization (EI) type [46, 47, 53, 54, 59, 66, 70, 73–76, 85, 106, 108], which are very suitable for molecules of polar nature. Sources with chemical ionization (CI) are less used [46]. The analysis in the collecting system is executed in the mode of selective ion monitoring (SIM) [16, 45, 46, 53, 54, 56–58, 64, 67, 70, 74–76, 90, 98, 106, 108, 121]. Some equipment uses high sensitivity detectors [16, 32, 70, 74, 78] that offer detection limits of the order of pgL^{-1} [78]. Spectra libraries that assist the researcher in the identification of compounds [123] are on the market.

GC-FID In addition to the GC-MS combination, GC with flame ionization detectors (FID) has also been used in combination with the DLLME [119] with good results.

Liquid Chromatography Although the advantages of GC-MS are unquestionable, HPLC is widely used for the detection of NSAIDs in water, especially in recent times [121]. This technique does not require derivatization, and it offers the possibility of

using several types of detectors although those of MS/MS are the most used [5, 7, 17, 19, 24, 28, 30, 42, 47, 50, 51, 57, 60, 69, 71–73, 78, 80, 81, 83, 84, 103, 107, 110]. Less frequently, HPLC-MS is used [4, 13, 105]. UV (UV) and UV diode array (DAD) spectrophotometric detectors are used alone (HPLC-UV) [55, 61, 89, 95, 109, 115], (HPLC-DAD) [8, 43, 45, 86, 91, 92, 96, 102, 104] or are combined with MS (HPLC-DAD-MS [43, 105], HPLC-UV-MS/MS [65]. Fluorescence detectors (FI) [102] are also used. The advent of ultra HPLC (UPLC), which allows operating at pressures much higher than normal HPLC, has made it possible to significantly shorten the analysis times (~10 min), improve resolution, and minimize the matrix-associated effects [13, 57, 65, 103, 111, 123].

HPLC-MS/MS HPLC-MS/MS is a sensitive, selective technique that allows to analyze concentrations in the order of ngL^{-1} and less, but unlike GC, it requires an interface for the introduction of the sample into the MS detector. The interface mostly used for polar molecules, such as NSAIDs, is electrospray ionization (ESI) [17, 19, 24, 28, 42, 43, 47, 50, 57, 60, 63, 69, 71–73, 78, 81, 84, 103, 105, 107, 110]. This interface ionizes in solution by evaporation of electrically charged drops that are obtained by nebulization. It can work in positive or negative ion modes [43, 60, 69, 83, 88, 104, 105, 110] or in multiple separation mode (MRM) [7, 17, 19, 24, 28, 42, 50, 57, 63, 65, 69, 71, 72, 78, 80, 81, 84, 103, 107], which allows collection of positive or negative ions depending on the analytes to be detected. NSAIDs are best detected in negative mode [7, 17, 19, 57]. The atmospheric pressure chemical ionization interface (APCI), which ionizes in the gas phase, is less used [30, 110].

The columns most commonly used in HPLC or UPLC equipment for the determination of NSAIDs in water samples are reverse phase type C_{18} [7, 17, 19, 24, 28, 43, 45, 50, 57, 60, 63, 69, 71–73, 80, 83, 84, 86, 91, 105, 121] or C_8 [17, 81, 91] of different sizes and diameters. The mostly used diameter is 2 μm. In order to adjust the pH of the mobile phase, formic acid [7, 19, 50, 65, 69, 73, 91, 103, 107, 110] acetic acid [42, 50, 55, 60, 78], ammonium hydroxide [24, 28, 84], and ammonium acetate [7, 50, 69, 73] have been used. The mobile phase uses methanol [17, 42, 50, 65, 69, 71, 73, 78, 83, 110], acetonitrile [7, 24, 28, 57, 80, 84, 103, 107], or combinations thereof [19, 47, 60, 91]. In order to improve detection sensitivity, tri-n-butyl amine (TrBA) [7, 42, 50, 78] and butylammonium acetate [7] are used. High-purity N_2 is used as a nebulizing gas [17, 19, 28, 42, 47, 50, 71, 84, 103] and as a carrier gas [17, 19, 50], although, for the latter purpose, Ar is more frequently used [28, 71, 103, 110, 121].

MS coupled to HPLC for tandem spectrometry is generally the triple quadrupole method [7, 17, 28, 42, 50, 57, 60, 68, 71, 72, 80, 81, 84, 103, 107] although ion traps have also been used [24]. The sources of ions are desorption and non-gaseous, similar to those used in GC.

HPLC-DAD HPLC-DAD is used when the expected concentrations of analytes are in the order of μgL^{-1} [8, 43, 65, 86, 91, 96, 102, 104] as occurs in plant effluents. The columns can be C_{18} [8, 86, 91, 92, 102, 109], C_{30} [91], or C_{16} [104]. The most

commonly used mobile phases are acetonitrile [8, 45, 65, 86, 91, 92, 96, 102, 104, 109, 111] and methanol [8, 91, 102, 111]. pH adjustment is achieved with formic acid [86, 91], trifluoroacetic acid [91], H_3PO_4 [96], $K_2H_2PO_4$ [8, 45, 104], acetic acid [65, 83, 109], or phosphate buffer [92]. The measurement is performed at wavelengths ranging from 200 to 300 nm [8, 65, 86, 91, 92, 96, 109, 111].

By attaching the HPLC-DAD system to an HPLC-DAD-MS mass spectrometer, detection limits of the order of ngL^{-1} [42, 70] can be achieved. These detection limits are also reached by coupling microextraction systems with HPLC-UV (SPME-HPLC-UV) in a single module while achieving greater selectivity [55, 89, 95].

Capillary Electrophoresis Although less used than GC and HPLC, capillary electrophoresis (CE) has also proven to be a powerful analytical tool for the determination of NSAIDs in water samples. It is based on the different migration speeds under the influence of an electric field, and can separate analytes previously distributed between the mobile and the stationary phase within a capillary column, depending on their different charge/mass relationships and affinity with the selected buffer solution.

The most commonly used variants of CE for the detection of NSAIDs in aqueous samples are capillary zone electrophoresis (CZE) [100, 125, 127, 129], capillary electrokinetic microemulsion chromatography (MEEKC) [3, 125, 130, 131], capillary electrochormatography (CEC) [125, 128, 129], and chromatography micellar electrokinetics (MEKC) [125, 132].

In the CE, the extraction and preconcentration stages are crucial [3]; hence, this technique gained importance as on-line equipment were developed to perform the extraction and concentration phase [127]. Although the method is less sensitive than GC and HPLC, there are different strategies for improving its sensitivity, which allow reaching limits of detection of the order of ng L^{-1}, particularly when coupled with SPME or LPME systems [100, 126].

For CE, columns of fused silica 30–80-cm-long [3, 126–131] with internal diameters of 50–100 m [3, 100, 126–131] (usually 75 μm) covered with polyamide with a window that allows the passage of UV light for detection are used. Buffer solutions are nontoxic phosphate solutions [3, 125, 130–132], borates [3, 100, 125, 127, 130], citrates [125], and acetates [100, 125, 126, 129, 130], over a pH range of 7–9. Reference [125] gives a list of the most used columns for the determination of NSAIDs. As background electrolytes (BGE), organic molecules such as methanol [126–128], *n*-octane [130, 131], *n*-heptane [130, 131], acetonitrile [3, 128], and mixtures thereof [125, 126] are used. In the case of MEEKC and MEKC, surfactant solutions are also added to the BGE [3, 125, 130–132]. The sample is injected in hydrodynamic form [3, 100, 126, 128–132] or electrokinetic [3, 127, 130]. The analytes are separated inside the capillary by applying an electric field for which 10–30 kV sources are used. The most commonly used detectors are UV with and without diode array [3, 100, 125, 127–130] although electrochemical detectors (ECD) [125] and MS detectors [125, 128] have also been used. For degassing of

Table 3 Techniques used in the identification and quantification of NSAIDs in water

Method	Advantages	Disadvantages	Detectors	References
Gas chromatography	High resolution power High sensitivity and selectivity. Fast, simple, low-cost	Very expensive ultrapure gases Requires derivatization to achieve volatile samples	MS	[16, 30, 32, 44, 45, 48, 49, 53, 54, 56, 58, 59, 62, 66, 67, 75, 76, 79, 85, 87, 88, 90, 98, 106, 113, 114, 123]
			MS/MS	[93, 123]
Liquid chromatography	It applies to any organic analyte. Versatility in the mobile phase/stationary phase mix and in the detectors. Efficiency and accuracy	Toxic reagents Expensive equipment Detection limits higher than GC	MS/MS	[5, 7, 17, 19, 24, 28, 30, 42, 47, 50, 57, 60, 63, 69, 71–73, 78, 81, 83, 84, 103, 107, 110]
			MS	[4, 13, 105].
			UV	[55, 89, 95, 106, 109, 115]
			DAD	[8, 43, 45, 86, 91, 92, 96, 102, 104]
			DAD-MS	[43, 45]
Capillary electrophoresis	Miniaturization Low reagent consumption High separation efficiency Shorter analysis times Low-cost and low environmental impact	Less sensitive and selective than GC and HPLC	EC-UV	[3, 128, 129]
			EC-DAD	[127, 130]
			EC-ECD	[121]

the BGE, sonication [127, 130, 132] or vacuum microfiltration [127] can be used. The determination is performed at wavelengths of 200–300 nm [3, 100, 126–132].

The main advantages of CE are the use of microvolumes and faster speeds compared to HPLC and GC. It consumes only microliters of sample and nanoliters of electrolytic solutions, making it a very economical technique. A comparison of the described techniques is shown in Table 3.

4 Conclusions

The concentrations of NSAIDs found in natural and wastewater in different countries are worrying due to the scarce knowledge about their potential long-term toxic effects. The predominant methods for routine determination in water are GC and HPLC in particular coupled to MS or MS/MS. These methods, together with microextraction techniques, require a small amount of sample, are environmentally safe friendly, and are not excessively expensive. However, the need to continuously monitor the presence of NSAIDs and their degradation products in waters of diverse

origin in areas of interest exists. Establishment of appropriate regulations demands the development of new, reliable, and low-cost methodologies, which allow an increase in sampling points and more precise evaluation of the concentrations of these products, in particular, in environmental waters with the purpose of adopting adequate measures to achieve a higher chemical quality of the water.

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DNA Alterations and Cellular Damage Induced by Non-steroidal Anti-inflammatories on Different Species of Fish

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Contents

Abstract Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of drugs used to reduce inflammation, pain, and fever by inhibiting the enzyme cyclooxygenase (COX 1 and COX 2). These drugs have been positioned among the most consumed worldwide. After their biotransformation in the body, they are eliminated as metabolites, and also in the environment they can undergo transformations,

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generating products that are more toxic than the original molecule. Several studies have shown that NSAIDs are not eliminated in conventional treatments used by wastewater treatment plants and represent a continuous contribution to the environment, causing significant effects on biota. However, there has been little attention given to the study of its toxic effects on aquatic organisms. The objective of this chapter is to review, compile, and analyze the oxidative damage induced by NSAIDs in different aquatic organisms, to evaluate the ecotoxicological effects of this type of drugs.

Keywords Aquatic species, Drugs, Toxic effects

1 Non-steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) are drugs commonly used to treat pain, inflammation, and fever and occupy the first position among the most widely used drugs worldwide [1, 2]. These drugs are available in a variety of doses and formulations making them more accessible to the population. These include acetaminophen, acetylsalicylic acid, diclofenac, ibuprofen, ketorolac, and naproxen, among others. Its mechanism of action consists in the inhibition of cyclooxygenase, COX-1 and COX-2 isoforms, involved in the synthesis of different prostaglandins from arachidonic acid [3, 4].

Once they have fulfilled the purpose for which they were designed, these drugs are discharged and can reach the bodies of water through municipal, hospital, and industrial effluents, where transformation products that are formed are molecules that form in the environment as a result of abiotic processes such as photolysis and hydrolysis and which may be more toxic than the original drug. The presence of drugs in the environment is a function of multiple variables, among which the quantity manufactured, dose, and frequency of elimination are highlighted, as well as the effectiveness of wastewater treatment plants for their removal [5].

Pharmaceuticals are designed to persist in the environment and have a long half-life; they can accumulate by continuous releases to the environment. NSAIDs have been detected worldwide in the aquatic environment at concentrations of ng L^{-1} to μg L^{-1} [6], and these concentrations have been shown to have a toxic effect on aquatic and terrestrial organisms at different trophic levels and consequently generate damage to the ecosystem [1].

2 Oxidative Stress, Geno- and Cytotoxicity

Reactive oxygen species (ROS) are generated in the mitochondria as a result of cellular respiration; they are also produced as a result of the metabolic processes that are carried out [7]. At normal physiological levels, they play a role in the regulation of signaling pathways and gene expression, and therefore, their production is of vital importance [8]. In addition, these species are formed during the biotransformation of various drugs including NSAIDs [9], and among these ROS are the superoxide anion radical ($O_2\bullet^-$), its conjugate acid, the hydroperoxide radical ($HO\bullet_2$), hydroxyl radicals (OH•), and hydrogen peroxide (H_2O_2) [10, 11]. The main enzymes that catalyze the generation of ROS include nitric oxide synthase, NADPH oxidase, prostaglandin synthase, xanthine oxidase, lipoxygenase, ribonucleotide reductase, glucose oxidase, myeloperoxidase, cyclooxygenase, and cytochrome P450 [12, 13].

Oxidative stress (OS) is a biochemical imbalance between the production of reactive species and antioxidant systems [8, 14]. Several studies have shown that high levels of free radicals or ROS in conjunction with reactive nitrogen species such as peroxynitrite anion (ONOO-), which is formed by reaction of nitric oxide that is derived from metabolism of arginine with $O_2\bullet$, whose reaction is catalyzed by the enzyme nitric oxide synthase [15, 16], generate damage to biomolecules such as lipids, proteins, and DNA. ONOO- can also affect the state of cellular energy by inactivating mitochondrial enzymes and can trigger the release of calcium from mitochondria [8].

Lipid damage occurs in lipids that contain carbon-carbon double bonds, especially polyunsaturated fatty acids; additionally lipids can also be oxidized by enzymes such as lipoxygenases, cyclooxygenases, and cytochrome P450. The main primary products of lipid peroxidation are lipid hydroperoxides, and secondary products are malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal, which can modify membrane permeability [17–19], altering their fluidity and finally inactivating membrane proteins. MDA is an important cytotoxic product and has a high reaction capacity with multiple biomolecules such as proteins and DNA that lead to adduct formation [17].

Proteins are also susceptible to oxidation by ROS; the main oxidative modifications of the protein occur in amino acid side chains, which include oxidation of the thiol group, aromatic hydroxylation, and formation of carbonyl groups [11, 20]. Besides, ROS can lead to the formation of protein-protein cross-links and the oxidation of the main protein chain, resulting in protein fragmentation [10]. Cysteine and methionine are the most susceptible to oxidation because they contain sulfur atoms which are very reactive [13].

To regulate excess ROS, the cell can (a) restrict breathing in the mitochondrial compartment, thus protecting other cellular components, (b) protect DNA by complexing it with histones, and (c) activate antioxidant enzymes [21–23]. Among the latter are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), among others [9]. SOD catalyzes the conversion of $O_2\bullet^-$ to H_2O_2; the

latter is metabolized to O_2 and water by the action of CAT and GPx enzymes [24]. The balance between prooxidant and antioxidant molecules and ROS sequestration is crucial to maintain cell homeostasis [25].

Genotoxicity can be defined as the damage generated to DNA; DNA damage includes chain breaks, sugar damage, modifications, or loss in the bases and cross-links [7, 26]. 8-Hydroxydeoxyguanosine, an oxidized form of guanine, is the main oxidative product of DNA damage that can cause mutations [27]. However, to counteract DNA damage, cells activate at least five specific repair pathways, including base excision repair, nucleotide excision repair, mismatch repair, homologous recombination, and nonhomologous end joining, which are activated at different stages of the cell cycle, allowing cells to repair DNA damage [7]. Micronuclei are extranuclear chromosomal fragments, which are caused by defects in cell division and errors in DNA replication or repair [28, 29]. When DNA damage is persistent, programmed cell death or apoptosis, a regulatory response to DNA damage, is activated to eliminate cells with genomic instability [30].

Cellular cytotoxicity refers to the ability of certain chemical substances to alter basic cellular functions and cause the destruction of living cells [31]. DNA damage induces expression of the tumor suppressor protein p53. The increase in this protein causes the expression of pro-apoptotic proteins of the Bcl-2 family (Bax and PUMA), which promote changes in the mitochondria with the release of cytochrome c. This compound leaves the mitochondria and activates the cascade of caspases [26, 32]. Caspases are a family of proteins belonging to the group of cysteine proteases, essential mediators of apoptosis processes. Caspase-3 activation is the point where the intrinsic or mitochondrial pathway and the extrinsic or death receptor pathway converge, resulting in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, protein cross-linking, formation of apoptotic bodies, ligand expression for phagocytic cell receptors, and absorption by phagocytic cells [30].

3 Biomarkers and Bioindicators

A biomarker is defined as a quantifiable change in the biological response (cellular and molecular), as well as physiological and histopathological alterations and even behavioral changes, which may be related to the toxic effects or exposure of chemicals that are present in the environment [33]. These can be classified into three groups [24, 34]:

(a) Exposure biomarkers: they contemplate the detection and calculation of exogenous substances or their metabolites or the product of an interaction between a xenobiotic and some target molecule or cell, which is measured in a

compartment within an organism. This type of biomarker is used to confirm the exposure of individuals or populations to a particular substance.

(b) Susceptibility biomarkers: they indicate the inherent or acquired capacity of an organism to respond to changes produced by exposure to a specific xenobiotic. This type of biomarker helps elucidate variations in the degree of responses by exposure to a toxic, observing differences between individuals.

(c) Biomarkers of effect: they include the biochemical, genetic, physiological, behavioral, and other alterations within a tissue or body fluids of an organism, which can be recognized and associated with a deterioration of health status.

A bioindicator is defined as a living organism (i.e., plants, plankton, animals, and microbes) that is used to detect the health of the natural ecosystem in the environment and control the presence of pollution and its effect on the ecosystem in which it lives [35, 36].

Some advantages of the use of bioindicators are the following: (1) biological impacts can be determined, (2) monitor the synergistic and antagonistic impacts of various pollutants, (3) diagnosis of toxic effects at an early stage, (4) can be easily counted, due to their prevalence, (5) they are an economically viable alternative compared to other specialized measurement systems [36].

4 Studies of Oxidative Damage on Aquatic Organisms

The effects of NSAIDs on different aquatic bioindicators have been reported worldwide. Table 1 shows some studies that have been carried out in recent years.

5 Conclusions

Oxidative damage to biomolecules are useful indicators of the effects of pollutants (drugs) on aquatic ecosystems, since these types of effects can be associated with organic disorders that can affect the life of the organisms involved. Although there

Table 1 Effects of NSAIDs on different aquatic organisms

Bioindicator/ exposure time	NSAID	Results reported	Country	References
Rhamdia quelen/ 14 days	ACT	ACT at environmentally relevant concentrations (0.25, 2.5, and 25 μg L^{-1}) induced oxidative damage and genotoxicity. In gills, all ACT concentrations reduced the activity of GST and GSH. CAT activity was not altered, and GPx activity increased at higher concentrations. SOD activity decreased to 25 μg L^{-1} and LPX levels increased to 2.5 μg L^{-1}. In the kidney, the activities of GST (2.5 μg L^{-1}), CAT (2.5 μg L^{-1} and at 25 μg L^{-1}), GPx, and GSH increased in all concentrations. SOD activity and LPX levels did not change. ACT caused genotoxicity in the blood and gills at concentrations of 2.5 μg L^{-1} and in the kidney at 2.5 and 25 μg L^{-1}	Brazil	Perussolo et al. [37]
Tinca tinca/ 35 days	IBP DCF	NSAIDs evaluated individually at 60 μg L^{-1} significantly influenced the activity of antioxidant enzymes (GST, GPx, and CAT). In addition, it was observed that at environmentally relevant concentrations (IBP of 0.02 and DCF of 0.2 μg L^{-1}), there were significant changes in the activities of GR, GPx, and GST	Czech Republic	Stancova et al. [38]
Cyprinus carpio 12, 24, 48, 72, and 96 h	IBP DCF	The drugs evaluated (17.6 mg IBP L^{-1} and 7.10 mg DCF L^{-1}) individually and in a mixture induced OS in the brain, blood, liver, and gills, as there were significant alterations in antioxidant enzymes (SOD, CAT, and GPx) and the LPX. Further, the drugs generated geno- and cytotoxicity (significant increase in the number of MNi, DNA damage, and alterations in the specific activity of caspase-3)	Mexico	Islas-Flores et al. [39]
Rhamdia quelen/ 21 days	DCF	The organisms were exposed to environmentally relevant concentrations of DCF (0, 0.2, 2, and 20 μg L^{-1}); in the liver there was a significant decrease in the activity of CAT and EROD to 2 μg L^{-1}. The activity of the SOD enzyme was decreased at all exposure concentrations, while there was an increase in GSH and GST in all concentrations tested. LPX was reduced in the groups exposed to 0.2 and 20 μg L^{-1}. In the testicles, the concentration of 0.2 μg L^{-1} caused the inhibition of SOD, GPx, and GST and also the decrease of LPX. On the other hand, DCF was not genotoxic since the	Brazil	Guiloski et al. [40]

(continued)

Table 1 (continued)

Bioindicator/ exposure time	NSAID	Results reported	Country	References
		MNi showed no significant differences in the exposed groups		
Rhamdia quelen/ 21 days	ACT	The fish were exposed to environmentally relevant concentrations of ACT (0.25, 2.5 μg L^{-1}). The highest concentration of ACT caused PC and an increase in SOD. In addition, it led to an inhibition of EROD and GST activities in both concentrations. ACT also caused liver genotoxicity at 0.25 μg L^{-1}	Brazil	Guiloski et al. [41]
Cyprinus carpio/4 and 24 days	DCF	DCF induced OS in the blood, muscle, gills, liver, and brain of the carp at a concentration of 7.098 mg L^{-1} by exposure to 4 and 24 days. There were significant increases in HPC, LPX, and PC in blood, muscles, gills, brain, and liver. The activity of SOD, CAT, and GPx also increased in these organs. The organism exposed to DCF was affected in the first days of the study (at 4 days), exhibiting a greater response at 24 days in the blood and liver. In contrast, a decrease in the muscle, gills, and brain was observed at 24 days compared to 4 days	Mexico	Saucedo-Vence et al. [42]
Hoplias malabaricus/ after 24 h	DCF ACT IBP	The study was conducted on primary culture of monocytic lineage of *H. malabaricus* anterior kidney. The cells were exposed to DCF (0.2, 2, 20, 200, and 20,000 ng mL^{-1}), ACT (0.025, 0.25, 2.5, 25, and 250 ng mL^{-1}), and IBP (0.1, 1, 10, 100, and 1,000 ng mL^{-1}). DNA damage occurred in monocytic cells at the concentration of 20 ng mL^{-1} of DCF, at concentrations 0.25, 2.5, and 25 ng mL^{-1} of ACT and at concentrations 0.1, 1, 10, and 1,000 ng mL^{-1} of IBP	Brazil	Ribas et al. [43]

ACT acetaminophen, *CAT* catalase, *DCF* diclofenac, *EROD* 7-ethoxyresorufin-O-deethylase, *GR* glutathione reductase, *GSH* reduced glutathione, *GST* glutathione-S-transferase, *GPx* glutathione peroxidase, *HPC* hydroperoxide content, *IBP* ibuprofen, *LPX* lipid peroxidation, *MNi* micronuclei, *NSAIDs* non-steroidal anti-inflammatory drugs, *OS* oxidative stress, *PC* protein carbonyls, *SOD* superoxide dismutase

are reports of the damage generated by exposure to NSAIDs on aquatic organisms, these are not sufficient to determine the impact of these drugs on the aquatic environment.

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Teratogenesis and Embryotoxicity Induced by Non-steroidal Anti-Inflammatory Drugs in Aquatic Organisms

Itzayana Pérez-Alvarez, Hariz Islas-Flores, Leobardo Manuel Gómez-Oliván, and Octavio Dublán García

Contents

Abstract The continuous elimination of pharmaceutical products to water sources has become a worldwide problem and has been getting considerable attention due to the effects that this compounds have induced in aquatic organisms, specifically non-steroidal anti-inflammatory drugs (NSAIDs), one of the most representative group of medications and the most consumed around the world, highlighting the teratogenic and embryotoxic effects induced by NSAIDs on early life stages of different organisms being this the most vulnerable stages in development; the main representants of NSAID group (diclofenac, ibuprofen, naproxen, ketoprofen, paracetamol, acetylsalicylic acid) have induced adverse embryonic effects, which can be consider for the development of strategies for an appropriate disposal of pharmaceutical residues, as well as establish maximum permissible limits for its emission to the environment.

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1 Introduction

The presence of pharmaceutical products in the environment has become one of the main causes of concern worldwide; since the 1970s, interest in the determination of organic substances of these drugs in the environment began [1]; however, it was until the 1990s when analytical methodologies allowed the detection of this kind of products at concentration in the order of μg/L. Pharmaceuticals have been considered as emerging pollutant substances of diverse origin and nature that do not have an established environmental regulation but may be candidates for a future regulation depending on the data of the effects they generate on health and incidence; another important feature is that they do not need to persist in the environment to generate negative effects, because their high rates of transformation can be compensated by their constant introduction in to the environment, and also data regarding their impact on the environment and health risks are scarce [2–4].

The main sources of pharmaceuticals to bodies of water are derived from anthropogenic activities such as effluents from wastewater treatment plants, effluents from hospital, domestic activities (including pharmaceuticals and their metabolic products of phases I and II in feces and urine, waste and inappropriate disposal of expired pharmaceuticals), and effluents from industrial activities as well as livestock activities [5–8]. So that, the increase in the use and consumption has led to the continuous elimination of these products or their transformation and biotransformation products towards the aquatic environment [9], and due to are substances that were designed with the purpose of having a biological effect, either preventing or treating a disease, their presence in the environment can generate various acute and chronic adverse or toxic effects in non-target organisms.

Non-steroidal anti-inflammatory drugs are a group of pharmaceuticals that have analgesic, antipyretic, and anti-inflammatory activity; they are usually weak acids that owe their pharmacological activity to the inhibition of the enzymatic systems of cyclooxygenases (COX) 1 and 2 (Fig. 1); COX-1 catalyzes the conversion of prostaglandins and thromboxane A2 responsible for controlling the mucosal barrier in the gastrointestinal tract, renal homeostasis, and platelet aggregation among other physiological functions, while COX-2 is induced in inflammatory cells as a response to stimuli [11]. Its therapeutic uses are diverse, and some of them are over-the-counter drugs and are available to consumers without medical prescription; so they are positioned as one of the highest consumption groups worldwide [6], the most common ones are diclofenac, ibuprofen, naproxen, ketoprofen, acetaminophen, acetylsalicylic acid, and indomethacin; main characteristics of NSAIDs are described on Fig. 2.

NSAIDs have demonstrated their ability to generate toxic effects in different organisms inducing oxidative stress alterations on growth, development, and energy storage on *Limnodynastes peronii* [13], also oxidative stress in the brain, gill, liver, and blood of *Cyprinus carpio*, increasing lipoperoxidation and enzymatic activity

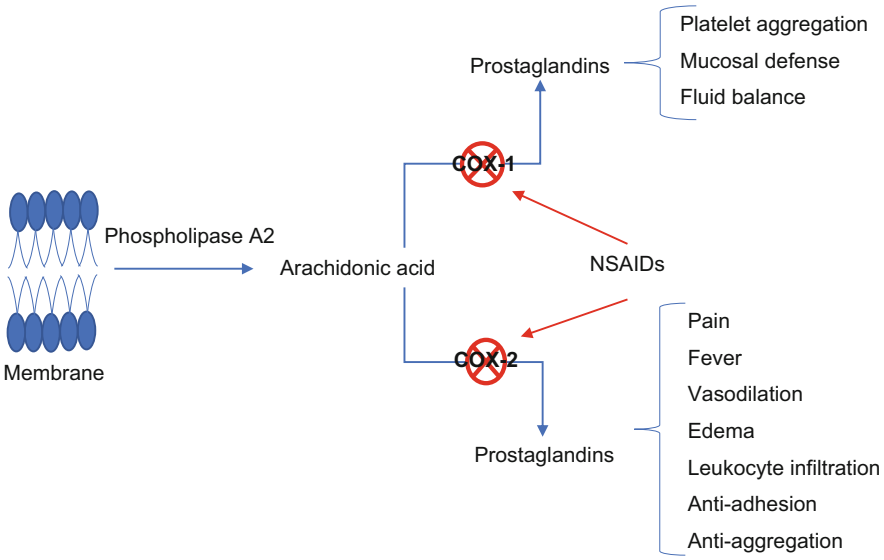

Fig. 1 General NSAIDs mechanism of action [10]

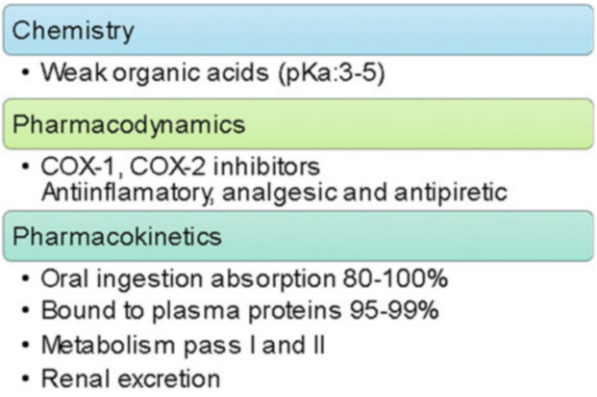

Fig. 2 Main characteristics of NSAIDs [12]

[14], increased oxidative damage and lipid catabolism in *Dreissena piolymorpha* [15], cytotoxicity and genotoxicity in *Daphnia magna* [16], stunting or growth inhibition on *Xenopus laevis* and *Lithobates catesbeianus* [17], and adverse effects on reproduction and offspring in *Danio rerio* [18] to mention some; however, to evidence this effects, bioassays must be done.

Biological methods or bioassays have been used for the determination of toxic on early life stages; these tests assess acute toxicity of chemicals or effluents to embryo and early life stages with lethality as the main endpoint [19].

Bioassays that focus on the evaluation of the development are important to identify if a substance or mixture of them can generate alterations in development;

these effects can be subtle or severe and can be manifested during embryonic development or subsequently throughout the life of the organisms. For the most part, embryotoxicity and teratogenicity studies focus on the development of mammals; however, contaminants such as pharmaceuticals manage to reach the bodies of water and come into contact with aquatic organisms; therefore, toxicity tests in early development stages of aquatic organisms are important since these organisms have its entire life cycle in aquatic environments and are frequently exposed to multiple stressors; this kind of studies can be useful for the identification and prioritization of development toxic substances [20].

Aquatic organisms are more sensitive during early stages of development; this may be because organisms in early stages of development have highly permeable membranes as well as different rates of absorption distribution and detoxification. Immature detoxification mechanisms can increase sensitivity to toxic agents, due to diverse physiological, morphological, and biochemical characteristics; since in the early life stages these responses are underdeveloped or have not yet fully developed, this contributes to a greater sensitivity compared to adult organisms [21].

Teratogens can affect morphogenesis, development, differentiation, and cell death; generate failures in cell interactions and cell movement; and affect cellular processes and different tissues; this can generate abnormalities and necrosis and can cause birth defects [22]. The effects on the development occur due to different mechanisms, depending on the teratogen agent will be the mechanism of action, and there may be more than one of them involved in the generation of adverse effects; there are some mechanisms described that can cause developmental alterations, some are disruptions in the central nervous system, modifications to DNA, enzymatic inhibition, hormonal alterations, cell membranes disruption, proteins or cellular organelles disturbances, and oxidative stress; Fig. 3 illustrates briefly how in one way NSAIDs can cause alterations in development and therefore teratogenesis [24, 25].

Since the toxicity of NSAIDs has been proven, special attention has been paid to the study of the possible toxic effects that these can generate in early development, in aquatic organisms; therefore some embryotoxic and teratogenic effects reported are described below.

1.1 Diclofenac

Diclofenac is one of the most widely used non-steroidal anti-inflammatory pharmaceuticals worldwide [26] and has been frequently detected in surface waters and effluents from wastewater treatment plants in concentrations in order of μg/L [27]. Some adverse effects caused by diclofenac have been reported previously, and herein are described some effects detected in early life stages of aquatic organisms.

The exposure of two Argentina native amphibians *Trachycephalus typhonius* and *Physalaemus albonotatus* to diclofenac at concentrations ranging from 125 to 4,000 μg/L for 96 h resulted in an LC_{50} of 2,828.43 μg/L and 2,462.29 μg/L,

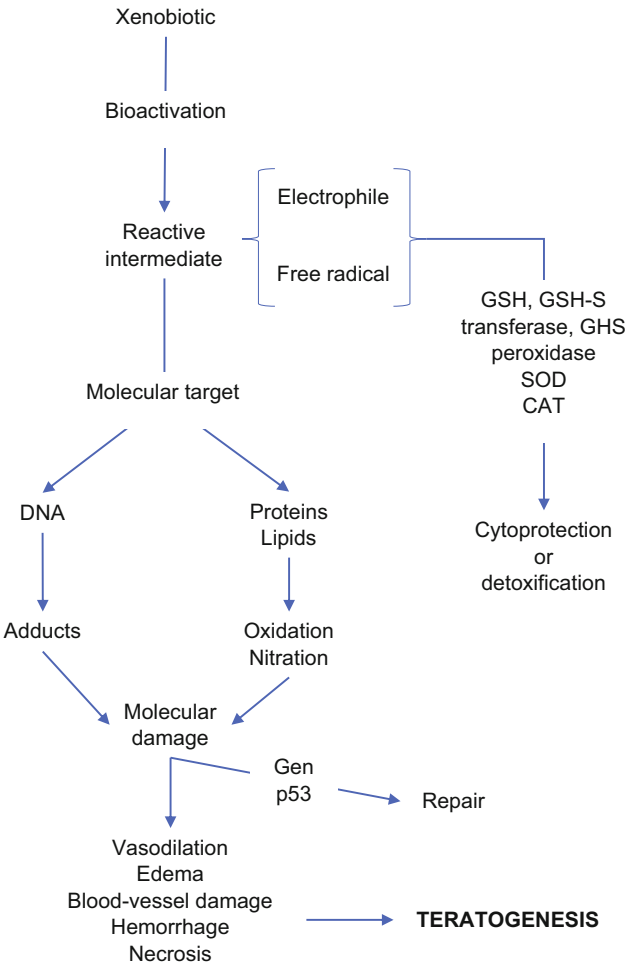

Fig. 3 Possible mechanisms by which NSAIDs can induce teratogenesis [23]

respectively; statistically significant differences were found in the size of the larvae with respect to the control group in both species, in addition to a small size and emaciation. Regarding the development and growth, there were also differences between the larvae; *T. typhonius* had a smaller size and lower degree of development than *P. albonotatus*; meanwhile, when evaluating the malformations for *T. typhonius*, the following were identified, absence of chondrocranium structures, absence of left hyobranchial skeletons, microcardia, increased gallbladder, and asymmetric pattern of gut, whereas for *P. albonotatus*, abdominal edema and altered axis, bilateral external body asymmetry, swollen body, absence of chondrocranium structures, partial hyobranchial skeleton, microcardia, and asymmetric pattern of gut were identified; microcardia was also observed in both species, and in

P. albonotatus, heart rhythm alterations were detected compared to the control group. Finally, a teratogenic index of 22.62 for *T. typhonius* and 19.69 for *P. albonotatus* was obtained, which indicate that diclofenac is a teratogenic agent to both species. Enzymatic activity of acetylcholinesterase and glutathione S transferase was also affected in both species showing different behaviors according to the concentrations tested; at low concentrations (125 μg/L), enzymes were inhibited, meanwhile at high concentrations, they were induced (2,000 μg/L); swimming behavior followed the same trend; at lower concentrations, a lower frequency was observed in the swim and less activity, while at higher concentrations, the frequency of activity and swimming was higher. These results show that diclofenac is a teratogenic drug for *Trachycephalus typhonius* and *Physalaemus albonotatus* triggering effects on embryogenesis and larval development; diclofenac is able to interfere with different biological functions affecting processes such as growth and development as well as generating abnormalities in different organs [28].

When *Mytilus galloprovincialis* were exposed to diclofenac at concentrations of 1 and 10 μg/L, the percentage of malformed embryos was approximately 30%; the malformations with the highest incidence were convex shell hinges, mineralization failures, transcription effects of several genes involved in biomineralization, biotransformation, antioxidant defense, and apoptosis; this demonstrates that diclofenac is capable to induce effects on the development of *Mytilus galloprovincialis* [29].

Danio rerio embryos were exposed to diclofenac at concentrations of 1.01, 3.38, 10.13, and 15.2 μM for 4 days; highest concentrations reached the maximum mortality effect at the fourth day of exposure, manifesting several abnormalities, mainly axial malformations and pericardial edema; abnormalities increased in severity as the concentration increased; at lower concentrations, malformations observed were shorter body length; smaller eye; muscle degeneration; lack of liver, intestine, and circulation; pericardial and body edema; and abnormal pigmentation. Diclofenac has the ability to be absorbed through non-covalent junctions and easily interact with embryos and thus generates developmental damage resulting in malformations such as curvature of the trunk and tail, as well as failure to regulate certain genes; such failures can lead to alterations in cardiogenic differentiation, which can generate pericardial edema as well as failures in the nervous system [30].

The exposure of *Salmo trutta* embryos to diclofenac at concentrations 0.1, 0.5, 1, 10, and 100 μg/L showed no toxic effects, and statistical differences to the control group were determined after the morality, hatching, development, or heart rhythm test through the embryonic development of *Salmo trutta* when it is exposed to these concentrations [31].

Danio rerio was exposed to diclofenac at 3.8, 7.5, and 15 mg/L; different malformations were observed, and the most recurrent were pericardial and yolk sac edemas and restricted systemic circulation; at 15 mg/L, a decrease in heart rate and a 100% inhibition of hatching were observed; at 3.8 mg/L, no severe effects were observed, and the hatching rate was not affected, nor were behavioral or in the swimming activity effects [32]. In another research, *Danio rerio* was exposed to 1, 20, 100, 500, 1,000, and 2,000 μg/L of diclofenac diluted with DMSO; no significant effects on the development of this organism were observed, even though

at concentrations of 1,000 and 2,000 μg/L; a decrease in the hatching rate was observed; however, the development was not affected, and no malformations were observed, nor significant adverse effects in early life stages, nor substantial changes were detected in stress proteins [33].

The evaluation 0.01, 0.05, 0.1, 0.5, 1 μg/L of diclofenac using *Mytilus galloprovincialis* as a bioindicator, changes in the larval development were observed, from the lowest concentration of barley (0.01 μg/L). Several malformations were observed, as well as deformations of the dorsal margin line in the shell of D-larvae; the LOAEC determined value was 0.01 μg/L. Diclofenac can seriously disturb the development of mollusks in the larval state at concentrations as low as 0.01 μg/L, without showing effects at higher concentrations. This research shows that bivalves are sensitive to environmentally relevant concentrations of diclofenac demonstrating the capacity of this pharmaceutical of generating irregularities in the formation of the shell [34].

Xenopus laevis embryos were exposed at diclofenac 1, 4, 16, 32, and 64 mg/L; the frequency of malformations increased from 16 mg/L and higher concentrations; at 24 h of exposure, 100% mortality was generated in embryos exposed to 64 mg/L. LC_{50} of 30.32 mg/L and MC_{50} of 12.25 mg/L were obtained, and a teratogenic index of 2.64 was determined, demonstrating that diclofenac has teratogenic potential, and as the degree of mortality increases, the degree of malformations increases as well. The most commonly observed malformations were axis, gut, heart, head, and eye abnormalities as well as blistering (edema); as the diclofenac concentration increased, larval length decreased; during stage 36, malformations observed were cardiac and intestinal. Effects on gene expression were generated; these failures indicate that the damage caused by diclofenac may be related with some proteins; it also generated neurological development failures [35]. The exposure of *Xenopus laevis* and *Lithobates catesbeianus* to 1, 4, 8, 16, 32, and 62.5 mg/L diclofenac resulted in a LC_{50} for *X. laevis* of 12.11 mg/L and LC_{50} 9.56 mg/L for *L. catesbeianus*; the highest concentration (62.5 mg/L, 100%) of mortality was reached in both organisms; all concentrations generated a decrease in the larvae size in both organisms; *X. laevis* was more sensitive than *L. catesbeianus*. The teratogenic index for *X. laevis* was 3.5 and for *L. catesbeianus* was 4.2. The most frequently observed malformations were axial malformations in the tail and notochord, edema, and hypopigmentation. Thus, this drug is a teratogenic agent for *Xenopus laevis* and *Lithobates catesbeianus* [17].

1.2 Ibuprofen

It is the third over-the-counter anti-inflammatory drug with the highest consumption worldwide, which is why it has been constantly detected in many bodies of water, rivers, and wastewaters, and the concentrations in which it has been detected are in the range of ng/L–μg/L [36, 37].

The exposure of adult organisms of *Danio rerio* to ibuprofen 1 μg/L induced alterations in reproduction, decreased the number of spawned eggs, 10 μg/L

exposure, as well decreased hatching rate of the progeny; this pharmaceutical is able to increase the mortality, decline maturation of sperm and gametogenesis, and produce developmental abnormalities; the most observed abnormalities due to ibuprofen exposure were cardiac edema and spinal malformations, and exposed embryos were also manifested [18].

Danio rerio embryos were exposed to ibuprofen 1 and 5 μg/L, mortality increases, and a decrease in swimming was identified, as well as failures in the response to stimuli. At 10 and 50 μg/L, ibuprofen increased mortality to 50%, and developmental damage was manifested; the hatching rate was also affected, and the most frequent malformations observed were failure in the organization of tail bud, optic vesicle, brain, and somites, as well as a decrease in body weight, size, and heart rate. At 100 μg/L, ibuprofen reached the highest degree of mortality to 57%, several malformations were observed, cardiac abnormalities could be seen visibly, and the most frequent malformations were cardiac edema, smaller size, absence of movement, and absence of response to external stimuli [38].

Ibuprofen concentrations of 0.01, 0.1, 1, 10, 100, and 1,000 μg/L were tested in *Mytilus galloprovincialis*; the exposure generated a dose-dependent behavior in terms of embryonic development; at 100 and 1,000 μg/L, several malformations were generated, the main one was convex hinge shells, and a LOAEC of 100 μg/L was obtained; this pharmaceutical can adversely affect embryonic development of bivalves at higher concentration than those detected in the environment [34].

Rana catesbeiana embryos were exposed to ibuprofen for 96 h; the first step was to obtain the premetamorphic LC_{50}, and it was 41.5 mg/L; then the specimens were subsequently exposed to 15 μg/L which generated effects on hepatic transcriptome and altered the levels of RNA in the liver; a reduction in the production of prostaglandins leads to effects in the metabolic state of this amphibian in the larval and post-embryonic stages; also, this pharmaceutical can act as an endocrine disruptor, disrupting the activity of certain genes that are involved in the metamorphosis processes of *Rana catesbeiana* [39].

After the exposure to ibuprofen LC_{50}, 56.7 mg/L, EC_{50} 39.9 mg/L, CMIC 30 mg/L, and IT 1.4 were obtained; the main abnormality observed was thoracic edema, and these results suggest that ibuprofen is a teratogenic pharmaceutical for *X. laevis* [40].

1.3 Naproxen

Naproxen is one of the anti-inflammatory drugs most commonly detected in bodies of water, wastewater treatment plants, rivers, and surface water in concentrations ranging from ng/L to μg/L [41, 42].

Danio rerio larvae were exposed to naproxen 0.1, 1, 10, and 100 μg/L, to subsequently evaluate the bioconcentration and evaluate effects such as thyroid disruption, as well as the mechanisms involved in the metabolism of this drug in zebrafish. At 0.1 and 1 μg/L, naproxen did not induced significant toxic effects on mortality compared to control group; however, at 10 μg/L, a 5% decrease in survival

was observed and at 100 μg/L a decrease of 7.5%; likewise at these concentrations (10 and 100 μg/L), there was a decrease in the larvae size and a reduction in the general weight. Regarding bioconcentration, values of 2052.69 ng/g were determined. Naproxen can generate a decrease in the growth of zebrafish and affect early life development remarkably [43].

Cyprinus carpio exposed to naproxen 10, 50, 100, and 200 μg/L showed a delay in embryo hatching; a mortality of 24% was determined, and growth was delayed. Some abnormalities were observed, and mainly pigmentation failure was observed; at 6-day postfertilization, in addition to malformations, gill cells were affected, alterations in the larval growth were generated, there was also a reduction in the body weight in all treated groups, the enzymatic activity was evaluated, and glutathione reductase activity declined; likewise variations were observed in glutathione transferase levels at 100 and 200 μg/L; finally the LOEC concentration was determined to be 10 μg/L [44].

The exposure of *Danio rerio* to naproxen at 0, 10, 20, 50, 75, 100, 125, 150, 175, 200, and 240 mg/L for 120 h induced a LC_{50} of 115.2 mg/L; for embryos at 96 h, a LC_{50} 147.6 mg/L was obtained, and a decrease in the hatching rate of the embryos was observed at 240 mg/L and also generated the greatest delay in embryonic hatching. The heart rate was decreased as the pharmaceutical concentration increased, and it was significantly inhibited at concentrations of 100 and 125 mg/L. The most frequently observed abnormalities were pericardial edema, yolk sac edema, and hemagglutination, weak pigmentation, hemorrhage, yolk condensation, and trunk abnormalities including without somites, tail not detached, axial malformation, and tail twisting. The most frequent sublethal effect was pericardial edema at a concentration of 20 mg/L; in addition, this malformation aggravated according to the increase in naproxen concentration, and also exposure to this pharmaceutical induced liver damage during the larval stage [45].

1.4 Ketoprofen

It is one of the first-line anti-inflammatory drugs for the treatment of several diseases, and its consumption for human and veterinary use is high; its production in Taiwan reached 7.9 kilotons in 2006 [46]. It has been detected in many bodies of water, surface water, groundwater, wastewater, and drinking water and has also been detected in solid atmospheres around the world [47].

Danio rerio embryos were exposed at 1, 10, and 100 μg/L of ketoprofen through 96 h; several malformations were observed in all concentrations tested, and the most relevant ones were edema, spinal curvature, slow heartbeat, an elongation of the heart, yolk sac edema, pericardial edema, and delayed hatching. After 48 h, a high mortality rate was observed and a decrease in the heart rate at 10 and 100 μg/L, whereas at 96 h, heart rate decreased significantly in comparison with the control group. According to these results, it is possible that ketoprofen produces abnormalities in the pericardium that cause alterations in heart rate and blood flow [48].

1.5 Celecoxib

Celecoxib is a non-steroidal anti-inflammatory drug that selectively inhibits COX-2 and is used as an anti-inflammatory and analgesic used to treat rheumatic diseases [49].

Xenopus laevis frog embryos were exposed to celecoxib, and LC_{50} of 8.99 mg/L, EC_{50} of 5.8 mg/L, and an IT of 1.54 were obtained; mortality and malformations increased according to the increase of celecoxib concentration. This pharmaceutical generated several malformations being the most frequent in the intestine, edema, hemorrhage, and abnormalities in the heart and blood vessels; mainly affected systems were cardiovascular due to the induction of effects on vascular cutting during development, which culminated in hemorrhage and edema, and digestive system due to its important effects. According to the results obtained, celecoxib is a teratogen pharmaceutical for this species [50].

1.6 Paracetamol

Paracetamol is a commonly used pharmaceutical; it is ubiquitous in the natural environment, and it easily accumulates in the aquatic environment; paracetamol has been detected in surface waters, sewage, and drinking water worldwide [51].

Daphnia magna exposure to paracetamol induced a significant increase in toxicity, with a dose-dependent behavior. The exposure of *Scenedesmus subspicatus* algae gave as a result an impaired growth of 50% at 134 mg/L of paracetamol. There were not significant effects in the exposure of *Brachydanio fish* embryos even at concentrations of 1 g/L [52].

In the damage assessment of paracetamol in *Xenopus laevis*, the following parameters were obtained, an LC_{50} of 191.1 mg/L, EC_{50} of 143.3 mg/L, and an IT 1.3. Based on the results obtained, paracetamol can be classified as an agent with low teratogenic potential; however, it can generate malformations in the absence of mortality. Malformations observed were intestinal, craniofacial, cardiac, pericardial, and ophthalmic edema [53]. Another research reports that no statistically significant differences were observed regarding the control group; however, all embryos showed malformations such as tail bending edemas and abnormalities in bowel curl; as for growth, no differences were found regarding the control group [40].

1.7 Acetylsalicylic Acid

It is a frequently used anti-inflammatory that is detected in the environment and contributes to environmental pollution and has been detected in surface waters at concentrations up to 340 ng/L [54].

Daphnia magna exposure to acetylsalicylic acid gave as a result an effective concentration for malformations EC_{50} of 118 mg/L, and for *Brachydanio fish*, the biological response to acetylsalicylic acid showed a more sensitive behavior, with an LC_{50} of 37 mg/L and a pulse reduction at 50 mg/L [52].

Cyprinus carpio was exposed to 0.004, 0.04, 0.4, 4, and 20 mg/L of acetylsalicylic acid; the hatching rate was significantly higher in the exposed embryo group compared to the control in concentrations of 0.004, 0.04, and 0.4 mg/L. In terms of mortality, it remained lower than 17% in the exposed groups as well as in the control group; a reduction in development was observed after day 6 of exposure to 20 mg/L and on day 13 in groups exposed to 0.004, 0.04, and 0.4 mg/L; while at the end of the experiment, a stimulation was observed in the development of organisms exposed to 0.004, 0.04, and 0.4 mg/L compared to the control group; in contrast, at 20 mg/L, a diminution in development was observed. Numerous abnormalities in development were observed axial hyperpigmentation and/or lateral curvature of the spine as well as dermal alterations and an increase in mucous cells to name some; also there was a decrease in body weight at 20 mg/L; however, as the concentration of pharmaceutical was minor, body weight was increased. With regard to the oxidative stress tests, an increase in lipoperoxidation and a decrease in the activity of antioxidant enzymes CAT, GPX, and GR were determined; finally a LOEC of 0.004 mg/L was obtained, and at this concentration, histopathological damage was observed [55].

2 Conclusions

Pharmaceutical products are substances that were designed to have a biological effect, to either prevent or treat a disease; however, their constant use and elimination towards the aquatic environment during several years have generated an alarming problem; due to its effects on most aquatic organisms still unknown, on the other hand, scientific groups have special attention in the study of the effects that pharmaceuticals can generate in different organisms. NSAIDs are the most consumed and eliminated group of drugs worldwide, and numerous effects have been evidenced in aquatic organisms and in the environment; however, research in early stages of development are scarce; nevertheless some research showed toxic effects that these pharmaceuticals can generate at environmentally relevant concentrations in early stages of development; this stage of life is important because it is the stage in which organisms are more susceptible to damage. More studies related to drug toxicity in organisms at early stages of development are necessary since it is the most critical period of development because embryos are at the topmost in cell division and differentiation and in the process of tissue and organ formation.

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Ecotoxicological Effects of the Drug Paracetamol: A Critical Review of Past Ecotoxicity Assessments and Future Perspectives

Bruno Nunes

Contents

Abstract Paracetamol (also designated as acetaminophen) has been systematically reported to occur in the aquatic environment, giving rise to serious concerns related to its ecotoxicological profile, final environmental fate, and potential biological interactions. In fact, the existing data concerning the toxicology of such drug shows its involvement in multiple adverse effects at several organs and tissues, a reality that also occurs in aquatic organisms of varied types, trophic levels, and habitats. From such data, it is possible to ascertain about the putative environmental risk posed by such drug, namely, by exerting deleterious irreversible effects in non-target organisms at low levels of exposure. The present article intends to critically present a comprehensive series of studies addressing the ecotoxicity of paracetamol, evidencing its deleterious nature, the extent of the problem, and alternative methods to determine the potential threat that it may constitute.

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1 Introduction

Paracetamol is one of the most valued therapeutic responses in modern medicine, given its analgesic properties, and its massive use as a painkiller started during the 1960s of the twentieth century [1]. Analgesics are a therapeutic class in which use has been rising for the last decades, as shown by Diener et al. [2], and paracetamol is among the most used pharmaceutical preparations used to treat and reduce pain of different natures and origins. Retrospective data show that, among the most common drugs consumed in developed countries, paracetamol is frequently among the top five [3]. Consequently, paracetamol has been recently classified as a priority compound, whose toxicity characterization is urgently required [4]. In addition, paracetamol is often formulated in over-the-counter pharmaceutical preparations, a factor that also contributes for its frequent use [5]. Considering its long history of use, versatility, safety, and efficacy in common therapeutics, paracetamol is used by millions of human patients, being of fundamental importance in pediatrics also [6]. Despite this massive use, and quite paradoxically, paracetamol is frequently associated both to unintentional poisoning and also to suicide among humans, due to its severe toxicological effects when used in overdosage [7, 8]. In fact, recent retrospective analysis about the concerns of users of this specific drug shows that paracetamol effects are not entirely acknowledged and that a large amount of questions on its safety still exist [9]. A long list of recent publications also shows that paracetamol poisoning is a common subject in modern toxicology, as demonstrated by Zyoud et al. [10]. This reinforces the correct notion that despite being safe, paracetamol is also toxic. In humans, paracetamol toxicity is usually evidenced with the involvement of liver alterations, since the most frequently targeted locations for the bioactive metabolites of this drug occur in the liver [1, 11–13]. Paracetamol intoxication results in centrilobular hepatic necrosis [14], liver failure, and death. Such outcomes are not restricted to humans, and similar effects were also reported to occur in mammals ([15, 16]; Hadi et al. [17]), suggesting a similar (putatively evolutionary conserved) mechanism of toxic action among a large number of species.

Paracetamol has a number of features that are common to most non-steroid anti-inflammatory drugs (NSAIDs), especially its mechanism of therapeutic action and resulting effects. In fact, paracetamol seems to act by inhibiting the peroxidasic activity of both cyclooxygenase forms (I and II), resulting in a significant impairment of prostaglandin biosynthesis, as summarized by Graham et al. [7]. However, this is not the mechanism that is responsible for paracetamol's toxicity. In fact, paracetamol is prone to be promptly metabolized in normal, therapeutic dosages,

without the establishment of any toxic effect. Low levels of paracetamol end up usually in its conjugation, namely, by sulfation and also glucuronidation [11, 18, 19], resulting in water-soluble intermediates whose presence in the organism is short, being excreted. A small portion of the administered paracetamol is metabolized by hepatic cells via the oxidative pathway with the involvement of cytochrome P450 (especially CYP2E1 and CYP1A2), as described by [20]. This portion of paracetamol is then bioactivated, giving rise to a highly reactive intermediate, designated N-acetyl-p-benzoquinone imine (NAPQI). NAPQI, despite its reactivity, may be conjugated with glutathione, with the involvement of the isoenzymes glutathione S-transferases, being then excreted as cysteine or mercapturic acid [21]. However, all the described conjugation pathways may be exhausted if the ingested amount of paracetamol exceeds the conjugation capacity, i.e., the intracellular available amount of each cofactor. At toxic doses, the sulfate, glucuronic acid, and reduced glutathione pools are depleted, and NAPQI accumulates resulting in toxicity. Being metabolized and bioactivated mostly in the liver, NAPQI concentration in this organ increases, impairing the biosynthesis of ATP, triggering DNA and RNA damages and binding to proteins and subcellular structures, thereby inducing rapid cell death and necrosis [22]. NAPQI accumulation is also responsible for an excessive accumulation of intracellular peroxide (due to glutathione depletion), a main factor that is the basis of damages by reactive species of oxygen (ROS) via Fenton mechanism, causing oxidative stress and extensive lipid peroxidation of cellular membranes [1, 23, 24]. Considering that the metabolic pathways involved both in the metabolism and in the bioactivation of paracetamol are evolutionary conserved, it is expectable that the previously described toxicity features may also occur in a large number of organisms, namely, those that are exposed via the environment. In addition, oxidative stress may result in the establishment of adverse effects as a consequence of the denaturation of specific proteins by ROS, as shown to occur in several mollusk, crustacean, and fish species. These effects included deleterious modifications in enzymes such as cholinesterases, with significant behavioral alterations, as evidenced in *Phorcus lineatus* [25], *Daphnia magna* [26], *Anguilla anguilla* [27], and *Phalloceros harpagos* [28]. The importance of the causal relationship between oxidative stress and cholinesterasic inhibition is ecologically significant. Since it shows that some compounds may alter behavioral traits by modulating redox imbalances and not by acting directly on the nervous system of exposed organisms. In turn, behavioral disturbances may be held accountable for loss of common responses, including reflexes (escape, sexual interaction, aggression, camouflage) that are determinant for the survival of species and for the ecological balance.

1.1 Environmental Presence and Fate of Paracetamol

Given its massive use, paracetamol is released into sewage systems by human patients, a factor that justifies its frequent detection in wastewater [29–31]. Despite

being usually effectively eliminated at sewage treatment plants (STPs; [32]; Falås et al. [33]; [29, 34]), paracetamol is persistent in the aquatic environment [35], being consequently found in receiving waters. Even advanced techniques and procedures of wastewater treatment are not completely efficient in removing paracetamol from such matrices [36], and paracetamol ends up being released into receiving waters, where it may attain considerable levels. Paracetamol is also prone to be degraded by bacterial metabolism, as reviewed by Żur et al. [37]. Bacteria from the genera *Pseudomonas*, *Bacillus*, *Acinetobacter*, and *Sphingomonas* are particularly effective in degrading this drug [38], which might in the future decisively contribute for an increased efficacy of wastewater treatment solutions for this specific drug. Nevertheless, the efficacy of such degradation processes, in the wild, is not effective enough to prevent paracetamol from being somewhat abundant in aquatic ecosystems. In fact, the generalized environmental presence and high levels of paracetamol have been considered particularly troublesome in some locations. The work by Ashfaq et al. [39] established that paracetamol presented the higher environmental risk among a series of pharmaceutical drugs (paracetamol, naproxen, diclofenac, ibuprofen, amlodipine, rosuvastatin, ofloxacin, ciprofloxacin, moxifloxacin, sparfloxacin, and gemifloxacin), which resulted from the released of contaminated effluents from Pakistan pharmaceutical industry units. Specific locations, where the network of sewage treatment facilities does not exist or is not entirely functional, have levels of paracetamol well above those reported to occur in countries where most sewage is effectively treated. This is the case of several African countries [40], evidencing that human excreta are the major sources of paracetamol in the wild. Values of paracetamol levels determined in Kenya reach 106,970 ng/L, as determined by K'oreje et al. [41], but this corresponds to an extreme value that may be interpreted as a worst-case scenario of contamination. Despite being considerably lower, paracetamol is ubiquitous in aquatic ecosystems. The presence of paracetamol in freshwater systems has been already documented, in levels up to 653.5 pg/L (Dal River, Sweden; [35]), 1,289 ng/L (Lobregat River, Spain; [42]), 10 µg/L (freshwater streams, USA; [43]), above 65 µg/L (Tyne River, UK; [44]), 30.421 ng/L (Monjolinho River, Brazil; [45]), and 610 ng/L (Savar River, Serbia; [46]). Paracetamol presence was also reported in marine waters (76.9 ng/L, determined at Vila do Conde, Portugal; [47]) and even drinking water (211 ng/L, water sample collected in France; [48]).

1.2 Ecotoxicological Effects Caused by Paracetamol

Considering the already found levels of paracetamol in the wild, its persistence, and the described patterns of metabolism and toxic effects, it is not surprising that paracetamol may exert deleterious alterations in exposed biota. In fact, a considerable number of studies has focused on characterizing adverse effects caused by paracetamol on multiple species, and the common conclusion is that it may exert significant toxicity in most organisms, namely, with the involvement of oxidative

alterations and with the triggering of antioxidant mechanisms. Despite the large metabolic differences that occur among distinct aquatic species, generally paracetamol exposure results in oxidative stress or in adaptive responses aiming at preventing the establishment of oxidative stress. Despite the common toxicological and mechanistic basis, toxicity of paracetamol may be highly variable among distinct species. According to the study by Nunes et al. [49], toxic effects measured in terms of EC_{50} values for different organisms yielded a remarkable variation. According to this study, the most sensitive organism was *Daphnia magna* (EC_{50} = 4.7 mg/L), followed by another crustacean species, namely, *Daphnia longispina* (EC_{50} = 65.9 mg/L). The bacterial species *Vibrio fischeri* was also sensitive to this drug, with a calculated EC_{50} = 92.2 mg/L. Algal species were somewhat tolerant to this pharmaceutical since the EC_{50} values calculated for *Raphidocelis subcapitata* were of 317.4 mg/L, and for *Cylindrospermopsis raciborskii*, of 192.9 mg/L. Aquatic plants were the least sensitive tested model species, with EC_{50} values of 429.9 mg/L for *Lemna minor* and exceeding 1,000 mg/L for *Lemna gibba*.

The presence of paracetamol in the already determined levels is not life threatening for most aquatic organisms, as shown by Trombini et al. [50], after determining the toxic effects of this drug in terms of lethality of the copepod *Tisbe battagliai*. This study demonstrated that concentrations that occur nowadays in the wild are not high enough to cause mortality of this marine species. However, a similar assumption can be established for most anthropogenic compounds, since toxic effects are likely to be reflected by subtle changes in the physiology of exposed organisms, rather than resulting in mortality. Consequently, toxicity of drugs must be always assessed by analyzing subindividual traits that are in close proximity with impacted pathways, metabolic routes, or pharmacological receptors, whose activation/deactivation may occur as a consequence of the presence of a specific drug. In this sense, a considerable number of studies have been published demonstrating that paracetamol exposure can indeed result in significant changes in exposed species. These studies, in general, report alterations in physiological, biochemical, and metabolic alterations in key features of selected species.

1.3 Paracetamol Toxicity in Plants

Plant physiology seems to be altered in specific cases by paracetamol exposure, as a consequence of its uptake and absorption [51] and metabolism [52]. However, this is not the general rule. The study conducted by Rede et al. [53] assessed the acute effects of paracetamol on several parameters (germination and growth) of *Lactuca sativa*. Paracetamol alone was not capable of altering any of the analyzed parameters (viz., percentage of seed germination, root elongation, shoot length, and leaf length), in levels reaching 100 mg/L. These data show that the selected species was refractory to the presence of this drug. The emergence time of maize (*Zea mays* L.) was not affected by paracetamol acute exposure, as demonstrated by Hammad et al.

[51]. This is in line with the study by Pino et al. [54], which calculated an EC_{50} value for the parameter of root elongation inhibition in this same plant species of 2,820 mg/L. Similar trends were observed by An et al. [55] after exposing the plant *Triticum aestivum* L (wheat) to paracetamol. In this case, the obtained acute EC_{50} value for the parameter of root elongation inhibition was of 668.8 mg/L, which is well above environmental levels. However, prolonged exposures to this substance yielded distinct profiles of toxic effects, evidencing the pro-oxidative nature of paracetamol. Periods of 7 and 14 days of exposure were enough to alter the antioxidant status of exposed plants, and following a 21-day period of exposure, growth was compromised, as well as chlorophyll levels and protein biosynthesis. In a study conducted with *Lemna minor* by Kummerová et al. [56], it was possible to observe that parameters such as plant number, biomass production, and leaf area size were only slightly changed by paracetamol, in contrast with biochemical and histological traits that were severely affected by this drug. Among the most impacted biomarkers, one could find a significant decrease in levels of photosynthetic pigments, and an increase in non-photochemical quenching; consequently, the relative chlorophyll fluorescence was strongly compromised. These effects were associated to increased levels of reactive oxygen and nitrogen species and to the activation of antioxidant defensive mechanisms. The study conducted by Nunes et al. [57] showed that the comparative sensitivity toward paracetamol may vary considerably, even when studying phylogenetically close plant species, such as *Lemna minor* and *Lemna gibba*. The obtained results clearly indicated that *L. minor* was more sensitive than *L. gibba* in terms of the pro-oxidative effects, since this species was capable of activating an antioxidant response involving the amino acid proline, which is known for its antioxidant properties.

1.4 Toxic Effects of Paracetamol on Polychaetes and Mollusks

Despite the general absence of studies focusing on this specific issue, invertebrates such as polychaete and mollusk species seem also to respond to paracetamol exposure, with the activation of metabolic and antioxidant mechanisms. This was the case identified by Brandão et al. [58], when studying the responses elicited by this chemical on the freshwater bivalve *Corbicula fluminea*. The obtained results evidenced an antioxidant response triggered by paracetamol, following both short- and long-term exposures, with the involvement of two of the tested biomarkers, namely, glutathione-S-transferases and glutathione reductase activities, which were significantly depressed. The study by Antunes et al. [59] was also demonstrative that bivalves, despite being from distinct species and habitats, could also respond to the presence of paracetamol. In fact, the pattern of responses deployed by two species of estuarine/marine clams, namely, *Ruditapes decussata* and *Ruditapes philippinarum*, were somewhat similar to the one described for *C. fluminea*. Changes in GST

activities were observed in individuals of *R. decussata*, with strong impairments at low levels of exposure, while high levels of paracetamol elicited sharp increases in this parameter. The same organisms also showed higher levels of glutathione reductase activity. Changes in *R. philippinarum* only involved increased GSTs activity. Despite the interspecific changes, it was possible to conclude that oxidative stress conditions were in place in both species after paracetamol exposure, despite the extent of the metabolic response. Again the problematic of paracetamol toxicity was studied, but under the scope of global changes scenarios. By being an estuarine species, *R. philippinarum* may be subjected to strong salinity fluctuations, whose amplitude may even increase in the future as a consequence of droughts. The study by Correia et al. [60] evidenced not only the response toward pro-oxidative conditions caused by paracetamol, and the extent of the defensive biological response, but also the modulation of such effects by salinity variations. In general, the activity of antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione-S-transferases, glutathione reductase), nonenzymatic defenses (glutathione levels), and peroxidative damage (lipoperoxidation) strongly fluctuated according to the salinity values. This set of results is indeed significant, since it implies that contamination, biological responses, and consequent damages are likely to be impacted and altered, with unpredictable consequences, but changes in abiotic conditions such as salinity, which are likely to vary under a scenario of global change in the future. *Gibbula umbilicalis* is a marine mollusk which showed to be responsive to paracetamol [61]. When exposed to ecologically relevant amounts of paracetamol for a short period of 96 h, individuals of this species responded by decreasing their catalase activity and lipoperoxidation levels, evidencing the antioxidant nature of this response, which is similar to other changes reported for other marine organisms. A limited but significant antioxidant response toward the presence of paracetamol was also established in the marine mollusk *Phorcus lineatus*, as shown by Almeida and Nunes [25]. In this study, individuals of this species chronically exposed to this drug were able to trigger an activation of their antioxidant mechanism catalase, thus preventing the occurrence of oxidative damage. Oxidative stress was the main underlying mechanism suggested to be the causal factor for the delay in the regeneration of injured tissues of the polychaete *Diopatra neapolitana*, as described by Freitas et al. [62]. In this study, mechanical injuries inflicted in individuals of the mentioned species were allowed to regenerate in the presence of several concentrations of paracetamol. For organisms exposed to the higher levels, the onset and progress of the tissue regeneration were significantly compromised. To discuss this finding, authors suggest that the excess of ROS produced during the metabolism of paracetamol, which is one of the main causative agents of the toxicity by this drug, can compromise the efficacy of the healing process. To support this assumption, authors state that oxidative alterations are of paramount importance for the onset of physiological processes of recovery in many organisms, given the role of regulators of cell proliferation and tissue differentiation attributed to ROS and also to nitric oxide (NO) intermediates. Following tissue injury, inflammatory processes with the involvement of immune cells are likely to be established, and such conditions are prone to the occurrence of such reactive oxygen species. When in excess, the

presence of such species may be deleterious, if the cells do not activate compensatory scavenging mechanisms. In this case, such conditions result in cellular and tissue damage [63], a process also known to occur in some species of annelids [64], which seems to be counteracted when animals are exposed to antioxidant compounds [65].

1.5 Toxic Effects of Paracetamol on Crustaceans

Crustaceans seem also to share most mechanisms described so far that are prone to be activated by paracetamol. The study conducted by Daniel et al. [26] evidenced that even short-term periods of exposure of the crustacean *Daphnia magna* to paracetamol could elicit significant defensive mechanisms, such as the increase of the phase II metabolic enzymes of conjugation, glutathione-S-transferases, closely followed by the augment of catalase activity, suggesting the onset of an antioxidant response. This oxidative effect culminated also in the decrease of cholinesterasic activity, establishing a link between oxidative effects and other physiological levels. Despite the occurrence of such effects, no behavioral traits were significantly compromised. This same study also showed that chronic responses to the presence of paracetamol did not result in any significant response in terms of the same parameters, evidencing the transient nature of the toxic effects and the responsiveness of the adopted species. These results seem to be somewhat validated by the results obtained by Masteling et al. [66], after exposing individuals of *D. magna* to paracetamol for a sub-chronic period (8 days of exposure). The obtained data evidenced once again the responsiveness of this species, since the activities of both catalase and glutathione-S-transferases were significantly increased for generically all tested concentrations. The assumptions about the occurrence of oxidative alterations were reinforced considering the establishment of peroxidative damage in exposed individuals. The toxicity of paracetamol toward crustacean species is not limited to exposed organisms, since transgenerational effects seem also to be possible, according to the study by Castro et al. [67]. The authors observed that prolonged paracetamol exposures not only caused deleterious effects in the reproductive performance of exposed *D. magna* but were responsible for a general decrease in the fitness of nonexposed, daughter (first-generation neonates) organisms. These data are clear about the putative ecotoxicological effects of paracetamol toward freshwater organisms, since toxic effects are likely to occur after short-, middle-, and long-term exposures but can also surpass generations and compromise the health condition of offspring born from exposed parental organisms.

1.6 Toxic Effects of Paracetamol on Fish

Realistic levels of exposure to paracetamol seem to significantly activate the antioxidant response in fish, mechanistically similarly to what was described for other taxa. In fact, fish species such as *Oncorhynchus mykiss* (rainbow trout) were shown to be extremely responsive to paracetamol in ecologically relevant levels, as described by Ramos et al. [68]. This study showed that acute and chronic exposure of these trouts to paracetamol resulted in the activation of glutathione peroxidase, glutathione reductase, and glutathione-S-transferases that was not however efficient enough to prevent the establishment of oxidative damage, reflected by a significant increase of lipid peroxidation. It is important to stress that such results, especially those obtained following the chronic exposure, were attained at low, realistic levels of exposure, increasing the ecological relevance of the entire set of results. On the contrary, individuals of the European eel (*Anguilla anguilla*) seemed to be more refractory to paracetamol, as demonstrated by Nunes et al. [27]. Despite the occurrence of biological responses, none of the tested metabolic or oxidative stress biomarkers signaled the occurrence of significant modifications after a 48 h exposure to ecologically relevant levels of paracetamol. Indeed, these conditions were not able to alter catalase or glutathione-S-transferase levels, suggesting the absence of an antioxidant response. The combination of low levels (albeit relevant) + short duration of duration may not have been enough to attain the conditions required to establish such a condition of oxidative stress. However, cholinesterasic activity of exposed fish was significantly impaired, suggesting a new, previously unsuspected manifestation of toxicity by paracetamol: neurotoxicity due to the direct denaturation of enzymatic forms by ROS resulting from paracetamol metabolism. This possibility opens new possibilities, considering that neurotoxic effects may be in direct relationship with behavioral alterations, which are always of extreme ecological relevance. In fact, this finding was again suggested as a main toxicological mechanism underlying the effects observed in the neotropical freshwater fish species *Phaloceros harpagos*, as shown by Matus et al. [28]. In this study, authors reported a significant behavioral alteration, namely for fish exposed to the highest levels of paracetamol (80 mg L^{-1}), which showed an altered preference in terms of scototaxis, i.e., preference for dark/light compartments in the aquaria. Fish exposed to such high levels of paracetamol were more prone to place themselves in a light area, which is a nonnatural behavior, considering that dark areas provide better refuge from predators. Despite being attained at high and thus non-ecologically relevant level of paracetamol, this altered trend evidenced the association between alteration in cholinesterasic activity (reported in previous studies) and behavioral modifications. The acute and chronic effects of paracetamol on cholinesterases of fish were also demonstrated by Pereira et al. [69]. In addition to antioxidant and metabolic responses (viz., with the increase of glutathione-S-transferases activity, after acute exposure), cholinesterasic activity was significantly increased in exposed fish. This was assumed as a surprising result, since the most frequently used effect criteria involving cholinesterases are their inhibition, not their increase. Among others,

authors pointed to the possibility that unspecific serum cholinesterases, present in the blood of the fish, may have been overexpressed as a consequence of paracetamol exposure. However, it is important to point that despite the type of interaction (inhibition or enhancement) in some cases, paracetamol seems to disturb the correct functioning of the central nervous system, affecting behavior. A conjugation of biochemical, developmental, behavioral, and epigenetic effects in the freshwater model fish *Danio rerio* after paracetamol exposure was reported by Nogueira et al. [70]. Paracetamol exposure resulted in the increase of embryos with deformations, in an increase in the locomotor activity, increase in DNA methylation (especially around the head and near the eyes of exposed embryos), increase in acetylcholinesterase activity, and higher levels of catalase, glutathione peroxidase, and glutathione-S-transferases. This set of results evidences the oxidative nature of the reported alterations and the onset of epigenetic alterations never before reported, along with a potential neurotoxicity indication that may have been reflected in altered behavioral traits. This is a highly comprehensive set of results that unequivocally shows the multilevel toxicity exerted by paracetamol on fish, with the impairment of several key functions with putative relationships among them.

2 Conclusions

The text above corresponds to a thorough selection of scientific data that shows the involvement of paracetamol in a large number of biochemical, metabolic, and cellular processes, whose impairment or modification may result in significant adverse effects to exposed organisms. Much of the cited studies were undertaken exposing aquatic organisms of different taxa and habitat to low, realistic levels of paracetamol, which allows interpreting the toxicological data as ecologically relevant. In some cases, known mechanisms of toxic action were observed and/or inferred from the measured alterations; in other situations, it is possible to assume that some effects may happen as a result (or modulated by) of others. Considering the entire alignment of studies, it becomes clear that paracetamol poses pertinent ecotoxicological concerns, even in low already reported levels in the wild, usually connected to its capacity of altering the redox balance of cells. However, its effects are not confined to oxidative stress, antioxidant defenses, and peroxidative damage; since other traits were shown to be involved as well, including histological development, tissue regeneration, embryogenesis and development, metabolism, and neurotoxicity and behavior. We can now conclude that despite the ever increasing number of studies, published toxicological data, and covered scientific areas, paracetamol is still an ecotoxicological challenge for years to come.

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Adverse Effects Induced by Nonsteroidal Anti-inflammatory Drugs on Freshwater Invertebrates

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Contents

Abstract Nonsteroidal anti-inflammatory drugs (NSAIDs) are a group of molecules representing one of the most relevant therapeutic class found in the aquatic ecosystems worldwide. NSAIDs are commonly and extensively used for their analgesic, antipyretic and anti-inflammatory properties to cure pain and inflammation in both human and veterinary therapy. Because of the huge, continuative and increasing use, as well as their specific pharmacokinetic properties, after medical use they are excreted in their native form or as metabolites and enter the aquatic ecosystems. A number of monitoring surveys has reported levels of NSAIDs exceeding 1 μg/L in influent and effluents of Wastewater Treatment Plants (WWTPs), while lower concentrations have been found in surface waters, ranging in the ng/L – μg/L range. Among NSAIDs, paracetamol, diclofenac, and ibuprofen are the most detected therapeutics found in aquatic ecosystems. Although the concentrations of these molecules in surface waters are quite low, their high biological activity might confer them a potential toxicity towards non-target aquatic organisms. The present chapter aims at reviewing the adverse effects induced by paracetamol, diclofenac, and ibuprofen towards different freshwater invertebrates belonging to different *taxa*. Although acute toxicity of paracetamol, diclofenac, and ibuprofen occur only at

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high, unrealistic concentrations, sublethal effects were caused by low, environmentally relevant concentrations of these drugs. For these reasons, further studies represent a priority in order to enlarge the knowledge on NSAID toxicity towards aquatic organisms and to shed light on their real ecological hazard towards aquatic communities.

Keywords Diclofenac, Ibuprofen, Invertebrates, Paracetamol, Toxicity

1 NSAIDs in Freshwater Ecosystems

In the last two decades, pharmaceuticals have been identified as emerging contaminants for aquatic ecosystems. Emerging contaminants are synthetic or natural compounds that has recently been found in natural ecosystems and for which environmental or public health risks are limited or yet to be established. These molecules are not routinely monitored, and, even if their environmental concentrations are low, they are suspected to cause adverse effects towards ecosystems [1, 2]. The presence of pharmaceutical compounds in aquatic ecosystems represents one of the main concerns that ecotoxicology has to face in recent years [3–5]. Pharmaceuticals are extensively and increasingly being used both in human and veterinary medicine, as well as in agriculture and aquaculture [5]. After the use, these molecules are excreted in their native form or as active metabolites entering the sewage, which has been individuated as the main spreading pharmaceuticals after therapeutic use or improper disposal of unused medicines to the environment. As traditional wastewater treatment plants (WWTPs) have a limited efficiency for the removal of several therapeutic drugs, these molecules are discharged in WWTP effluents in unneglectable concentrations, resulting in contamination of surface waters and, rarely, groundwater and drinking water [4]. Moreover, sewage sludge originated from WWTPs and manure from zootechnical breeding farms have been identified as a secondary source of pharmaceuticals, contributing to aquatic contamination as a consequence of their use in agriculture and the subsequent runoff. Pharmaceuticals have been designed to have a specific mode of action, targeting specific organs, metabolic pathways, or receptors in order to modulate physiological functions of the orgnism, to treat a desease and to restore the health of the organism. Thus, because of their usefulness, pharmaceuticals play a pivotal role in our society and are commonly used, and often abused, worldwide. For instance, in the European Union (EU) alone, it has been estimated that about 3,000 different substances are commonly used in human therapy such as anti-inflammatory drugs, contraceptives, antibiotics, β-blockers, lipid regulators, neuroactive compounds, and many others [3]. Similarly, a large number of these molecules are used also in veterinary applications. Following the trend of production and use, several therapeutics commonly used in human and veterinary therapy as contraceptives, β-blockers,

antiepileptic, anti-inflammatory, antidepressants, or antibiotics have been found at concentrations ranging from a few ng/L to few mg/L in wastewater, surface water, and groundwater worldwide [4, 6]. Although the environmental concentrations measured in aquatic ecosystems are often quite low, pharmaceuticals are designed to be biologically active at low concentrations; for this reason pharmaceuticals revealed in environment might represent a potential risk for chronically exposed, non-target organisms [3, 5]. Considering the potential hazard of pharmaceuticals towards ecosystems some international actions have been planned. For instance, the European Union has included 17α-ethinylestradiol, 17β-estradiol, and diclofenac to the list of the Water Framework Directive (2013/39/EU) as priority molecules to be monitored in aquatic ecosystems.

Nonsteroidal anti-inflammatory drugs (NSAIDs) represent one of the most relevant therapeutic class found in the aquatic ecosystems. NSAIDs are largely used for their analgesic, antipyretic, and anti-inflammatory properties to cure pain and inflammation. They inhibit the synthesis and the release of prostaglandins from arachidonic acid, acting as non-selective inhibitors of cyclooxygenase enzymes, including both the cyclooxygenase-1 (COX-1) and the cyclooxygenase-2 (COX-2) isoforms [7]. Different NSAIDs have been prescribed extensively throughout the world. For instance, more than 70 million prescriptions are written each year in the United States, while considering the over-the-counter use, more than 30 billion NSAID doses are consumed annually in the United States alone [8]. Because of the huge, continuative and increasing use, as well as their specific pharmacokinetic properties, NSAIDs can reach detectable concentrations both in sewage and in surface water [9], accounting for 15% of pharmaceuticals measured in aquatic ecosystems worldwide [4]. Diverse monitoring surveys have reported levels of NSAIDs exceeding 1 μg/L in influent and effluents of WWTPs, while lower concentrations have been found in surface waters [4, 10]. Among NSAIDs, paracetamol, diclofenac, and ibuprofen are the most detected therapeutics found in aquatic ecosystems [4].

Paracetamol (PCM; N-(4-hydroxyphenyl)acetamide) is an analgesic and antipyretic agent. Although PCM does not own a proper anti-inflammatory action, it is usually considered in the NSAID group by a toxicological point of view because of its mode of action, similar to that of NSAIDs [11]. As PCM is considered a safe drug at therapeutic doses, it can be purchased as an over-the-counter drug in most countries. According to its extensive use, PCM is one of the most frequently detected pharmaceuticals in surface waters, wastewaters, and drinking water. For instance, Kolpin et al. [12] detected PCM in 24% of samples during a survey performed in 139 US streams, at a median concentration of 0.11 μg/L, with concentrations up to 10 μg/L. The median concentration of PCM measured in surface waters worldwide was 0.055 ± 0.051 μg/L [13, 14], while in wastewaters PCM was detected at a median concentration of 48 ± 75 μg/L [14, 15].

Diclofenac (DCF; 2-[(2,6-dichlorophenyl)amino] phenylacetic acid) is a phenylacetic acid NSAID used to reduce inflammation and pain associated with arthritis, osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis [16]. According to its huge over-the-counter sale, coupled with the great number

of medical prescriptions, DCF has been identified as one of the main pharmaceuticals contaminating the aquatic ecosystems. Dermal application results the main source of DCF in water [17]; in fact, because of the relative small absorption on skin (5–10%), the most of the pharmaceutical is released to water by washing [18]. Moreover, as traditional WWTPs have a limited efficiency of DCF removal, this drug was commonly detected at low μg/L range in WWTP effluents of Europe and North and South America [19, 20]. Accordingly, DCF was commonly detected also in surface waters, in concentrations ranging between low ng/L up to low μg/L [10–14]. Despite these findings, the information of the environmental fate and the adverse effects of DCF towards non-target aquatic organisms is still limited.

Ibuprofen (IBU; ((+/−)-2-(p-isobutylphenyl) propionic acid with R and S isomers) is used to relieve the symptoms of arthritis, rheumatic disorders, pain, and fever [21]. IBU represents one of the core pharmaceuticals included in the "Essential Drug List" of the World Health Organization (WHO), and it is therefore produced in large amounts worldwide [22]. Because of its huge over-the-counter sale, large prescription volume, and high excretion rate (estimated as 70–80% of the ingested therapeutic dose), IBU has been identified as one of the main pharmaceuticals in aquatic ecosystems. Moreover, IBU has relatively high mobility into aquatic environments but a lower persistence in comparison with other pharmaceuticals [23]. IBU has been detected in moderate concentrations (up to tens of μg/L) both in the effluents of WWTPs and in surface waters during surveys carried out in both Europe and North America [10, 12, 24].

Although the occurrence of low to moderate concentrations of paracetamol, diclofenac, and ibuprofen has been demonstrated in aquatic ecosystems worldwide, the information concerning their potential toxicity towards non-target aquatic organisms is still limited. For this reason, the aim of the present study is to review the adverse effects induced by the exposure to paracetamol, diclofenac, and ibuprofen towards freshwater invertebrates that was performed in order to shed light on the potential hazard of these pharmaceutical compounds towards non-target organisms and to lay the foundations for further ecotoxicological investigations.

2 Adverse Effects of NSAIDs of Freshwater Organisms

The studies of the adverse effects induced by the exposure to paracetamol, diclofenac, and ibuprofen towards freshwater invertebrates have been performed on different model species belonging to different taxa, from algae to mussels. Thus, the effects induced to different organisms by the exposure to each single molecule are discussed in the paragraphs below.

2.1 Effects Induced by Paracetamol Exposure

The toxicity of paracetamol (PCM) towards non-target, freshwater invertebrates has been investigated on algae (*Pseudokirchneriella subcapitata*), cyanobacteria (*Cylindrospermopsis raciborskii*), cnidarian (*Hydra vulgaris*), rotifers (*Plationus patulus*), crustaceans (*Daphnia magna, Daphnia longispina*, and *Moina macrocopa*), bivalves (*Dreissena polymorpha* and *Corbicula fluminea*), as well as plants (*Lemna minor* and *Lemna gibba*) (Table 1).

Acute toxicity of PCM to *D. magna* was calculated as 5.32 ± 0.73 mg/L [25]. A study performed by Nunes and coauthors [26] investigated the toxicity of paracetamol towards different freshwater species, from algae to plants. This study assessed the growth inhibition of the microalga *P. subcapitata* after the exposure for 72 h to seven PCM concentrations, ranging from 87.8 and 1,000 mg/L and the growth inhibition of the cyanobacterium *C. raciborskii* exposed to eight paracetamol concentrations, ranging from 48.4 to 510.2 mg/L. Moreover, acute and chronic toxicity of PCM was assessed in the crustaceans *D. magna* and *D. longispina.* Acute toxicity of PCM towards *D. magna* and *D. longispina* was assessed through static exposures to five (range 48.6–85 mg/L) and eight (range 4.0–8.9 mg/L) PCM concentrations, respectively. Chronic toxicity was assessed by a reproduction test exposing *D. longispina* 7.9, 11.8, 17.8, 26.7, 40.0, and 60.0 mg/L, while *D. magna* to 0.53, 0.79, 1.2, 1.7, 2.7, and 4.0 mg/L of PCM. Lastly, acute toxicity of increasing PCM

Table 1 List of studies investigating the adverse effects induced by paracetamol (PCM) exposure towards freshwater invertebrates

Model species	Phylum/ subphylum	Concentration range	Effect	References
Daphnia magna	Crustacea	4–972 mg/l	Acute	[25]
Pseudokirchneriella subcapitata	Chlorophyta	87.8–1,000 mg/L	Acute	[26]
Cylindrospermopsis raciborskii	Cyanobacteria	48.4–510.2 mg/L	Acute	[26]
Daphnia magna	Crustacea	4.0–8.9 mg/L	Acute	[26]
Daphnia longispina	Crustacea	48.6–85 mg/L	Acute	[26]
Lemna minor[a]		62.5–1,000 mg/L	Acute	[26]
Lemna gibba[a]		62.5–1,000 mg/L	Acute	[26]
Daphnia magna	Crustacea	0.53–4.0 mg/L	Chronic	[26]
Daphnia longispina	Crustacea	7.9–60 mg/L	Chronic	[26]
Plationus patulus	Rotifera	2–32 mg/L	Chronic	[27]
Moina macrocopa	Crustacea	2–32 mg/L	Chronic	[27]
Hydra vulgaris	Cnidaria	0.001–10 mg/L	Chronic	[28]
Corbicula fluminea	Mollusca	0.05–532.78 mg/L 3.88–61.95 µg/L	Chronic	[29]
Dreissena polymorpha	Mollusca	30–450 µg/L	Chronic	[30]
Dreissena polymorpha	Mollusca	0.154–1.51 µg/L	Chronic	[31]

[a]*Lemna minor* and *Lemna gibba* belong to the Kingdom Plantae

concentrations (five concentrations ranging between 62.5 and 1,000 mg/L) towards *L. minor* and *L. gibba* was investigated. Paracetamol toxicity was widely variable among species, even among phylogenetically related ones. Paracetamol was toxic to all test organisms in the tested concentration range, with the exception of *L. gibba*, whereby no acute effects occurred also at concentrations up to 1,000 mg/L. Considering acute toxicity in terms of EC_{50}, the scale of toxicity, from the most sensitive to the most tolerant model organism, was the following: *D. magna* < *D. longispina* < *C. raciborskii* < *P. subcapitata* < *L. minor* < *L. gibba*. PCM caused mortality in the reproduction test with *D. magna* at the highest tested concentrations (between 1.2 and 1.7 mg/L), so that no organisms survived over the whole duration of the experiment, although they generated offspring. Differently, *D. longispina* showed a significant delay in the first reproductive event and a reduction in the fecundity. A study by Sarma and coauthors [27] exposed the rotifer *Plationus patulus* and the cladoceran *Moina macrocopa* to increasing concentrations of PCM (2, 4, 8, 16, and 32 mg/L) in order to assess changes in population growth. Population growth curves of both the species were affected by the exposure to increasing concentrations of PCM, showing a decrease in organism density with increasing levels of drug. Moreover, the daily rate of population increase was negatively and significantly affected by PCM exposure in both the zooplanktonic species. A 7-day exposure to 10, 100 μg/L, 1.0 and 10 mg/L of PCM did not affect the survival of *Hydra vulgaris* specimens at concentrations up to 1.0 mg/L, while after 17 days neither feeding nor bud formation was adversely affected. Moreover, the ability of dissected polyps to regenerate a hypostome, tentacles, and foot was not inhibited [28]. Biochemical effects of PCM exposure were investigated in the freshwater clam *Corbicula fluminea* following short- (96 h) and long-term (28 days) exposures to 0.05, 0.48, 4.82, and 532.78 mg/L of PCM and 3.88, 7.74, 15.49, 30.98, and 61.95 μg/L of PCM, respectively [29]. Effects of PCM exposure on some oxidative stress endpoints, namely, catalase (CAT), glutathione S-transferases (GSTs), glutathione reductase (GRed), and lipid peroxidation were investigated. No mortality was observed in clams over short- or long-term exposures. PCM did not modulate CAT activity but induced a significant decrease of GSTs activity following both short- and long-term exposure (LOEC values of 532.78 mg/L and 30.98 μg/L, respectively). Moreover, PCM treatment induced a significant dose-dependent decrease of GRed activity in both short- and long-term exposures. A significant increase of lipid peroxidation was noted at the end of short- and long-term exposure to the highest PCM tested concentrations. These results indicated that the exposure to increasing PCM concentration caused notable changes in the cellular redox status of *C. fluminea.* The cytogenotoxicity of PCM was investigated through an in vitro approach by exposing the hemocytes collected from the zebra mussel *D. polymorpha* for 1 h to 30, 150, and 450 μg/L [30]. Cytotoxicity was evaluated by the neutral red retention assay (NRRA) while genotoxicity by SCGE (single cell gel electrophoresis) and DNA diffusion assay. Significant cytotoxic and genotoxic effects were after the exposures to all the tested concentrations according to a dose-dependent relationship. PCM exposure induced significant alterations of the oxidative status of the zebra mussel *D. polymorpha* [31]. Zebra mussels were exposed for

96 h to three PCM concentrations (0.154, 0.75, and 1.51 μg/L), and cytogenotoxicity was assessed in mussel hemocytes through the application of a suite of eight different biomarkers, namely, the lysosomal membrane stability (neutral red retention assay), the single cell gel electrophoresis (SCGE) assay, the micronucleus test (MN test), and assessments of the apoptotic frequency (DNA diffusion assay). The alteration of mussel oxidative status was assessed by measuring the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and the detoxifying enzyme glutathione S-transferase (GST). No mortality of zebra mussel over the test or changes in hemocyte viability were induced by PCM exposure. Although PCM did not induce primary genetic damage in zebra mussel hemocytes at all the tested concentration, a significant increase of fixed genetic damage, in terms of both micronuclei and apoptotic frequency, was noted at the end of the exposure to the highest tested concentrations. Moreover, a significant destabilization of lysosomal membranes and significant modulation of CAT, GPx, and GST activity was induced by the exposure to high PCM concentrations. All these data suggested that the exposure to environmental concentrations of PCM might modulate the oxidative status of freshwater invertebrates, leading to oxidative stress situation and genetic damage.

2.2 *Effects Induced by Diclofenac Exposure*

Acute and chronic toxicity of diclofenac (DCF) towards non-target, freshwater invertebrates has been investigated on rotifers (*Plationus patulus*), crustaceans (*Daphnia magna* and *Moina macrocopa*), diptera (*Chironomus riparius*), bivalves (*Dreissena polymorpha*), and gastropods (*Lymnaea stagnalis*) (Table 2).

Complete mortality of *D. magna* specimens was caused after only 24 h exposure to high levels of DCF (486 mg/L). DFC exposure caused 50% mortality in *D. magna*

Table 2 List of studies investigating the adverse effects induced by diclofenac (DCF) exposure towards freshwater invertebrates

Model species	Phylum/subphylum	Concentration range	Effect	References
Daphnia magna	Crustacea	2–486 mg/L	Acute	[25]
Daphnia magna	Crustacea	29.5–75 mg/L	Acute	[32]
Daphnia magna	Crustacea	5–5,000 μg/L	Chronic	[33]
Plationus patulus	Rotifera	2–32 mg/L	Chronic	[27]
Moina macrocopa	Crustacea	2–32 mg/L	Chronic	[27]
Daphnia magna	Crustacea	5–50 mg/L	Chronic	[34]
Dreissena polymorpha	Mollusca	60–250 μg/L	Chronic	[30]
Dreissena polymorpha	Mollusca	0.001–10 mg/L	Chronic	[35]
Dreissena polymorpha	Mollusca	95–637 ng/L	Chronic	[36]
Lymnaea stagnalis	Mollusca	100–1,000 μg/L	Chronic	[37]
Chironomus riparius	Arthropoda	34.0 μg/g	Chronic	[38]

after 21 days exposure to 2.00 ± 0.30 mg/L and a significant reduction of egg production at the lowest exposure concentrations of 0.50 mg/L [25]. A study by de Oliveira and coauthors [32] calculated a diclofenac (EC_{50} = 123.3 mg/L) in *D. magna*, but no effect on population increase was noted after the exposure to increasing DCF concentrations (range 29.5–75 mg/L). Toxic effects of 21 days exposure to DCF (5, 50, 500, and 5,000 μg/L) on survival, growth rate, and reproduction, as well effects on the expression of the genes related to the detoxification metabolism, growth, development, and reproduction, such as HR96, P-gp, CYP360A8, CYP314, GST, EcR, and Vtg after 96 h exposure, were investigated in *D. magna* specimens [33]. Significant toxic effects of DCF to *D. magna* were observed at 50 μg/L, whereby the expression of the selected genes was inhibited after 24 h of exposure, while they were increased after 48 h. Despite modulation of gene expression, no significant effects were observed in molting frequency, number of eggs produced in the first brood, total number of eggs per individual, total number of broods per individual, body length, and growth rate. In contrast, the exposure to increasing concentrations of DCF (2, 4, 8, 16, and 32 mg/L) affected the population growth curves of the rotifer *Plationus patulus* and the cladoceran *Moina macrocopa,* leading to a decrease in organism density with increasing levels of drug, as well as a negative effect on the daily rate of population increase [27]. Another research investigated the toxicity of DCF at biochemical level in *D. magna* by assessing the modulation of hsp70 level as a biomarker for proteotoxicity [34]. Hsp70 induction occurred at high levels of DCF, as the LOEC was calculated at 40 mg/L. The cytogenotoxicity of DCF was investigated through an in vitro approach by exposing hemocytes from the zebra mussel *D. polymorpha* for 1 h to 60, 126, and 250 μg/L [30]. Cytotoxicity was evaluated by the neutral red retention assay (NRRA) while genotoxicity by SCGE (single cell gel electrophoresis) and DNA diffusion assay. A significant cytotoxic effect was noted only after the exposure to 250 mg/L of DCF, while genotoxicity occurred after the exposures to all the tested concentrations. A further in vitro experiment [35] investigated the toxicity of increasing DCF concentrations (0.001, 0.01, 0.1, 1, and 10 mg/L) on three different cell typologies from the zebra mussel (*Dreissena polymorpha*), namely, hemocytes, gill, and digestive gland cells. At the end of the exposure (i.e., 96 h), viability of DCF treated gill cells was significantly reduced already at the lowest concentration with respect to baseline levels. Viability of DCF-treated digestive gland cells was significantly reduced already after 48 h exposure to 0.01 mg/L, while hemocyte viability was affected already at the lowest concentration (0.001 mg/L). Zebra mussels specimens were exposed for 96 h to increasing concentrations (95, 318, and 637 ng/L) of DCF through an in vivo approach [36]. Cytogenotoxicity was assessed by means of the single cell gel electrophoresis assay, the apoptotic frequency, the micronucleus test (MN test), and the lysosomal membrane stability (neutral red retention assay) in mussel hemocytes. Moreover, the activity of catalase, superoxide dismutase, glutathione peroxidase, and the phase II detoxifying enzyme glutathione S-transferase was measured as oxidative stress biomarkers. Negligible cyto- and genotoxicity of DCF was noted towards the zebra mussel hemocytes; in fact only a slight decrease of lysosomal membrane stability was observed at the end of exposure to the highest

tested concentration (637 ng/L). DCF toxicity of gastropods was assessed by exposing *Lymnaea stagnalis* specimens for 3 days to environmental realistic (1–10 μg/L) and therapeutic concentrations (100–1,000 μg/L) of DCF [37]. Effects on immune parameters of individual snails were measured, namely, hemocyte density and viability, hemocyte phagocytosis capacity, and hemocyte-related oxidative activities (basal and NADPH-oxidase). Diclofenac induced immune responses, while no immunosuppression was observed. DCF significantly affected the immunocapacity and the immunoefficiency of the snails' hemocytes. This effect is typical of an inflammatory response, confirmed by the increase of the NADPH-oxidase activity, mainly at 1,000 μg/L. The effects of exposure to DCF towards the *Chironomus riparius* was assessed through an experiment using spiked sediment [38]. A 10-day chronic toxicity test with *C. riparius* was performed to assess effects on survival, growth, and developmental stage, in terms of biomass, as well as emergence rates and *sex ratio* after 21 days of exposure. No effects on survival and no change in the sex ratio was induced by DCF exposure. In contrast, DCF decreased the emergence ratio in organisms exposed at concentrations of 34.0 μg/g of DCF.

2.3 *Effects Induced by Ibuprofen Exposure*

Acute and chronic toxicity of ibuprofen (IBU) towards non-target, freshwater invertebrates has been investigated on crustaceans (*Daphnia magna*), cnidarian (*Hydra vulgaris*), bivalves (*Dreissena polymorpha* and *Corbicula fluminea*), and gastropods (*Planorbis carinatus*) (Table 3).

Acute toxicity on *D. magna* occurred at lower concentrations compared to DCF. In fact, complete mortality of *D. magna* specimens was caused after only 24-h exposure to high levels of IBU (200 mg/L), while EC_{50} was calculated as

Table 3 List of studies investigating the adverse effects induced by ibuprofen (IBU) exposure towards freshwater invertebrates

Model species	Phylum/subphylum	Concentration	Effect	References
Daphnia magna	Crustacea	1–200 mg/L	Acute	[25]
Daphnia magna	Crustacea	20–80 mg/L	Acute/chronic	[22]
Daphnia magna	Crustacea	20–80 mg/L	Acute/chronic	[21]
Daphnia magna	Crustacea	0.5–50 μg/L	Chronic	[39]
Hydra vulgaris	Cnidaria	0.001–10 mg/L	Chronic	[28]
Hydra vulgaris	Cnidaria	0.1–100 mg/L	Chronic	[40]
Dreissena polymorpha	Mollusca	45–909 μg/L	Chronic	[30]
Dreissena polymorpha	Mollusca	0.2–8 μg/L	Chronic	[41]
Dreissena polymorpha	Mollusca	0.206–206 μg/L	Chronic	[40]
Corbicula fluminea	Mollusca	0.1–50 μg/L	Chronic	[42]
Planorbis carinatus	Mollusca	0.1–100 mg/L	Acute/chronic	[43]

3.97 ± 0.43 mg/L [25]. A 14-day exposure of *D. magna* to IBU (concentration range 20, 40, and 80 mg/L) measuring chronic effects on life history traits and population performance was performed by Heckmann and coauthors [22]. Population growth rate was significantly reduced at all the IBU tested concentrations, while *D. magna* survival was affected only by the exposure to 80 mg/L of IBU. Reproduction was influenced by the exposure to low concentrations of IBU, whereby the 14-day EC_{50} was calculated as 13.4 mg/L but was utterly inhibited at 80 mg/L. Similar results were obtained by Hayashi and coauthors [21], who exposed *D. magna* (5-days old) to the same range of IBU concentrations than [22] (i.e., 20, 40 and 80 mg/L) for 10 days. Individuals exposed to higher concentrations produced significantly fewer offspring than controls, while no reproduction occurred at 80 mg/L. Moreover, at first reproduction was delayed at all the tested IBU concentrations. *D. magna* survival was affected after the exposure to 80 mg/L during the 10-day exposure, while the population growth rates were >1 after the exposure to control, 20 and 40 mg/L of IBU, suggesting and increasing population, <1 at 80 mg/L of IBU, suggesting a decreasing population trend [39]. A recent study by Wang and coauthors [39] investigated the modulation of the expression of CYP360A, CYP314, and GST genes involved in the detoxification process and the responses of their associated enzymes activity, as well as in some physiological parameters (e.g., growth and reproduction) in *D. magna* exposed to environmentally relevant concentrations of IBU (0.5, 5, and 50 μg/L). IBU did not affect the total amount of eggs produced per female, total number of brood per female, and body length of *D. magna* specimens. By a molecular and biochemical point of view, IBU treatment inhibited the expression of CYP360A gene at 0.5 μg/L while induced its expression at 50 μg/L. Similar trend was also noted for GST gene, while the gene CYP314 showed an inhibition after short time exposure (6 h). Conversely, the gene CYP314 showed an overexpression after prolonged exposure time (48 h at 0.5 μg/L). Erythromycin N-demethylase (ERND) and aminopyrine N-demethylase were both inhibited after short time exposure (6 h). However, they were both overexpressed after prolonged exposure time (48 h) at 0.5 μg/L. Moreover, an induction of glutathione S-transferase (GST), superoxide dismutase (SOD), and catalase (CAT) activity was observed in short-term exposure to IBU, while EROD and methane dicarboxylic aldehyde (MDA) content increased in a dose-dependent manner [41]. A 7-day exposure to 10, 100 μg/L, 1.0, and 10 mg/L of IBU did not influence the survival of *H. vulgaris* at concentrations up to 1.0 mg/L, while after 17 days neither feeding nor bud formation nor the ability of dissected polyps to regenerate a hypostome, tentacles, and foot was affected [28]. However, a further study showed that regeneration was significantly inhibited at 5 mg/L of IBU, while the 96-h IC_{50} (i.e., the concentration that inhibits 50% of the embryos to develop) was calculated as 3.84 mg/L (confidence interval 2.36–6.26 mg/L) [44]. IBU exposure also induced sublethal effects towards mollusks. The cytogenotoxicity of IBU was investigated through an in vitro approach by exposing zebra mussel hemocytes for 1 h to 45, 450, and 909 μg/L [34]. A significant decrease in the stability of lysosomal membranes was noted after the exposure to 450 and 909 μg/L of IBU, while genotoxicity occurred after the exposures to all the tested concentrations. A further in vivo

exposure of the zebra mussel showed that the 96 h treatment with 0.2, 2, and 8 mg/L of IBU induced a slight cytogenotoxicity (i.e., NRRA, SCGE assay, apoptosis, and MN test) on hemocytes at the IBU concentration of 0.2 mg/L, while higher IBU concentrations (2 and 8 mg/L) cause a significant increase of both cellular and primary and fixed genetic damage. In addition, IBU significantly altered the activity of antioxidant and detoxifying enzymes at all the tested concentrations, suggesting the imbalance of oxidative status and a possible onset of oxidative stress [41]. A study performed on the zebra mussel exposed for 7 days to increasing IBU concentrations (0.206, 2.06, 20.6, and 206.3 μg/L) investigated the effects of this NSAIDs at molecular level, assessing the mRNA changes of enzymes and other proteins involved in the prevention of protein damage (heat shock protein 70) and oxidative stress (superoxide dismutase, catalase, metallothionein), biotransformation (glutathione S-transferase, aryl hydrocarbon receptor), elimination (P-glycoprotein), and reversible protein posttranslational modification (protein phosphatase 2A). Mussels exposed to the lowest tested concentrations of IBU experienced an oxidative stress situation as showed by induced mRNA levels in the digestive gland of mussels recorded for catalase and metallothionein, as well as superoxide dismutase, after 1 and 4 days of exposure, respectively. At higher concentrations, an increase in transcript levels of glutathione S-transferase occurred, suggesting the activation of biotransformation processes of IBU or by-products deriving from oxidative stress [40]. Moreover, responses induced by 21-days exposure to increasing IBU concentrations (0.1, 1.5, 10, 15, 50 μg/L), in terms of general stress (lysosomal membrane stability), biomarkers of phase I and II (ethoxyresorufin-O-deethylase, dibenzylfluorescein dealkylase, glutathione S-transferase), oxidative stress (glutathione reductase, glutathione peroxidase, lipid peroxidation), and DNA damage were investigated in the clam *Corbicula fluminea*. IBU induced a destabilization of lysosomal membrane at all the tested concentrations. Moreover, IBU activated both phase I and II enzymes, including glutathione reductase and glutathione peroxidase, at the highest tested concentration (50 μg/L). Moreover, an increase of lipid peroxidation, but not of DNA damage, was observed at the end of the exposure to 50 μg/L [42]. Individuals of the freshwater Keeled rams horn snails (*Planorbis carinatus*) were exposed for 72 h to 0.1, 1.0, 10, and 100 mg/L of IBU and to 0.32, 1.0, 3.2, and 10 mg/L of IBU for 21 days. The 48 and 72 h LC_{50} values were both 17.1 mg/L (95% confidence intervals 5.9–72.3 mg/L), while the 21 days LOEC and NOEC based on individual survival were calculated as 45.36 and 5.36 mg/L, respectively. The 21-day LOEC and NOEC calculated for snail reproduction (i.e., hatching success) were 5.36 and 2.43 mg/L, respectively, while the LOEC and NOEC calculated for growth were 2.43 and 1.02 mg/L, respectively [43].

3 Conclusions

The results reported in the present review show that three of the most common NSAIDs found in the aquatic ecosystems worldwide might represent a serious hazard towards non-target, freshwater invertebrates. In fact, although acute toxicity of PCM, DCF, and IBU occurs only at high concentrations, much higher than those measured in freshwaters, sublethal effects due to chronic exposures cannot be neglected. In fact, studies performed on different model species belonging to different *taxa* showed that the exposure to low, environmentally relevant concentrations of PCM, DCF, and IBU can induce notable adverse effects at molecular, biochemical, and cellular level, while effects at individual level (e.g., growth, survival, reproduction) seem to be improbable. The sublethal effects pointed out by short- and mid-term exposures might be also more worrisome considering that freshwater invertebrates are exposed to NSAID concentrations for their whole lifespan. In addition, considering the increasing production and use of NSAIDs might lead to a notable increase in freshwater environmental levels and, consequently, to an enhancement of the hazard of these pharmaceuticals towards non-target, freshwater invertebrates. For these reasons, further studies should be needed to enlarge the knowledge on NSAID toxicity towards aquatic organisms, considering long-term exposures and the use of alternative and innovative assays to shed light on the mechanisms of action of these pharmaceuticals. Lastly, considering that NSAIDs occur in aquatic ecosystems in complex "cocktails," studies of toxicity of NSAID mixture toxicity should be a priority in environmental risk assessment for this molecules in order to explore their real ecological hazard towards aquatic communities.

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Ibuprofen and Diclofenac: Effects on Freshwater and Marine Aquatic Organisms – Are They at Risk?

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Contents

Abstract Pharmaceuticals are included in the group of emergent pollutants due to their characteristics and potential negative effects. They remain mostly unregulated or are undergoing currently some sort of regularization process. Diclofenac, for instance, has been included in a watch list of substances for European Union-wide monitoring and the priority list of the Water Framework Directive (WFD) of the

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European Union. Nonsteroidal anti-inflammatory drugs (NSAIDs) are a varied and chemically heterogeneous group of mainly anti-inflammatory, analgesic, and antipyretic drugs, reducing symptoms of inflammation, pain, and fever, respectively. They are widely employed and have been detected in freshwater, seawater, and sediment. Nevertheless, they are found as mixture instead of single compounds. In this chapter, we have tried to summarize how to assess the risk due to the occurrence of pharmaceuticals in aquatic ecosystems. We have focused on the mixture of diclofenac and ibuprofen using acute and sublethal toxicity data for different aquatic species. It has been presented new strategies as adverse outcome pathway to improve the understanding of the toxicity of these compounds. Although gaps of the information are pointed out, the risk levels associated with the occurrence of these compounds in aquatic ecosystems will range between no risk or high risk, depending on concentrations and environmental conditions.

Keywords Aquatic pollution, Diclofenac, Ibuprofen, Lethal and sublethal effects, NSAIDs, Pharmaceuticals, Risk evaluation

1 Introduction

Over the last 15 years, pharmaceuticals have been receiving increasing attention as potential bioactive chemicals in the environment [1]. They are considered emerging contaminants in water bodies because they still remain mostly unregulated or are undergoing currently some sort of regularization process. Diclofenac (DF), for instance, is included in a watch list of substances for European Union-wide monitoring according to Directive 2008/105/EC (EU Commission Implementing Decision 2015) or the priority list of the Water Framework Directive (WFD) of the European Union (EC, Directive 2000/60/EC) by means of a proposal for a Directive amending the WFD with respect to priority substances (COM [2] 876).

One of the first studies that evidences the presence of pharmaceutical compounds in surface waters has been published by Kolpin et al. [3] who detected up to 95 pharmaceuticals, hormones, and other organic contaminants in water samples from a network of 139 US streams across 30 states in 1999 and 2000, including the anti-inflammatory drug Ibuprofen (IB). Since then, more and more reports have been published on the increased occurrence of pharmaceutical residues at concentrations at the ng-μg L^{-1} range in different environmental matrixes [3–6]. Although being present at relatively low concentrations, pharmaceutical compounds are bioactive molecules especially designed to carry out a certain function which they continue to do once discharged into receiving waters with only small amounts required to produce effects in exposed organisms. Nonsteroidal anti-inflammatory drugs (NSAIDs) are a varied and chemically heterogeneous group of mainly anti-inflammatory, analgesic, and antipyretic drugs, reducing symptoms of inflammation, pain, and fever, respectively [7, 8]. NSAIDs

work by inhibiting the activity of cyclooxygenase enzymes (COX-1 and/or COX-2). In cells, these enzymes are involved in the synthesis of key biological mediators, namely, prostaglandins, which are involved in inflammation processes, and thromboxanes, which are involved in blood clotting. Side effects in humans depend on the specific drug but largely include an increased risk of gastrointestinal ulcers and bleeds, heart attack, and kidney disease.

In the case of NSAIDS, the growing use and abuse mainly due to the fact that no prescription is required for their purchase in combination with their limited removal during wastewater treatment has dramatically increased their concentration in receiving waters and has triggered scientific research into the effects that exposure to environmentally relevant concentrations may cause in nontarget organisms in order to evaluate the risk that their presence may have on populations and ecosystems.

2 Risk Assessment

Environmental risk assessment comprises a series of steps in order to identify acceptable or unacceptable levels of environmentally present chemical substances to human health and the environment. These steps include:

(a) Identification of a substance able to cause adverse effects (hazard identification)
(b) Estimation of the relationship between the dose and the incidence and severity of an effect (dose-response assessment)
(c) Assessment of the levels to which humans or ecosystems are exposed to the compound to
(d) identify if there is a risk or not

Once detected in the environment and its effects of exposure evaluated in laboratory experiments, risk is evaluated as proposed by the Technical Guidance Document on Risk Assessment [9] of the European Commission. This procedure is based on the comparison of predicted environmental concentration (PEC) or measured environmental concentration (MEC) of a compound and the concentration that is supposed to not represent any hazards for exposed organisms (Predicted No Effect Concentration (PNEC)), through the calculation of the PEC/PNEC ratio:

$$\mathrm{RQ} = \frac{\mathrm{PEC\ or\ MEC}}{\mathrm{PNEC}} \tag{1}$$

where $\mathrm{RQ} > 1$ implies that the presence of the compound at the considered concentration represents a potential risk for the ecosystem and $\mathrm{RQ} < 1$ implies no risk for the ecosystem.

The ultimate objective of this procedure is to provide a basis for possible regulatory decisions in case a risk has been identified.

PNEC (concentration in the environment below which an unacceptable effect will most likely not occur) values are usually determined on the basis of results from

Table 1 Assessment factors to derive a PNEC for the aquatic environment. Adapted from EU TGD [9]

Toxicity data	Assessment factor
At least one short-term L(E)C_{50} from each of three trophic levels including fish, *Daphnia*, and algae	1,000
One long-term NOEC from fish or *Daphnia* tests	100
Two long-term NOECs representing two trophic levels including fish, *Daphnia*, and algae	50
Long-term NOECs from at least three species, normally fish, *Daphnia*, and algae	10
Species sensitivity distribution (SSD) method	5–1

single-species laboratory tests from which toxicity parameters such as LC_{50}, EC_{50}, or NOECs can be derived using generally an assessment factor (AF) approach.

$$\text{PNEC} = \frac{\text{NOEC or } LC_{50} \text{ or } EC_{50}}{\text{AF}} \tag{2}$$

Several different assessment factors have been proposed, depending on the nature of the toxicity parameters derived in the laboratory study, with higher values for obtained LC_{50} values and lower for EC_{50}s and NOECs (Table 1). Assessment factors lie usually in the range of 10–1,000, and their application accounts for the degree of uncertainty when extrapolating from laboratory toxicity test data for a limited number of species to the "real" environment or human health. This AF takes into account that laboratory tests cover only a small part of the variety of responses that may occur in ecosystems. Thus, the higher the assessment factor, the lower the derived PNEC, expressing a more cautious approach to the studied chemical. Lower PNECs articulate the idea that more organisms are protected. In the same context, assessment factors applied for long-term tests are smaller as the uncertainty of the extrapolation from laboratory data to the natural environment is reduced. For this reason, long-term data are preferred over short-term data.

As PECs vary from site to site due to temporal and local emission characteristics, environmental risk assessment for the same compound can produce different risk quotients. i.e., environmental concentrations of a compound generally depend on localization of their producing industry and agglomerations with high consumption and discharge which can vary along the year. As a consequence, the comparison of PNEC values for the different sites and compartments with different PEC values for different exposure scenarios can lead to different levels of hazard.

The guidance provided by the EU TGD [9] is one of the most employed principles for risk assessment. Other methods such as the guideline provided by the OECD [10] propose similar assessment factors under similar conditions of data availability.

To date, the above mentioned procedure is mainly applied to acute and sublethal toxicity test results in form of LC_{50} (derived from mortality data) and EC_{50} (derived from tests assessing endpoints such as growth or reproductive success) respectively.

Table 2 Acute toxicity data of Diclofenac and Ibuprofen for aquatic species

Compound	Taxa	Species	LC_{50}/EC_{50} (mg L^{-1})	References
Diclofenac	Algae	*Dunaliella tertiolecta*	185.7	[15]
		S. subspicatus	71.9	[16]
		L. minor	7.5	[17]
	Crustaceans	*A. salina*	>100	[18]
		A. desmarestii	6.3	[19]
		T. battagliai	15.8	[20]
			9.5	[21]
		D. magna	68	[16, 17]
			80.1	[22]
			22	[23]
		C. dubia	23	[23]
	Bacteria	*V. fischeri*	27.8	[20]
			11	[23]
		A. fischeri	11.79	[24]
			16.31	[24]
Ibuprofen	Algae	*S. subspicatum*	342.2	[16, 17]
		L. minor	22	[16, 17]
	Cnidarian	*Hydra vulgaris*	22.36	[25]
	Platyhelminthes	*Dugesia japonica*	128.5	[25]
	Crustaceans	*T. battagliai*	49.7	[21]
		A. desmarestii	13.3	[19]
		T. platyurus	19.59	[26]
		D. magna	101.2	Cleuvers
		D. magna	124.4	http://cfpub.epa.gov/ecotox
	Molluscs	*P. carinatus*	17.1	[27]
	Fish	*Cirrhinus mrigala*	142	[25]
		O. latipes	>100	[27]
	Bacteria	*A. fischeri*	39	[24]

These approximations are useful for range definition of effects to be observed and to perform an initial risk evaluation of the compound in question. In this sense, the recent and increasing detection of pharmaceuticals in aquatic environments [5, 11] has triggered an increase in the studies on the effects of various pharmaceuticals in nontarget organisms [12–14] in acute exposure experiments.

In the case of DF and IBU, some acute toxicity values are available for different species. Acute toxicity values obtained in laboratory toxicity tests employing different test species are comprised between 6.3 and >100 and 13.3 and 342 m/L^{-1} for DF and IB, respectively (Table 2).

Table 2 has not included the data for *Paracentrotus lividus* [28] with EC_{50} 0.01 μg L^{-1} for IB. Application of the highest AF (1000) results in PNECs ranging between 6.3 and >100 and 13.3 and 342 μg L^{-1}, respectively, representing this factor the most conservative and protective approach. With the condition that PEC/PNEC > 1 for a compound to be environmentally safe, acceptable environmental concentrations must not exceed 100 and 342 μg L^{-1} for DF and IB, respectively.

Considering different test organisms, we can observe that there are significant differences in the obtained LC_{50} values and, hence, sensitivities of the organisms toward the pharmaceuticals. Within the same species, susceptibility to suffer effects of exposure to contaminants also varies, being generally the developing and early-life stages the most vulnerable.

3 Species Sensitivity Distributions DF and IB

The species sensitivity distribution (SSD) is an ecotoxicological tool which has been employed to establish safe levels for risk assessment. The basic premise of the SSD concept is to consider that sensitive species can be described using a parametric distribution (e.g., logistic) [29]. Figures 1 and 2 plot the SSD distribution of aquatic

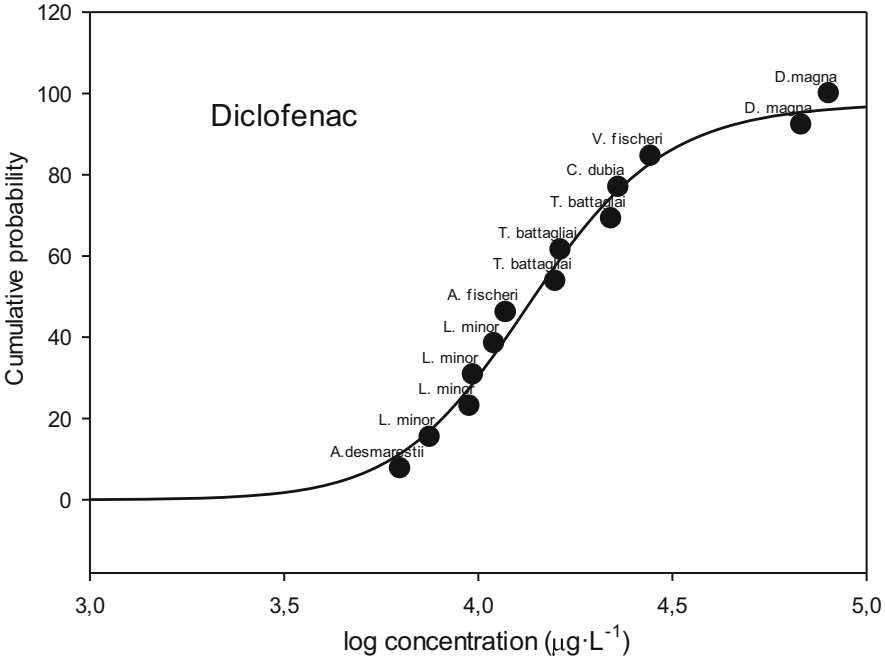

Fig. 1 SSD distribution of toxicity acute data for Diclofenac in aquatic species

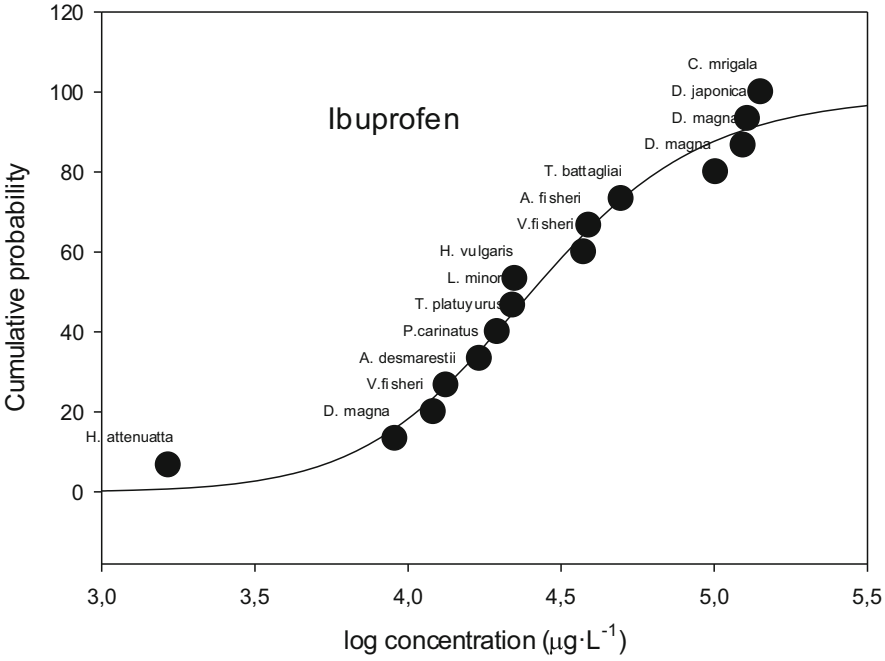

Fig. 2 SSD distribution of toxicity acute data for Ibuprofen in aquatic species

species for DF and IB. The collected data correspond to the acute toxicity values of Table 2 for species from different phyla. Hazard effect concentration (HEC5) values have been calculated for both compounds; for DF and IB, HEC5 were 4.5 and 4.4 mg L^{-1}, respectively. These plots have been carried out using acute toxicity data, if an assessment factor (AF) of 5 can be employed to calculate the PNEC, obtaining values close to 0.8 mg L^{-1}. This value is very high, and some concerns about its use as benchmark should be considered. Chronic values for many species are lower than HEC5; thus Schwaiger et al. [30] reported alteration in the digestive tract histology at concentrations of 5 μg L^{-1}. Posthuma et al. [29] pointed out the limitations of this approach (e.g., lack of use of community data, data set, uncertainty data, etc.).

To carry out a site risk assessment, PEC or MEC. However, the use of MEC allows to reduce the uncertainty. Nevertheless, the available information about the occurrence of pharmaceuticals in environmental compartment is limited, and the geographical data distribution is biased, with more available information for EU and the USA. Table 3 shows the range of concentrations for IB and DF in three environmental compartments (seawater, freshwater, and sediment) summarizing scientific literature. This is not an exhaustive database, which is beyond of the objective of this chapter, but it gives information about the field data campaigns. Wide variations are observed for both compounds in the three compartments, with the highest range for freshwater and the lowest for sediment. The lack of wastewater treatment plants in many developing or emerging countries leads that extreme values

Table 3 Range of concentrations for Ibuprofen and Diclofenac in seawater, freshwater, and sediment[a]

Environmental compartment	Ibuprofen (ng L^{-1})	Diclofenac (ng L^{-1})
Seawater	0.01–2,370	0.06–843
Freshwater	0.10–17,600	0.04–10,200
Sediment[b]	5.83–24.93	0.67–11.02

[a]References corresponding to extreme values (freshwater [31, 32], seawater [33, 34], sediment [35, 36])
[b]The results are expressed as ng g^{-1}

recorded there. However, the calculation of the ratio between PEC and PNEC can be considered as initial approach to establish the potential risk.

4 Drug Mixture: Prediction of Effects and Risk Assessment

The contaminants in natural systems rarely occur as individual chemicals, but usually as complex mixtures. The joint presence of various compounds implies toxic effects different from those associated with the individual compounds because interactions between them can alter the magnitude of their impacts on exposed organisms. The main toxicological interactions are synergism (when the mixture effect is greater than the effect estimated by the sum of the individual effects of each mixture component), antagonism (when the combined effect of different chemicals is less than the sum of each chemical considered individually), potentiation (when a chemical that does not have toxic effect alone, increase the effect of a second chemical), inhibition (when a component that does not have a toxic effect alone reduce the apparent effect of a second chemical), and masking (when the components produce opposite or functionally competing effects on the same system and reduce the effects of each other, or one overrides the effect of the other) [37, 38]. Consequently, mixtures may have high toxicity even when their components are present at very low concentrations; below their individual no observed effect concentration (NOEC) [39] and water quality investigated by individual substances may lead to underestimation in aquatic environmental risk assessment [40].

To analyze the possible effects of a pollutant mixture, models based on toxicity data obtained in studies with individual compounds are used. Two classical models which are widely accepted in pharmacology and whose use has been extended to the field of ecotoxicology are based on the concepts of concentration addition (CA) and independent action (IA) [38, 39, 41, 42]. CA model is based on the assumption that all mixture components have similar mode of action acting on the same biochemical pathways and target sites (mixture toxicity increases each time a component is added, additive effects) and is computed by equation [43]:

$$\mathrm{EC}x_{\mathrm{mix}} = \left(\sum_{i}^{n} \frac{p_i}{\mathrm{EC}_{xi}} \right)^{-1} \tag{3}$$

where $\mathrm{EC}x_{\mathrm{mix}}$ is the effect concentration of the mixture provoking x% effect, EC_{xi} is the concentration of the component i provoking the same effect (x%) as the mixture when applied individually, and p_i is the fraction of the component i in the mixture.

The IA model assumes that mixture components have dissimilar mode of action, interacting with different molecules or target sites. As a result, the relative effect of a compound in the mixture can remain unchanged in the presence of other compounds, and total toxicity can be produced only by some elements of the mixture (e.g., the most active compounds). The following equation applies for IA [43]:

$$E(c_{\mathrm{mix}}) = 1 - \prod_{i=1}^{n} (1 - E(C_i)) \tag{4}$$

where $E(c_{\mathrm{mix}})$ is the effect of the total concentration of the mixture and $E(c_i)$ is the effect generated by the i component at the concentration c_i at which it is present in the mixture [44].

For the aquatic compartment, the CA model has shown to provide good predictions for biocidal and pesticide products, herbicides, pharmaceuticals, and estrogen active substances [45–50]. Various studies pointed out that CA slightly overestimates the toxicity of the mixture, whereas IA model underestimates it, making the first a more conservative and protective model and therefore more suitable for regulatory purposes (environmental risk assessment) [51, 52].

The simplicity of both models, based essentially on the primary mode of action (MoA) of chemicals, is a point in favor for their use within regulatory purposes. However, this is also their greatest weakness. Under environmental conditions, due to interactions between mixture components, aquatic medium, and biological systems, basic assumptions for CA and IA models are likely to be violated, and therefore the predictive power of the concepts will decrease [53]. Neither CA nor IA models take into account the complexity of biological systems and the specific properties and pathways of mixture components [54, 55], which casts doubt on their suitability in terms of accuracy in predicting the joint effects of real environmental pollutants.

Alternative models were created to overcome the limitations of CA and IA models, between them, the two-stage prediction (TSP) model [56, 57], integrated fuzzy concentration addition-independent action model (INFCIM) [58, 59], toxic equivalency factors (TEF) [60, 61], mixture toxicity indices (MTI) (median-effect/combination index (CI)-isobologram equation, sum toxic units, additivity index, etc.) [62, 63], and quantitative structure-activity relationship (QSAR) method [64, 65].

The risk associated with a selected mixture can be calculated using the risk quotient (RQ). According to Backhaus and Faust [55], the RQs of mixtures could be calculated by summing up the individual pollutant PEC/PNEC ratios as follows:

$$RQ_{PEC/PNEC} = \sum_{i=1}^{n} \frac{PEC_i \text{ or } MEC_i}{PNEC_i} \quad (5)$$

The final risk for a mixture can be calculated for a specific organism, using data from individual experiments or in a general way integrating data from different trophic levels according to the equation:

$$RQ = \sum_{i=1}^{n} \frac{PEC_i \text{ or } MEC_i}{\min(EC_{50}\text{algae}, EC_{50}\text{daphnids}, EC_{50}\text{fish}) \times (1/AF)} \quad (6)$$

where min(EC_{50}algae, EC_{50}daphnids, EC_{50}fish) is the minimum EC_{50} value for each mixture component selected between data existing in the bibliography for the three trophic levels.

4.1 *Case Studies: Acute Toxicity of DF and IB and Their Mixture on the Marine Copepod* Tisbe battagliai *and the Freshwater Shrimp* Atyaephyra desmarestii

Acute effects of DF and IB, both individually and in mixture, were studied by Trombini et al. [21] on the harpacticoid copepod *Tisbe battagliai*. Copepods are one of the dominant taxa in aquatic zooplankton communities being the principal and essential link between the primary phytoplankton producers and higher trophic levels in aquatic systems [66, 67]. Because of their ecological importance, since the 1970s, harpacticoid copepods, particularly *Tisbe battagliai*, have been successfully used as model species in marine ecotoxicological studies and widely applied in laboratory toxicity tests [68–71]. In this study, neonate nauplii (<24-h-old), considered the most sensitive developmental stage, were exposed to pharmaceuticals using 48-h acute toxicity tests according to the protocol indicated by the UK Environment Agency [72]. The LC_{50} obtained in this work were 9.5 and 49.7 mg L^{-1}, respectively, for DF and IB indicating a higher toxicity of the first compound. These results are consistent with most of studies found in the bibliography that indicate DF as the compound with the highest acute toxicity within the class of nonsteroidal anti-inflammatory drugs (NSAID) [73]. Schmidt et al. [20] studied the acute toxicity of gemfibrozil and DF on the same copepod *T. battagliai* obtaining, for this second compound, a LC_{50} value of 15.8 mg L^{-1} (Table 2), similar to the value obtained for copepod nauplii by Trombini et al. [21]. Cleuvers [16, 17] studied different acute endpoints in the crustacean *Daphnia magna* (immobilization), the green alga *Scenedesmus subspicatus* (growth inhibition), and the duckweed *Lemna minor* (growth inhibition) exposed to DF and IB; the median effect concentrations (EC_{50}) obtained in this study once again were lower for DF than for IB in both aquatic

organisms (68.8, 71.9, and 7.5 mg DF L^{-1} and 101.2, 342.2, and 22 mg IB L^{-1} for *D. magna*, *S. subspicatus*, and *L. minor*, respectively) (Table 2).

The acute effect of DF and IB was also studied by Nieto et al. [19] in the widely distributed freshwater shrimp *Atyaephyra desmarestii* [74, 75]. Assays were realized according to the US EPA Penaeid Acute Toxicity Test protocol (96 h assays), and results obtained were used to calculate LC_{50} for both compounds: 6.3 and 13.3 mg L^{-1}, respectively, for DF and IB (Table 2). These results highlight once again the higher toxicity of DF.

The acute toxicity data (LC_{50} or EC_{50}) can be used by referring to the EU Directive 93/67/EEC [76] to classify the substances in different risk classes: "extremely toxic" to aquatic organisms (LC_{50} or $EC_{50} < 0.1$ mg L^{-1}), "very toxic" (0.1–1 mg L^{-1}), "toxic" (1–10 mg L^{-1}), "harmful" (10–100 mg L^{-1}), and "no toxic" (>100 mg L^{-1}). According to this criterion, both for naupliar stage of *T. battagliai* and for *A. desmarestii*, DF and IB can be classified as toxic and harmful, respectively. However, observing the LC_{50} values reported in Table 2, we can see that toxicity level varies from "no toxic" to "extremely toxic" for DF and from "no toxic" to "harmful" for IB depending on the species considered (sensitivity, developmental stage, etc.). The LC_{50} (or EC_{50}) values can be used to estimate the risk associated with pharmaceuticals by the calculation of a risk quotient (RQ) using the equation seen above: in line with the above, the risk associated with the exposure to DF and IB will be different for different species. According to the classification indicated in the EU TGD [9], the risk can be defined as low environmental risk when $0.01 < RQ \leq 0.1$, medium risk when $0.1 < RQ \leq 1$, and high risk when $RQ > 1$. Equation (1) can be used to derive the environmental concentrations associated with a low risk for aquatic organisms from the PNEC values. In Table 3, LC_{50} obtained for *T. battagliai* and *A. desmarestii* were used to calculate the PNEC values (applying an assessment factor of 1,000 recommended for toxicity data obtained from short-term assays and considered as a conservative and protective value, [9]) and successively to estimate the environmental concentrations of DF and IB associated with a low risk for both species ($0.01 < RQ \leq 0.1$). A concentration range of 95–950 ng L^{-1} for DF and 497–4,970 ng L^{-1} for IB represents a low risk for the copepod *T. battagliai* (no risk for environmental concentrations lower than 95 and 497 ng L^{-1} for DF and IB, respectively). For the freshwater shrimp, the exposure to DF in the range of 63–630 ng L^{-1} or to IB in the range of 133–1,330 ng L^{-1} would be associated with a low risk for this species (no risk for environmental concentrations lower than 63 and 133 ng L^{-1} for DF and IB, respectively).

Similarly, environmental concentrations associated with low or no risk can be calculated for other organisms, particularly for the most sensitive species, extrapolating limit values below which the protection of aquatic organisms is guaranteed (Table 4).

As indicated above, aquatic organisms are exposed to mixtures of chemicals that can interact with each other producing greater effects than expected; therefore, the risk assessment based on the effects of individual chemicals can significantly underestimate the degree of risk. The acute effects of the mixture of DF and IB were studied by Trombini et al. [21] and Nieto et al. [19] both on *T. battagliai* and

Table 4 Calculation of environmental concentrations of Diclofenac (DF) and Ibuprofen (IB) associated with low risk for the copepod *T. battagliai* and the shrimp *A. desmarestii*

	DF	IB
LC_{50} *T. battagliai* (mg L^{-1})	9.5	49.7
PNEC (AF = 1000) (μg L^{-1})	0.0095	0.0497
Environmental concentration associated with low risk (ng L^{-1})	95–950	497–4,970
LC_{50} *A. desmarestii* (mg L^{-1})	6.3	13.3
PNEC (AF=1000) (μg L^{-1})	0.0063	0.0133
Environmental concentration associated with low risk (ng L^{-1})	63–630	133–1,330

A. desmarestii, checking the acute effect of increasing concentrations of the mixture. For both species, an RQ was calculated according to Eq. (5). In the case of *T. battagliai*, the risk associated with DF and IB individually and to their mixture was 0.0205, 0.0152, and 0.0357, respectively. Similarly, RQs obtained for *A. desmarestii* were 0.127, 0.752, and 0.879, respectively, for DF, IB, and their mixture. We can observe that in both cases, the values obtained for the mixture were higher than that obtained for the individual compounds, and this result can be interpreted as a higher risk associated with the exposure to the mixture. However, due the fact that the difference between the RQ values is not very large, the risk associated with the mixture, according to the classification system used above, is the same as the risk observed for individual compounds: low risk for *T. battagliai* and medium risk for *A. desmarestii*. Similar results were found by Sathishkumar et al. [77] who in their review work on the occurrence, effects, and ecological risk of DF in various environmental matrices indicate that this drug represents a low to medium risk for organisms in surface waters, having RQ values between 0 and 1.0. Additionally, the authors indicated a potential greater impact of DF in seawater organisms (RQ values higher than one). The RQ values found in the literature for IB vary between <0.01 and >1 depending on environmental concentrations (MEC or PEC) used in the RQ calculation, model organism, and endpoint selected [31, 78–81]. Therefore, data found for both anti-inflammatories indicates that the RQ values and the associated potential risk depend on the site-specific concentration and the specific species considered.

Laboratory assays can help evaluate the toxic effects of drugs in the more complex case of the real aquatic environment where many compounds are present simultaneously. However, the study of the mixture effects implies a significant investment in economic and time terms, in addition to the ethical implications, due to the number of organisms that would be necessary to sacrifice. In this context, it is therefore useful to use models to predict mixture toxicity, extrapolating toxicological parameters such as LC_{50} and consequently the potential risk (by RQ calculation) associated with different mixtures. Trombini et al. [21] and Nieto et al. [19] used the models CA and IA to predict toxic effects of the mixture of DF and IB on *T. battagliai* and *A. desmarestii* and compared experimental and predicted results to check the effectiveness of both models. According to the theoretical basis of

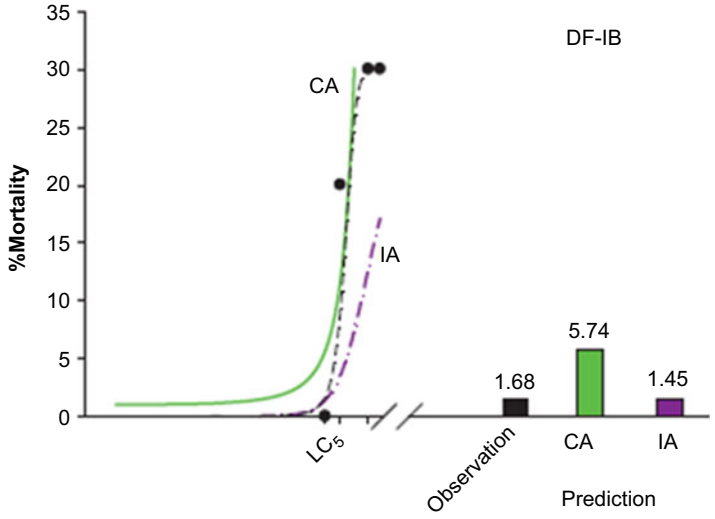

Fig. 3 Observed and predicted mortality for the freshwater shrimp *Atyaephyra desmarestii* exposed to the mixture of Diclofenac (*DF*) and Ibuprofen (*IB*). Comparison between observed toxicity (filled circles) and predicted mixture effects by the models CA (green solid line) and IA (violet dash dot). Graph on the right represents the observed combined effects and predicted effects of the mixture at LC_5 (mg L^{-1}). Source: Nieto et al. [19] (with permission)

classical models (both pharmaceuticals have similar MoA), CA model should provide the best prediction about the toxic effects of the mixture of IB and DF.

In the case of the freshwater shrimp (Fig. 3), the CA model allows the best prediction of the lethal effect of the mixture of DF and IB, although the adjustment was more obvious at higher concentrations. Similarly, a better adjustment at higher exposure concentrations was also observed in the case of the copepod *T. battagliai*, but in this case, both models CA and IA provided very similar toxicity predictions (Fig. 4). In this work, the authors tested a third model, a modification of the CA model which includes a combination index that takes into account deviations from additivity (CI in the Fig. 4): this model provided better predictions at lower exposure concentrations.

The results of the works of Nieto et al. [19] and Trombini et al. [21], like those of other authors [52], question the accuracy of the classic models (and those that derives from them) for assessing toxicity of pharmaceutical mixtures, especially at low exposure doses, making it difficult to assess the risk associated with the presence of these two drugs in conditions closer to real scenarios.

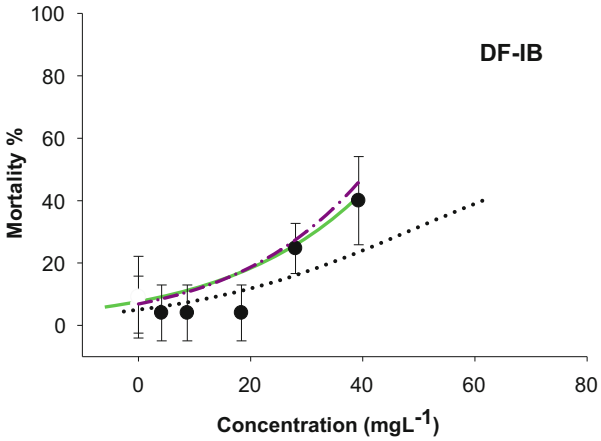

Fig. 4 Observed and predicted mortality for the copepod *Tisbe battagliai* exposed to the mixture of Diclofenac (*DF*) and Ibuprofen (*IB*). Comparison between observed toxicity (filled circles) and predicted mixture effects by three models: CA (green solid line), IA (violet dash dot), and CI (dotted line). Empty circles represent controls. Source: Trombini et al. [21]. With permission

5 Sublethal Effects

Although acute toxicity tests are of regulatory interest to derive PNECs for posterior risk characterization, environmentally relevant concentrations of isolated compounds are generally not likely to cause mortality in exposed organisms; thus lethal effects are unlikely to occur in the aquatic medium [73, 82] unless in cases of accidental spills. Thus, recent developments in risk assessment are undergoing a shift toward the observation of effects produced at the longer term in environmentally relevant concentrations. Chronic low-level exposure has shown to be able to induce sublethal responses that in the long term represent a hazard to natural populations by reducing their relative fitness and competitiveness, altering behaviors or inducing other subtle changes that make the persistence of populations difficult. When the focus is directed toward endpoints that only describe lethality, an opportunity to capture broader exposure-related sublethal effects is neglected although these generally precede mortality, providing a linkage between sublethal and lethal toxicities [83]. If chronic effects are more appropriate to the questions being asked within a risk assessment context, then alternative test endpoints must be developed and standardized. Chronic bioassays should measure ecologically relevant endpoints which enable effects at the population level to be predicted.

Effect evaluations based on sublethal endpoints most frequently comprise behavioral and morphological observations to evaluate chronic effects associated with contaminant exposure. Chronic, sublethal exposure to contaminants, however, must not be underestimated due to the lack of immediate, alarming effects, as their subtler establishment is able to cause effects on fitness, performance, and reproduction in the

longer term. Thus, today, toxicity tests are carried out more and more at environmentally relevant concentrations focusing on physiological, biochemical, and molecular endpoints. In this context, responses such as growth are evaluated under increasing exposure conditions for the derivation of EC_{50} values which can also be used in traditional risk quotient approaches also considered in the EU TGD, by applying a correspondently lower AF than to mortality data-derived LC_{50} values.

In the case of biochemical and molecular responses, however, dose-response curves are generally scarce, and derivation of a toxicity parameter is more complicated. Often, exposure experiments are carried out only at one or two environmentally relevant exposure concentrations to examine if a certain response occurs; however, follow-up tests in a dose-response-dependent manner are generally lacking, and a concrete value of the concentration affecting 50% of the individuals is not derived. Thus, no toxicity parameter is available to perform practical risk assessment in a traditional way employing the risk quotient approach. In this case, it is important to define at what threshold concentration of the compound these sublethal biochemical and molecular alterations translate into death or reproductive failure affecting populations in the long term. Little research has attempted to identify exposure thresholds at which observation of sublethal effects becomes a practical predictor of toxicity, so far. To date, there is no established procedure for risk evaluation relying on sublethal data, and scientists are still searching for a way to incorporate these data in the risk evaluation process. Therefore, it is important to characterize threshold responses to provide reasonable guidance for risk management measures. These sublethal alterations must not be underestimated, as aquatic invertebrates are generally very sensitive components of aquatic ecosystems, and a long-term exposure to bioactive compounds at low concentrations impairing basic functions can reduce fitness and performance of the exposed organisms, with important consequences on population level. Evaluation of nonconventional sublethal endpoints is imperative to assess the overall condition of the organism, and although in some cases the exposed individual might be able to maintain homeostasis, in other cases, especially when the stress persists, the organisms' mechanisms may not be effective enough to protect against the insult. Therefore, although sublethal endpoints are often identified in toxicity tests, they are rarely used to inform future testing decisions or to establish relevant exposure thresholds for complex substances, primarily due to the shortage of a standardized methodology [84].

5.1 *Case Study: Sublethal Toxicity of DF and IB in the Freshwater Shrimp* Atyaephyra desmarestii

In order to assess the sublethal effects of exposure to the pharmaceuticals DF and IB, Nieto et al. [19, 85] exposed the freshwater shrimp *Atyaephyra desmarestii* under environmentally relevant exposure conditions.

Only one concentration comprised in the range of tens of μg L^{-1} was assayed [19] to evaluate if the compounds had an effect on a series of selected physiological endpoints including feeding- and respiratory-related parameters that affect directly the fitness of exposed organisms such as ingestion rate, osmoregulatory capacity, and hemolymph osmolality.

At the concentrations tested, neither ingestion rate (exposed to 13.3 and 70.6 μg L^{-1} DF and IB, respectively) nor osmoregulatory capacity and hemolymph osmolality (exposed to 14.6 and 47.24 μg L^{-1} DF and IB, respectively) were statistically affected (Fig. 5). However, specific oxygen consumption showed a decreasing respiratory independence trend for all treatments (Fig. 5c) within the range of environmental oxygen concentration tested. The degree of respiratory independence versus oxygen concentration indicated that a shrimp's oxyregulation increased from well-oxygenated water to moderate hypoxia and decreased under severe hypoxic conditions. Specific oxygen consumption was not affected, but shrimps exposed to DF showed lower respiration rates under severe hypoxic conditions (1 mg O_2 L^{-1}) allowing the assumption that DF reduces the respiration rate of *A. desmarestii* under increasingly anoxic conditions that are more likely to occur under a global change scenario.

Thus, the selected physiological endpoint ingestion rate, osmoregulatory capacity, and hemolymph osmolality seemed to not significantly affect *A. desmarestii* when confronted with the selected exposure concentrations. Other crustaceans such as the crab *Carcinus maenas* under similar exposure conditions do have shown significant changes in osmoregulatory capacity and hemolymph osmolality after exposure to only 10 ng L^{-1} of DF and 17.5 psu of salinity [86]. Namely, DF produced an increased osmoregulatory capacity, suggesting an ability of these organisms to compensate for adverse osmotic conditions and an increase in hemolymph osmolality due to the impairment of its osmo- and iono-regulatory ability.

5.2 *Case Study: Acute and Sublethal Toxicity of DF in the Shrimp* Palaemon longirostris *and* Palaemon serratus

When looking at the joint action of salinity and temperature changes combined with exposure to environmentally relevant concentrations of DF (40 and 750 μg L^{-1}) on the selected endpoint survival, development, and growth of exposed larvae of the shrimps *Palaemon longirostris* and *Palaemon serratus*, González-Ortegón et al. [87] observed no effect on larval survival, development, and growth. However, a slight but significant interactive effect of salinity and pharmaceutical on intermolt duration was reported. However, in combination with another pharmaceutical compound, clofibric acid, a metabolite of the cholesterol-lowering pharmaceutical drug clofibrate as well as a commercially available herbicide, DF, showed to increase duration of development for 6 days at the higher exposure concentration tested. Thus, although DF alone did not produce effects on the examined sublethal endpoints,

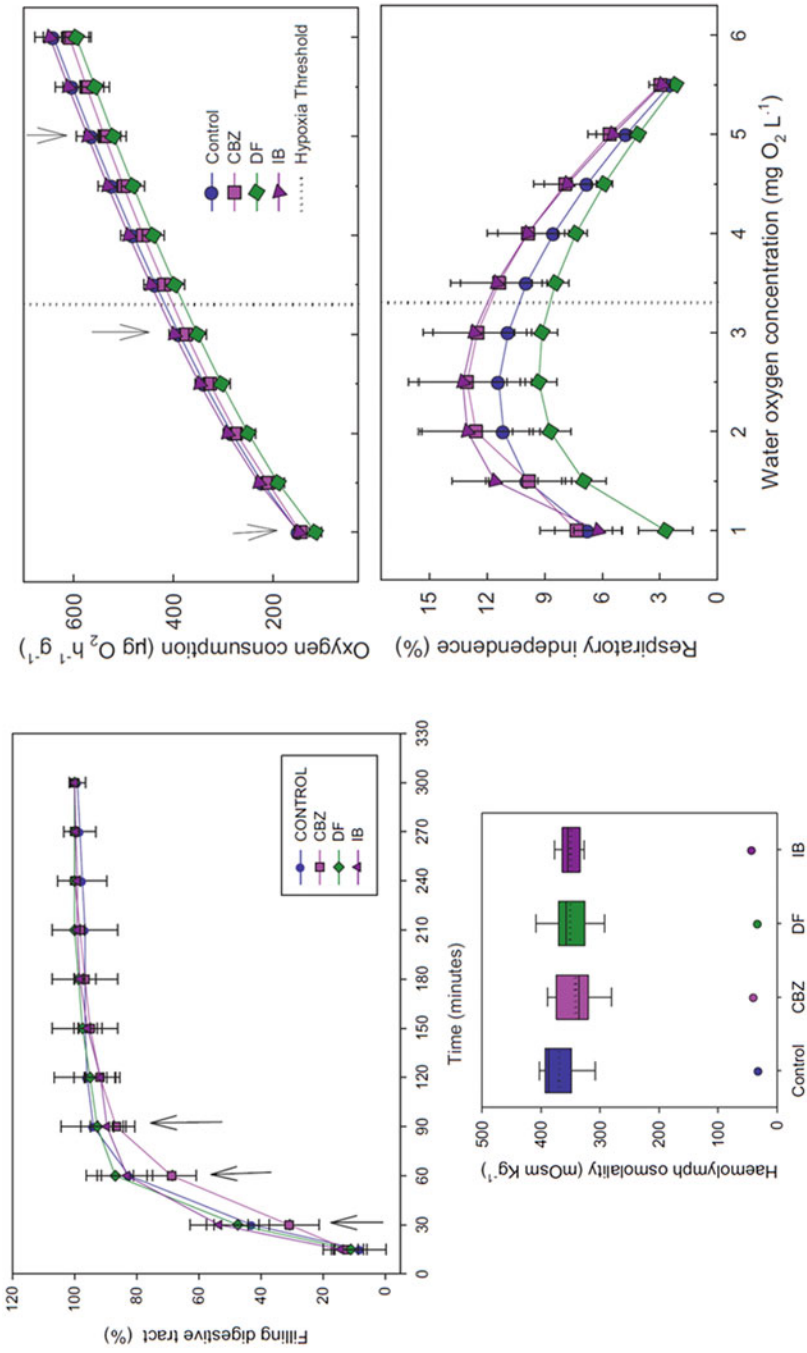

Fig. 5 (**a**) Ingestion rates in *A. desmarestii* exposed to DF, IB, and CBZ compared to control. Arrows indicate the times at which rates were statistically tested. (**b**) Hemolymph osmolality of shrimps exposed to DF, IB, and CBZ. Within boxes, solid and dotted lines represent medium and mean values, respectively. Circles represent water osmolalities. (**c**) Specific oxygen consumption rates (top) and oxygen independence degrees (bottom) after CBZ, DF, and IB exposure. Arrows indicate the times at which rates were statistically tested. (Source: Nieto et al. 2012, with permission)

under environmentally relevant conditions where an indeterminable number of compounds co-exist together and which are able to enhance or reduce the effects of individual compounds, their concentration can contribute to the overall effect of environmentally available contaminants.

5.3 *Case Study: Acute and Sublethal Toxicity of Sediment Sorbed DF and IB in the Midge* Chironomus riparius

In order to perform environmentally relevant risk assessment, the final fate of contaminants has to be taken into account. Apart from being dissolved within the water column, chemical compounds may tend to be sorbed onto sediments or particulate matter affecting the organisms dwelling within. The factors governing this tendency are generally compound-specific and are defined by its molecular structure, but also by the nature of the sediment onto which the compound is sorbed. Thus, the extent of sorption of the compound is related to its physicochemical properties and other factors such as the sediment organic matter content, surface sorption to mineral constituents, ion exchange capacity, pH value, and complex formation with metal ions such as Ca, Mg, Fe or Al, and H bonding [88].

An idea about the lipophilicity is given by the octanol-water distribution coefficient K_{ow}, with very soluble compounds (hydrophiles) generally having a $\log K_{ow} < 3$ indicating they preferentially remain in water and low-soluble compounds (hydrophobes) having a $\log K_{ow} > 3$ and a tendency to associate with the sediment. The range of K_{ows} within the group of pharmaceuticals varies significantly, with $\log K_{ows}$ from negative values up to values of 6. The $\log K_{ows}$ of DF and IB are comprised between 1.90 and 2.48, respectively, indicating a relatively low tendency to potentially adsorb onto sediments. A small proportion of the environmentally available DF and IB, however, are able to be sorbed onto sediments.

Within the group of sediment-dwelling organisms, the amount of sorbed chemical affects species differently depending on their living and feeding habits. Sediment exposure can occur through the fraction reversibly bound to the sediment which is available through the interstitial water, whereas those organisms that ingest sediments have to affront exposure through two different ways and are generally more sensitive [89]. In the past years, the number of studies that have reported the negative effects of sediment sorbed pharmaceutical compounds on sediment-dwelling organisms has increased, but only a limited number of standardized tests are available. Standardized protocols for sediment toxicity covering a wide range of sublethal, environmentally relevant responses have been developed for the midge *Chironomus riparius* (OECD 218/219: Sediment-Water Chironomid Toxicity Test Using Spiked Sediment/Spiked Water; OECD 233: Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment; OECD 235: *Chironomus* sp., Acute Immobilisation Test). *Chironomus* species occur in aquatic habitats in high abundance and diversity and are easily bred in laboratory cultures, with their larval

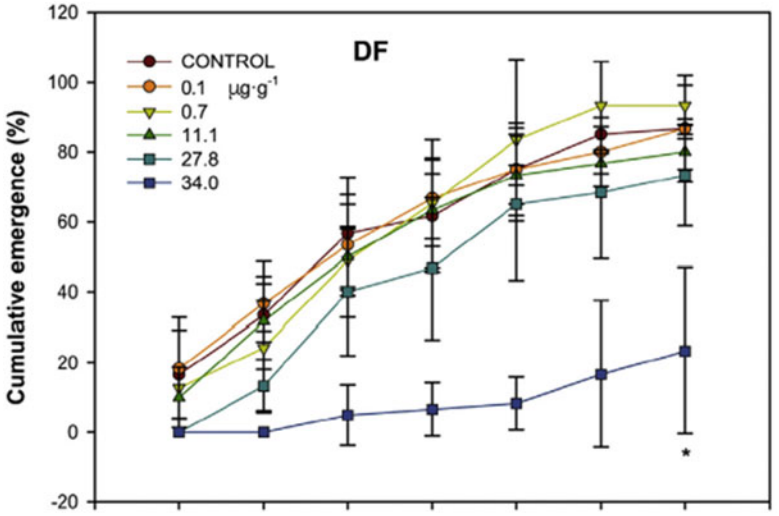

Fig. 6 Cumulative emergence curves (%) of midges exposed to increasing concentrations of sediment sorbed DF. Asterisks indicate significant differences between means compared with the control ($p < 0.05$). (Source [96], with permission)

development happening typically in the sediment. In the past years, the number of studies that have reported the negative effects of pharmaceutical compounds on benthic macroinvertebrates from contaminated sediments employing midges has increased significantly [90–95]. Among the analyzed responses in these studies, the authors observed decrease of emergence, reduced growth, increase of the biomass, and increase of the female/male ratio in spiked sediment exposure experiments at low concentrations with different pharmaceuticals. Nieto et al. [96] carried out chronic toxicity experiments (21 days) with DF spiked sediments analyzing the endpoints survival, growth (biomass) and developmental stage (day 10), emergence rate, cumulative emergence, and sex ratio (male/female) (days 15–21) in low-level dose-response exposures comprised between 0.1 and 34.04 µg g^{-1}.

At these concentrations, no significant mortality was observed nor was development having reached all organisms the fourth instar [96] at the end of the experiment. However, percentages of cumulative emergence varied between 23% and 87% in the case of DF where at 34.0 µg g^{-1}, a significant difference was found with respect to the control ($p = 0.003$) (Fig. 6). Although not significant, a continuous decreasing trend in growth of the organisms with increasing exposure concentrations was also observed, with all exposed organisms being bigger than the control organisms suggesting the stimulation of the larval growth, with the highest relative increase in growth occurring at the lowest DF exposure concentration (Fig. 7).

To calculate the RQ for DF with this data, the highest concentration where no significant difference was reported compared to the control was used as PNEC (close to 30.0 µg g^{-1}), whereas for the environmental DF sediment concentration, we have

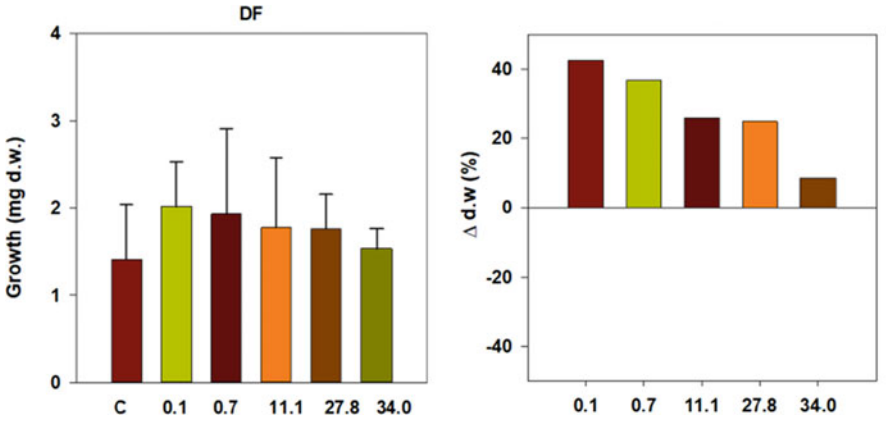

Fig. 7 Growth measured as mean dry weight (d. w. ± SD) and percentage of increase of dry weight with respect to control (Δ d. w. %) after 10 days of exposure to DF spiked sediments

employed the measured environmental concentration reported by Ebele et al. [97] where the current state of knowledge about global pharmaceutical levels has been reviewed. Using a PEC proposed by Ebele et al. [97] of 57 ng g^{-1} corresponding to worst-case scenarios, the resulting RQ for DF was 0.19, indicating medium risk.

6 New Approaches: Mechanistic Risk Assessment

The currently widely employed assessment factor approach is increasingly criticized for being not sufficiently ecologically relevant and uncertain but also little comprehensive and coherent, apart from being practically and economically impossible to test all existing and new chemicals that are on the market in different test species and developmental stages. In this context, the World Health Organization (WHO) works to establish, through the International Programme on Chemical Safety (IPCS), the scientific basis for the sound management of chemicals, proposing to integrate weight mechanistic evidence in hazard and risk assessment. In a time where regulatory agents are facing legislative imperatives which require greater efficiency in chemical assessment and management with progressively less reliance on animal testing, recent developments in risk identification are now taking advantage on evolving technologies able to provide high-throughput biological data at lower levels of organization (e.g., transcriptomics, proteomics, and metabolomics) and increasing computational capacity for data assimilation and prediction.

The adverse outcome pathway (AOP) conceptual framework [98] is a logical sequence of events or processes within biological systems which can be used to understand adverse effects and refine current risk assessment practices with the purpose to develop predictive methods for human and environmental toxicology.

The thought behind this principle is that a chemical, when comes into contact with the organism at a sufficiently high dose, triggers a molecular initiating event (MIE), i.e., receptor binding, which then develops via several key events into a pathology considered as an adverse outcome. This framework is explored on the basis of investigating the initial interaction between a chemical and a biomolecule or biosystem that can be causally linked to an outcome via a pathway. AOPs were first outlined for environmental risk assessment by Ankley in 2010 (Fig. 8) and can be defined as a sequence of events from the exposure of an individual to a chemical through to an understanding of the adverse effect at the population level. AOPs span multiple levels of biological organization but always contain an initial molecular interaction between a compound and the organism that triggers subsequent effects at higher levels of biological organization.

The predictive principle of this method is based on the fact that the chemistry of the molecule allows it to have specific MIEs. Therefore, once the chemical has reacted with the biomolecule of the exposed organism in the MIE in a compound-specific way, the development of the pathology throughout the different levels of organization is independent of the chemical, sharing different MIE common disease outcomes. A single MIE could be the cause of multiple toxicological endpoints, or a single endpoint may be the result of several MIEs. Thus ideally, knowing how the chemical interacts with the organism at the first time allows to predict the pathology that the organism is likely to develop. Because of the chemical-specific MIE(s), links between chemical structure or chemical property and MIE will undoubtedly be stronger than links to toxicological endpoints, due to a smaller "jump" between chemical exposure and MIE. With the help of structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) models, the prediction of effects of a certain chemical shall be vastly simplified, allowing the grouping of compounds on the basis of an understanding of their MIEs, predicting their expected disease outcome, and thus reducing experimental effect evaluation. Thus, by understanding the individual key events, one can better understand what the health outcome will be. The identification of the MIE of a chemical has greatly been aided by the development of sensitive, high-throughput molecular techniques such as transcriptomics, proteomics, and metabolomics in environmental toxicology which has helped to understand biological processes in exposed organisms, shedding a light on the mechanistic mode of action and adverse outcome pathways. By combining knowledge about a certain MIE of a compound and dose-response data and an understanding of adverse outcomes downstream in the AOP, quantitative predictions for new compounds could be made. Thus, mechanistic insights feed into a combination of approaches that can help to reduce reliance on animal methods [99].

From a practical point of view, AOPwiki (https://aopwiki.org/) is an AOP knowledge database that aims to serve as the central repository for all AOPs developed as part of the OECD AOP Development Effort by the extended Advisory Group on Molecular Screening and Toxicogenomics. Exploring available information for DF and IB reveals the existence of a common AOP, which is renal failure

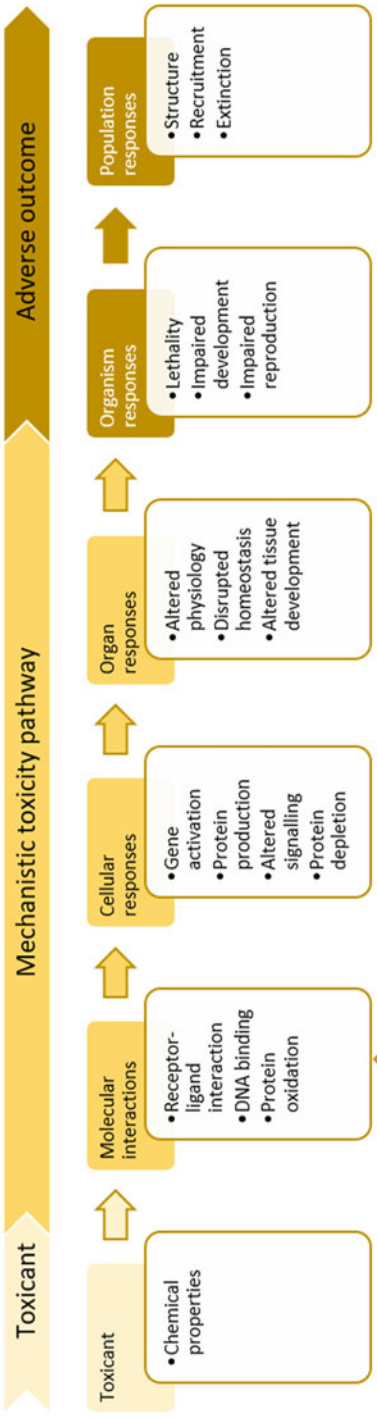

Fig. 8 Ankley's conceptual diagram of an adverse outcome pathway (AOP) including the molecular initiating event (MIE). Image adapted from Ankley et al. [98]

and mortality mediated by the inhibition of cyclooxygenase 1 (COX-1) activity. While for DF the MIE is unknown, in the case of IB, this COX-1 inhibition is mediated through the inhibition of prostaglandin-endoperoxide synthase (prostaglandin G/H synthase 1) activity, the inhibition of the organic anion transporter 1 (OAT1) resulting in decreased signaling mediated by the solute carrier family 22 member 6, and the inhibition of the IKK complex, an enzyme complex involved in propagating the cellular response to inflammation through the caspase-8 pathway, leading finally to liver injury. However, no information is provided on the concentrations activating these events in representative organisms or human, making its use for practical risk assessment not useful so far.

7 Conclusions and Final Remarks

Both pharmaceutical compounds IB and DF show a wide occurrence and range of concentrations in aquatic ecosystems from different geographical areas. Their fate and behavior and distribution are related to chemical structure. These compounds can interact with wild species and provoke unwanted effects. To perform a preliminary environmental risk assessment, PEC or MEC data are needed jointly with PNEC. Currently, the available information is biased with a bigger database for environmental concentrations and acute toxicity data species for temperate than tropical regions. However, the chronic toxicity data can reflect in a better and realistic way the negative effects of these pharmaceuticals, including mechanistic information. Adverse outcome pathways are a conceptual framework that links the processes occurring between the first contact of the organism with the stressor at molecular level and the establishment of some sort of pathology that may cause death or reduce its fitness in comparison with other unexposed individuals. In this context, to use mechanistic data within the AOP framework to support effective risk assessment, there is the essential need to translate this mechanistic information into endpoints representing ecological risk, such as survival, reproduction, etc. It is also essential to relate this translation with concentration values, at which the organism is not able to overcome the challenge by its own defense mechanisms. Therefore, although promising, the effective use of the AOP framework still requires traditional dose-response testing at which molecular and apical endpoints are explored simultaneously. In real ecosystems, the pharmaceuticals can interact with other emergent or legacy pollutants or be affected by other nonchemical stressors as temperature, salinity, etc., many of them with unknown effects. In fact, to predict the environmental risk for different scenarios, we need to improve the knowledge of response mechanisms and toxicity in multi-stressed systems. In summary, although gaps of the information are pointed out, the risk levels associated with the occurrence of these compounds in aquatic ecosystems will range between no risk or high risk, depending on concentrations and environmental conditions.

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Toxicity Assessment of Acetylsalicylic Acid to a Freshwater Fish *Cyprinus carpio*: Haematological, Biochemical, Enzymological and Antioxidant Responses

Subramaniam Siddeswaran, Sathisaran Umamaheswari, and Mathan Ramesh

Contents

Abstract Pharmaceutical pollution is a global threat to the biosphere causing significant environmental health concern. A wide range of pharmaceuticals (antibiotics, nonsteroidal anti-inflammatory drugs, beta-blockers, etc.) are widely used in

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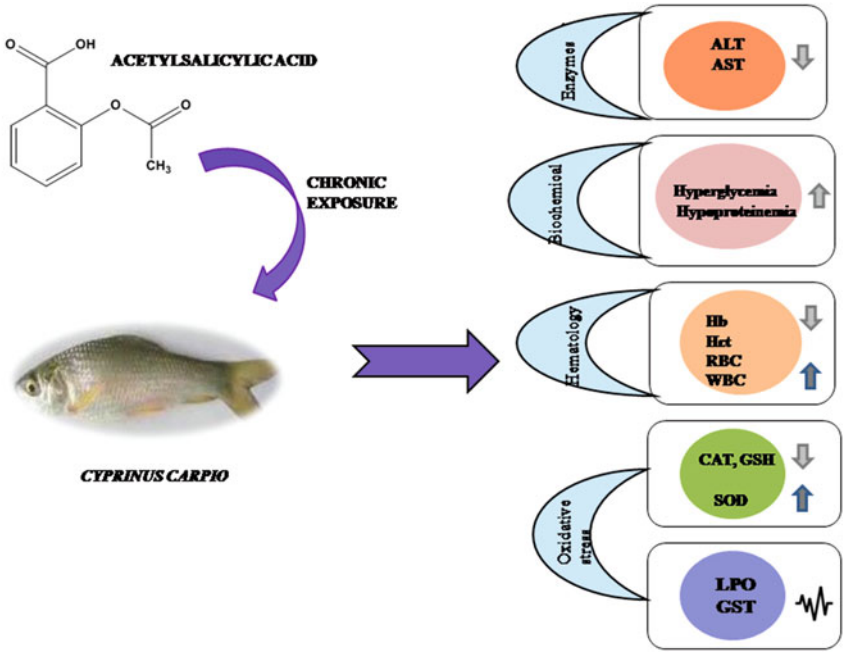
human and veterinary medicine, agriculture and aquaculture purposes to protect the life against various diseases and to improve human health. The extensive use of these compounds may enter the environment through discharge of domestic waste waters, excretion via water and sewage treatment systems which may affect the aquatic organisms. Aspirin (acetylsalicylic acid, ASA) is one of the most commonly used nonsteroidal anti-inflammatory drug (NSAIDs) worldwide and has been detected in aquatic bodies. Therefore, it is important to gain knowledge about the toxicity of acetylsalicylic acid in aquatic organisms. Here we have administered 100 and 200 mg L^{-1} of acetylsalicylic acid, to a freshwater fish *Cyprinus carpio* fingerlings, and have studied its effects on haematological, enzymological biochemical and antioxidant parameters. When compared to control, acetylsalicylic acid-treated fish showed a significant ($P < 0.05$) decline in haemoglobin (Hb), haematocrit (Hct) and red blood cell (RBC) levels throughout the study period (12 days). On the other hand, a significant ($P < 0.05$) increase was observed in white blood cell counts (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values. Acetylsalicylic acid induced a hyperglycaemic condition compared to control, whereas the level of proteins was declined. A significant decrease in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity was noted in acetylsalicylic acid-treated groups (except 21st day in ALT activity and 21st day in AST activity). Significant alterations in various antioxidant parameters such as superoxide dismutase (SOD), lipid peroxidase (LPO), catalase (CAT) glutathione (GSH) and glutathione S-transferase (GST) were observed in ASA-treated groups compared to the control group. From the results, it is noteworthy that the drug ASA even at considerable environmental concentrations causes negative impacts on the health of aquatic organisms. The alterations of these parameters can be effectively used to monitor the impact of pharmaceutical drugs in the aquatic environment.

Graphical Abstract



Keywords Acetylsalicylic acid, Biomarkers, *Cyprinus carpio*, NSAIDs

1 Introduction

Over the past 30 years, environmental pollution has been increasing at an alarming rate. Industrialization, modern agricultural practices and infinite use of pharmaceuticals in medicine release xenobiotics into the environment [1]. Pharmaceuticals are bioactive chemical compounds used in diagnosis, treatment or alleviation of disease, improving health status as well as to revamp normal physiology in organisms [2]. Besides human medication, veterinary sectors (livestock, poultry and aquaculture) rely on the application of pharmaceutical products such as antibiotics and hormones, to promote growth, biological functions and health conditions [3]. Ramesh et al. [4] have reported that about 200 million kg of antibiotics used all over the world flow into water bodies (rivers, lakes and sea) annually causing water pollution.

The indiscriminate use of pharmaceuticals in human and veterinary medicine has caused contamination of aquatic ecosystem [5], perhaps affecting the health of aquatic organisms. Fish and marine mammals which occupy the upper trophic

level of the aquatic food chain are prone to higher levels of toxicity present in the marine or freshwaters [6]. The existence of pharmaceuticals along with their metabolites in water bodies has been reported in many countries [7–9]. Several of these compounds act as endocrine disrupting chemicals interfering with the normal hormonal balance system [10] by mimicking a natural hormone or blocking the binding of endogenous hormone to certain receptors [11] and also induce bone marrow, reproductive and nervous system disorder in living organisms [12, 13].

The major group of pharmaceuticals includes nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, analgesics, lipid regulators, beta-blockers, steroids and related hormones [14, 15]. NSAIDs act by inhibiting cyclooxygenase (COX) enzyme which is responsible for the biosynthesis of various active lipid compounds called prostaglandins [16]. Among the various classes of NSAIDs reported, aspirin, ibuprofen, naproxen and diclofenac are widely prescribed. NSAIDs are weak organic acids having a high affinity for lipids and plasma proteins with various therapeutic potentials.

Aspirin (acetylsalicylic acid, ASA) is one of the most commonly used NSAIDs worldwide [2, 17]. It is medicated to treat minor pains, cardiovascular thrombosis [18], arthritis and related musculoskeletal disorders [19]. ASA with the IUPAC name 2-acetoxy benzoic acid is the NSAID with salicylate chemical group. ASA is widely used as an analgesic and antipyretic agent as it causes cyclooxygenase (COX) inhibition on administration [20]. Kerola et al. [21] reported that ASA is COX-1 specific; COX-1 enzymes are found on the surfaces of platelets and gastric mucosal cells. The acetyl and the salicylate portions of the ASA molecule produce analgesic, antipyretic and anti-inflammatory effects when consumed [22]. The activity of cyclooxygenase (COX-1) is hindered by ASA molecule to decrease the synthesis of precursors of prostaglandins and thromboxanes from arachidonic acid. Therefore, this helps to regulate the production and release of prostaglandins preventing the symptoms of inflammatory responses such as swelling, increased blood vessel dilation, immune response and blood coagulation [23].

The body does not detain the entire dose of drug consumed, as a major portion are transformed to one or more drug metabolites and are defecated as metabolite conjugates or parent compounds through urine and faeces [24, 25]. After consumption, acetylsalicylic acid is rapidly hydrolysed into salicyluric acid and glucuronic acid in the liver and eliminated via urine [26, 27]. The plasma half-life of ASA is dose-dependent and lengthens as the dose increases [28]. Despite its biodegradable potential, it is found in river waters [29, 30]. Schulman et al. [31] reported the presence of ASA in sewage effluents and surface water at maximum loading levels of 1.5 and $\geq$ 3.1 g L^{-1}, respectively. The concentrations of ASA reported by Philip et al. [32] in surface waters of South Indian zone is about 660 ng L^{-1}, whereas samples of pharmaceutical effluents had about 2,270 mg L^{-1} of salicylic acid.

ASA exert negative effects on the aquatic ecosystem. Fishes on exposure to salicylates showed hormonal aberrations and delayed response to an acute stressor [33, 34]. ASA is teratogenic in rats [35] and also caused maternal toxicity in rabbits [36]. Exposure of tilapia to ASA induced altered plasma thyroid hormone levels and cortisol levels [34]. Similarly, the freshwater fishes *Cyprinus carpio* and *Danio*

rerio, exposed to salicylic acid, showed an induction in oxidative stress indices [37, 38]. Bioassays performed with acetylsalicylic acid in *Daphnia magna* showed immobilization [39], whereas Cleuvers [40] documented growth inhibition in green alga *D. subspicatus.*

Fishes are potent bioindicators as they occupy different trophic levels in the food chain [41], and the response of fish to pollutants is monitored using several biomarkers [42]. Chronic toxicity analysis using a fish model facilitates better understanding of the impact of any compound on health status of aquatic organisms [43]. Responses of fish biomarkers such as haematology [44], biochemical profile [4], antioxidant status [45], behavioural perturbations [46], neuronal responses [47], metabolomics [48], transcriptomics [49] and toxicokinetics [50] serve as reliable markers in toxicity researches. Marques et al. [51] have reported that chronic effects are much more toxic than acute effects.

Indian drug industry holds third place in terms of volume and 14th in terms of value in and around the globe [52]. In India, analgesics and anti-inflammatory drugs are the two largest groups of over-the-counter drugs in urban areas. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most extensively used class of pharmaceutical agents worldwide [43]). Shanmugam et al. [53] reported the presence of nonsteroidal anti-inflammatory drugs in Indian River waters. The present study focuses on the chronic (100 and 200 mg L^{-1}) effects of acetylsalicylic acid (ASA) in a common carp *Cyprinus carpio* for the period of 21 days by using haematological, enzymological biochemical and antioxidant parameters in gill and liver.

2 Materials and Methods

2.1 Experimental Animal

The handling and testing of individuals were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Organization of Economic Co-operation and Development (OECD). Specimens of *Cyprinus carpio* with an average weight of 6.0 ± 0.5 g and length of 7.5 ± 0.5 cm were procured from Aliyar Fish Farm, Aliyar, and Tamil Nadu in India. Fishes were taken to the laboratory and stocked in a 6′ × 4′ × 3′ size cement tank [42]. Only dechlorinated tap water was used throughout the study period. The physicochemical parameters of water 25.0 ± 0.5°C, pH 7.2, hardness 17.8 mg L^{-1} (as $CaCO_3$), alkalinity 18.5 ± 7.0 mg L^{-1} (as $CaCO_3$) and dissolved oxygen concentration 6.2 ± 0.02 mg L^{-1} were monitored and maintained throughout the study period. The stocked fishes were acclimatized to laboratory conditions for a period of 20 days. During acclimatization, period fish were with fed rice bran, corn flour, and wheat flour and groundnut oil cake once in the day. The water in the aquarium was renewed daily and aerated mechanically. Feeding was ceased 24 h before the commencement of the experiment.

2.2 Test Compounds

The drug acetylsalicylic acid of 99.0% purity (CAS Number: 50-78-2) was purchased from Sigma-Aldrich. AST and ALT kits were purchased from Coral Clinical Systems, India. All the other chemicals and reagents (of analytical grade) used in the present investigation were purchased from HiMedia Laboratories Pvt. Ltd., India. 1 g of acetylsalicylic acid (ASA) was dissolved in 0.9 mL of dimethyl sulfoxide (DMSO) was used to prepare stock solution (1,000 mg L^{-1}) of ASA. From this stock solution, appropriate quantity was taken and dissolved in experimental tanks to get the desired concentration of toxicity solution.

2.3 Chronic Toxicity Assay

Two different concentrations were selected for testing the chronic toxicity of acetylsalicylic acid such as 100 and 200 mg L^{-1}, and they were grouped as Treatment I and Treatment II, respectively. Three glass aquaria of 100 L capacity were taken and filled with 80 L of water in which one is maintained as control and the other two as Treatment I and Treatment II. The glass tanks were aerated. From the stocking tank, 40 healthy fish fingerlings were randomly selected and introduced into each tank. The toxicant was renewed and the water in the tanks was changed daily, in order to prevent accumulation of faecal matter.

2.4 Blood and Organs Sampling

Fish from control and ASA-treated groups were sacrificed without anaesthetizing, and blood was collected in a heparinized syringe by puncturing dorsal aorta. The collected blood was maintained in sterilized plastic vials. A freshly pooled whole blood sample was used for haematological analysis. The remainder of the blood were centrifuged at 10,000 rpm for 20 min to separate the plasma and transferred to clean vials for biochemical (plasma glucose and protein) and enzymological (AST and ALT) analysis. Subsequently gill and liver tissues were excised out for the study of antioxidant parameters (SOD, LPO, CAT, GST and GSH). The collected tissue samples (liver and gill) were washed with 0.9% NaCl solution dried with filter paper and maintained at $-80^{\circ}C$.

2.5 Blood Chemistry Analysis

RBC and WBC count were performed following the methods of Rusia and Sood [54]. Hb count was estimated by the cyanmethaemoglobin method, and Ht was determined by the micro-Ht method of Nelson and Morris [55] using the diagnostic reagent kit (Monozyme India, Ltd., India) at 540 nm using UV spectrophotometer. Erythrocyte indices of fish, viz. MCV, MCH and MCHC, were calculated using standard formulas:

$$\text{MCV (cubic micra)} = \left[\text{Hct (\%)}/\text{RBC}\left(\text{millions} \times \text{Cu} \times 10^6\right)\right] \times 100$$

$$\text{MCH (picograms)} = \left[\text{Hb (g/dL)}/\text{RBC}\left(\text{millions} \times \text{Cu} \times 10^6\right)\right] \times 100$$

$$\text{MCHC (g/dL)} = [\text{Hb (g/dL)}/\text{Hct (\%)}] \times 100$$

2.6 Biochemical Analysis

2.6.1 Estimation of Plasma Glucose and Protein

Plasma protein was estimated following the method of Lowry et al. [56] using bovine serum albumin as standard. Briefly, 0.10 mL of plasma sample from control and ASA-treated groups were added to the reaction mixture (0.90 mL of distilled water and 5 mL of copper tartrate solution (5% copper sulphate, 10% sodium potassium tartrate, 10% sodium sulphate in 0.5 M sodium hydroxide solution)) and kept at room temperature for 30 min. Subsequently, Folin-Ciocalteu phenol reagent diluted in 0.1 N sodium hydroxide was added and incubated at room temperature for 10 min, and the absorbance was read at 720 nm by using UV spectrophotometer. For the preparation of 'Standard' (S) 1.0 mg of bovine serum albumin was added to 10.0 ml of 1N NaOH and made up to 100.0 ml in a solution standard flask. From this, 1.0 ml of solution was taken in 'Standard' tube and mixed with 0.5 ml of Solution- C, kept for 10 min, and then 0.5 ml of Folin phenol reagent was added. The optical density of the 'Standard' (S) was read as mentioned above.

Plasma glucose estimation was performed following the method of Cooper and Mc Daniel [57]. In brief, after exposure, 0.1 mL of plasma samples from each treatment were taken with 5 mL of O-toluidine reagent, and the aliquots were incubated in boiling water bath for 10 min. After incubation, the aliquots were cooled under running tap water. The absorbance was read against blank at 630 nm using UV spectrophotometer.

2.6.2 Estimation of Enzymological Parameters

The enzyme activities of AST and, ALT, were determined by Diagnostic Reagent Kits (Coral Clinical Systems, A Division of Tulip Diagnostics (P) Ltd., India) following the manufacturers' instructions.

2.7 Oxidative Stress Parameters Analysis

2.7.1 Superoxide Dismutase (SOD)

Superoxide dismutase activity was measured by Marklund and Marklund [58]. After the homogenization of tissues in 100 mM Tris-HCl buffer (pH 7.4), the contents were centrifuged at 12,000 rpm for 15 min at 4°C. The obtained supernatant of 50 mL was added to the reaction solution (50 mM Tris-HCl buffer, pH 8.4 with 1 mM EDTA and 2.64 mM pyrogallol), and the absorbance was read at 420 nm in UV spectrophotometer.

2.7.2 Lipid Peroxidation (LPO)

LPO was measured following thiobarbituric acid reactive substances (TBARS) assay [59]. Pooled tissue samples were homogenized in 100 mL ice-cold potassium phosphate buffer (pH 7.4), and 100 mL of 5% trichloroacetic acid (TCA) was added. Shortly the contents were kept undisturbed on ice for 10 min, and 100 mL of 0.67% thiobarbituric acid was added. After centrifugation (2,200 g, 10 min at 4°C), 250 mL of supernatant was incubated in boiling water bath for 10 min preceded by cooling, and the absorbance was determined at 535 nm in UV spectrophotometer.

2.7.3 Catalase (CAT)

The catalase activity was estimated by adapting the method of Sinha [60]. Briefly, the sampled tissues were homogenized manually in Tris-HCl buffer (100 mM, pH 7.4) and cold centrifuged at 12,000 rpm for 15 min. After centrifugation, 100 mL of supernatant with 3 mL of reaction mixture (containing 5% potassium dichromate and acetic acid (1,3) and phosphate buffer (10 mM, pH 7.0)) was incubated in water bath for 20 min, and the absorbance was read at 570 nm in UV spectrophotometer.

2.7.4 Glutathione S-Transferase (GST)

GST activity was measured by the method of Habig and Jakoby [61]. From each treatment, the sampled tissues were homogenized in potassium phosphate buffer (0.1 M, pH 6.5). Subsequently the homogenate is centrifuged at 9,000 g for 30 min at 4°C, and 50 mL of the obtained supernatant is added to 100 mL of the reaction solution (10 mM GSH and 60 mM 1-chloro2, 4-dinitrobenzene). The absorbance of the samples was read at 340 nm in UV spectrophotometer for 5 min.

2.7.5 Reduced Glutathione (GSH)

Reduced glutathione levels were determined by following the method of Ganie et al. [62]. In brief, the tissues were homogenized in 100 mL of ice-cold potassium phosphate buffer (0.1 M, pH 6.5) followed by adding 100 mL of 25% TCA to precipitate the homogenate. Then the precipitate was centrifuged at 3,000 g for 10 min, 4 °C, and 150 mL of supernatant were transferred to aliquots containing reaction mixture (60 mM DTNB and 450 mL of 50 mM potassium phosphate buffer (pH 7.4)). The absorbance of the samples was read at 412 nm in UV spectrophotometer.

2.8 Statistical Analysis

A statistical analysis was performed using SPSS software – Ver.16 statistical package. The results of ASA -treated groups were compared against the control followed by one-way ANOVA and the Duncan's multiple range test at $p < 0.05$ level.

3 Results

3.1 Haematology

The changes in the Hb level of fish *C. carpio* exposed to chronic concentrations (100 mg L^{-1}- Treatment I and 200 mg L^{-1}- Treatment II) of acetylsalicylic acid (ASA) for a period of 21 days were illustrated in Fig. 1. During the above exposure period, Hb level was decreased in both the treatments throughout the study period. A similar trend was also noted for Ht and RBC count in ASA-treated group when compared with their respective control groups ($P < 0.05$). A maximum percent decrease of Hb level was recorded at the end of 14th day in Treatment II (Fig. 2). Similarly a maximum percent decrease of HCT level was noted on 14th day in Treatment II. There was a significant ($P < 0.05$) decrease in the RBC count at the end of 7th, 14th and 21st days of exposure in ASA-treated groups when compared to control groups (Fig. 3). In both the treatments, WBC level of *C. carpio* was elevated when compared to the control group (Fig. 4). Compared with the control groups, ASA-treated *Cyprinus carpio* had significantly higher MCV (Fig. 5), MCH (Fig. 6) and MCHC values (Fig. 7) throughout the study period.

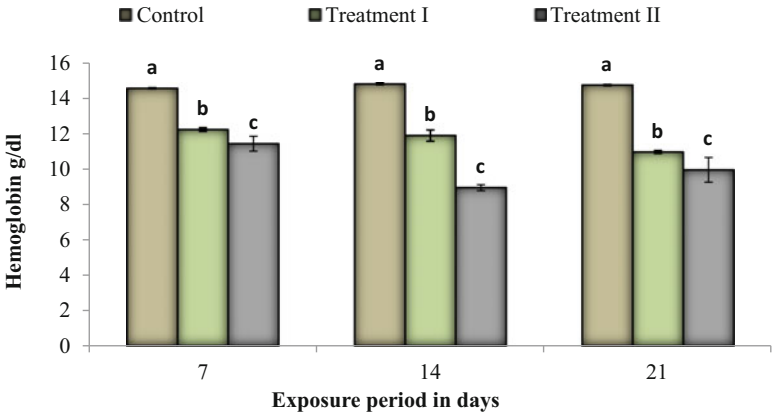


Fig. 1 Hb content of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

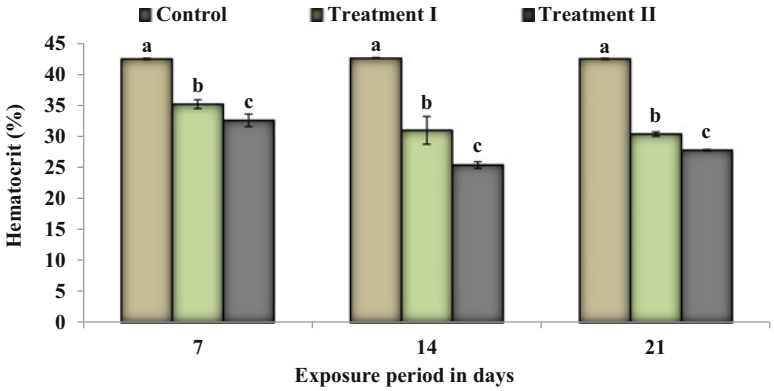


Fig. 2 Hct content of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

3.2 Glucose and Protein Content

Fish exposed to ASA showed a significant increase ($p < 0.05$) in plasma glucose level throughout the study period when compared with the control groups (Fig. 8). However, plasma protein level was found to be significantly lower in ASA-treated fish throughout the study period when compared with the control groups (Fig. 9).

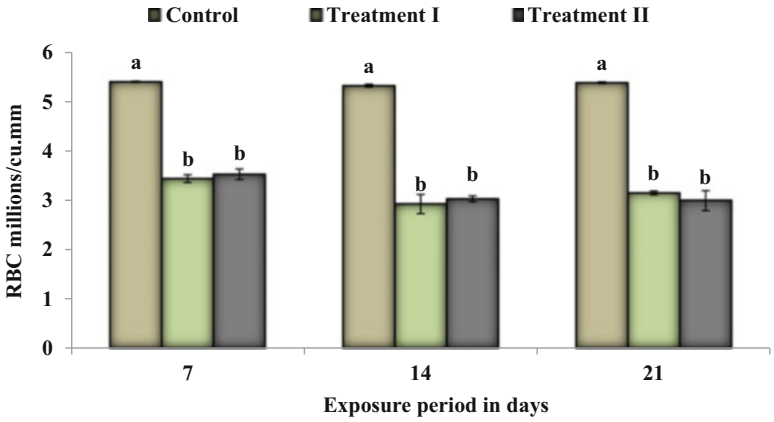


Fig. 3 RBC count of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

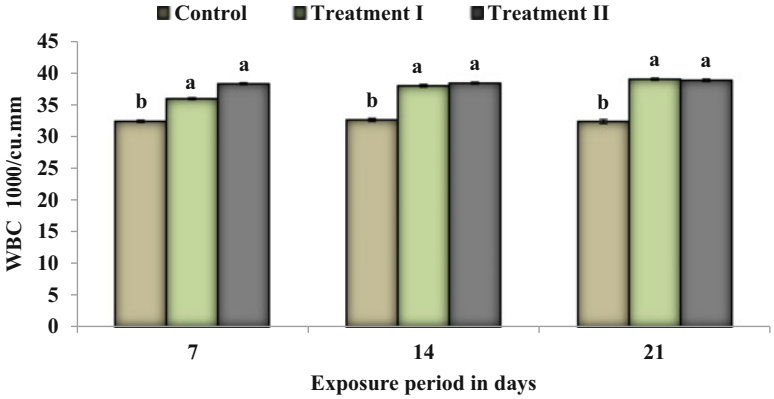


Fig. 4 WBC count of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

3.3 Enzymological Parameters

The changes in the transaminase activities of fish *C. carpio* exposed to chronic concentrations (100 mg L^{-1}- Treatment I and 200 mg L^{-1}- Treatment II) of acetylsalicylic acid (ASA) for a period of 21 days were illustrated in Figs. 10 and 11. AST activity reflected a significant decrease in ASA-treated fish comparable to those obtained in unexposed fish 9 except 21st day in Treatment I. ALT activity was

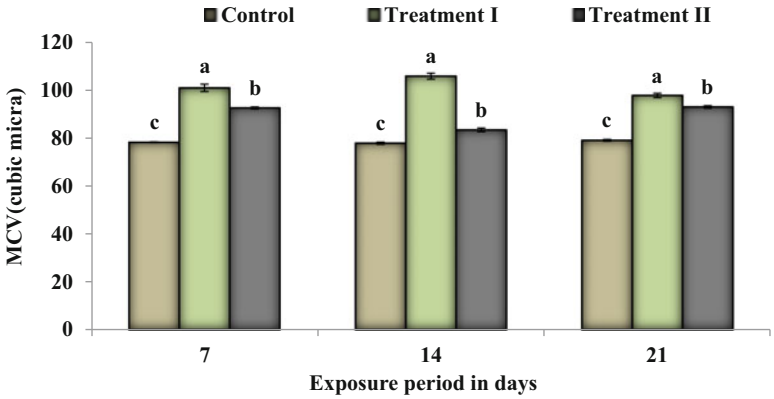


Fig. 5 MCV value of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

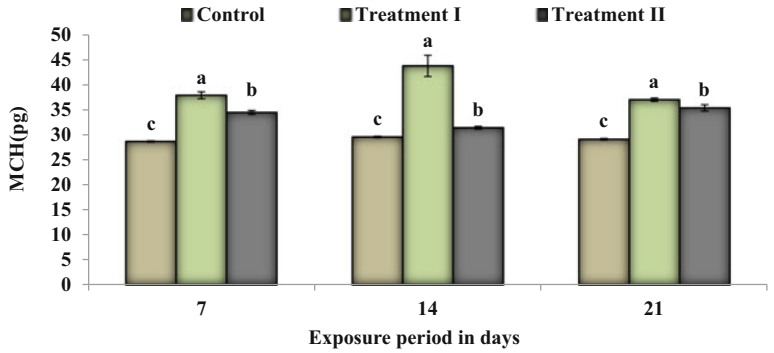


Fig. 6 MCH value of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

also found to be lower in ASA-treated fish throughout the study period when compared with the control groups ($p < 0.05$) except in 21st day of Treatment II.

3.4 Antioxidants

Variations in the antioxidant responses such as SOD, LPO, CAT, GST and GSH levels were presented in Table 1. The SOD activity in gill and liver of ASA-treated group was significantly ($p > 0.05$) increased over the control groups in both the treatments. The LPO activity was found to be elevated in gill at the end of the 14th day, and then it suddenly decreased in the 21st day of Treatment I, whereas it

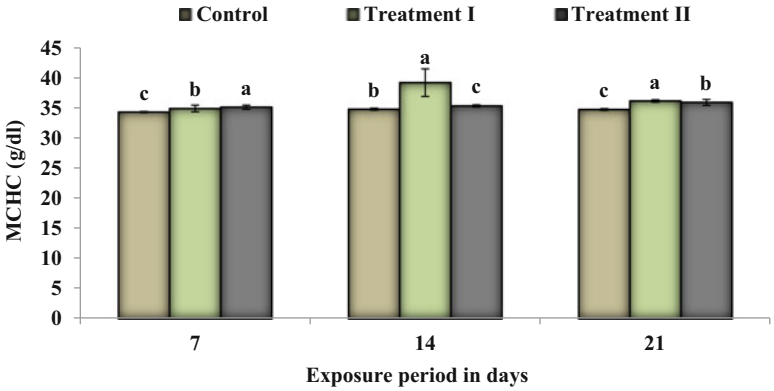


Fig. 7 MCHC value of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

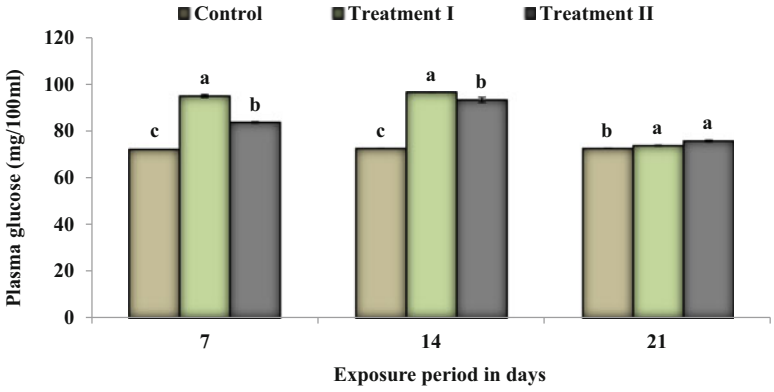


Fig. 8 Plasma glucose level of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

exhibited a biphasic response in Treatments I and II of the liver. Compared with the control group, the chronic exposure to ASA resulted in significantly lower ($p < 0.05$) CAT activity in gill and liver of *C. carpio*. GST level in gill was significantly ($p > 0.05$) decreased at the end of 21st day exposure in Treatment I and II. However, the gill tissue of fish exposed to Treatment I showed an elevated GST level at the end of the 7th and 14th day. The level of GST in the liver of ASA-treated groups was significantly decreased ($p < 0.05$) compared to the control group except at the end of 7th day in the gill of exposed to Treatment I. The GSH activity in gill and liver of ASA-treated groups was significantly decreased ($p < 0.05$) compared to the control group except at the end of 14th day in Treatment I.

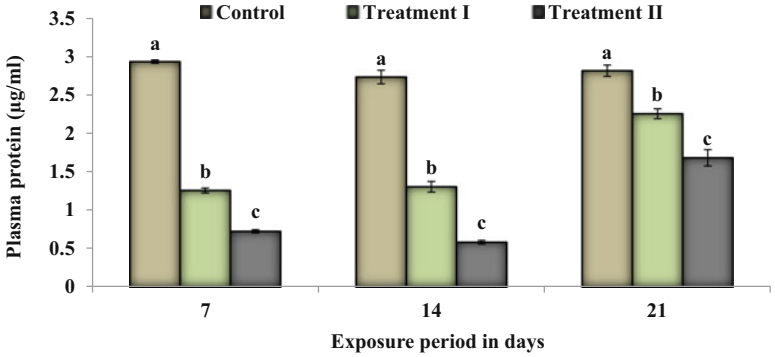


Fig. 9 Plasma protein content of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

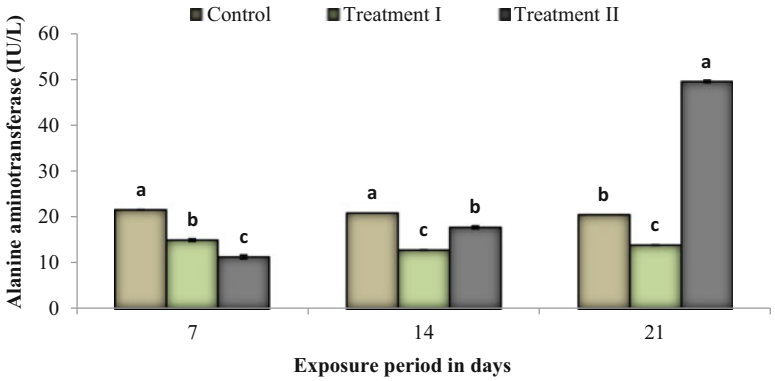


Fig. 10 Plasma ALT activity of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

4 Discussion

Increased production and elevated use of pharmaceuticals in human and veterinary medications lead to the discharge of more pharmaceutical compounds into the environment [63]. Despite their presence at very low concentrations, pharmaceuticals are nevertheless preferred for their potency to hinder specific biologic pathways at low levels [64]. Nonsteroidal anti-inflammatory drugs (NSAIDs) and antibiotics are widely prescribed, and their utilization in developed countries is higher than hundred tons per year [65].

Acetylsalicylic acid (ASA) is a nonsteroidal anti-inflammatory drug with analgesic, anti-pyretic and anti-thrombotic properties [20]. Acetylsalicylic acid constitutes about 81% of STPs effluents, whereas salicylic acid constitutes about 99%

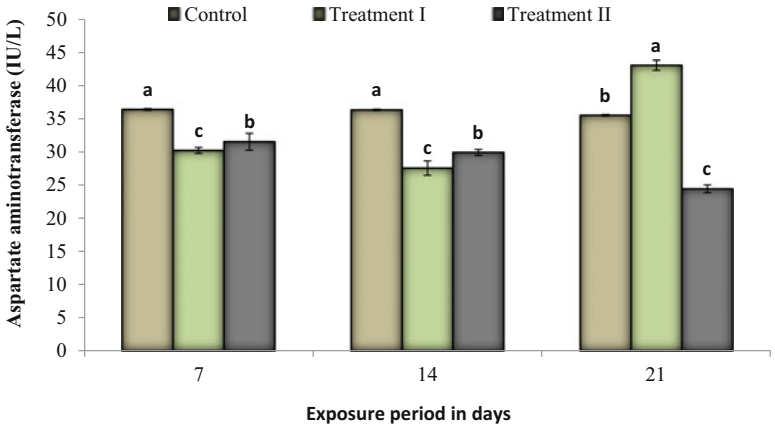


Fig. 11 Plasma AST activity of control and ASA (Treatments I (100 mg L^{-1}) and II (200 mg L^{-1}))-exposed fish *C. carpio* for a period of 21 days. Means in the bars followed by common letters for the experiment are not significantly different ($P < 0.05$) according to DMRT

[66]. Traces of acetylsalicylic acid have been reported in samples of municipal waste waters at levels ranges from13 μg L^{-1} [67] to 59.6 μg L^{-1} with median levels of 3.6 μg L^{-1} [68]. The adverse reactions of ASA include ulceration, hematemesis, melena [69] and development of ASA resistance in organisms [70]. In addition to the above data, the impact of ASA on fish stress performance was also reported by Van Anholt et al. [34] in Mozambique tilapia, Gravel and Vijayan [71] in rainbow trout and Praskova et al. [72] in zebrafish.

In the current study, the chronic effects of the nonsteroidal anti-inflammatory drug acetylsalicylic acid to the fish *C. carpio* were evaluated under laboratory conditions. During the investigation period (21 days), haematological, biochemical, enzymological and oxidative stress parameters of fish were altered upon exposure to ASA. The two concentrations (100 mg L^{-1} and 200 mg L^{-1}) of acetylsalicylic acid (ASA) are taken as the Treatment I and II based on the tested concentrations of ASA in *Danio rerio* reported by Praskova et al. [72].

A study of blood biochemistry plays a crucial role in monitoring fish health status, pollution load, stress and disease [73]. In clinical diagnosis laboratories, haematological variables such as Hb, Hct, RBC and WBC counts are extensively used in the prediction of health status [74]. The exposure of *C. carpio* to ASA caused a significant decline in RBC, haemoglobin and haematocrit values. This might have resulted from the inhibition of erythropoiesis process by the drug ASA leading to the anaemic condition of the fish. Similarly, Hemalatha et al. [75] reported that significantly lower values of RBC count and the Hb and Ht levels in *Labeo rohita* exposed to an antimicrobial agent, triclosan. Elevated WBC count indicates the production and circulation of antibodies in the bloodstream which might be due to the stress induced by the drug to the fish [76, 77].

Inflated MCV, MCH and MCHC levels observed in *Cyprinus carpio* exposed to ASA might have resulted from the macrocytic anaemia [78, 79]. Increased red cell

Table 1 Changes in the antioxidant enzymes in a freshwater fish *C. carpio* treated (Treatment-I: 100 mg L^{-1}; Treatment-II: 200 mg L^{-1}) with acetylsalicylic acid

Parameter	Tissue	Exposure period in days	Control	Treatment I	Treatment II
SOD (units/µg protein)	Gill	7	01.2 ± 0.60^{b}	01.59 ± 0.32^{b} (+32.50)	03.07 ± 1.74^{a} (+155.83)
		14	01.24 ± 0.65^{c}	02.23 ± 0.37^{b} (+79.83)	03.52 ± 0.14^{a} (+183.87)
		21	01.29 ± 0.75^{c}	02.06 ± 1.84^{b} (+59.68)	04.48 ± 0.44^{a}(+247.28)
	Liver	7	0.46 ± 0.09^{b}	0.91 ± 0.28^{b}(+97.82)	02.41 ± 3.60^{a} (+423.91)
		14	0.47 ± 0.09^{c}	01.57 ± 0.50^{a} (+234.04)	01.28 ± 0.04^{b} (+172.34)
		21	0.48 ± 0.10^{c}	01.99 ± 0.84^{b} (+314.58)	01.27 ± 0.68^{a} (+164.58)
LPO (mole of MDA/g protein)	Gill	7	05.43 ± 0.15^{c}	07.56 ± 0.07^{b} (+39.22)	14.74 ± 4.14^{a} (+171.45)
		14	05.61 ± 0.15^{c}	08.01 ± 0.20^{b} (+42.78)	09.74 ± 0.92^{a} (+73.61)
		21	05.26 ± 0.15^{a}	02.16 ± 0.20^{c} (−58.93)	06.96 ± 0.27^{a} (+32.31)
	Liver	7	04.52 ± 0.10^{b}	04.54 ± 0.14^{b} (+0.44)	05.72 ± 1.15^{a} (+26.54)
		14	04.44 ± 0.10^{a}	05.49 ± 1.01^{c} (+23.64)	04.04 ± 1.21^{b} (−9.00)
		21	04.54 ± 0.10^{b}	03.49 ± 0.42^{c} (−23.12)	06.09 ± 0.21^{a} (+34.14)
CAT (µmol of H_2O_2 consumed/min/mg protein)	Gill	7	14.34 ± 0.10^{a}	09.61 ± 0.12^{b} (−32.98)	05.44 ± 0.11^{c} (−62.36)
		14	14.19 ± 0.09^{a}	08.06 ± 0.21^{b} (−43.19)	04.02 ± 0.15^{c} (−71.67)
		21	14.45 ± 0.10^{a}	03.08 ± 0.13^{b} (−78.68)	01.23 ± 0.07^{c} (−91.48)
	Liver	7	20.38 ± 3.31^{a}	17.26 ± 0.47^{b} (−15.30)	04.83 ± 1.03^{c} (−76.30)
		14	20.26 ± 1.48^{a}	16.04 ± 1.67^{b} (−20.82)	03.35 ± 0.21^{c} (−83.46)
		21	20.48 ± 1.48^{a}	16.17 ± 2.53^{b} (−21.04)	02.43 ± 0.05^{c} (−88.13)
GST (µmoles of CDNB-GSG/min/mg protein)	Gill	7	08.12 ± 0.05^{b}	09.41 ± 0.04^{a} (+15.88)	08.47 ± 0.39^{b} (+4.31)
		14	08.09 ± 0.05^{b}	09.02 ± 0.19^{a} (+11.49)	07.60 ± 0.81^{c} (−6.05)
		21	08.12 ± 0.05^{a}	07.35 ± 0.89^{b} (−9.48)	05.80 ± 1.01^{c} (−28.57)
	Liver	7	09.54 ± 0.06^{a}	09.61 ± 0.11^{a} (+0.73)	08.04 ± 0.06^{b} (−15.72)
		14	09.51 ± 0.05^{a}	04.65 ± 0.10^{c} (−51.10)	07.89 ± 0.46^{b} (−17.03)
		21	09.59 ± 0.06^{a}	02.56 ± 0.03^{b} (−73.30)	01.64 ± 0.03^{c} (−83.33)

GSH (nmole GSH/mg protein)	Gill	7	02.34 ± 0.06^{a}	02.35 ± 0.10^{a} (+0.42)	01.05 ± 0.06^{b} (−55.12)
		14	02.25 ± 0.06^{a}	02.50 ± 0.11^{a} (+11.11)	01.50 ± 0.07^{b} (−33.33)
		21	02.21 ± 0.06^{a}	01.17 ± 0.01^{b} (−47.05)	0.94 ± 0.03^{b} (−57.46)
	Liver	7	03.62 ± 0.19^{a}	02.29 ± 0.05 $(-36.74)^{b}$	02.29 ± 0.11 $(-36.74)^{b}$
		14	03.45 ± 0.18^{a}	02.17 ± 0.04 $(-37.10)^{b}$	01.43 ± 0.11 $(-58.55)^{c}$
		21	03.72 ± 0.19^{a}	01.80 ± 0.11 $(-51.61)^{b}$	01.62 ± 0.08 $(-56.45)^{b}$

Values are expressed as the mean ± S.E five individual observations. Values in the parentheses represent % changes over control. Means within a row bearing same letters are not significantly different ($p < 0.05$) according to DMRT Different letters indicates significant difference at P. < 0.05.

destruction causes macrocytic anaemia with low haemoglobin content causing dysfunction of organs [80]. Similar results were recorded in *Oreochromis niloticus* exposed to drug sulfamethazine [74] and in *Pangasianodon hypophthalmus* exposed to triclosan [81].

Alterations of plasma carbohydrates levels act as a non-specific hallmark of disturbed physiology as they are considered as the foremost organic nutrients to be degraded in response to in fishes during stress conditions [82]. A rise in the plasma glucose concentration indicates high utilization of glucose to cope the metabolic stress caused by the drugs [83]. Furthermore, the increase in any of the stress hormones catecholamines or corticosteroids may also lead to elevated plasma glucose levels in fishes [84]. Similar observations were reported by Renuka et al. [43] in N-acetyl-p-aminophenol-treated rohu fingerlings, Ambili et al. [85] in oxytetracycline-treated rohu fingerlings and Umamaheswari et al. [86] in amoxicillin-treated rohu fish fingerlings.

Protein serves as the primary energy source to meet energy demand at some point in increased physiological and metabolic activities of fish under stress conditions [87]. The observed decline in plasma protein level in ASA-exposed fishes might be resulted from the free amino acid production and its utilization in TCA cycle for energy production. ASA molecules are weak organic acids having the high affinity for lipids and plasma proteins, and therefore they bind to the circulating free protein molecules causing hypoproteinaemic condition in ASA-treated fishes. Hepatocytes in liver synthesize most of the proteins. The liver is one of the core target organs for the detoxification of toxicants. ASA accumulation in liver causes impaired protein synthesis [88]. This observation is in accordance with the results of Saglam and Yonar [89] who reported a decline in plasma protein content in sulfamerazine-exposed *Oncorhynchus mykiss*.

Any stress condition in fish causes perturbations in enzymatic activities, thus resulting in decreased growth rate and metabolic rate in fishes. Glutamate oxaloacetate transaminase (GOT or AST) and glutamate pyruvate transaminase (GPT or ALT) are two important liver enzymes found in plasma and in various body tissues. Serum ALT and AST level and their ratio are universally accepted clinical biomarkers for analysing liver health [90, 91]. In the present study, there were significant changes in GPT and GOT activity in plasma of *C. carpio* exposed to the chronic concentration of ASA for 21 days. The depleted levels of GPT and GOT in plasma indicate the incapability of destructed hepatocytes to release aminotransferases into the circulatory system [92]. The sudden increase in GPT and GOT activity in plasma might be resulted from the hepatic tissue damage caused by the drug [93]. Similarly, Ramesh et al. [4] documented on the significant alterations in the freshwater fish *Cyprinus carpio* exposed to chloroquine.

Xenobiotics induce oxidative stress in aquatic animals mainly in fishes through free radical and reactive oxygen species (ROS) mechanisms [41]. The liver is the site of detoxification in fishes [94]. The detoxifying mechanism occurs in a sequence involving biotransformation enzymes such as GST and antioxidants such as CAT, SOD, and GPx [95]. The superoxide dismutase (SOD) mediates the first transformation by dismutation of superoxide free radicals (O_2^-) into hydrogen peroxide

(H_2O_2), while catalase (CAT) converts it into water (H_2O) and oxygen (O_2) [96]. In the present study, induction of SOD activity in gill and liver would help to avoid reactive oxygen species generation caused by oxidative damage [97]. Similarly, Matozzo et al. [98] observed an increase of SOD activity in gills of clam *Ruditapes philippinarum* exposed to triclosan treatments.

LPO activity in liver of Treatments I and II showed a biphasic trend, and the values range from 7.56 to 2.16 mol of MDA/g protein and 14.74 to 6.96 mol of MDA/g protein respectively. Significant changes were observed in the LPO activity of gill, liver, and kidney of triclosan-exposed rohu fingerlings [75]. The elevation of LPO level may be due to increased production of ROS, due to ASA stress leading to lipid peroxidation. The significant changes observed in liver LPO level of fish may be due to the persuaded activity of antioxidants, increasing the scavenging of free radicals and reducing MDA production [77].

The CAT and GPx are reactive oxidative species (ROS) reducing enzymes. CAT eliminates hydrogen peroxide, whereas GPx can detoxify hydrogen peroxide and degrades fatty acid peroxides [38]. The depleted levels of CAT may be due to its inactivation by (O_2^-) or due to the poor detoxifying mechanism as a result of the excess production of hydrogen peroxide. Perhaps Kono and Fridovich [99] explained the inhibition of CAT activity by (O_2^-) and the synergetic reaction between SOD and CAT. Similarly Ku et al. [100], Alak et al. [101] and Rangasamy et al. [102] observed changes in CAT activity in *Pelteobagrus fulvidraco* exposed to triclosan, in rainbow trout exposed to eprinomectin and in *Danio rerio* exposed to ketoprofen.

GST is one of the indispensable liver enzymes that defend the cell from the ROS toxicity by catalysing the reactive intermediates to reduced glutathione through the process of biotransformation [103, 104]. The fluctuations seen in gill and liver GST activity might be resulted from the defence mechanism developed by the fish against oxidative damage caused by the ASA [105, 106]. Comparably Liao et al. [107] and Zivina et al. (2013) observed an increase in liver GST activity of medaka fish on ketamine exposure and in developmental stages of zebrafish on ASA exposure, whereas Ajima et al. [108] reported for the decreased brain GST activity of the fish exposed to verapamil.

Glutathione (GSH) is one of the important antioxidants capable of preventing damage to cells caused by reactive oxygen species [109]. In the current study, GSH levels in the liver and gill were found to be depleted which indicates its utilization to meet the oxidative stress caused by the drug. Zhang et al. [110] reported that the decline in GSH level may be due to the lack of adaptive mechanisms and GSH oxidation to GSSG. Similar decrease was also noted in *Carassius auratus* after exposure to decabromodiphenyl ether and ethane or their mixture [111] and in *Channa punctatus* after exposure to thermal power plant effluents [112].

5 Conclusion

The present study concludes that acetylsalicylic acid induced alterations in the haematological, biochemical, enzymatic and antioxidant activities of the freshwater fish at chronic concentrations. Therefore it is more rational to affirm that a rise or fall in any of these biomarkers in ASA-exposed fish provides significant information on the overall physiology, metabolism and health status of fish under examination. As a future outlook, we suggest evaluating biodistribution and biotransformation of ASA in fishes as it would provide insight into the biomagnifying potency of pharmaceuticals in aquatic biota.

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Contemporary Methods for Removal of Nonsteroidal Anti-inflammatory Drugs in Water Reclamations

Luong N. Nguyen, Audrey S. Commault, Donna Sutherland, Galilee U. Semblante, Seungdae Oh, and Long D. Nghiem

Contents

Abstract Global water quantity and quality are anticipated to decrease in the coming decades, as a result of both increasing global populations and the effects of climate change. Reusing and recycling water is a key part of reducing the pressure on our existing water supplies and the aquatic environment. However, the occurrence of nonsteroidal anti-inflammatory drugs (NSAIDs) in secondary, and in some tertiary, treated effluents- and sewage-impacted water bodies is one of the major obstacles for the implementation of water reuse. For several decades, NSAIDs have

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been extensively used for therapeutic purposes in both humans and domestic livestock. The negative effects of NSAIDs on aquatic biota are just beginning to be realized. Currently, intensive treatments are required to remove effectively NSAIDs from recycled treated effluent in order to minimize or eliminate risks to human health and aquatic environment. In this chapter, we focus the discussion on contemporary methods for NSAID removal including biological, physical, chemical, and combined process that may provide a more effective and efficient alternative.

Keywords Advanced oxidation process, Integrated process, Membrane process, NSAIDs, Water reuse

1 Introduction

Water reclamation refers to the treatment of used water, or wastewater, to the quality suitable for either potable (e.g., drinking) or non-potable (e.g., irrigation, agricultural applications, and toilet flushing) use. Water reclamation provides an alternative source of water that gives an extra level of certainty and security to water supplies in the face of a changing climate. In recent years, there has been an upward trajectory in both technology development and full-scale implementation of water reclamation. For example, NEWater, the trade name of reclaimed water produced in Singapore, now operates five full-scale NEWater plants that supply up to 40% of Singapore's water demand (i.e., water fabrication processes, non-potable applications in manufacturing processes as well as aircon cooling towers in commercial buildings). Despite recent advances, there are several barriers to acceptance of water reclamation, including capital and operation costs, presence of emerging contaminants (ECs), as well as community attitudes. Research efforts to reduce the cost, treat and remove ECs, and enhance the community awareness are ongoing.

One group of EC of particular concern is the nonsteroidal anti-inflammatory drugs (NSAIDs), which include aspirin, ibuprofen, naproxen, diclofenac, and paracetamol. NSAIDs are commonly used in our daily life to reduce pain, decrease fever, prevent blood clots, and decrease inflammation [1]. As a result of this usage, the presence of NSAIDs in the environment is beginning to receive considerable attention from the scientific community, public health, and ecological conservation authorities [2, 3]. The concerns are mainly due to their potential physicochemical toxicological properties on aquatic biota, although there are currently no environmental protection limits for NSAIDs [2]. NSAIDs have been reported in both wastewater and the receiving environment at trace levels of ng/L to μg/L, and while these concentrations may not always be harmful to humans, they are still considered to be undesirable with regard to the "precautionary principle" [2–4].

Currently, there are no statutory requirements for wastewater and water reclamation plants to monitor the concentrations of NSAIDs in the water which, in most instances, are not routinely monitored for. However, with increased application of

water reclamation and improved understanding on the impacts of NSAIDs in reclaimed water, technologies for the treatment and/or removal of NSAIDs will need to be developed. In this chapter, contemporary technologies for the treatment and/or removal of NSAIDs are reviewed and discussed. In particular, integrated processes (i.e., combination of biological, physical, and chemical process) for NSAID removal are described.

2 Contemporary Methods for Nonsteroidal Anti-inflammatory Drug Removal

2.1 Biological Methods

2.1.1 Bacterial-Based Process

Microbial consortia currently play a significant role in conventional activated sludge (CAS), carrying out soluble organic matter removal, nitrification–denitrification, luxury uptake of phosphorus, and volatile fatty acid degradation. Bacteria (the major component of the microbial consortia) with such diverse metabolic capacities are employed in a series of (or single) chambers with differential redox conditions (e.g., anaerobic, anoxic, and aerobic). The potential role of wastewater treatment bacterial consortia in biodegrading NSAIDs is discussed below and conceptualized in Fig. 1.

CAS is not designed and operated for the treatment and removal of emerging contaminants, including NSAIDs. The removal rates of NSAIDs in CAS are, therefore, often incomplete and significantly variable [5]. For example, CAS remove 10–50% of diclofenac from influents at concentrations of 100–5,000 μg/L [6, 7],

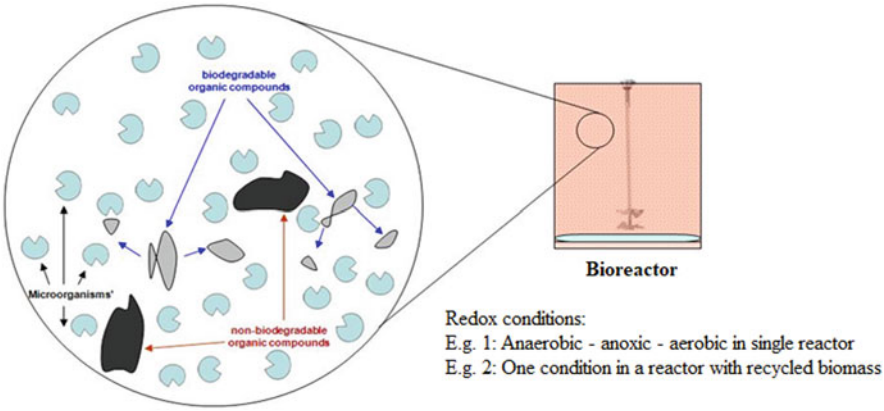

Fig. 1 Biodegradation and adsorption concept of NSAIDs by bacteria in conventional activated sludge (CAS), sequencing batch reactor (SBR), and membrane bioreactor (MBR)

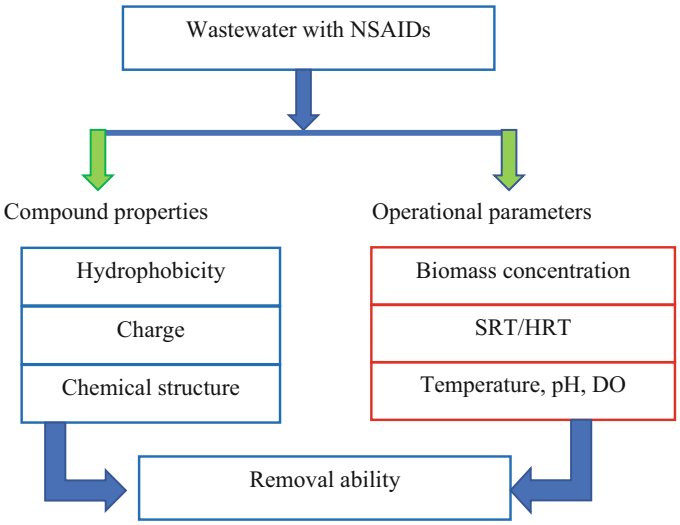

Fig. 2 Factors affecting the removal of NSAIDs in the biological process. SRT/HRT stands for sludge retention time and hydraulic retention time, respectively

while Kimura et al. [8] found that ketoprofen and naproxen are not eliminated at all in CAS. In contrast, Tran et al. [9] found that nitrifying bacteria cultures achieved 10–30% improvement in the removal of diclofenac, ibuprofen, and naproxen in comparison to CAS. Biodegradation of NSAIDs in CAS can be affected by microbial community structure and their associated metabolic capabilities [4, 10, 11]. Microbial community composition and functionality are influenced by various operating conditions, such as pH, temperature, dissolved oxygen concentration, hydraulic/solid retention time, and the type and concentration of growth substrates. For example, high removals of NSAIDs were observed in CAS systems with higher nitrifying activity [12]. Higher removal efficiency of some NSAIDs could also be attributed to adsorption to sludge biomass [13]. However, compounds which are relatively hydrophilic (log $D < 3.2$, acetaminophen, naproxen, ibuprofen, diclofenac) show limited sorption to sludge [14]. Therefore, physicochemical properties of a compound can greatly influence its fate and removal during CAS treatment.

Membrane bioreactor (MBR) is a combination of a membrane filtration process with a suspended growth bioreactor. MBR provides effective removal of both organic and inorganic contaminants from municipal and/or industrial wastewaters. MBR produces a more consistent effluent quality compared to that of CAS. The combination of activated sludge and membrane filtration has made MBR a reliable and popular technology for treating many types of wastewaters, particularly those that contain emerging contaminants such as NSAIDs [15–19]. However, the removal efficiency of ECs such as NSAIDs during MBR treatment depends on the physicochemical properties of the compound and the operational conditions of the wastewater treatment plant (Fig. 2) [20–23]. Physiochemical properties such as

hydrophobicity, chemical structure, and compound polarity are likely to be important factors affecting the removal of NSAIDs in MBR systems. Understanding to what extent and how each property affects the removal of NSAIDs would help better design and operate MBR-based WWTPs for controlling NSAIDs-bearing waste streams.

Hydrophobicity is a major factor affecting the sorption of NSAIDs by MBR. Of the many NSAIDs present in wastewater, some are highly hydrophobic and can be readily removed by MBR treatment via biosorption. For example, 80% of nonylphenol was eliminated in a pilot-scale MBR process treating landfill leachate [24], largely due to the high hydrophobic nature of nonylphenol (log $D = 6.19$ at pH 8). In a laboratory-scale study, the removal of hydrophobic compounds (log $D > 3.2$), such as amitriptyline 17β-estradiol, androsterone, and simvastatin, by MBR was greater than 85% at pH 8 [19]. However, in the same study, the authors found that less than 20% removal was achieved for hydrophilic and moderately hydrophobic compounds (log $D < 3.2$).

The chemical structure of the NSAIDs can be another major factor affecting their removal by MBR. Compounds with simple chemical structures (e.g., the absence of a branched alkyl chain) are likely easily degraded, whereas compounds with complex structures, or with toxic functional groups (e.g., halogens and nitro group), have a higher resistance to biodegradation, resulting in incomplete degradation [24]. In addition, simple structure (e.g., not containing multiple rings) compounds with chloride groups (e.g., diclofenac) are less removable by MBR [25]. Cirja et al. [24] reported a decrease in the degradation rate of aromatic compounds when the number of nitro and chlorine groups increases. Therefore, MBR can represent a promising technology, but further research on the removals of NSAIDs in relation to MBR-based water reclamation processes is highly desired.

2.1.2 Enzyme-Based Process

The enzymatic treatment process is at the border of traditional chemical and biological processes, where enzymes are the biological catalysts of chemical reactions. The use of enzymes purified from various plant and microbial sources for wastewater treatment has been actively studied in recent years. Enzymatic treatment processes have various advantages over conventional biological and chemical processes such as high substrate specificity, effective degradation of recalcitrant xenobiotic compounds, high reaction rate, and biodegradability in discharged water [26–29]. Despite all these advantages, the deployment of enzyme-based technologies in wastewater treatment is impaired by their relatively high production costs, limited scalability, sensitivity to inhibitors, and low stability under harsh environmental conditions [30].

Among the different oxidative enzymes of interest for wastewater treatment, laccases are the most studied [30]. Laccases are multicopper oxidases produced in fungi, bacteria, and some algae, which can oxidize phenols and similar substrates and have been shown to degrade NSAID compounds [9, 31, 32]. Laccases catalyze the ring cleavage of aromatic compounds using oxygen as an electron acceptor. Kim

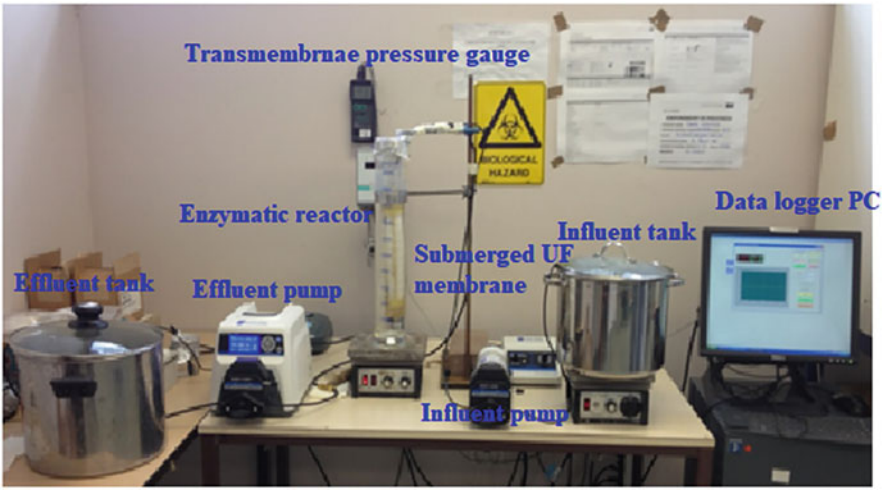

Fig. 3 An example of laboratory-scale enzymatic membrane reactor

and Nicell [33] reported 71–100% degradation of triclosan (20 μM), while the measured laccase activity increased from 0.3 to 3 U/mL. Tran et al. [9] reported that 2 mg/L of fungal laccase can degrade 60% of diclofenac and naproxen in the effluent after a 3 h reaction.

Enzyme washout and inactivation are the limitation of enzyme application in water treatment process [26, 34]. Depending on the origin of the laccase (fungal or bacterial) and the reaction conditions, the half-life of the enzyme can vary from minutes to days [35, 36]. The recovery of the enzyme and its reusability are key factors for the feasibility of continuous-mode enzymatic reactors because the high cost of the enzyme may limit their application [37]. Enzyme immobilization on a support is one of the approaches to tackle this major limitation. Different supports, namely, polyacrylonitrile, polystyrene, SiO_2 (celite), chitosan, and sol-gel, have been used to immobilize laccase [29]. For example, Cabana et al. [38] immobilized laccase on SiO_2 that degraded nonylphenol, bisphenol A, and triclosan in a packed bed reactor. The use of membranes with pore size smaller than the molecular weight of an enzyme is another approach to prevent enzyme washout from a continuous flow enzymatic reactor. Enzymatic membrane reactor (EMR) allows for continuous feeding and product withdrawal without loss of the enzyme. Depending on the EMR design, the enzymes may be freely circulating in the retentate or immobilized onto the membrane surface or inside its porous structure [26, 28, 39]. An example of enzymatic membrane reactor is presented in Fig. 3.

Nguyen et al. [32] reported 60% removal of diclofenac at influent concentration of 0.5 mg/L. However, enzymatic denaturation continuously occurred despite of a complete retention by the membrane, requiring the periodic addition of enzyme. Indeed, the authors proposed a strategy to maintain enzymatic activity by adding 200 μL of the commercial laccase solution per L of the reactor volume every 12 h

(equivalent to a laccase dose of 23 mg/L.d). Apart from enzyme reinjection, different methods have been reported to minimize the loss of enzyme during operation of an EMR. For example, ethylenediaminetetraacetic acid and polyethylene glycol, which are believed to possess a protecting role for enzymes, especially under oxidative stress, may be added to an EMR [40, 41]. While enzymes hold a great potential, more research is needed to increase their stability before they can be implemented at industrial scale.

2.1.3 Algae-Based Process

Microalgae have demonstrated potential for detoxifying a wide range of organic and inorganic compounds at a range of scales, from laboratory through to full scale [2]. Such detoxification typically occurs via three main pathways: bioadsorption, where the compound is adsorbed to cell wall components or onto organic extracellular excretions; bio-uptake, where the compound is actively transported into the cell; or biodegradation, where the compound is broken down into simpler molecules through catalytic metabolic degradation [2]. While coupling NSAID bioremediation with technologies such as microalgal wastewater treatment could potentially be economically viable, there are several research challenges associated with microalgal NSAID biodegradation that need to be overcome before this becomes a viable option.

Detoxification via microalgal bioadsorption is dependent on the chemical structure of the compound, with hydrophobic, cationic compounds being attracted to the microalgal cell surface through electrostatic interactions, whereas hydrophilic compounds are repelled [42]. Once at the cell surface, a number of chemical interactions between the compound and the functional, charged groups on the cell surface may occur, including adsorption reactions, ion exchange reactions with functional groups on the microalgal surface, surface complexation reactions, chelation, and microprecipitation. However, NSAIDs are hydrophilic compounds, meaning that they are anionic, or negatively charged, and have low bioadsorption affinity values with microalgal cells due to the cells also being negatively charged [2]. This means that the use of live microalgal cells for NSAID bioadsorption is not a viable option, but the use of either physically or chemically modified nonliving cells may potentially be a viable treatment option. Physical or chemical modifications can be made to the microalgal cell surface that permits hydrophilic interactions between the hydroxyl and carbonyl functional groups of the cell surface and the amino and carbonyl groups in the molecules [2]. This can result in increased adsorption onto the cell surface for hydrophilic compounds such as NSAIDs [2].

Adsorption of a non-NSAID hydrophilic drug (Tramadol) onto nonliving microalgal cells was enhanced by 70% through simple chemical treatment (0.1 N NaOH) of microalgal cell surfaces, compared to living microalgae [2]. Similarly, Coimbra et al. [43] demonstrated that physically damaged (freeze-drying and grinding) nonliving microalgal cells were able to remove between 20 and 28 mg of

diclofenac from water per gram of algal biomass, although no live microalgal biomass was used as a comparison.

Microalgal biodegradation involves the transformation or breakdown of complex compounds into simpler molecules either through direct catalytic metabolic degradation, in which the compound serves as the carbon source or electron donor/acceptor, or by co-metabolism, in which the compound is degraded by enzymes that are catalyzing other substrates present [2]. Microalgal biodegradation can occur either intracellularly, where the compound is taken up by the cell; extracellularly, where enzymes are excreted into the EPS to function as an external digestive system; or a combination of them. The intracellular biodegradation of compounds involves a complex enzymatic process involving both Phase I and Phase II enzyme families. The main role of Phase I enzymes in biodegradation is to make the compound more hydrophilic, while the main role of Phase II enzymes is to catalyze the degradation of the compound [42]. Microalgal-mediated biodegradation is regarded as being highly complex, and the exact role of the multiple enzymes in both the Phase I and Phase II enzyme families is not fully understood [42], and both the enzymes involved and their respective roles are likely to differ, at least in part, between different microalgal species [2].

There are few studies that have assessed microalgal biodegradation of a limited number of NSAID compounds, and despite NSAIDs being hydrophilic, the reported rates of microalgal-mediated biodegradation are low. For example, reported microalgal biodegradation rates of the NSAID diclofenac range from $<7\%$ to 22% and require at least a 9-day exposure to microalgal culture [44, 45]. However, the authors did not state whether the microalgal culture was axenic or not, or if any associated bacteria could have played a role in the reported degradation. Similarly, Ding et al. [46] found varying rates of degradation of the NSAID naproxen between different microalgal species, with *Cymbella* sp. enhancing naproxen degradation by 27% above that in the control while *Scenedesmus quadricauda* inhibiting degradation by 23%, following 30 days of incubation. One of the challenges to successful microalgal biodegradation of NSAIDs is ensuring that the microalgae can uptake the hydrophilic compounds into the cell in the first place or that extracellular enzymes are expressed in sufficient quantity to induce extracellular degradation. Fungal biodegradation of NSAIDs has been attributed to extracellular ligninolytic enzymes (e.g., peroxidases, laccases), often in coordination with an internal detoxification process, involving both Phase I and Phase II enzymes, which is mediated by the cytochrome P450 family (CYP), epoxidases, and transferases [47, 48]. These enzymes have also been reported as being present, to some degree, in some microalgae, but their efficacy and mode of action (redox mediator) may vary, and the exact role these enzymes play in microalgal biodegradation of compounds is unknown [42, 48–50]. There are several strategies that may potentially improve microalgal-mediated biodegradation of NSAIDs. Firstly, the conditions of the growth media can be optimized to enhance the secretion, activity, and stability of native laccases; for instance, Otto et al. found increased laccase production in microalgae was achieved through the simple addition of copper sulfate. Secondly, the catalytic performance of the native enzymes can be increased by random

mutagenesis and/or site-directed mutagenesis [51]. Finally, exogenous enzymes, such as fungal enzymes with high biodegradation capacity, can be recombinantly expressed in microalgae. Microalgae have higher growth rates than fungi, minimal growth requirements (phototrophy), and therefore potentially lower bioremediation costs [52]. For example, Chiaiese et al. [53] succeeded in producing the fungal laccase POX A1b in *Chlamydomonas pitschmannii*, *Chlorella emersonii*, and *Ankistrodesmus braunii* for the remediation of phenolic compounds from olive oil mill wastewaters. However, the genetic engineering of microalgae is in its infancy, and numerous limitations such as low transformation efficiencies and low recombinant protein yields still need to be overcome [52]. In addition to the current technology limitations, for many countries, legislation around the limited use, or the total ban, of genetically modified organisms (GMO) due to the risks and potential impact on the environment means that, at present, genetically modifying microalgae for NSAID biodegradation is not a viable option.

Microalgae play a role in enhancing bacterial biodegradation of NSAIDs. In microalgae-bacteria coupled treatment systems, microalgal photosynthesis provides the necessary oxygen, a key electron acceptor, for aerobic bacterial degradation of the organic compounds, while microalgal released dissolved organic matter (DOM) provides the necessary substrates for bacterial co-metabolism of compounds such as NSAIDs [2]. For example, Matamoros et al. [54] successfully demonstrated microalgal enhancement of bacterial biodegradation of the NSAID ibuprofen. The authors found that, in the presence of microalgae, bacterial degradation of ibuprofen increased from 15 to 60%, following 3 days of incubation under laboratory conditions [54]. However, the exact mechanism for microalgal enhancement of bacterial degradation of NSAIDs and other organic compounds is not fully understood. Investigations into the interactions between the two organisms and conditions that further enhance coupled degradation would help to enable the development of biological-mediated NSAID remediation.

Microalgae may also enhance the photodegradation of NSAIDs through the release of DOM, which is comprised of a range of molecules such as hydrophilic organic acids, hemicellulose, humic acids, and fulvic acids. This released DOM is thought to enhance photodegradation through various mechanisms, including catabolic processes, redox cycling, production of hydroxyl radicals, or inhibiting photooxidation by competitive reaction with radicals, resulting in the photosensitized transformation of NSAIDs [55]. Photodegradation of the NSAIDs diclofenac [54, 56] and ibuprofen [54] in the presence of microalgal-derived DOMs has been successfully demonstrated in both wastewater treatment high rate algal ponds and photobioreactors, with reported removal rates between 82 and 99% compared to 7% for biodegradation.

Options for cost-effective microalgal degradation of NSAIDs are limited due to the hydrophilic nature of the compounds and the negatively charged cell surface of the microalgae. The most promising options include coupled microalgal-bacterial

degradation or enhancement of photodegradation. Further research into the mechanisms behind microalgal-assisted degradation is needed in order to optimize the treatment system.

2.1.4 Fungi-Based Process

Considerable research has been devoted to test the performance of different white-rot fungi (WRF) for the removal of NSAID compounds. For example, Tran et al. [9] observed the complete removal of the NSAID compounds ibuprofen, naproxen, diclofenac, and ketoprofen by a white-rot fungus *Trametes versicolor* over 7 days of inoculation. Cajthaml et al. [57] investigated the performance of eight different strains of WRF for the removal of several NSAID compounds, including diclofenac, ibuprofen, and ketoprofen; while almost all tested fungal strains were able to degrade the selected NSAIDs, to some degree, the strains *Irpex lacteus* and *Pleurotus ostreatus* provided the highest removal efficiency of NSAIDs (i.e., 90% and 80%, respectively), after 7 days of incubation. Marco-Urrea et al. [47] found that four different strains of WRF were able to completely remove the NSAID ibuprofen from culture but were ineffective at removing carbamazepine and clofibric acid. In another study by Marco-Urrea et al. [47], the WRF strain *T. versicolor* was capable of removing diclofenac (70%) from the culture. The authors suggested that at least two different mechanisms were involved in the degradation of diclofenac: (1) cytochrome P450 system and (2) laccase catalysis. However, to date the application of fungi for wastewater treatment is still at laboratory-scale studies as scale-up of fungal cultures is challenging.

2.2 Advanced Oxidation Process

Advanced oxidation processes (AOPs) aim at chemically generating strong oxidants (e.g., hydroxyl radicals) to transform persistent organic compounds such as NSAIDs into biodegradable substances. The hydroxyl radicals ($^{\bullet}OH$) can be generated using catalysts (electrodes, metal oxides), irradiation (UV light, solar light, ultrasounds), and strong oxidizing agents like hydrogen peroxide (H_2O_2) or ozone (O_3). These methods can be used separately or in combination. AOPs have been used to remove organic pollutants from reclaimed effluent and groundwater [58]. Numerous studies in the literature have demonstrated the effectiveness as well as limitation of AOPs for the removal of trace organic contaminants from wastewater [59–63]. In this chapter, we focused mostly on ozone and UV oxidation.

2.2.1 Ozonation

Ozonation process involves two reaction mechanisms: (1) direct reaction by ozone and (2) indirect reaction by OH radicals during ozone reactions [64]. While ozone reacts selectively with electron-rich moieties compounds, the OH radicals can react with a wide range of aromatic compounds including NSAIDs [65]. Regardless of the reaction mechanisms, the required ozone treatment dose is proportional to the bulk organic content in the wastewater. Ozone (O_3) has been shown to degrade trace organic contaminants during wastewater treatment and water reuse applications [44, 66]. Ozone reacts with TrOC either through direct reactions or through the formation of free radicals, including the hydroxyl radical ($^{\bullet}OH$) [67]. Oxidation using ozone can achieve >92% removal of a number of pharmaceuticals, including NSAIDs, and pesticides such as ofloxacin, sulfamethoxazole, propranolol, carbamazepine, clofibric acid, diclofenac, atrazine, and diuron [59, 63, 68]. However, a number of other pharmaceuticals and personal care products (e.g., ibuprofen, naproxen, caffeine, and tonalide) could not be oxidized using the same process [63].

2.2.2 UV Oxidation

UV oxidation generates hydroxyl radicals by photolysis. Huber et al. demonstrated that UV treatment alone resulted in 75, 13, and 7% removal of diclofenac, iopromide, and sulfamethoxazole, respectively. Complete removal of several pharmaceuticals (e.g., ofloxacin, sulfamethoxazole, propranolol, carbamazepine, clofibric acid, and diclofenac) was achieved using the combination of hydrogen peroxide and UV radiation [59], although only 30–40% of ibuprofen, diphenhydramine, phenazone, and phenytoin could be removed using this method [69].

The AOPs are effective at treating NSAIDs, but the operating cost of AOPs is high due to the requirements in chemicals and energy [70]. It therefore limits their applications as a widespread solution of NSAID remediation.

2.3 Membrane Separation Process

High-pressure membrane filtration, including nanofiltration (NF) and reverse osmosis (RO), has been widely used to remove organic pollutants including NSAIDs [71–75]. NF/RO membranes can reject TrOCs mainly due to size exclusion, electrostatic exclusion, and adsorption on the membrane [76, 77]. In a full-scale study, Verliefde et al. [78] reported a high rejection (>95%) of most investigated NSAIDs by the Triseps (X20 and ACM5) and Hydranautics (ESPA1 and ESPA4) RO membranes. In another study, an NF-270 membrane achieved a high rate of rejection for charged pharmaceuticals, i.e., 96% for ibuprofen, where removal of NSAIDs was enhanced

by charge repulsion [79]. However, the rejection rate of some uncharged and small molecular weight organic contaminants by NF/RO membrane can be low [80].

The low rejection of some small molecular weight and uncharged NSAIDs by NF/RO membranes, as mentioned above, has been widely reported in the literature [8, 80–84]. For example, at extended stages of filtration, there was poor rejection of chloroform and bromoform by RO (e.g., TFC-HR and XLE) and NF membranes (e.g., NF-90 and TFC-SR2) [84]. Chloroform and bromoform are both neutral and have a molecular weight of 119.4 and 252.7 g/mol, respectively. The charge of the trace organic contaminants and that on the membrane can play a significant role in the rejection of TrOCs. For example, rejection of a charged compound by NF/RO membranes is usually higher than for a neutral compound with the same molecular weight or size [84]. Since most pharmaceuticals are negatively charged particularly at neutral pH, a considerable number of these compounds may be completely rejected by charge repulsion between the compound and membrane charges [79]. Xu et al. [84] reported that highly negative surface charge membranes such as the loose NF-200 membrane, with a molecular weight cutoff (MWCO) of 300 g/mol, could reject more than 89% of low molecular weight negatively charged compounds such as ibuprofen. A high rejection of other pharmaceuticals such as dichloroacetic acid (91%) and trichloroacetic acid (94%) was also achieved using the ESNA (NF) and RO-XLE (RO) membranes [8].

Membranes with a high degree of desalting showed the highest rejection of most NSAIDs [81]. A UTC60 aromatic polyamide membrane (an NF membrane) which has a low NaCl rejection (55%) demonstrated a poor rejection of several trace organics such as 47% for bisphenol A and 5% for chloroform [71]. Moreover, higher membrane roughness has been highly correlated with a lower rejection of large organic contaminants [81].

The hydrophobicity and charge of an active layer of the membrane can also affect the rejection of various NSAIDs [84]. The surface hydrophobicity of a membrane can be determined by measuring the contact angle. The rejection of some organics could be improved by increasing the hydrophobicity of the membrane because it reduces the affinity between the neutral organic solute and the surface of the membrane [85]. Furthermore, the amount of charge in the surface of the membrane affects the degree of electrostatic repulsion and rejection of negatively charged solutes that are subjected to dynamic property changes during the membrane process [84]. For example, Bellona and Drewes [86] studied the rejection of negatively charged organic acids (2-naphthalenesulfonic acid and 1,4-dinaphthalenesulfonic acid) by negatively charged NF membranes (e.g., NF-90 and NF-200). According to their findings, the rejection was larger than expected based on steric exclusion and was mainly driven by the surface charge of the membrane and correlated with the degree of ionization of these compounds [86].

Operational parameters such as feed solution pH, salinity, temperature, pressure, and cross-flow velocity can influence the rejection of NSAIDs by NF/RO membranes. The feed solution pH can govern the speciation of ionizable NSAIDs (and to a lesser extent, the membrane surface charge) and thus their rejection. For instance, Bellona et al. [81] claimed that when using NF/RO at pH values between 3 and

9, more than 90% of trace organics such as estrone can be rejected. Sulfamethoxazole and ibuprofen are also highly soluble at high pH (in the alkaline region) where the compounds are negatively charged, but when the solution pH decreases, their solubility decreases sharply [87]. Nghiem and Hawks reported an almost complete rejection of sulfamethoxazole using the NF-270 membrane at a pH above 8.

Temperature is another parameter that can affect the water flux and rejection of NSAIDs [83]. Increasing the feed temperature can lead to a change in the structure and morphology of the polymer matrix, causing an increase in the mean pore radius and MWCO [88]. An increase in the solubility of some NSAIDs can occur due to the increase in the temperature of the surrounding solution [89].

Operating pressure and cross-flow velocity are important factors which can affect the volume and quality of a product. An increase in the operating pressure can reduce the shielding of negative charges on the surface of a membrane, which makes repulsion more effective and enhances the rejection of negatively charged contaminants by NF/RO membranes [90]. Also, the permeate flux increases with cross-flow velocity over a range of operating conditions because increasing the cross-flow velocity increases the flux and rejection of NSAIDs due to a reduction in concentration polarization [90, 91].

The hydrophobicity of both contaminant and membrane can affect the rejection of NSAIDs by NF/RO membranes. Contaminants such as steroid hormones with a high hydrophobicity ($\log D > 3.2$) can adsorb onto the surface of the membrane due to hydrophobic–hydrophobic interactions [78, 92]. Nghiem et al. [82] reported that the rejection of natural hormones by the NF-270 and NF-90 membranes was lower than that expected based on steric hindrance. They explained this phenomenon by the adsorption of these hydrophobic compounds onto the surface of the membrane followed by diffusion through its polymeric matrix [83]. The membrane separation processes (i.e., NF/RO) have demonstrated excellent capacity in removing NSAID compounds; however, their inherent operation conditions (high pressure, membrane fouling) require pretreatment process.

2.4 Integrated Process

Biological-based processes are the most pragmatic approach for wastewater treatment. However, the biological treatment alone is not effective for NSAID compounds (Sect. 2.1) for water reuse purposes. Because the biological treatment processes can reduce large bulk of organic content, research reports a significant synergy when it is integrated with other physical (i.e., membrane filtration and adsorption)- and chemical (i.e., advanced oxidation)-based processes.

2.4.1 Biological Treatment Coupled with Membrane Filtration

A complementation between membrane filtration and biological degradation of NSAIDs in hybrid systems such as MBR coupled with NF/RO has been successfully demonstrated [77, 93, 94] (Fig. 4). Alturki et al. [93] reported that hydrophilic NSAIDs, which passed through the MBR, were effectively removed by the following NF/RO membranes. For example, the MBR–RO removed naproxen at 100% of which MBR and RO contributed 40 and 60%, respectively. The authors also reported the removal of 40 compounds to below the analytical detection limit (10 ng/L); thus, the final effluent may meet the reuse water quality standard [93]. Nguyen et al. [77] reported that MBR and NF/RO removed NSAIDs based on different mechanisms. Thus, hybrid systems are very effective at removing NSAIDs. Apart from NSAID removal, the hybrid system also offers a stable permeate flux of NF/RO membranes over extended operating periods [95–99].

2.4.2 Biological Treatment Coupled with Activated Carbon Adsorption

It is well-known that activated carbon (AC) is one of the most effective adsorbents for the removal of taste-, color-, and odor-causing organic pollutants from aqueous or gaseous phases. Activated carbon is widely applied as a commercial adsorbent in the purification of water and air [100]. It is also widely used for treatment of taste and odor. Treatment with activated carbon has proved to be efficient for removal of geosmin and 2-MIB [101]. Zhang et al. demonstrated that granular activated carbon (GAC) is an excellent adsorbent for two algal odorants dimethyl trisulfide and β-cyclocitral. Activated carbon has been widely studied for treating landfill leachate wastewater. AC has been also investigated intensively for treatment of dye

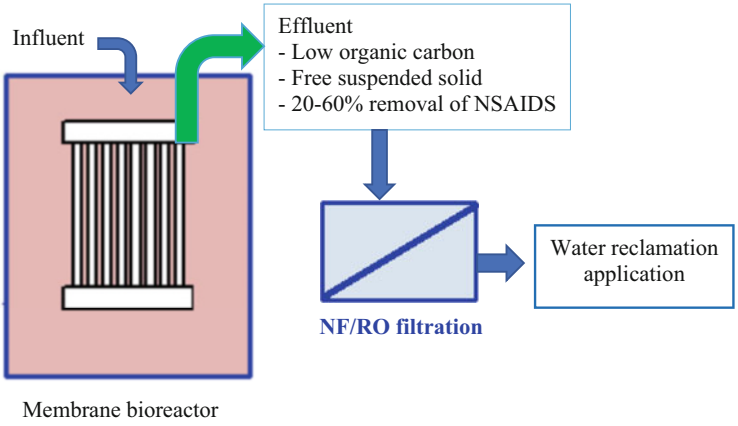

Fig. 4 Schematic diagram of the combined membrane bioreactor (MBR) and nanofiltration (NF)/reverse osmosis (RO) process

wastewater [102–104]. The results indicated that activated carbon could be employed for efficient removal of dyes from wastewater [104–106].

PAC (powdered activated carbon) and GAC are frequently applied in drinking water treatment for removal of natural or synthetic organic compounds (SOCs), e.g., pesticides [107]. Recently several studies have evaluated adsorption of other trace organics (PhACs, EDCs) on activated carbon both under laboratory conditions and surveys at full-scale drinking water treatment plants [108, 109]. For example, Hernández-Leal et al. [110] reported complete adsorption of all studied trace organics (bisphenol-A, benzophenone-3, hexylcinnamic aldehyde, 4-methylbenzylidene-camphor (4MBC), triclosan, galaxolide, and ethylhexyl methoxycinnamate) onto PAC in batch tests with Milli-Q water spiked with 100–1,600 μg/L of trace organics at a PAC dosage of 1.25 g/L and contact time of 5 min.

GAC has a relatively larger particle size compared to PAC and, consequently, presents a relatively smaller surface area. Nevertheless, GAC has long been used in the removal of traditional organic contaminants such as pesticides [107]. GAC has been proposed as a potential treatment method to aid in the effective removal of emerging contaminants, particularly EDCs in wastewater treatment. A significant reduction in the concentration of steroidal estrogens (43–64%) and mebeverine (84–99%) has been achieved in a full-scale granular activated carbon plant [111]. In a study by Hernández-Leal et al. [110], three GAC columns were operated to treat aerobically treated gray water which was spiked with the above emerging contaminants in the range of 0.1–10 μg/L at a flow rate of 0.5 bed volumes (BV)/h. They observed more than 72% removal of all compounds (bisphenol-A, hexylcinnamic aldehyde, 4-methylbenzylidene-camphor (4MBC), benzophenone-3 (BP3), triclosan, galaxolide, and ethylhexyl methoxycinnamate). Tanghe and Verstraete [112] reported that at least 100 mg/g of nonylphenol is adsorbed on GAC in an adsorption test. A few studies have investigated GAC adsorption as an option for tertiary treatment of conventional biologically treated wastewater [111, 113]; for example, Grover et al. [111] reported that a full-scale GAC plant could reduce above 60% of steroidal estrogens in sewage effluent.

Activated carbon adsorption can be coupled with a biological treatment in two different configurations: (1) addition of powdered activated carbon (PAC) directly in the bioreactor [114–117] and (2) posttreatment of the bioreactor (e.g., MBR) permeate using either a granular activated carbon (GAC) column [118, 119] or a continuously mixed reactor containing a slurry of PAC [120]. Research results have suggested that addition of PAC enhanced NSAID removal by initial adsorption and subsequently enhanced contact time with biological agents in the reactor for biodegradation. While the removal by initial adsorption has been easily demonstrated in a number of studies, the enhancement of biodegradation is an assumption. Nguyen et al. [21] observed an immediate improvement in naproxen, diclofenac, ketoprofen, and ibuprofen removal after PAC addition to the MBR. The NSAID adsorbed onto PAC can be efficiently removed by the PAC–MBR system because of the complete retention of the sludge by the membrane [21, 114].

In the second configuration, a GAC posttreatment can specifically target the residual NSAID compounds in the MBR permeate without significant competition or interference from the bulk organics [21]. Nevertheless, periodic regeneration/replenishment of the activated carbon is necessary, because over an extended operating period, fouling and substrate deterioration are inevitable.

2.4.3 Biological Treatment Coupled with Advanced Oxidation Process

Advanced oxidation processes (UV or ozonation) are very effective at oxidizing NSAID compounds but are mostly used as a polishing or disinfection step. Packer et al. [121] observed a rapid and mild photodegradation of diclofenac and ketoprofen, respectively. Nguyen et al. [77] reported almost 100% removal of pentachlorophenol and triclosan within 7.5 min of UV 254 nm exposure. These compounds are quite recalcitrant to biological treatment. The benefit of combining biological treatment (e.g., MBR) with UV oxidation therefore can be shown by examining the removal of these compounds. For example, diclofenac was poorly removed by the MBR (40%). By contrast, treatment by UV system following MBR attained exceptionally high removal efficiency (i.e., 98%). The MBR also provides a low background organic matter content and suspended solids-free influent which is highly suitable as influent for UV oxidation process.

The efficiency of a combined MBR and ozonation process for NSAID removal has been assessed in different operational modes. de Wilt et al. [122] reported the limitation of removing ibuprofen, naproxen, and diclofenac by individual biological and ozonation process. In details, 14 and 80% removal of diclofenac was achieved by biological and ozonation process, respectively. However, their combination resulted in >99% removal, indicating the complementary impact. The combination also reduced the ozone dose due to the decrease in organic matter of the influent [122]. Ikehata et al. [123] also reported that diclofenac was reactive toward ozone.

Apart from the MBR–ozonation, studies have reported the integrated MBR with ozonation (i.e., ozone is dosed directly in the reactor) [124]. Positive results include virtue of higher removal of ozonation by-products and lower ozone treatment dose requirement. A similar observation was reported by Laera et al. [125], where the ozonation by-product was 20-fold lower in the final effluent of the integrated process than in that of MBR–post-ozonation process. Mascolo et al. [126] achieved a similar removal of an antiviral drug (acyclovir) by both configurations; however, the integrated process again was more beneficial in terms of removal of specific ozonation by-products.

3 Conclusions and Outlooks

The necessity of water reclamation is growing, driven by a stress on water supply and increased statutory regulations with respect to wastewater effluent quality. More water reclamation schemes in regions with restricted freshwater resources for both non-potable and indirect potable purpose are on trial at full-scale operation (e.g., in Singapore). As of today, membrane filtration processes such as NF and RO continue to play a central role in propagating the success of water reclamation due to the robust performance on the removal of emerging contaminants (e.g., NSAIDs). In the future, integrated processes (i.e., to combine the advantages of biological and chemical/mechanical processes) should be at the forefront of research considerations as such processes have the potential to reduce the cost and enhance the application of water reclamation.

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Photo-Fenton Treatment of a Pharmaceutical Industrial Effluent Under Safe pH Conditions

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Abstract This chapter aims to present the effect of treating a pharmaceutical industrial effluent by photo-Fenton catalyzed with a Fe-pillared bentonite. XRD proved the pillaring process successful, and by N_2 physisorption, it was established that the specific surface area of bentonite (34 m^2/g) increased to 277 m^2/g and pore volume increased from 0.058 to 0.106 cm^3/g. Active Fe species were identified by Mössbauer spectroscopy. The effect of reaction variables such as catalyst loading, pH, H_2O_2 concentration, and initial concentration of total organic carbon (TOC) is also presented. It was concluded that to reach near 100% mineralization, an acidic pH (2.7) should be observed. A high mineralization under these conditions, however, does not directly correlate with a low toxicity. Actually, the oxidative stress biomarkers only decreased when pH was not modified (pH = 8) albeit the attained mineralization was only 51%. It is worth noticing that the use of pillared clays allows carrying out photo-Fenton treatment under pH conditions other than acidic. The synthesized catalyst exhibited magnetism and this can be used for an easier recovery.

Keywords AOPs, Emerging contaminants, Mineralization, Photocatalysis, Toxicity, Wastewater

1 Introduction

The pharmaceutical production is one of the biggest problems related to water pollution. In Mexico, it has been shown that pharmaceutical industry effluents are a mixture of a variety of compounds frequently toxic [1], which include excipients, pharmaceutical drugs, and washing products. In this context, the effluents of production processes of emerging contaminants, such as nonsteroidal anti-inflammatory drugs (NSAIDs), are receiving special attention. Among NSAIDs, paracetamol or acetaminophen stands out because worldwide it is highly consumed and therefore highly produced. The importance of effective treatment of paracetamol containing effluents is related to toxicological effects in aquatic environment [2], since the degradation products of paracetamol are potentially toxic, causing on indicator species oxidative stress and cellular damage or death and inhibition of reproduction [3]. This has motivated the study and development of processes that contribute to the treatment of theses pollutants. Particularly attractive options are the advanced oxidation processes (AOPs). Among these, there are the well-known Fenton and photo-Fenton processes. The Fenton process involves the reaction of Fe(II) with H_2O_2 to produce hydroxyl radicals ($HO^{\cdot}$) via reaction 1. Under suitable conditions, the process is considered catalytic due to the reduction of Fe(III) by reactions 2 and

3. Yet with this, the regeneration of Fe(II) is not efficient enough, and sludge is generated by precipitation of species of Fe(III). Although this sludge can contribute to the removal of organic matter, it is desirable to degrade it and not only change its phase. In order to reduce this problem and increase the efficiency of this process, UV radiation is added. By this means, more hydroxyl radicals are produced by photoreducing Fe(III) to Fe(II) (reactions 4–6), and this process is called photo-Fenton [4–6].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^- O_2 \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe - OOH^{2+} + H^+ O_2 \quad (2)$$

$$Fe - OOH^{2+} \rightarrow Fe^{2+} + HO_2^{\cdot} \quad (3)$$

$$Fe^{3+} + H_2O \rightarrow Fe(H_2O)^{3+} O_2 \quad (4)$$

$$Fe(H_2O)^{3+} \rightarrow Fe(OH)^{2+} + H^+ \quad (5)$$

$$Fe(OH)^{2+} + hv \rightarrow Fe^{2+} + OH \quad (6)$$

Importantly, in this process, it is necessary to control the pH of the medium because the Fenton and photo-Fenton reactions exhibit high activity at pH about 2.8 [7]; at pH greater than 3.0, the reaction is slower because the generation of insoluble iron hydroxides decreases the concentration of the Fe(III) ion in solution and thus radiation transmission [8]. In spite of its high efficiency [9, 10], some undesirable features of the homogeneous photo-Fenton process are high hydrogen peroxide consumption, radiation field diminishment, the need of a separation step to remove the added iron, and the addition of chemicals to maintain an acidic pH for iron ions to be in solution [5]. These disadvantages have led to the investigation of solid supports capable of maintaining iron immobilized. In this sense, recent studies have shown that bentonite clay promises to be good catalyst support by modifying its surface [11, 12]. Among the different ways of modifying bentonite, the pillaring has been considered as a good alternative since the resulting material exhibits a high catalytic activity for removal of organic contaminants, stability against pH changes in the solution, high specific surface area, and relatively easy separation from treated effluents [11].

Herein, the toxicity reduction of a pharmaceutical industry effluent treated by photo-Fenton process catalyzed with an iron-pillared clay (Fe-PILC) is described. The synthesis of this catalyst as well as its characterization and mineralization results is also included.

2 Photo-Fenton Catalyst

2.1 Fe-PILC Synthesis

The catalyst used to conduct the study described here was an iron-pillared clay (Fe-PILC), and it was prepared by the method reported by Martin del Campo and Valverde [11, 13] as follows: 300 mL $FeCl_3{\cdot}6H_2O$ (aqueous solution 0.2 M) was slowly added to 600 mL of NaOH aqueous solution 0.2 M at room temperature under continuous stirring. The so obtained mixture was stirred for 4 h at room temperature and pH between 1.78 and 1.8 using hydrochloric acid 5 M. These pH values are important in order to generate the corresponding iron hydroxides in solution. The pillaring solution was slowly added to the 0.1 wt% aqueous bentonite suspension under stirring. The next step was to recover the clay by centrifugation and washed with distilled and deionized water in order to remove the chlorides. The product was dried overnight at 75°C and calcined for 2 h at 400°C. For this synthesis, the following reagents were employed sodium hydroxide (NaOH), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$ with purity of 99%), and hydrochloric acid (37%). Moreover, deionized and distilled water were provided by HYCEL and bentonite (pure grade) supplied by Thermo Fisher Scientific. This clay has a particle size >2 μm and a cation exchange capacity of 94 meq/100 g.

2.2 Fe-PILC Characterization

Figure 1 is the diffractogram of iron-pillared clay (Fe-PILC). This XRD pattern was obtained by a Bruker Advance 8 instrument using *Cu-Kα* radiation at 35 kV and 30 mA and was collected from 0 to 40° (2θ) with a step of 0.04°/min. At 4° (2θ), a small reflection that is commonly associated with pillaring processes can be observed [11, 14]. Three reflections of interest are observed, the first at 20° corresponding to (FeO(OH), the other two at 26 and 35 related to hematite (Fe_2O_3) [15].

Figure 2 shows the room temperature Mössbauer spectrum of pure bentonite, and it was fitted with two doublets. One doublet with isomer shift $\delta = 0.32$ mm/s and a quadrupole splitting $\Delta Q = 0.44$ mm/s corresponding to Fe^{3+} in octahedral site and the other one with $\delta = 1.02$ mm/s and $\Delta Q = 2.95$ mm/s corresponding to Fe^{2+} [16–19]. The ratio Fe^{2+}/Fe^{3+} in this bentonite is unusually high.

Figure 3 shows the Mössbauer spectrum of Fe-PILC at room temperature. We can see that there is no contribution of Fe^{2+} to the signal probably because Fe^{2+} ions migrated to Fe^{3+} into the pillared clay layers [20, 21] or because Fe^{2+} stabilizes the formation of Fe_3O_4. The Fe-PILC Mössbauer data was fitted with three doublets, one of them corresponding to Fe^{3+} in octahedral site of bentonite with $\delta = 0.36$ mm/s and $\Delta Q = 0.43$ mm/s, another with $\delta = 0.36$ mm/s and $\Delta Q = 0.69$ mm/s corresponding to Fe^{3+} in γ-FeOOH [22–24], and the doublet with the major contribution, with

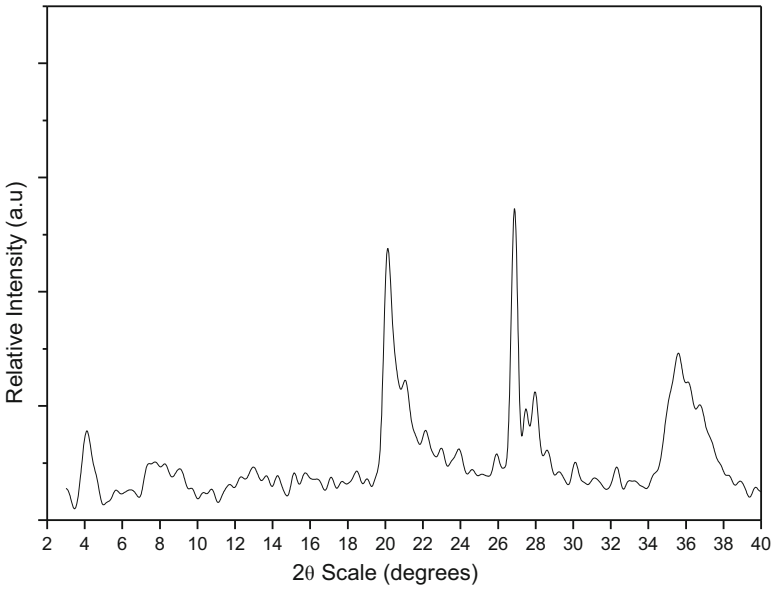

Fig 1 X-ray diffractogram of Fe-PILC

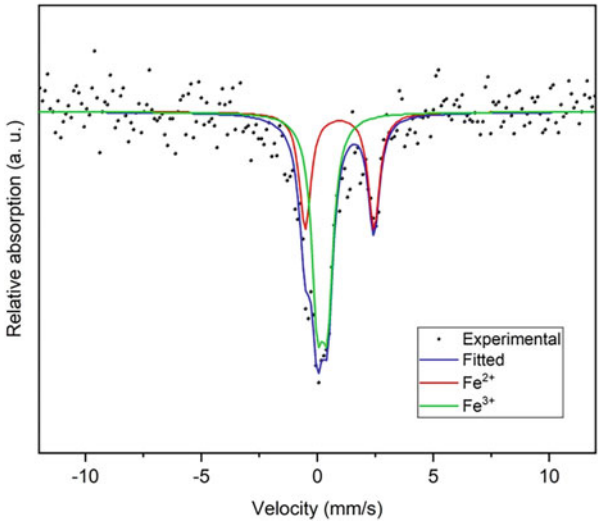

Fig 2 Mössbauer spectrum of bentonite

$\delta = 0.329$ mm/s, $\Delta Q = 0.88$ mm/s assigned to nanoparticles of magnetite (Fe_3O_4) [25–27]. The doublet associated to Fe_3O_4 has a FWHM = 0.71 mm/s suggesting that there is a size distribution of magnetite nanoparticles in Fe-PILC and probably this oxide is forming the pillars [20]. The presence of lepidocrocite (γ-FeOOH) in Fe-PILC is not a surprise since there are reports indicating that lepidocrocite is an intermediate product in the magnetite synthesis [28–30]. Table 1 shows the

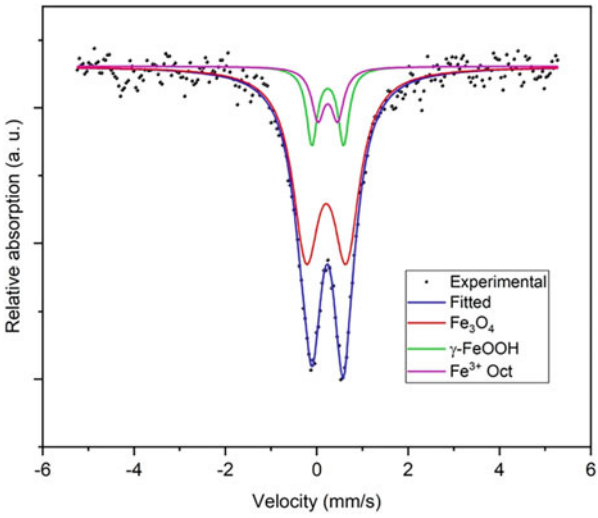

Fig. 3 Mössbauer spectrum of Fe-PILC at room temperature

Table 1 Mössbauer parameters of pure bentonite and Fe-PILC at room temperature

Site	δ (mm/s)	ΔQ (mm/s)	Γ (mm/s)	%
Bentonite				
Fe^{3+}	0.340 ± 0.072	0.44 ± 0.01	0.6[a]	60.0 ± 0.8
Fe^{2+}	1.079 ± 0.094	2.96 ± 0.02	0.6[a]	40.0 ± 1.0
Fe-PILC				
Fe_3O_4	0.329 ± 0.030	0.88 ± 0.03	0.710 ± 0.072	76.0 ± 0.5
γ-FeOOH	0.362 ± 0.037	0.69 ± 0.01	0.28 ± 0.02	14.0 ± 0.2
Fe^{3+} Oct	0.363 ± 0.052	0.43 ± 0.02	0.34 ± 0.02	10.0 ± 0.3

δ is the isomer shift respect to metallic iron, ΔQ the quadrupole splitting, and Γ the FWHM
[a]Fixed

Mössbauer parameters of pure bentonite and Fe-PILC at room temperature. It is important to mention that the powder of Fe-PILC is attracted by a magnet confirming the presence of magnetite, and this is in agreement with XPS results [31].

Regarding textural properties, the clay pillaring process implies an increase in specific surface area and pore volume. The specific surface area of bentonite (34 m^2/g) increases to 277 m^2/g, and pore volume increases from 0.058 to 0.106 cm^3/g. This is attributed to the formation of iron oxides between clay layers [32]. For N_2 physisorption studies, a Quantachrome Autosorb analyzer was used with N_2 adsorption relative pressure $P/P_o = 0.99$ and 77°K, and degassing condition clay were achieved at 250°C for 2 h under vacuum of 6.6×10^{-9} bar. The specific surface area was calculated according to Brunauer-Emmett-Teller method (BET). The iron content of the Fe-PILC used in this work was 17%, and this was established by atomic absorption using an AA240FS VARIAN spectrometer with a calibration curve of a standard solution of Fe.

3 Effluent Characterization

The treated effluent was obtained from an NSAID-manufacturing plant in Lerma (State of Mexico) and was sampled according to the official Mexican norm for wastewater sampling (NMX-AA-003-1980). Sampling point was in the production area that connects directly to the municipal sewer. The following physicochemical characteristics of the effluent were determined: total organic carbon (TOC) with a Shimadzu TOC analyzer, TOC-L CPN with integrated autosampler Shimadzu ASI-L, chemical oxygen demand (COD) according to the NMX-AA-030-SCFI-2001 using a HACH DR/5000 and a digestive solution HACH, total suspended solids (TSS) and temperature according to NMX-AA-034-SCFI-2001 and NMX-AA-007-SCFI-2013 norms, and turbidity and dye with the NMX-AA-0038-SCFI-2001 and NMX-AA-45-SCFI-2001 methods in a HACH DR/4000. The quantification of paracetamol (PAR) in water was determined by the method reported by San Juan [33] using liquid chromatography tandem-mass spectrometry (LC-MS/MS) and employing an Agilent 1290 Infinity HPLC unit (Santa Clara CA) and an RRHD Plus C_{18} (2.1 × 50 mm, 1.8 μm) chromatography column.

The official Mexican norm responsible for regulating the discharge of wastewater to sewage systems (NOM-002-SEMARNAT-1996) establishes limits only for COD, TSS, and temperature, these being 500 mg/L for COD and 220 mg/L for TSS and temperature of 40°C. The lack of inclusion of more physicochemical characteristics has led to increase water pollution and therefore damage to aquatic systems. Turbidity, TOC, and COD are key to the efficiency of the effluent treatment with photo-Fenton, being turbidity a limitation in the use of light [34]. The photo-Fenton process is used with low values of TOC and COD. Therefore, it is important to mention that the effluent COD and TOC are very high in comparison with effluents studied by Klamerth and Michael [2, 35]. Actually, it is worth clarifying that the TOC values of the industrial effluent were so high that mineralization was not observed at all and therefore it was decided to test different dilution degrees. It was found that the minimum dilution degree to observe mineralization was 1:100. The characterization of such diluted effluent is shown in Table 2.

4 Effluent Treatment

The results presented here were obtained in a reaction system consisting of a Pyrex glass batch reactor with a volume of 100 mL (2.5 cm in diameter and 20 cm of height), equipped with a UVP Pen-Ray Lamp of mercury of 5.5 W UV light (UVP) placed inside at the center of reactor. This lamp emits primary energy at 254 nm with a typical intensity of 4,400 μW/cm^2 and uses a UVP Pen-Ray power supply of 115 V/60 Hz. Also, there was inside the reactor an electrode Boeco Germany BA 17 connected to a pH meter accumet XL15, Fisher Scientific. Stirring was conducted using a magnetic stirrer and temperature was kept constant with a water bath. The

Table 2 Effluent characterization prior treatment

Parameters	
TOC (mg/L)	178.0 ± 0.14
COD (mg/L oxígeno)	181 ± 0.7
pH	7.95 ± 0.005
Conductivity (μS/cm)	192 ± 0.1
Temperature (°C)	20
Turbidity (NTU)	193.0 ± 0.7
Dye (mg/L (Pt-Co))	0.45 ± 0.007
Paracetamol concentration (mg/L)	1.7 ± 0.03
TSS (mg/L)	12

photoreactor was intermittently operated using a reaction volume of 30 mL of effluent to be treated, and the experiments were performed using the following methodology: Initially, the effluent was charged, and thereafter, the catalyst was added and then stirred. After the pH was measured and adjusted as required (2.7, 5, or 8), the UV lamp was turned on and finally H_2O_2 (30%) was added. The temperature (T) and stirring were kept constant at 30 ± 2°C and 800 rpm. Samples taken at different reaction times were subjected to a separation process in a centrifuge BOECO M-240 to remove the Fe-PILC. Sulfuric acid (96.9%) and hydrogen peroxide (30%), both from Fermont, were used to conduct the photo-Fenton process.

The studied variables were (1) initial catalyst concentration, (2) pH, (3) initial concentration of H_2O_2, and (4) initial concentration of total organic carbon of effluent. Given the diversity of materials used for the cleaning of areas coupled with those used in the manufacturing process, it was decided to use as primary response variable the total organic carbon (TOC) content.

In order to make evident the effect of photo-Fenton, initially, the effluent was separately treated with hydrogen peroxide (H_2O_2), catalyst (Fe-PILCs), and UV light. Experiments combining all of them (photo-Fenton) were also carried out. Total organic carbon (TOC) was used as response variable. It is worth pointing out that the H_2O_2 stoichiometric amount used in all experiments was calculated based on the TOC content of the effluent [36]. Figure 3 shows that in terms of mineralization, the best treatment of the effluent is the photo-Fenton process, since it shows a significant difference regarding TOC values with the other essayed treatments after 3 hours of reaction. These results showed that the photo-Fenton process can be carried out without changing pH effluent. At the same conditions, however, and only decreasing pH to 2.7, a greater mineralization of 51.0% was attained. Therefore, the other variables were studied under a pH 2.7, and further below the results of a systematic study of pH are also presented.

The results shown in Fig. 4 allow to discard the adsorption of contaminants onto the catalytic surface. Furthermore, from Fig. 4, it can also be inferred that the hydroxyl radical generation by photolysis of hydrogen peroxide via reaction 7 [37] is rather slow.

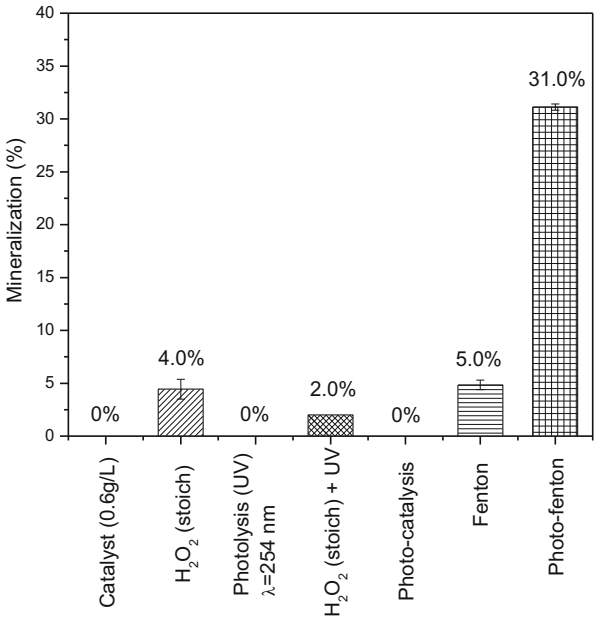

Fig. 4 Effluent mineralization percentage (%) after each treatment. Experimental conditions: catalyst loading = 0.6g/L; temperature = 30°C; pH = 8; stirring speed = 800 rpm; reaction time = 180 min

$$H_2O_2 + hv \rightarrow 2HO^{\cdot}O_2 \quad (7)$$

4.1 Effect of Catalyst Loading

The photo-Fenton process was performed with five different loadings of Fe-PILC, i.e., 0.4, 0.5, 0.6, 0.7, and 0.8 g Fe-PILC/L with a reaction time of 180 min. It can be observed in Fig. 5 that the smallest mineralization degree and rate were with a catalyst loading of 0.4 g/L. This can be ascribed to a low generation of hydroxyl radicals due to a small amount of Fe to catalyze hydrogen peroxide dissociation [38]. There were not significant differences with other catalyst loadings indicating that degradation exhibits the same oxidation resistance [39]. Still, if initial reaction rates are calculated (Fig. 5b), it can be observed that the best initial reaction rate is attained with a loading of 0.5 g/L and that initial reaction rate decreases after this catalyst dosage. This can be ascribed to an increase on turbidity that causes a reduction in UV light absorption, consequently decreasing the photoreduction of Fe(III) (reaction 6) [34, 40, 41]. The effect of this variable gives evidence that resistance to mass transfer from liquid to solid is negligible.

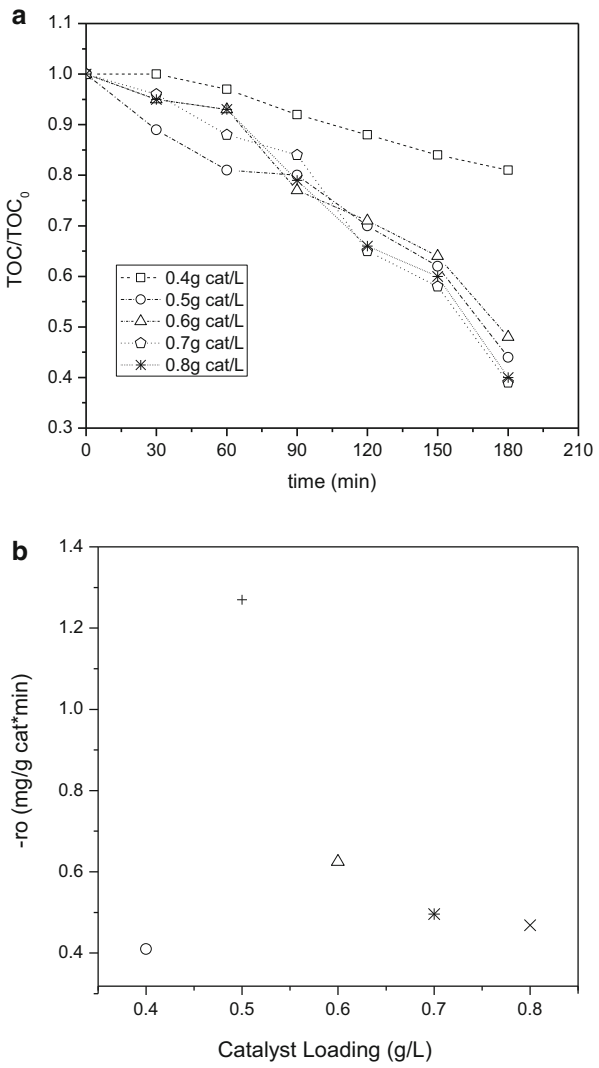

Fig. 5 (**a**) and (**b**) Effect of catalyst loading on TOC normalized content. Experimental conditions: temperature = 30°C; pH = 2.7; stirring speed = 800 rpm

4.2 Effect of pH

A key variable to efficiently perform homogeneous Fenton and photo-Fenton processes is pH. This should be kept around 2.8 in order to minimize the formation of iron complexes. This pH value, however, is considered as a limiting factor [35, 42], and therefore, it is an important variable to be assessed in heterogeneous processes. This variable was studied in the photo-Fenton process at three values (2.7, 5.0, and 8.0). To achieve the acidic values, 0.1 M H_2SO_4 was added. The results plotted in Fig. 6 show that both mineralization degree and rate are significantly

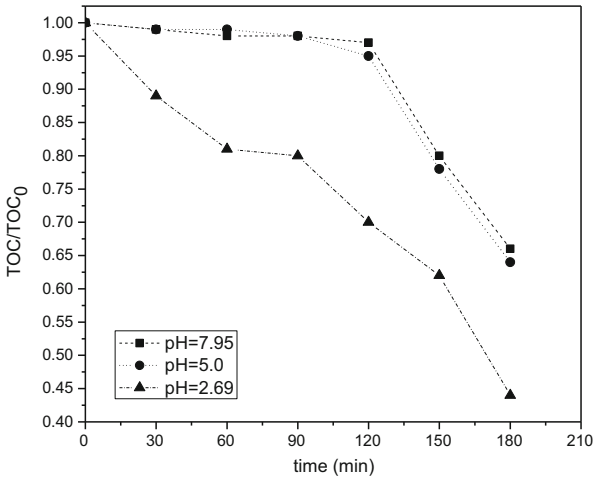

Fig. 6 Effect of pH on normalized TOC profile. Reaction conditions: catalyst loading = 0.5g/L; temperature = 30°C; stirring speed = 800 rpm

affected by pH. After 180 min of reaction, the mineralization percentages were 34, 32, and 56 at pH values 8, 5.0, and 2.7, respectively. The mineralization enhancement can be attributed to the iron oxides in the pillars of clay [43].

Iron leaching was quantified by atomic absorption and was found to be dependent on pH. At high pH, 5 and 8, an iron leaching of 1.3% was measured. At acidic pH, the iron leaching was greater than 4.4%. This suggests that the increase on mineralization rate can partially be ascribed to homogeneous photo-Fenton being promoted due to the higher leaching. Also, it is likely to be more rapid mineralization by reaction of iron oxides in the pillars with H^+ (reactions 8 and 9) increasing the production of radicals HO [44]. Despite pH 2.7 leading to a faster and higher mineralization, the photo-Fenton process without changing the pH of the effluent has the advantage of avoiding the use of additional reagents to neutralize the treated effluent.

$$2FeOOH + 4H^+ + H_2O_2 \leftrightarrow Fe^{2+} + O_2 + 4H_2O \quad (8)$$

$$Fe^{2+} + H_2O_2 \leftrightarrow Fe^{3+} + HO^{\cdot} + HO^{-}O_2 \quad (9)$$

4.3 *Effect of H_2O_2 Concentration*

This variable was studied at three values (0.5∗stoich, stoichiometric, and 2∗stoich). The stoichiometric amount was calculated based on the TOC content of the effluent to be treated. In Fig. 7, it can be seen that the effluent mineralization was significantly affected by this variable. It can be observed that an H_2O_2 concentration above and below the stoichiometric one leads to a plateau after only 60 min of reaction. The

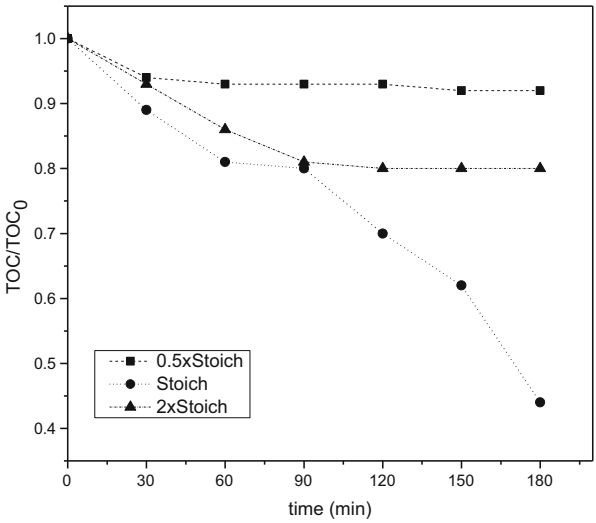

Fig. 7 Effect of H_2O_2 concentration on TOC normalized content. Experimental conditions: pH = 2.7; catalyst loading = 0.5 g/L; temperature = 30°C; stirring speed = 800 rpm

attained mineralization was 8% and 20%, with half and twice the stoichiometric amount of H_2O_2, respectively. The lowest achieved value (8%) can be attributed to the lack of H_2O_2 that limits the hydroxyl radical concentration and therefore is insufficient to react with organic molecules [45]. It was expected that increasing the concentration of H_2O_2 favored effluent mineralization due to the relationship with production of $HO^{\cdot}$ radicals. This was not observed though. An excess of H_2O_2 probably caused a scavenging of HO radicals by reactions 10, 11, and 12 [12, 40, 46]. The best results were obtained by using the stoichiometric H_2O_2 amount, and an increase on the initial rate and mineralization percentage (56%) after 180 min of reaction was observed.

$$H_2O_2 + HO\cdot \rightarrow HO_2^{\cdot} + H_2OO_2 \quad (10)$$

$$HO_2^{\cdot} + HO\cdot \rightarrow H_2O + O_2O_2 \quad (11)$$

$$HO\cdot + HO\cdot \rightarrow H_2O_2 \quad (12)$$

4.4 Effect of TOC Initial Concentration

The effect of TOC initial content is shown in Fig. 8. The experiments were performed with the following contents: 59, 95, and 178 mg/L with 0.5 g/L Fe-PILCs, stoichiometric concentration of hydrogen peroxide from 178 mg/L TOC, and pH between 2.65 and 2.69. It can be seen that the percentage of mineralization increases from 56% to 91% when decreasing the TOC content from 178 to 95 mg/L. Moreover, the maximum effluent mineralization percentage is not affected

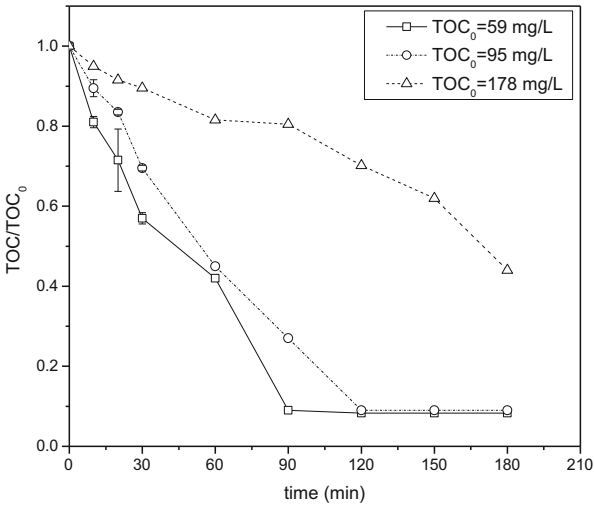

Fig. 8 Effect of initial TOC on mineralization extent. Experimental conditions: pH = 2.7; catalyst loading = 0.5 g/L; temperature = 30°C; stirring speed = 800 rpm

by initial TOC lower than 95 mg/L. Feij Ji et al. also evaluated this variable and reported a similar behavior [38]. At this concentration, it might be that organic molecules absorb less photons in such a way that the photoreduction of Fe(III) is less affected [34, 37, 39, 40]. Thus, the concentration of H_2O_2 dissociation catalyst is higher.

4.5 Effluent Characterization After Photo-Fenton Treatment

In Table 3, a decrease in all parameters after photo-Fenton treatment can be observed. The treatment conditions for such an effluent were as follows: 0.5 g/L of Fe-PILC, stoichiometric concentration of hydrogen peroxide, and pH 2.7. These

Table 3 Effluent characterization after treatment

Parameters	
TOC (mg/L)	78 ± 0.2
COD (mg/L)	56 ± 0.7
pH	2.69 ± 0.01
Conductivity	111 ± 0.7
Temperature	30 °C
Turbidity (NTU)	77 ± 0.7
Dye mg/L (Pt-Co)	0.09 ± 0.007
Concentration (mg/L)	0.006 ± 0.0001
TSS (mg/L)	0

The results are shown as mean ± standard deviation of two replicate samples

results and those from the acute toxicity study (see Sect. 5) demonstrate that this process can be effectively used to treat wastewater from pharmaceutical industry.

5 Oxidative Stress Determination Prior and Posttreatment Using *Hyalella azteca* as Biomarker

5.1 Procurement, Culturing, and Maintenance of Specimens

Hyalella azteca was collected from its natural habitat in San Miguel de Almaya Lake, municipality of Capulhuac (State of Mexico), and transported to the laboratory under constant aeration in plastic bags. Breeding stock was transported to the laboratory using the water source in which the organisms were reared. Water used for transporting organisms was well oxygenated (90–100% saturated). Upon arrival at the testing laboratory, the organisms were gradually acclimated to the laboratory holding and testing conditions so they would not get stressed. Test organisms were in good health, and the mortality rate for juvenile *Hyalella* did not exceed 20% [47]. The collected organisms were morphologically identified [48]. To eliminate potential differences in sensitivity to contaminants due to acclimation to local conditions or maternal effects, we used organisms from the same clade that had been cultured under the same feeding conditions, temperature, and photoperiod for approximately 4 months (third-generation neonates obtained by sexual reproduction). During culture, specimens were maintained in reconstituted water ($NaHCO_3$ = 174 mg L^{-1}; $MgSO_4$ = 120 mg L^{-1}; KCl = 8 mg L^{-1}; and $CaSO_4.2H_2O$ = 120 mg L^{-1}; all reagents were obtained from Sigma-Aldrich, St. Louis MO), pH 7.5–8.5, room temperature with constant oxygen (6.4–6.6 mg L^{-1}, O_2), and 12 h/12 h light/dark photoperiod and were fed ground lettuce ad libitum. The estimated number of surviving adults and the production of young in each culture chamber, dates of culture renewals, numbers and age classes of transferred individuals, daily feedings, and water quality measurements were documented.

5.2 Artificial Sediment

The employed artificial sediment was 70% sand (0.2 mm), 20% kaolinite (<0.002 mm), and 10% organic matter (0.2 mm). The organic matter source was lamb compost inactivated by dry heating at 55–60°C for 3 days. The sediment was sterilized with three 15-min autoclave cycles at 121°C and 15-lb pressure, separated by 1-h interval [49, 50].

5.3 Oxidative Stress

Test systems were set up by adding industrial effluent and artificial sediment in a 3:1 ratio to 50-ml polyethylene containers equipped with constant oxygenation and maintained under a 12-h/12-h photoperiod at room temperature. Light intensity adjacent to the surface of the overlying water was 500 lux. The test was conducted at a daily mean temperature (overlying water) of 23 ± 1°C. Static systems were used, the medium was not replaced, and no food was provided to specimens during exposure. These systems were added with 1 g of biomass (*Hyalella azteca*), and the exposure time of these organisms to such systems was 96 h. The oxidative stress of an industrial effluent containing paracetamol was determined prior treatment and posttreatment. Once the exposure time was over, 1 g of *Hyalella azteca* was homogenized with phosphate buffer solution. The oxidative stress of the homogenized system was established through lipoperoxidation (LPX) degree by a previously reported method [51]; carbonyl proteins content (CPC) by the modified method of Levine et al. [52]; cumene hydroperoxide (CHP) content by the method of Jiang [53]; activity of the SOD by the method of Misra and Fridovich [54]; and CAT by the method of Radi et al. [55]. This was conducted by triplicate. Also, the protein content was determined [56] in order to normalize the results of the assessed biochemical parameters.

The results in Table 4 show the *Hyalella azteca* oxidative stress biomarkers after 96 h of exposure to treated and untreated effluent samples. It can be observed that the cell oxidation biomarkers were reduced in an interval of 28.6–31.3% with the photo-Fenton treatment. Concomitantly, the antioxidant enzymes were reduced to 28.1–32.51% with the treatment. Based on the summarized results in Table 3, it can be concluded that the treated industrial effluent was less toxic than the untreated one and that, generally speaking, there was a reduction of 30% in all assessed oxidative stress biomarkers.

Regarding oxidative stress, the obtained results in this study are in concordance with those previously reported by Novoa-Luna et al. [57] although at 72 h exposure time. Different studies have pointed out that nonsteroidal anti-inflammatory pharmaceutical compounds like paracetamol are unsteady and photodegraded. Also, it

Table 4 Oxidative stress biomarkers in *Hyalella azteca* prior treatment and posttreatment

Biomarker	Before treatment	After treatment	Biomarker reduction (%)
Lipoperoxidation degree (LPx) [nM de MDA/mg protein]	0.16	0.11	31.3
Hydroperoxides content (CHP) [nM CPH/mg protein)	0.7	0.5	28.6
Carbonyl content in proteins (mM reactive carbonyls/mg proteins)	1.22	0.86	29.5
SOD activity (UI SOD/mg protein)	2.86	1.93	32.5
CAT activity (mM de H_2O_2/mg protein)	32	23	28.1

has been shown that their metabolites are actually more toxic than the parent compounds for aquatic organisms like *Hyalella azteca* [58, 59]. The increase in the oxidative stress biomarkers can be ascribed to the biotransformation of paracetamol (i.e., an NASAID present in the effluent) by the subfamily of cytochrome P450, CYPWC9, that allows the formation of reactive oxygen species (ROS). These ROS can be OH and oxygenated intermediates like the oxy-cytochrome P450 complex [P450 (Fe^{3+}) O^{2*}] as a result of the release of the superoxide anion by reaction decoupling. In both cases, ROS production is increased, which explains the observed increases in LPX and HPC. Also, Gómez-Oliván et al. [60] found similar effects when *H. azteca* was exposed to paracetamol at 770 mg kg^{-1}. This increase may be due to the formation of N-acetyl p benzoquinonimine which is able to bind to cellular membranes.

Thus, the results herein presented show that the photo-Fenton process conducted under pH 8 reduces the toxicity of the pharmaceutical industrial effluent and therefore the oxidative stress biomarkers are considerably reduced. Therefore, it can be concluded that the process is effective at achieving both chemical and biological efficiencies. It is worth pointing out that despite the high mineralization degree achieved under acid pH, the oxidative stress biomarkers were not reduced, thus indicating a high degree of toxicity, probably due to the acid condition of the treated effluent. This implies the need of further addition of chemicals to neutralize the treated effluent and represents a disadvantage of the process that can be overcome by using the proposed catalyst Fe-pillared clay.

6 Conclusions

An industrial effluent was mineralized by photo-Fenton process catalyzed with an iron-pillared clay. The use of this catalyst not only facilitates its recovery (e.g., by magnetism) and reuse after treatment but also allows the use of pH conditions different to those commonly required acidic for a Fenton process. This treatment becomes effective when the effluent has an initial TOC of approximately 200 ppm, otherwise must be diluted. Furthermore, in this process, it is essential to add hydrogen peroxide (H_2O_2) in a stoichiometric ratio with TOC from effluent to be treated; otherwise, other less efficient oxidation mechanisms are promoted. This process leads to a relatively good mineralization degree even without pH modification. A decrease of pH favored Fe leaching and the maximum observed was 4% at a pH 2.7. The results of oxidative stress biomarkers show that the applied process is not only chemically effective but also biologically at pH 8.

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Use of Membrane for Removal of Nonsteroidal Anti-inflammatory Drugs

Rosa María Gómez-Espinosa and Daniel Arizmendi-Cotero

Contents

Abstract Nonsteroidal anti-inflammatory drugs (NSAIDs) belong to most used pharmaceuticals in human and veterinary medicine, the emerge of drugs in the environment is a concern subject. The contamination is due to the consumption and the excretion of large quantities of pharmaceuticals via urine and feces in wastewaters. In this chapter, the reader will have an overview of the use of different types of membranes and their combined method in the removal of NSAIDs and demonstration that the use of membrane could be an environment-friendly methodology that enhances its efficiency in the removal of these compounds.

Keywords Membrane bioreactor, Nanofiltration, Nonsteroidal anti-inflammatory drugs, Reverse osmosis, Ultrafiltration

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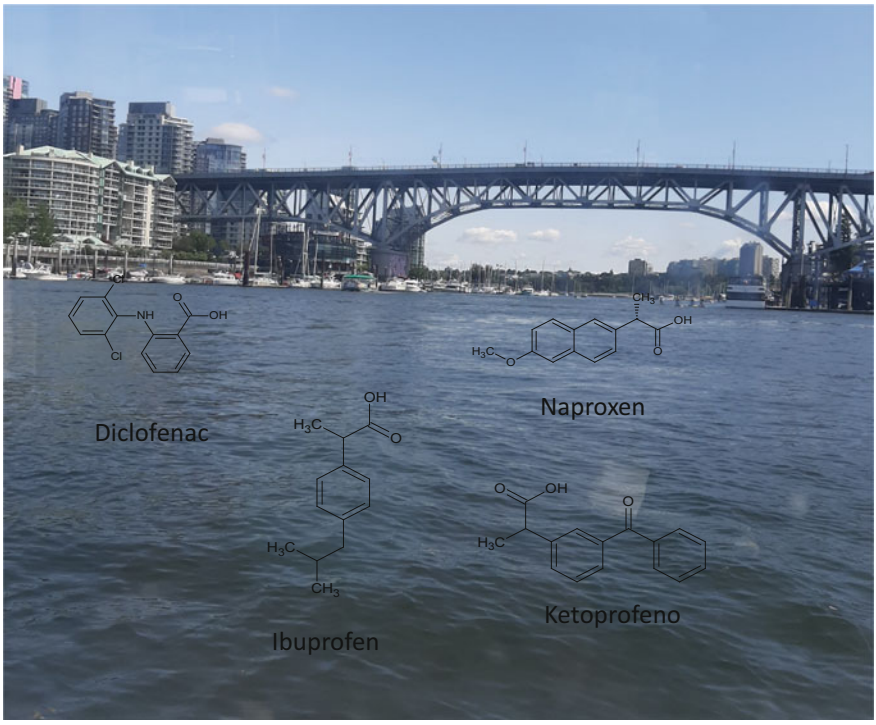
1 Introduction

In the past decades, there has been a growth in the number of published articles that have focused on the environmental monitoring of nonsteroidal anti-inflammatory drugs (NSAIDs). Among the emerging environmental contaminants, pharmaceutically active compounds have become a growing public concern because of their potential to cause undesirable ecological and human health effects. The concentrations of five common nonsteroidal anti-inflammatory drugs, diclofenac, ketoprofen, naproxen, ibuprofen, and acetylsalicylic acid, were determined in surface waters [1]. Nonsteroidal anti-inflammatory drugs are a drug class FDA approved for use as antipyretic, anti-inflammatory, and analgesic agents [2]. These effects make NSAIDs useful for the treatment of muscle pain, dysmenorrhea, arthritic conditions, pyrexia, gout, and migraines and used as opioid-sparing agents in certain acute trauma cases [3–5].



It is believed that the water sources are contaminated with a variety of pharmaceutical compounds due to the absence of wastewater separation and limitation of sanitation sewer systems. Most frequently, conventional treatment processes applied at domestic wastewater treatment plants fail to remove completely pharmaceutical substances; that is why it is very important to explore a new technology using

membranes and a combined membrane process in order to remove nonsteroidal anti-inflammatory drugs and improved the quality of water.

2 Consumption and Presence of NSAIDs in Wastewater

Worldwide acute or chronic pain and fever are the main symptoms of numerous disorders and are the main reasons for medical consultation. Inflammation is a pathogenetic factor in many diseases and also is an outcome of physical damage (blows and injuries, among others). In classical medicine are used drugs possessing antipyretic, analgesic and anti-inflammatory activities [6], to counteract these symptoms.

Among the most common pharmaceuticals are nonsteroidal anti-inflammatory drugs (NSAIDs), and the most consumed of these drugs in the world are diclofenac, ketoprofen, naproxen, ibuprofen, and acetylsalicylic acid. For example, in Germany in 2001, they reported a consumption of acetylsalicylic acid 836 tons, paracetamol 622 tons, ibuprofen 345 tons, and diclofenac 86 tons. In England in 2000, the use of naproxen was 35 tons [7]. The occurrence of several drugs has been reported in are sewage treatment plant (STP) effluent, as well as in surface and drinking water in Brazil, Canada, China, Germany, Italy, Spain, Switzerland and the United States [6, 8–15].

Various drugs have been extensively studied, such as ibuprofen (IBU), (RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid; naproxen (NPX), (+)-(S)-2-(6-methoxynaphthalen-2-yl) propanoic acid; and ketoprofen (KPF), (RS)-2-(3-benzoylphenyl) propanoic acid, not only in wastewater but also in drinking water sources [14, 16, 17]. Salgado et al. [18] identified 73 pharmaceutical active compounds of diverse families in a municipal wastewater treatment plant. Shanmugam et al. [1] report presence of diclofenac, ketoprofen, naproxen, ibuprofen, and acetylsalicylic acid in surface waters from 27 locations of the Kaveri, Vellar, and Tamiraparani Rivers in Southern India. Farré et al. [19] describe a work collaboration of 13 laboratories distributed in nine European Countries exercise for the analysis of nonsteroidal anti-inflammatory drugs (NSAIDs). The compounds selected in this study were ketoprofen, naproxen, ibuprofen, and diclofenac. Analyses samples were river water, wastewater, and artificial water (fortified environmental and distilled water) with different ranges of complexity. For its part, Petrovic et al. [20] also reports presence of analgesics and anti-inflammatory drugs. Table 1 summarizes the concentration ranges of NSAIDs detected in these papers.

Some research has focused on the NSAID residues in the environment. Nishi et al. [21] monitored the concentrations of seven NSAIDs in domestic wastewater in Japan. The concentration averages of diclofenac, ibuprofen, salicylic acid, ketoprofen, mefenamic acid, felbinac, and naproxen in wastewater were, in order, 259.7, 162.9, 55.3, 48.3, 39.7, 30.8, and 11.8 mgL^{-1}, respectively. Lolić et al. [22] recognize maximum concentrations between 5.34 mg L^{-1} and 1,227 ng L^{-1} for acetylsalicylic acid and carboxyibuprofen, respectively, in seawaters of Portugal.

Table 1 Range (maximum value–minimum value) of concentration of some drugs present in wastewater (μgL^{-1})

Diclofenac	Etofenamate	Ibuprofen	Ketoprofen	Naproxen	Acetylsalicylic acid	Salicylic acid	Acetaminophen	Reference
64.5	40.2	52.2	104.1	0	0	0	0	Salgado et al. [18]
0.103	0	0.2	0.1	0.028	0.66	0	0	Shanmugam [1]
3.248	0	2.717	1.175	0.228	0	0	0	Farré [19]
4.06	0	372.619	2.792	17.062	18.93	14.1	25.96	Petrovic [20]

For zero value, it means that the NSAIDs was not analyzed

He et al. [6] reported 17 mgL^{-1} of ibuprofen and 2 mgL^{-1} for propiphenazone as the highest and lowest concentrations, respectively, with respect to the other nineteen NSAIDs found in water samples, taken from six drinking water purification plants and two water purification plants in Japan. Among all detectable NSAIDs in the environment, diclofenac and ibuprofen often showed the highest concentration and detection rates [6].

3 Mechanisms of Elimination of NSAIDs and Influence Variables

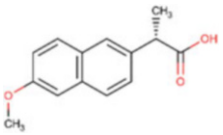
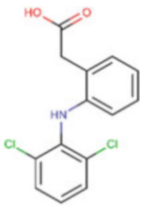
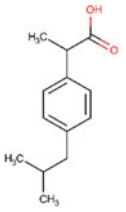
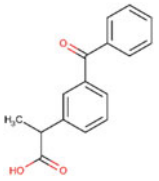
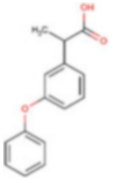
The elimination of NSAIDs can occur through various mechanisms in the activated sludge process, mainly by biodegradation, sorption, or volatilization [23]. Sewage sludge is designed to substantially degrade the organic compounds by microbial metabolism, which varies depending on operating conditions such as sludge retention time (SRT), hydraulic retention time (HRT), and temperature [16]. Longer HRTs involve mayor contact time between the activated sludge and organic compounds and thus better removal efficiency [24, 25]. Sorption onto sludge, referring to hydrophobic or electrostatic interactions with the biomass, is a common mechanism whose effectiveness depends on the physicochemical properties of the compounds and the biomass concentration [13]. Adsorption to the sludge of hydrophilic compounds is limited [26], and, consequently, their removal by sorption processes is inefficient and can impede the biodegradation of these compounds too [27].

The adsorption process also intervenes in operating conditions in the NSAID retention processes (e.g., temperature, pH, ionic strength, or porous characteristics of the adsorbent and aqueous matrix) [28]. As well, the physicochemical properties of NSAIDs interfere with the adsorption process. The NSAIDs are weak organic acids with a carboxylate moiety present in them [29, 30]. Their acid dissociation constants (pKa) range from 4.00 to 4.91, while their octanol-water partition coefficients (Kow) range from 1.10 to 3.97, implying that NSAIDs exist as dissolved neutral species under normal environmental conditions [31, 32]. Their high water solubility and polar nature lead to difficulty in their removal efficiency in wastewater treatment plants [33, 34]. The physicochemical properties relevant to their existence in water bodies are given in Table 2.

4 Filtration Method

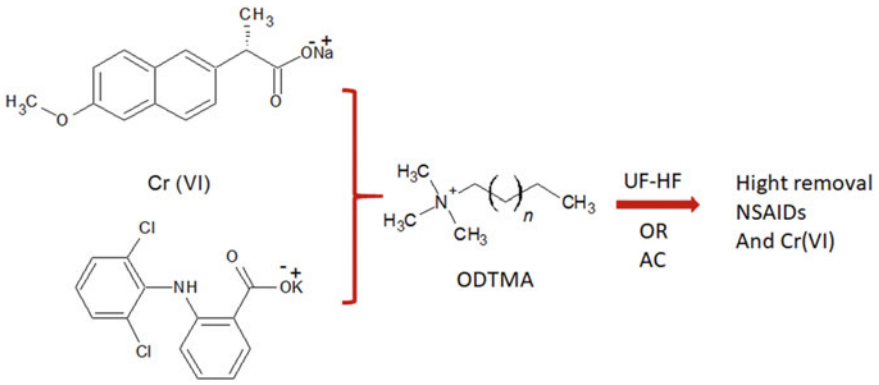
The filtration method using membranes as removal material has revolutionized the remotion of emerging contaminant from wastewater. Qurie et al. [36] reported in 2014 the combined use of membranes with adsorbent compounds had resulted as an efficient method for remotion of nonsteroidal anti-inflammatory drugs (NSAIDs);

Table 2 Chemical structures and physicochemical properties of NSAIDs

NSAIDs	Chemical structure	CAS number	Weight	Log know	pKa	Water solubility (mgL^{-1})
Naproxen		15,307–86-5	296.149	3.18	4.19	44
Diclofenac		15,307–86-5	296.149	4.51	4	10
Ibuprofen		15,687–27-1	206.2808	3.97	4.91	58
Ketoprofen		22,071–15-4	254.2806	3.12	4.45	51
Fenoprofen		29,679–58-1	242.2699	4.05	4.5	81

Information: (https://www.drugbank.ca/) assessed in the period between November and December (2019), [35]

for example, the cationic ODTMA-micelle-clay combined with ultrafiltration (UF) (hollow fiber HF and spiral wound SW) membranes, activated carbon (AC), and reverse osmosis (RO) has demonstrated high removal efficiency toward these two NSAIDs and naproxen metabolite (DMN). Besides, the ODTMA-micelle-clay complex has been found capable of completely removing the heavy metal Cr (VI) from its aqueous solutions at ambient pH and temperature.



The efficiency of filters is based on the use of a micelle-clay complex to polish the tertiary treated wastewater that is generated from ultrafiltration plants by using hollow fiber membranes with 100 kD cutoff filters. Solutions of UF-hollow fiber permeate were passed through the column filter performed with 100/1 or 50/1 (w/w) mixtures of quartz sand and ODTMA-clay complex with two flow rate modes at 1.2 mL min^{-1} and 50 mL min^{-1} [37].

The filtration experiment was performed by using a laboratory column (18 × 4 cm) prepared by mixing 3.0 g of micelle-clay complex and 147 g sand. Elution rate was 2 mL min^{-1}, and eluted volume used to investigate the removal efficiency of naproxen was 1,000 mL.

The summary of low flow rate (1.2 mL min^{-1}) indicates that filtration of tertiary treated water obtained from HF ultrafiltration by micelle-clay complex with excess sand reduced significantly the FC, TC, BOD, EC, turbidity, and COD of effluent.

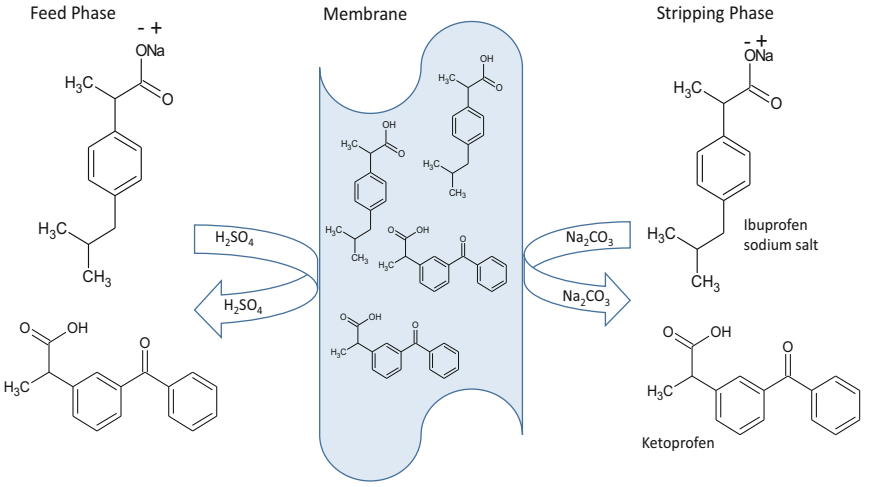
In this study, the effectiveness of ODTMA-micelle-clay complex for the removal of Cr(VI) anion from aqueous solutions has been investigated using either clay (montmorillonite) or micelle-clay complex. Batch experiments have showed the effects of contact time, adsorbent dosage, and pH on the removal efficiency of Cr (VI) from aqueous solutions. Filtration experiments, using columns filled with micelle-clay complex mixed with sand, were performed to assess Cr(VI) removal efficiency under continuous flow at different pH values.

Column experiments were performed using glass columns (18 × 4 cm) prepared by mixing 3.0 g of ODTMA-micelle-clay complex and 147 g sand. The results indicate that complete removal of chromium was achieved at all studied pH values. However, at pH 1 and 2, the breakthrough point was greater than 1,000 mL, whereas at pH 3, 4, and 6, the saturation point was significantly lower with a value of about 500 mL. These results are consistent with those obtained from batch experiments, indicating that the elution volume plays an important role during the adsorption process at pH values higher than 2 complete removal of Cr(VI) with possible reduction to Cr(III) after the breakthrough points.

The removal of the two NSAIDs, naproxen metabolite (DMN) and Cr (VI) using ODTMA-micelle-clay complex, was studied and compared with that of activated charcoal. The adsorption results revealed that ODTMA-micelle-clay complex was more efficient in removing these pollutants than activated carbon as judged by the calculated Qmax and k for both adsorbents.

5 Emulsion Liquid Membrane

Recently, emulsion liquid membrane or surfactant liquid membrane has gained attention as an advanced extraction process for the removal of emerging contaminants present in wastewater. The transport mechanisms of liquid membranes are not only an important technique for concentration, separation, and recovery but also are fundamental importance from an environmental engineering point of view. The emulsion liquid membrane process is carried out by combining extraction and stripping steps in one stage, which leads simultaneous purification and concentration of the solute. Emulsion liquid membrane treatment process represents a very interesting advanced separation process for the removal of nonsteroidal anti-inflammatory drugs from complex matrices such as natural water and seawater [38].



The extraction of IBP and KTP using liquid emulsion membrane involves three steps: preparation of liquid membrane emulsion, removal of the solute from the feed by contacting the emulsion, and separation of liquid emulsion from the external phase.

Volume ratio of internal phase to the membrane phase plays an important role in determining the effectiveness of ELM system. The effect of volume ratios of the

internal solution to membrane phase varied between 1:2 and 2:1, by maintaining membrane volume constant on the removal of IBP.

An emulsion liquid membrane was developed to remove NSAIDs ibuprofen and ketoprofen from water. The optimum experimental conditions for the extraction of IBP were summarized as follows: emulsion volume, 60 mL; external phase volume, 600 mL; volume ratio of internal phase to organic phase, 1:1; emulsification time, 3 min; stirring speed, 250 rpm; concentration of span 80, 3% (w/w); volume ratio of W/O emulsion to external phase, 60:600; internal phase concentration (Na_2CO_3), 0.1 N; diluent, hexane; and concentration of H_2SO_4 in the external phase, 0.1 N. Under the best operating parameters, it was possible to extract nearly all of IBP molecules from the feed solution even in the presence of high concentration of salt. At the optimum experimental conditions, about 97.4% KTP was removed in less than 20 min of contact time. This study demonstrates that ELM treatment in comparison with other techniques that are hindered by the presence of salts is a promising process for the elimination of NSAIDs IBP and KTP from complex matrices such as natural water and seawater.

6 Membrane Bioreactor

Membrane bioreactor (MBR) has become a technically and economically feasible alternative for remotion of emerging contaminant. The upgrading of wastewater treatment plants and implementation of sustainable technologies impose as possible solutions for the safe reclamation of high-quality treated effluent. The MBR technology integrates biological degradation of organic matter present in wastewater with membrane filtration, thus passing the limitations of the conventional activated sludge treatment. Membrane bioreactor has become a technically and economically feasible alternative for water and wastewater treatment [20].

For most of the investigated PhACs, membrane bioreactor effluent concentrations were usually significantly lower than in the effluent of a conventional treatment.

The membrane treatment is a promising process to be able to remove negative-charged NSAIDs from wastewater effluent in source waters due to the negative-charged membrane surface.

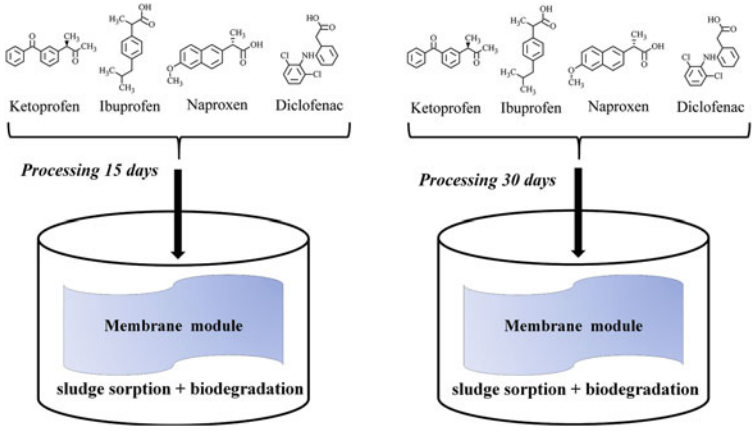
Different materials were compared in terms of rejection of ibuprofen and removal of effluent organic matter from membrane bioreactor (MBR). The membranes used in study by Park et al. [39] were polyethersulfone, polyamide TFC, and titanium oxide, because pharmaceutical compounds contain a potential risk and effluent organic matter is the precursor of carcinogenic disinfection by-products when reusing for drinking water source.

Membrane	NMW (datons)
Polyethersulfone	10,000
Polyamide	8,000
Titanium oxide	1 k, 3 k, 5 k,8 k

Note: NMW, nominal molecular weight

Filtration membrane with a molecular weight cut off of 8,000 Da exhibited 25 ~ 95% removal efficiencies of ibuprofen with a molecular weight of 206 with and without presence of effluent organic matter from membrane bioreactor. The membranes with different nominal molecular weight cut-offs a tight-Ultra. UF membrane could successfully remove ibuprofen at lower J0/k ratio range ($\leq$1) in organic free water.

The sludge retention time (SRT), sludge concentration (SC), and hydraulic retention time (HRT), in the treatment the waste water to scale of the pilot-plants were evaluated by Schröder et al. [24]. The membranes were evaluated during 15 days to 12 g/L and 9 h, and 30 days to 12 g/L and 13 h for. Both MBRs used in this study were equipped with 1.43 m^2 of hollow-fiber ultrafiltration (UF) membranes. To estimate the dilution factor in pharmaceutical compound removal, sodium chloride (NaCl) was aggregated on the tank containing wastewater and membrane. The sludge retention time was performed in separate tanks, for the membranes evaluated during 15 and 30 days.



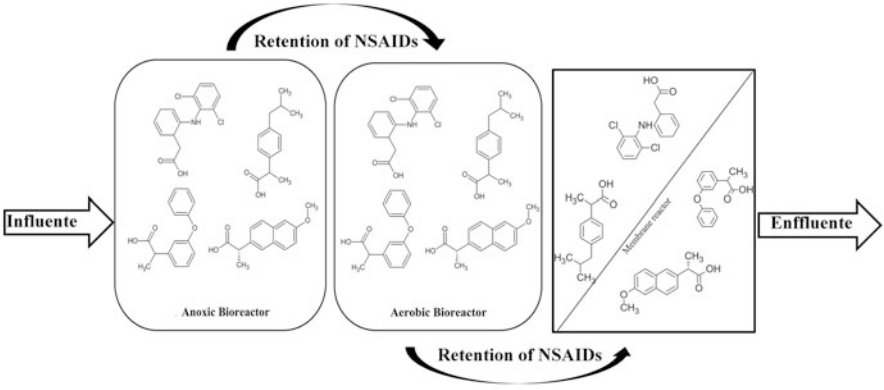
The remotion of each pollutant was the combination between the sludge sorption + biodegradation + membrane retentión. The order of remotion of NSAIDs and antibiotics on both times of treatment, 15 and 30 days, was acetaminophen > ketoprofen > trimethoprim > naproxen > roxithromycin > sulfamethoxazole. The elimination of pharmaceutical compounds can occur in various ways. Sorption onto sludge is one of the mechanisms that take into account the absorption and adsorption factors. The absorption refers to the hydrophobic interactions of the aliphatic and aromatic groups of a compound, while adsorption refers to the

electrostatic interactions of positively charged groups of dissolved chemicals with the negatively charged surfaces of the microorganisms.

The elimination of pharmaceutical compounds can occur in two ways. By absorption because of the hydrophobic interactions of the aliphatic and aromatic groups of a compound, and by adsorption through the electrostatic interactions of positively charged groups of dissolved chemicals with the negatively charged surfaces of the microorganisms.

Another mechanism responsible for the removal of pharmaceutical compounds in MBRs is the physical retention by the membranes. The retention of the pharmaceutical depends on the molecular weight cutoff (MWCO) of MBR membranes. Sorption onto the membranes is also limited by the available membrane surface area. Pharmaceutical compounds which are nonpolar will sorb onto the biomass and will therefore be removed indirectly during the retention of the solids by the membranes. Polar pharmaceuticals, with a low tendency to adsorb to the lipophilic sludge surface, will be eliminated neither by adsorption nor by biodegradation because the interaction with the wastewater biocoenosis essential for the biodegradation process will be too short [4, 11]. A better performance in the treatment of wastewater contaminated by drugs could be achieved by the application of additional treatments, e.g., activated carbon adsorption, ozone oxidation, advanced oxidation processes (AOP), nanofiltration (NF), or reverse osmosis (RO).

González-Pérez et al. [16] report a system of wastewater treatment composed of an anoxic bioreactor (3.6 m^3), aerobic bioreactor (8.8 m^3), and membrane reactor (3.5 m^3). The membrane reactor was equipped with hydrophilicized microfiltration flat-sheet membranes (0.4 μm nominal pore size) made of chlorine polyethylene.



All NSAIDs studied by Gonzalez-Pérez (ibuprofen, diclofenac, ketoprofen, and naproxen) were eliminated from the contaminated water inflow. The ibuprofen was almost eliminated (98%) with removal. Naproxen removal efficiency was similar or slightly higher than the best removal efficiency values previously reported in MBR systems. The average removal of ketoprofen for the experimental MBR was at least

between 80 and 95% depending on the influent concentration. Thus, the removal efficiency was consistently high compared with the effectiveness found for other MBR treatment. Diclofenac, as opposed to the previous substances, was resistant to MBR treatment, demonstrating that DCF was only partially removed by the MBR (21%).

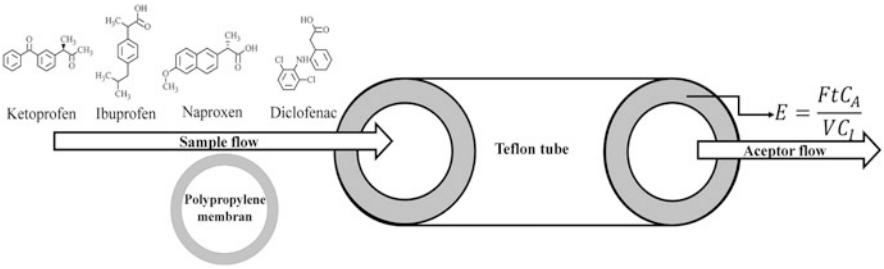
IBU, NPX, and KTP are hydrophilic compounds, so their removal by adsorption to biomass can be considered negligible [11, 40]. Several studies have attributed the high removal effectiveness in MBR of these compounds to biodegradation. The high biodegradability of these compounds causes the greater or lesser effectiveness in elimination to depend on the operational variables of the biological treatment, one of the most influential being sludge retention time.

7 Hollow Fiber Membrane

The hollow fiber membrane liquid-phase microextraction could be a good alternative to extract nonsteroidal anti-inflammatory drugs from aqueous samples. It has been reported that a supported liquid membrane in the pores in the wall of a small porous hollow fiber can be used on bioanalytical and environmental chemistry, where analytes are extracted through the supported liquid membrane by the application of electrical potentials [41].

Liquid-phase membrane extraction is based on passive diffusion, and the flux of analyte across the support liquid membrane is basically controlled by distribution ratios. Recently, LPME was reported with a direct-current electrical potential difference across the SLM as the driving force for extraction based on electrokinetic migration [42]. This technique was termed electromembrane extraction. For the extraction of basic drugs, pH in the sample (300 L) and in the acceptor solution (30 L) was adjusted to 2.0 with HCl to ensure full ionization of the target analytes.

Larsson et al. [33] developed a continuous flow system for the elimination of NSAIDs by liquid-phase microextraction (SPME). In a Teflon tube, polypropylene membranes placed 30 mm wall thickness, 240 mm id, and 0.1 mm by size. The tube was fed by a flow of stock solution of each sample (ibuprofen, ketoprofen, naproxen, and diclofenac) to 10 mgL^{-1} and mixes of the four analytes diluted in water. Ionizable analytes in neutral form were extracted through the membrane, and the extraction was selectively tuned, depending mainly on pH in sample and extract.



In their experiments, Larsson et al. [33] measured the enrichment (E) of each drug in the membrane, as a variable dependent on the feed flow (F) and the contact time (t) between the solution and the membrane. A flow of 30 mL min^{-1} allowed a longer contact time. However, the enrichment rate (ΔE) during the first 30 or 45 min of each analyte was constant when the concentration in the sample flow (C_A) remained approximately equal to the initial concentration (C_I). Enrichment max time was 45 min for ketoprofen and naproxen and 60 min for diclofenac and ibuprofen. The enrichment (E) for diclofenac and ibuprofen is probably because these analytes have somewhat higher log K_{ow} values and therefore are transferred more slowly from the membrane into the acceptor. Your results show that the method can be applied in sewage treatment plants' effluent matrix with linear extraction in an environmentally relevant concentration range.

8 Conclusion

Numerous studies show the presence of five common nonsteroidal anti-inflammatory drugs: diclofenac, ketoprofen, naproxen, ibuprofen, and acetylsalicylic acid in surface waters. High consumption of these medications and the absence and/or limitations of separating wastewater seem to be the most important causes of contamination. Besides, conventional water treatment processes fail to eliminate pharmaceutical substances.

The combined use of polymeric membranes, emulsion membranes, and/or liquid membranes, with adsorbent compounds such as clay, activated carbon, residual sludge, biomass has demonstrated the high efficiency of elimination toward NSAIDs. But due to the physical and chemical complexity of the compounds, there is no single method that is sufficiently effective against all types of contaminants.

The different variables in the separation processes by membranes also play an essential role in the efficiency of the treatment of wastewater contaminated by NSAIDs. Operating conditions, such as sludge retention time, hydraulic retention time, temperature, pH, ionic strength, or porous characteristics of the adsorbent and aqueous matrix, interfere with the adsorption process.

Drug retention also depends on the molecular weight cutoff (MWCO) of the MBR membranes. The absorption on the membranes is also limited by the surface area available. Nonpolar pharmaceutical compounds are absorbed in the biomass and, therefore, are removed indirectly during the retention of the solids by the membranes.

The results of various investigations show that performance in wastewater treatment could be improved by applying additional treatments, for example, activated carbon adsorption, ozone oxidation, advanced oxidation processes (AOP), nanofiltration (NF), or reverse osmosis (RO), among others.

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Nanotechnologies for Removal of Nonsteroidal Anti-inflammatory Drug from Wastewater

Monica Cerro-Lopez, Jorge Jiménez Cisneros, Miguel A. Méndez-Rojas, and Lucila I. Castro-Pastrana

Contents

Abstract Nowadays, our world faces one of the greatest challenges in terms of water consumption due to its growing population and demanding economic development. Water pollution is taking place at a rate and grade that make the advancement in water treatment technologies a research priority on several fronts, including those needed from the environmental and health standpoints. Today, one of the major concerns for allowing water reuse and providing safe drinking water supply is related to the presence of natural organic matter (NOM) and micropollutants in raw water. Among the latter, pharmaceutical compounds (PhCs) stand out, as they could partially or totally resist conventional removal treatments. Nonsteroidal anti-inflammatory drugs (NSAIDs) are especially ubiquitous PhCs due to their extensive prescription, and, consequently, they are often detected in hospital effluents, surface water bodies, sewage treatment plants (STP) effluents, and soil matrices. Therefore,

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NSAIDs wastewater removal is becoming a major concern in environmental protection. New technologies capable of efficiently removing them have been developed in the last few decades, and, within them, nanotechnology has risen as a promising tool to aid these technologies to accomplish their goal. In this chapter, the most common approaches to treat NSAIDs-containing wastewater are addressed, including adsorption, photocatalysis, and electrocatalysis; besides, recent advances on nanotechnological applications to improve their performance are covered.

Keywords Adsorbents, Electrocatalyst, Nanomaterials, NSAIDs, Photocatalyst, Removal

1 Introduction

NSAIDs are active pharmaceutical ingredients (APIs), which are among the most common molecules used in the treatment of rheumatic or degenerative joint diseases and in pain relief and muscle inflammation. This category includes ibuprofen, aspirin, indomethacin, ketorolac, naproxen, acetaminophen, sulindac, nimesulide, and diclofenac, among others (Fig. 1) [1–3]. However, several biologically active metabolization products are excreted into domestic effluents, easily reaching water effluents; furthermore, as they are over-the-counter drugs, these emerging pollutants are becoming a serious concern to public health due to their growing environmental presence. Diclofenac, ibuprofen, and naproxen are in the top ten of persistent pollutants found in wastewater; besides, they exhibit specific properties that draw concerns on their potential environmental and health impacts: they can passively diffuse across biological membranes, have low pK_a values, and are highly persistent in aquatic environments [4, 5]. Several methods have been assayed to remove NSAIDS from water effluents such as ozonation [6], chloride oxidation [7], coagulation [8], reverse osmosis [9], reusable ionic-liquid extraction [10], and activated sludges, [11] among others, but most of them are highly energy-intensive and present a low efficiency compared to their cost. Therefore, novel, high-performance alternatives are required.

Due to their inherent nature, pharmaceuticals are resistant to physical and chemical changes and persist after conventional wastewater treatment. In several conditions, most of them are not efficiently adsorbed on conventional adsorbents, and, what is even worse, some steps in the traditional treatment such as chlorination can generate more toxic degradation products. Therefore, there is a need to develop novel, low-cost adsorbents with high adsorption capacity and reusability in order to concentrate these contaminants and separate them from their aqueous matrices for further proper disposal. On the other hand, there is a need to finally destroy these compounds. As an alternative, several catalytic methods have been developed in the last decades; mostly oxidative, these methods intend to decompose pollutants into smaller, safer molecules, ideally into CO_2 and H_2O.

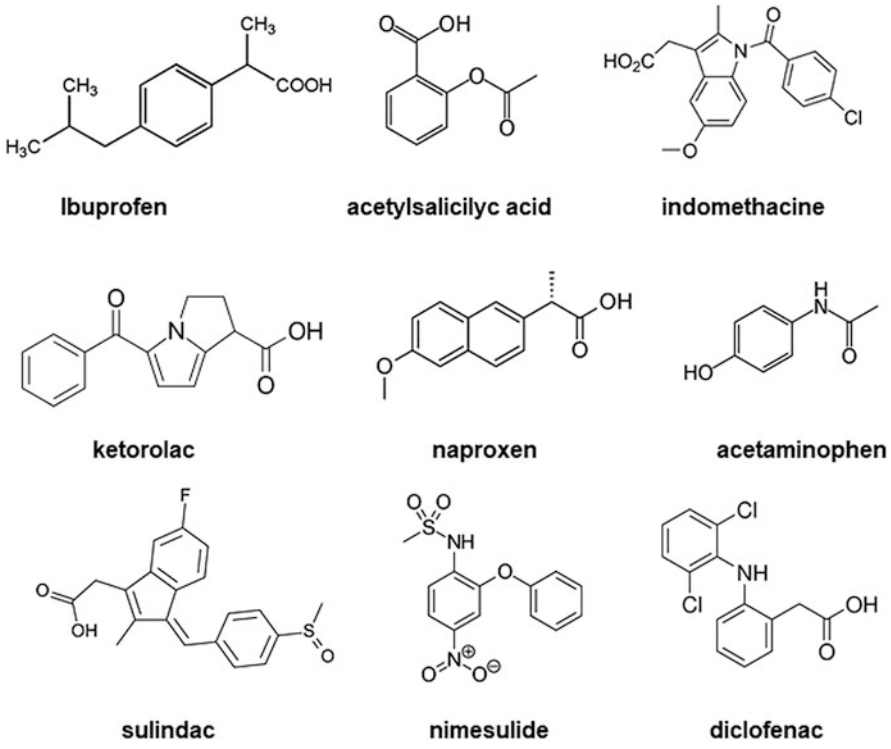

Fig. 1 Schematic representations of some of the most usually administered NSAIDs that can be found in water effluents as emerging pollutants

As an alternative, several catalytic methods have been developed in the last decades; mostly oxidative, these methods decompose pollutants into smaller, safer molecules, ideally into CO_2 and H_2O. Photocatalysis, electrocatalysis, Fenton, photo-Fenton, electro-Fenton, photoelectro-Fenton, sonolysis, and combinations among thereof are processes that have been used for organics water removal in the so-called advanced oxidation processes (AOPs). In these processes, an improvement in the catalyst performance is paramount for increasing their efficiency. This can be achieved by increasing their active surface area, by doping them to modify their electronic structure and their ability to move charge, and by blending them with other materials that confer them new chemical and physical properties which in turn may result in additive or synergistic effects that enhance their efficiency.

Nowadays, nanotechnology is considered a promising tool to tailor adsorbent properties to make them more efficient and versatile for removing NSAIDs from water. Moreover, nanostructured materials could be used in AOPs as catalysts themselves or be incorporated to catalysts to modify their structure and morphology, thus enhancing its catalytic activity and making AOPs more feasible and accessible. In this chapter, we will address some of the recent research approaches in the

development of nanomaterials to eliminate NSAIDs from water in the fields of adsorption, photocatalysis, and electrocatalysis, which have been in the spot of environmental research for these pharmaceutical wastewater treatments.

2 Nanomaterials for NSAIDs Adsorption

Recently, Mlunguza et al. reviewed the use of different adsorbent materials, which can become an efficient removal strategy for NSAIDs present in water effluents [12]. Among the systems reviewed, we can find activated carbon, ligninolytic enzymes, graphene-based adsorbents, molecularly imprinted polymers, electrochemical methods, sonochemical processes, and photocatalytic degradation. Nanomaterials are becoming an important class of adsorbing materials due to their small size, large active surface area, catalytic properties, and easy tunability through chemical functionalization of their surfaces. These so-called "nanoadsorbents" may be capable of removing the new emerging pollutants selectively, even at very low concentrations (μg/L) and under different conditions of pH, temperature, and wastewater composition [13]. Here, we will discuss and analyze some specific features, advantages, and limitations of selected examples of adsorbent materials recently reported in the literature.

Graphene has been revisited as a versatile nanomaterial that can be used as photocatalyst, disinfectant, and, due to its large surface area, a potentially useful adsorbent in water treatment technologies [14]. The efficiency of removal of ibuprofen, ketoprofen, naproxen, and sodium salt of diclofenac from an aqueous model and a real solution was investigated by Al-Khateeb et al. using high surface area graphene (HSAG) (Fig. 2a). They evaluated different operational parameters that may affect the adsorption process including solution pH, temperature, and adsorption time. Kinetic and thermodynamic parameters were also determined in order to understand the adsorption mechanism. Characterization of the HSAG showed that it was conformed by layered nanoplatelets (average thickness of 5.0 nm) and surface area of 677.5 $m^2\ g^{-1}$. The material was able to remove most of the studied NSAIDs after a few minutes using 10 mg of the HSAG at room temperature, with adsorption capacities of 11.9 mg/g (ibuprofen), 16.6 mg/g (ketoprofen), 17.8 mg/g (naproxen), and 19.3 mg/g (diclofenac sodium salt). Thermodynamically, the adsorption process was spontaneous, endothermic, and temperature-dependent (the higher temperature, the larger the adsorption capacities). When tested in a real water sample, the results showed a high removal efficiency for the mentioned NSAIDs [15]. Focusing on other type of carbon-based nanoadsorbent, Ahmed reviewed the performance of different types of activated carbons (ACs, Fig. 2b) as adsorbents for the water remotion of ibuprofen, ketoprofen, naproxen, and diclofenac [16]. The ACs were prepared by physical and chemical activation of lignocellulosic biomass and/or agro-industrial wastes. In general, ACs show better adsorption properties than zeolites, graphene-based adsorbents, and clays. The maximum adsorption capacities obtained from Langmuir isotherms for these drugs were of 417, 25, 290, and 372 mg/g for

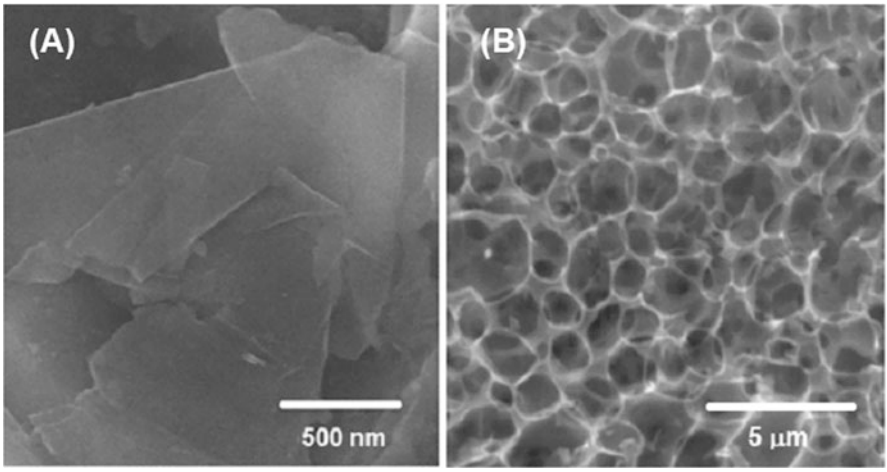

Fig. 2 Scanning electron micrographies of (**a**) high surface area graphene and (**b**) physically and chemically activated carbon (source: authors work)

ibuprofen, ketoprofen, naproxen, and diclofenac, respectively. In all cases, a spontaneous, low-temperature, nonlinear adsorption process was determined, with a pseudo-second-order kinetic and a mechanism not controlled by the pore diffusion step.

Magnetic nanomaterials are becoming an interesting alternative for the simple recovery of the adsorption system using an external magnet (Fig. 3). Originally designed for facilitating the extraction and analysis of drugs in different types of matrices (water, soil, and biological fluids) through magnetic solid phase extraction, these versatile systems have moved into the pipeline for the design of efficient wastewater treatment methods [17, 18]. For example, Kollarahithlu et al. prepared cysteine-modified silane-coated magnetic nickel ferrite nanoparticles using 3-glycidyloxypropyltrimethoxysilane and L-cysteine, and determined their ability to remove ibuprofen from an aqueous model system, optimizing pH, time, and concentration of ibuprofen as operative parameters. The cysteine-modified magnetic nanoparticles showed improved adsorption at acidic pH. One of the main advantages of this approach is the easy remotion of the nanocomposite, after ibuprofen adsorption, simply using an external magnetic field. Adsorption was found to occur due to the presence of the amino (-NH_2) groups of the L-cysteine; adsorption kinetics fitted to a pseudo-second-order kinetics, confirming that the rate-limiting step is the chemical adsorption [19]. In a different work, Nodeh et al. prepared silica-coated magnetite nanoparticles decorated onto graphene oxide (GO-MNPs-SiO_2) and studied their capacity for removing naproxen from wastewater [20]. The magnetic nanocomposite showed higher adsorption capacity and faster adsorption of naproxen with respect to other previously reported systems, which could be explained by electrostatic interactions among negatively charged naproxen and positively charged adsorbent. The maximum adsorption capacity of the magnetic nanocomposite was of

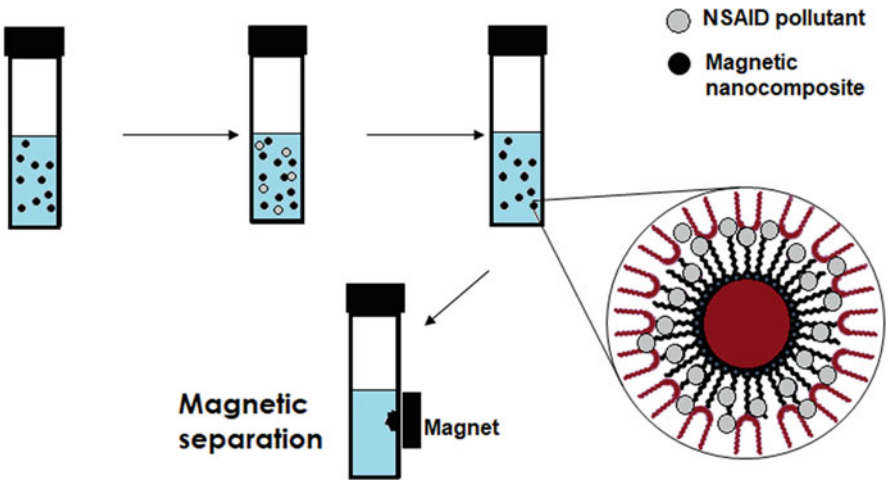

Fig. 3 Schematic representation of the use of magnetic nanocomposites as nanoadsorbents for the removal of pollutants in wastewater

31.25 mg/g at pH 5, after 60 min. A multilayer adsorption process was determined from Freundlich isotherm studies.

Following a similar strategy, Singh et al. prepared a magnetic nanocomposite for the water removal of ibuprofen [21]. A ferric nitrate solution containing a suspension of coconut shell was precipitated to iron oxide under alkaline conditions and then calcinated at 750°C to prepare the magnetic nanocomposite. It exhibited higher removal capacities than coconut-based activated carbon, with ibuprofen maximum removal of 60.4% and 14.7%, respectively. A four-factor Box-Behnken experimental design optimization model was designed for maximizing ibuprofen removal from water at optimum conditions (ibuprofen concentration of 80 mg/L; temperature of 48°C; pH 2.5; and dose of nanocomposite of 0.6 g/L). The model predicted a maximum removal of 65.8%, which was very close to the experimental value (65.1%). Furthermore, the nanocomposite was easily separated from the aqueous phase using an external magnet.

In a different approach, copper nanoparticles synthesized through a green method were evaluated as nanoadsorbents toward ibuprofen, naproxen, and diclofenac in wastewater [22]. Metallic copper nanoparticles with diameters in the range from 4.7 to 17.4 nm were obtained by using *Tilia* aqueous extracts to reduce a $CuSO_4$ solution at 90°C under stirring for 30 min. The removal capacities were of 36.0, 33.9, and 33.9 mg/g for diclofenac, ibuprofen, and naproxen, respectively, with a pseudo-second-order kinetic of adsorption, which was spontaneous, endothermic, and physical in nature. The best removal conditions were found to be at 298 K and pH = 4.5, using 10.0 mg of copper nanoparticles with a contact time of 60 min, with removal percentages of 74.4%, 86.9%, and 91.4% for diclofenac, naproxen, and ibuprofen, respectively.

Table 1 Selected examples of nanoadsorbents, based on composition

System	Characteristics	Ref.
Carbon-based nanoadsorbents		
High surface area graphene (HSAG)	10 mg of the material, efficiently removed ibuprofen (11.9 mg/g), ketoprofen (16.6 mg/g), naproxen (17.8 mg/g), and diclofenac sodium salt (19.3 mg/g)	[15]
Activated carbon (AC)	Efficient maximum adsorption capacities for removal of ibuprofen (417 mg/g), ketoprofen (25 mg/g), naproxen (290 mg/g), and diclofenac (372 mg/g)	[16]
Inorganic nanoadsorbents		
Copper nanoparticles (CuNPs)	Nanoparticles with size range from 4.7 to 17.4 nm with good removal capacities for diclofenac (36 mg/g), ibuprofen (33.9 mg/g), naproxen (33.9 mg/g)	[22]
Carbon/inorganic composite nanoadsorbents		
Magnetic iron oxide/activated carbon composite	Nanocomposite obtained from coconut-based carbon mixed with $Fe(NO_3)_3$ and calcinated at 750°C has an ibuprofen removal efficiency of 60.4%	[21]

As we have seen different types of nanoadsorbents are now being tested to remove NSAIDs. These adsorbents have shown to have a high and fast removal percentage in most of the cases. We have classified, based on their composition, the main types of adsorbents that have been used for NSAIDs removal; see Table 1.

3 Nanomaterials for Photocatalytic Degradation of NSAIDs

Photocatalysis as an alternative for eliminating organic pollutants in wastewaters has been extensively studied for a wide variety of compounds of environmental concern such as pesticides, petroleum-derived products, organochlorinated compounds, aromatics, and emerging contaminants like domestic-use chemicals and pharmaceuticals. Up to date, photocatalysis has shown to be promising for the removal of a variety of water pollutants, and in several cases it has proven to achieve complete oxidation of organic species [23]. Photocatalysis efficiency depends on several factors such as catalyst nature, catalyst load, initial concentration of substrate, pH, catalyst adsorption capacity and type of matrix, among others. However, improvement on photocatalytic processes strongly relies on the photocatalyst.

Among the photocatalysts that have been used to treat water polluted with persistent organic pollutants (POPs, e.g., pharmaceuticals, personal care products, and endocrine disruptors), we can mention TiO_2, ZnO, CdS, SnO_2, Fe_2O_3, SiO_2, Nb_2O_3, and g-C_3N_4. Among these materials, nano-TiO_2 photocatalyst (20–30 nm particle diameter) has been widely studied for POPs oxidation due to its high efficiency, low cost, good stability, and noncorrosive properties. However, this catalyst still faces important drawbacks to spread its application. These limitations

include (a) narrow solar light absorption spectrum limited to the UV spectrum (5%), (b) low adsorption capacity for hydrophobic compounds, (c) high aggregation tendency, and (d) difficult separation and recovery [24].

The TiO_2 nanomaterial Degussa P25 (P25 TiO_2) has proven to be a very efficient photocatalyst for oxidation of several pollutants including pharmaceuticals. Diclofenac, naproxen, and ibuprofen have shown a 100% removal within a 4 h photocatalysis using a catalyst load from 0.1 g/L for diclofenac and naproxen to 1.0/L g for ibuprofen when their initial concentration was 200 ppm [25], which is much higher than the naturally occurring concentration of these NSAIDs. However, the use of Degussa P25 TiO_2 has an important drawback: its fine particle size increases its dispersion in aqueous media and its eventual agglomeration. Also, these suspended particles can act as a screen that prevents irradiation from reaching other catalyst particles in the reaction vessel. Therefore, it has been supported in several substrates like glass and quartz materials with the aim to facilitate its separation and recycling. Different types of films are found in literature for photocatalysis on organic pollutants. It has been reported that photocatalysis with a heterostructured film based on P25 TiO_2 and TEOS (tetraethyl orthosilicate) supported on a glazed ceramic surface on salicylic acid, ibuprofen, naproxen, and diclofenac achieved, under optimum conditions, degradation of 76%, 85%, 94%, and 65%, respectively [26].

Nanotechnology on traditional photocatalysts promises to improve their properties not only in terms of the increase in active surface area but also in terms of their photoconversion efficiency and stability. Photoconversion efficiency has been improved by adding noble metal nanoparticles (Ag, Pt, Pd) to a metal oxide semiconductor surface to reduce charge carriers recombination [27, 28] and, more recently, by adding carbon nanomaterials such as graphene, reduced graphene oxide (RGO), and carbon nanotubes. These hybrid nanocomposites have attracted attention of researchers because, in principle, these carbon materials can carry charges, thus reducing electron-hole recombination [28]. However, Minella et al. have pointed out that for reduced graphene oxide (RGO), there are results that show a decrease in efficiency, which was attributed to the following: (a) the null electron-hole transfer between TiO_2 and RGO and vice versa; (b) RGO can become a recombination center by capturing both holes and electrons from TiO2; (c) it can behave as a competitive light absorber; and (d) it can act as a nonreactive phase that holds the substrate unchanged [29].

Graphene oxide has also been added to TiO_2 to make photocatalytic nanocomposites. In these nanocomposites, it can act as an adsorbent, electron acceptor, and photosensitizer. As a nanomaterial, GO has a large specific area that increases the surface area on the nanocomposite. Its adsorption properties rely on the possibility to form pi-pi conjugations between a benzenoid substrate and its aromatic rings; besides, it can develop ionic interactions with the substrate through its oxygen-containing functional groups at the edges or on the surfaces of carbon-based nanosheets [30].

Doping of TiO_2 with carbon [31, 32] nitrogen [33, 34], other nonmetals, and metals and also co-doping are mechanisms to reduce this oxide band gap in order to

harvest sunlight more efficiently [35]. N,S co-doped TiO_2 nanoparticles and nanosheets have been used in photocatalysis of ibuprofen and naproxen with a catalyst loading of 2 g/L, at pH 6. In this study, ibuprofen was removed up to an 85% with the nanoparticles, but only a 71.6% with the nanosheets, whereas for naproxen a similar degradation (99%) was achieved on both materials. The reusability of these catalysts was of six cycles and the mechanism of degradation proceeded through direct oxidation on the catalyst holes which leads to generation of reactive oxygen species. Another important feature is that TiO_2 nanosheets performance was independent from pH in the range 5–9 [36]. TiO_2 doping with potassium ferricyanide, prepared via sol-gel, was tested toward visible light degradation of paracetamol, and it proved to be about five times faster than pure TiO_2 to eliminate 99.1% of this pharmaceutical [37]. Another dopant that has been tested for paracetamol removal is potassium peroxodisulfate with a 100% removal at an initial concentration of 0.1 mM paracetamol, pH 9 and a catalyst load of 1 g/L [38].

Other nanocatalysts being developed to treat NSAIDs are NiO and NiS which have been supported on a substrate of Fe_3O_4 and polypyrrole to treat water polluted with naproxen. Immobilization reduced their band gaps from 2.23 to 2.1 eV for NiS and from 3.4 to 3.05 for NiO. In these experiments, the highest removal percentage was achieved with the immobilized catalysts. Naproxen from real water samples decreased its concentration in a 65% in tap water and in a 77% in pharmaceutical wastewater with the most efficient nanocatalyst, the supported NiS [39].

ZnO and g-C_3N_4 have also been investigated as photocatalysts that have been tested for NSAIDs degradation. Choina et al. investigated ZnO nanoparticles of 15–30 and 100 nm to treat tetracycline and ibuprofen. They found that higher ibuprofen removal percentages, 24% against 14%, were achieved with smaller catalyst particles. They also observed that ibuprofen abatement was about 60% with the lowest initial drug concentration, 5 ppm against 60 ppm [40]. In another study, maximum ibuprofen removal (83%) was obtained with a ZnO nanophotocatalyst (100 nm particle diameter) under 254 nm radiation with substrate initial concentration of 1.5 mg/L and a catalyst load of 0.58 g/L within 95 min of reaction [41]. In a comparative study between TiO_2 and ZnO nanoparticles, authors found that, under UV light irradiation, an optimum catalyst load was 1.5 g/L for TiO_2 and 1.0 g/L for ZnO. In this study, pH influence was stronger on TiO_2 than on ZnO, being pH 3 more favorable for complete removal in 20 min reaction on TiO_2 and pH 7 more favorable for total abatement in 30 min reaction on ZnO. The reason for this is attributed to the positive charge on both materials at these pH values. At pH 3 a protonated ibuprofen (pK_a 5) will be more attracted to protonated TiO_2, while at higher pH values both deprotonated materials, ibuprofen and TiO_2, will experience electrostatic repulsion that leads to a decreased degradation rate. On the other side, ZnO point of zero charge is between 7 and 9; therefore, at pH 7 it will be positively charged, while ibuprofen will be negatively charged which results in a stronger electrostatic interaction between substrate and catalyst that favors degradation [42]. Moreover, ZnO supported on clay mineral fibrous sepiolite has been used to photocatalytically remove ibuprofen, paracetamol, and antipyrine in an initial concentration of 10 mg/L and with a catalyst load of 250 mg/L under simulated solar

light. In this research, ibuprofen could be 100% eliminated from the model water solution within 10 h of reaction, while paracetamol was removed in an 85% and antipyrine in a 70% during the same reaction period [43].

In the search for better photocatalysts, graphitic carbon nitride g-C_3N_4, a metal-free organic polymeric semiconductor, is now being studied to remove organic pollutants from water due to its nontoxicity, stability, low cost, and narrow band gap (2.7eV) [44]. Polymeric graphite-like C_3N_4, prepared by the polycondensation of melamine at 500°C for 4 h, has been used as a photocatalyst for the degradation of pharmaceuticals including salicylic acid and ibuprofen. This material shows, through SEM analysis, the formation of irregular particles and microlayers. Also, adsorption-desorption isotherms indicate that it is mesoporous with a broad pore-size distribution in the range of 5–100 nm with a mean size of 20 nm. This photocatalyst, under UV-vis irradiation and after 4 h of reaction, achieved a low decomposition for both NSAIDs, a 20% degradation for ibuprofen and a 30% for salicylic acid. This low degradation suggests the formation of several intermediates identified in previous works; for ibuprofen some identified intermediates are 2-hydroxyl-propanoic acid, 1,4-benzenecarboxylic acid, hydroxyl-acetic acid, and phenol. For salicylic acid, some identified intermediates are 2,3-dihidroxybenzoic acid, 2,5-dihydroxybenzoic acid, and 2,6-dihydroxybenzoic acid. These intermediates suggest a mechanism where the first step involves the hydroxyl radicals attack to the aromatic ring and, later on, the ring opening of their hydroxylated products to form short-chain carboxylic acids. A second pathway that has also been suggested is the direct cleavage of the aromatic ring which is highly stable; but, in any case, this aromatic ring opening is the rate-determining step that leads to a low degradation rate [45].

In a comparative study, exfoliated g-C_3N_4 and P 25 TiO_2 were tested toward paracetamol, ibuprofen, and diclofenac photocatalytic degradation. Almost complete degradation of these pharmaceuticals was achieved with P25 TiO_2 under UV light, and a less effective degradation (about 50–75%) was achieved with g-C_3N_4 under visible light. An interesting result of this study is the fact that intermediates from both ibuprofen and paracetamol were completely removed; but for diclofenac, intermediates were detected even after 12 h of irradiation. Some of these, identified by GCMS, were carbazole-1-acetic acid, 2,6-dichloroaniline, and hydroxylated derivatives [46].

Despite its suitable properties, g-C_3N_4 photocatalytic performance still needs to be improved by increasing its specific surface area, diminishing charge carrier recombination, and broadening its visible-NIR light absorption spectrum. Photocatalytic activity of g-C_3N_4 is significantly enhanced when it is nanostructured or combined, via doping or coupling, with metals, nonmetals, or other semiconductors. To this respect, Wang et al. synthesized a novel ternary photocatalyst based on g-C_3N_4 loaded with single atom-dispersed silver and carbon quantum dots and studied its photocatalytic behavior toward naproxen. With this material, an 87.5% naproxen and a 52.1% TOC removal were observed after 24 min of visible light irradiation, and after 96 min of extended irradiation, a 65.2 % TOC removal was attained. In this research, about a 10% inhibition on naproxen removal was observed

depending on the water matrix, and it was attributed to high concentration of water solutes, such as dissolved organic matter, bicarbonates, transition metals, etc., that could compete for the photogenerated radicals or weaken the radiant flux reaching the catalyst [44]. In Table 2, we have summarized some of the nanostructured photocatalysts that have been used to remove NSAIDs.

Table 2 Selected examples of NSAIDs and specific photocatalysts used for their degradation

NSAID	Photocatalyst	Catalyst load	Concentration of NSAID	Vis or UV	% of removal	References
Naproxen	SDAg-CQDs/UCN	50 mg	4 mg/L	Vis	87.5% in 24 min	[44]
	$GO/LaVO_4$	–	–	Vis	45% in 6 h	[47]
	N, S-TiO_2	2 g/L	5 mg/L	Vis	99.3% in 90 min	[36]
Diclofenac	g-C_3N_4	0.9 g	25 g/dm^3	Vis	77% in 2 h	[46]
	10% wt. MWCNTox-TiO_2	0.5 g/L	8 mg/L	UV	100% in 30 min	[48]
Diclofenac sodium	$CuBi_2O_4/Ag_3PO_4$	0.25 g	10 mg/L	Vis	85.45% in 2 h	[49]
	N, S, C-doped ZnO	0.44 g/L	–	UV	98% in 4 h	[50]
Ibuprofen	3% wt. $GQD/AgVO_3$	0.01 g	10 mg/L	Vis	90% in 2 h	[51]
	PAN-MWCNT/ TiO_2-NH_2	15 mg	5 mg/L	UV	99% in 2 h	[52]
	g-C_3N_4	0.9 g	15 g/dm^3	Vis	71% in 2 h	[46]
	$POPD/Sb_2O_3$	50 mg	50 mg/L	Solar	91.49% in 1 h	[53]
	$BaBi_4Ti_4O_{15}$	–	10 mg/L	Vis	80% in 2 h	[54]
	$BaBiO_3$	–	10 mg/L	Vis	60% in 2 h	[54]
	N, S-TiO_2	2 g/L	5 mg/L	Vis	85% in 90 min	[36]
	Acidified g-C_3N_4/ PANI/RGO biochar	0.5 mg/ mL	20 mg/L	Vis	98.4% in 50 min	[55]
Ketoprofen	MWCNT-TiO_2	1.2 g/L	59 μM	UV	100% in 15 min	[56]

4 Nanomaterials for Electrocatalytic Degradation of NSAIDs

As it has been pointed out, conventional treatment methods have shown a deficiency to separate pharmaceuticals from wastewater effluents. For several reasons, these methods are not effective enough to eliminate these pollutants from disposal waters. For example, biological techniques can be very time-consuming [57]; adsorption processes and filtrations require further treatments in order to remove them and do not always show high efficiencies [58, 59]; and furthermore, some physicochemical methods involve a low removal percentage, generation of toxic by-products, and high operational costs [60].

Electrooxidation, as an AOP, offers several advantages that can overcome the deficiencies of previously mentioned methods: they are cheap and environmentally friendly and have a low energy demand and a high efficiency in wastewater treatment, all of which make it possible for electrochemistry to be utilized in the removal of NSAIDS and their intermediates [61, 62].

Furthermore, ever since the use of electrical techniques was proposed for water remediation in 1889, several methods have been developed, but all of them rely on the use of electrolysis for the removal of the compounds of interest. This can happen in either of two ways: direct or indirect electrolysis, being understood by electrolysis as the decomposition of a compound present in solution by applying an electrical current or voltage [63].

Direct electrolysis applies when electrons are exchanged directly between the compound of interest (pollutant) and the surface of an electrode at its interface [64], whereas indirect electrolysis refers to an oxidation-reduction process at the bulk of the solution by species that were generated at the electrode's surface. This implies that the pollutants' degradation process does not necessarily occur at the surface of an electrode, but it is initiated at it [65, 66]. For this reason, it is important to take into account the diffusion of species from the bulk of the solution to the electrode, and vice versa, to achieve the desired reaction [67].

It is known that when electrocatalysis of pollutants takes place by indirect electrolysis, the efficiency of this process strongly relies on the generation of a high concentration of oxygen reactive species at the electrode from water discharge, which mainly implies the production of hydroxyl radicals. This reaction takes place at the surface of the electrode as indicated in (1):

$$H_2O \rightarrow OH_{ads} + H^+ + e^- \quad (1)$$

In such a manner, and due to the strong oxidizing potential of these species, it is possible to degrade pharmaceuticals to CO_2 and water, and even though the oxidizing agents have short lifetimes, they are capable of promoting the formation of other oxidizing species present in the wastewater [66, 68, 69]. In this oxidation process, the electrode (M) where direct anodic electrolysis occurs (oxidation) is called an active anode, while the electrode where oxygen reactive species are generated to

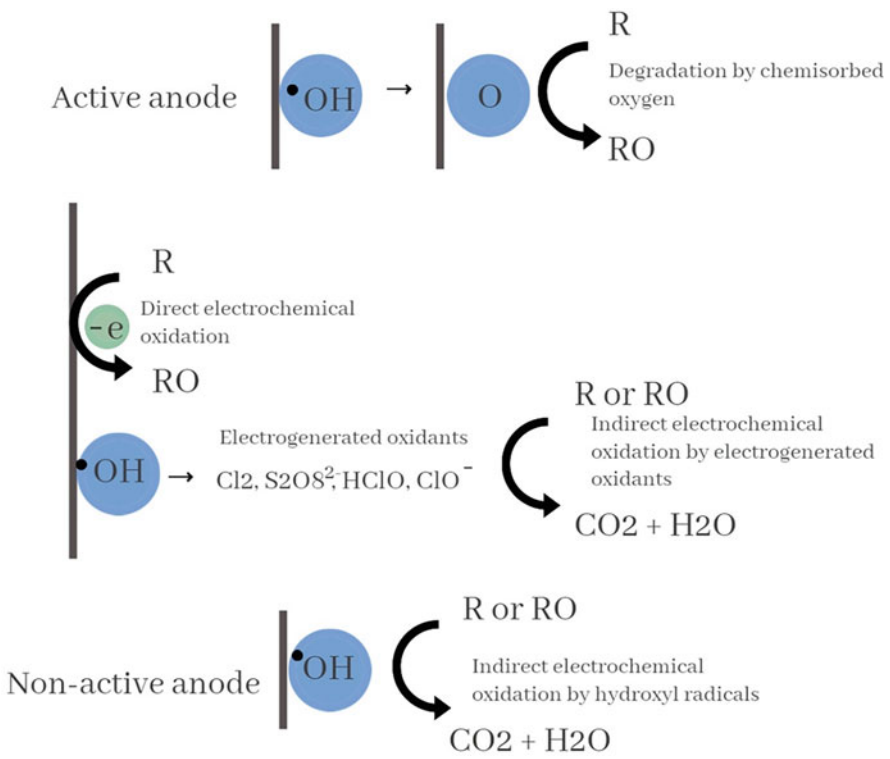

Fig. 4 Mechanism of oxidation of organic matter in active and non-active anodes

further oxidize species in solution is called a non-active anode. These processes can be pictured in Fig. 4.

We can see from this figure that in both types of anodes, the initial reaction is the generation of hydroxyl radicals that will be adsorbed onto the surface. The active anode will strongly interact with the adsorbed hydroxyl radicals, and, depending on the conditions, it can form chemisorbed active oxygen (2), which has a lower oxidizing capacity but is able to degrade organic compounds, R, oxidizing them to RO (3).

$$\mathrm{M}(\cdot\mathrm{OH}) \rightarrow \mathrm{MO} + \mathrm{H}^{+} + \mathrm{e}^{-} \tag{2}$$

$$\mathrm{MO} + \mathrm{R} \rightarrow \mathrm{M} + \mathrm{RO} \tag{3}$$

On the contrary, non-active anodes interact weakly with the hydroxyl radicals. This enables them to completely degrade the organic matter, producing carbon dioxide and water. Furthermore, both anodes are able to directly oxidize the organic compounds by electron transfer, and, also, depending on the solutions employed, other weak oxidizing species can be formed, such as peroxodisulfates and chlorine compounds [70].

Subsequently, electrochemical techniques do not require the addition of chemicals to take place, but they do require having a supporting electrolyte in solution which is already present in most of the effluents to be treated [57, 59, 71]. The electrolyte is an important factor to keep in mind in the implementation of electrochemical processes since its nature influences the type of chemical reactions that can take place in solution [68]. Other parameters that make electrochemical techniques feasible for the degradation of pharmaceuticals are the following: they (a) can be operated at room temperature [62], (b) are versatile and easily controlled [68], (c) use a low-cost equipment, and (d) can be scaled from the laboratory to the industry since they operate at room conditions [65].

As we have mentioned before, the catalysis performance relies on its properties. Thus, in electrooxidation, the anode material plays a key role on the oxidation reactions that can take place on its surface [62, 72]. As an example, Coria et al. showed that by using different electrodes, the efficiency of the degradation of naproxen varied depending on the type of electrode material [69].

Nowadays, electrochemical advanced oxidation processes (EAOPs) have been studied on a variety of electrode materials because of their chemical stability, low cost, and high electrocatalytic activity. These materials include Ti/IrO_2, boron-doped diamond (BDD), Ti/SnO_2, Ti/RuO_2, Ti/RuO_2–IrO_2, Ti/RuO_2–TiO_2, Pt, Ti/SnO_2–Sb, PbO_2, SnO_2, Ti/Pt/PbO_2, and Ti/SnO_2-Sb_2O_5 [60, 67]. These materials will perform in a different way depending on the conditions mentioned, such as pH, electrolyte, and substrates. Among them, BDD has shown a remarkable performance under a variety of environments.

Doping diamond with boron makes it conductive but preserves some of its characteristics such as being chemically inert and mechanically resistant. When doped with boron, it becomes conductive because the introduction of boron allows it to have intermediate states between the valence and the conduction gap, enhancing the electron-transfer ability [73]. As a result, BDD has been studied for the degradation of pharmaceuticals present in wastewater because it is able to resist aggressive conditions without corroding, it has a high durability, it is efficient and chemically inert, and it has a high oxygen overpotential. This is important because it favors the production of oxidizing species such as hydroxyl radicals, peroxodisulfates, hydrogen peroxide, and chlorine, without oxygen production, which permits a better degradation efficiency [68, 74]. In fact, it has been found that BDD, as an anode, allows the complete degradation of ketoprofen in a sodium sulfate medium [72]. However, BDD has the limitation of being expensive and having a weak adsorption capacity, limiting its application [75].

Therefore, with the aim of preparing cheaper but effective and stable electrode materials, nanotechnology has been applied in the area of electrocatalysis to build nanostructured-modified electrodes that show suitable electron transfer and own remarkable properties such as high surface area, thermal and chemical stability, tunable porosity, and biocompatibility. Some examples of nanomaterials implemented in this field are carbon nanomaterials, nanostructured metal oxides, and platinum nanoparticles which will be discussed here [76, 77].

4.1 Carbon Nanotubes (CNTs) for NSAIDs Removal

Carbon nanotubes show a great potential for applications involving the oxidation of compounds present in wastewaters due to their exceptional features which include a good electrical conductivity, chemical stability, high surface area, and mechanical strength. Consequently, they can be used to modify an electrode to increase its electroactivity and stability [57, 65].

Moreover, it has been shown that CNTs have a strong adsorption capacity due to reactive groups that are present at their surface, which enhance their capacity for the removal of the target molecules. Parameters such as the size of the nanotubes or the electrostatic interactions influence greatly their ability of adsorption [60, 78]. Montes et al. reported that a current increase was achieved by a sequestration of naproxen due to π-π interactions with the methoxy-naphthyl ring which is highly aromatic. This observation also explains why this strong adsorption effect was not observed with ibuprofen [79].

Furthermore, in the experiment performed by Díaz et al., an electrochemical signal was only observed after the addition of CNTs for the degradation of naproxen, which corroborates an electron transfer process. On top of that, it was established that by increasing the volume of CNTs from 5 to 15 μL, the current increased as well, due to more superficial area available for naproxen oxidation; but by increasing volume up to 20 μL of CNTs, the current decreased, which means that having an augmented thickness on the nanotubes film promotes its instability. The results showed that the removal of naproxen in water was 82.5% and 77% for 500 and 250 rpm stirring, respectively [57].

In another interesting study, multiwalled carbon nanotubes (MWCNTs) were dispersed in an electrolytic solution containing diclofenac. The purpose of this experiment was to investigate the degradation of this drug under a variety of conditions using three different electrode materials: Ti/RuO_2, Ti/TiO_2, and Ti/RuO_2-TiO_2. The addition of MWCNTs had a positive effect on the percentage removal of this pharmaceutical since a removal of about 75% was achieved with a dosage of 70 mg/L MWCNTs. This result was explained based on an increase in the hydroxyl radical production due the electrode-like behavior of the MWCNTs that can, at an appropriate current density, produce additional OH radicals. These particle electrodes reduce O_2 to H_2O_2 and catalyze hydrogen peroxide decomposition to hydroxyl radicals [60].

4.2 Titanium Dioxide (TiO_2) Nanostructures

TiO_2 nanomaterials are semiconductors that have been studied particularly as photocatalysts in water remediation because ultraviolet light can induce the formation of an electron-hole pair capable of producing oxygen reactive species that can oxidize organics in a high degree. Moreover, TiO_2 is cheap, chemically stable, and nontoxic [80]. In the field of electrocatalysis, under an applied current or voltage,

titanium dioxide nanotubes (TiO_2 Nts) are able to produce oxidizing agents that can degrade pharmaceuticals in the absence of light. However, its electrochemical hydroxyl radical production is scarce but can be improved by annealing the nanotubes in a reducing atmosphere that will create defects that allow the material to generate oxidizing species by applying a voltage. Notably Carlson et al. were able to degrade ibuprofen up to 50% in 15 min by using TiO_2 NTs annealed under a reductive atmosphere [81].

Titanium dioxide nanotubes can also be used as support materials to hold electroactive [82] or photoactive species. They have been employed to support TiO_2 nanoparticles (TiO_2 NPs), N,S decorated TiO_2 nanocrystallites (N, S-TiO_2 NCs), reduced graphene oxide (RGO), and Pd nanoparticles (Pd NPs). Among these, the photoelectrode that had shown the best photoelectrochemical performance, the N,S co-doped TiO_2 nanocrystallites decorated TiO_2 nanotube arrays (N, S-TiO_2 NCs/TiO_2 NTAs) was used to carry on the photoelectrochemical degradation of diclofenac. With this electrode, about 70% diclofenac was removed under a 35 W Xenon light irradiation, at an applied voltage of 0.4 V (vs SCE), at pH 5.0, 0.10 M Na_2SO_4 and with a 5 mg/L diclofenac initial concentration [83].

4.3 Zinc Oxide (ZnO) Nanostructures

Zinc oxide is a semiconductor suitable for electrochemical techniques and has shown to be effective in the degradation of dyes and pharmaceuticals. Its properties include great chemical stability, environmental friendliness, low cost, high adsorption coefficients, high electron mobility, and electron communication features [84, 85]. However, it has a high isoelectric point of 9.5; therefore in acidic conditions, the charged ZnO nanoparticles might repel the pharmaceuticals to be degraded, reducing the removal efficiency. On the other hand, experiments made by Tashkourian et al. evidence that the incorporation of ZnO nanoparticles and CNTs increase the surface area of the electrode and allow a greater electron transfer, therefore enhancing naproxen degradation [77].

Another example of implemented ZnO and TiO_2 nanostructures in the degradation of NSAIDS is the work published by Gomes et al. where it is shown that the effect of adding the semiconductors together increases the surface area, and, therefore, a better response is obtained, compared to electrodes consisting of just zinc or titanium oxide. In this work, a degradation of ibuprofen was achieved by a photoelectrochemical method, and the total organic carbon removal was of 23% [86].

4.4 Platinum Nanoparticles (PtNPs)

Platinum is a noble metal widely required as an electrocatalyst due to its incredible chemical durability, as well as its physical and chemical properties. Platinum nanoparticles in particular have attracted the attention of researchers for applications

in electrochemical sensors or energy conversion devices. Some reports expose that the electrocatalytic ability of carbon-based materials and metallic compounds can be enhanced upon the use of PtNPs because it allows to have a higher surface area. Also, it is important to mention that PtNPs are often supported on some material because otherwise they might agglomerate [87, 88].

Consequently, the PtNPs supported in fluorine-doped tin oxide (FTO) has been reported, and the use of the nanoparticles allows to increase the roughness, raise the resistance and the stability, and lower the oxidation potential, which enhances the catalytic performance of the electrode. This can be seen in the work published by Ching et al. who showed that PtNPs/FTO degraded 79.3% and 89.1% of naproxen at pH values of 4.6 and 3, respectively. Hence, a variation in the pH of the solution can also help in improving the degradation efficiency [58]. In a more recent study performed by Chang et al. the addition of MWCNTs to Pt on the FTO glass increased this electrode efficiency toward ibuprofen removal due to a higher electric conductivity and surface area for the adsorption and oxidation of ibuprofen [61].

4.5 Other Nanomaterials

Alumina nanoparticles (ANPs) are widely studied ceramic materials, because even when they are nonconducting and cannot transfer electrons, they can be used as catalysts or catalyst supports because they provide a high surface area that promotes the adsorption of organics present in wastewater [89]. This property can be used to concentrate organics that will be eletrooxidized. As an example, Tabeshnia et al. reported that the incorporation of ANPs onto a glassy carbon electrode allowed to obtain a better response for the voltammetric electrooxidation of selected NSAIDS: diclofenac, mefenamic acid, and indomethacin, which were mainly attributed to a higher adsorption surface [76].

Finally, as a summary of this chapter section, some examples of the nanomaterials currently used in electrooxidation with their respective removal percentages are shown in Table 3.

It is important to mention that the percentage of removal presented in this table makes reference to the degradation of the mentioned pharmaceutical; however, its oxidation generates other products that must be studied in order to determine if they represent a toxic threat or if they can be further degraded due to the unspecific chemical activity of hydroxyl radicals (Fig. 5).

For example, in the degradation of ibuprofen, several by-products have been identified, particularly 1-(1-hydroxyethyl)-4-isobutyl-benzene, which has a toxic effect on human erythrocytes; however, it was shown by Chang et al. that it can be completely eliminated in 60 min using a PtRu-FTO electrode. However, it must be kept in mind that larger amounts of reaction products are generated due to the higher electrocatalytic ability of certain electrodes [61]. Other intermediates of reaction include 4-isobutylacetophenone, 4′-(2-methylpropyl)-acetophenone, 4-isobutyl phenol, and 4-ethylbenzaldehyde, whose structures can be seen in the next picture [59, 66, 79].

Table 3 Selected examples of electrode materials and oxidation conditions used for NSAIDs remotion

NSAID	Electrode	Current density/ applied potential	Electrolytic solution/pH	% of removal	References
Naproxen	MWCNTs on glassy carbon	1.5 V vs SCE	Phosphate buffer/Ph 7.5	82.5% in 20 h	[57]
	Pt/MWCNTs-FTO	70 mA/cm^2	Sodium sulfate	96% in 2 h	[58]
Ibuprofen	PtRu-FTO	50 mA/cm^2	pH 3	99% in 2 h	[61]
	AgZMWCNT	1.75 V vs Ag/AgCl	Sodium sulfate	82%	[62]
	AgZMWCNT	1.25 V vs Ag/AgCl	Sodium sulfate	78%	[62]
	TiO_2 NTs	3.5 V vs Ag/AgCl	–	50% in 15 min	[81]
	Ti/ZnO-TiO2	1 V vs Ag/AgCl	Sodium sulfate/pH 6.2	23% in 3 h	[86]
Diclofenac	N, S co-doped TiO_2-TiO_2 NTS	0.4 V vs SCE	Sodium sulfate/pH 5	80%	[83]
	Pt/CNT	1.5 and 2 V vs SCE	Carbonate buffer	50% in 8 h	[90]

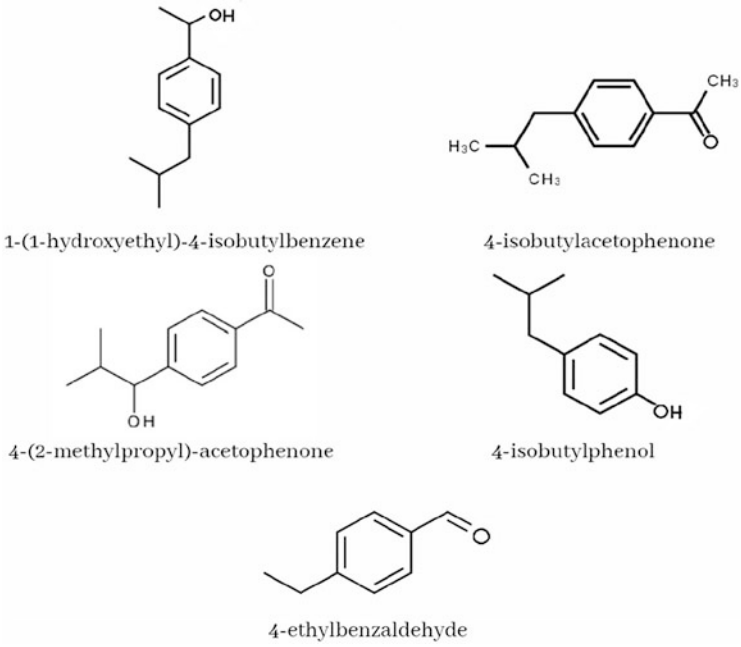

Fig. 5 Degradation products of ibuprofen

It is clear that at this stage, all the products contain an aromatic ring; however, the degradation continues with the cleavage of the ring and eventual mineralization of the products [59, 67]. This is important because according to Feng et al. the toxicity of these compounds can be reduced upon the mineralization of the aromatic intermediates, and they found out that the final oxidation products are carboxylic acids such as pyruvic, acetic, formic, and oxalic which can be mineralized in 92% upon the use of BDD with a photoelectro-Fenton method [66].

Similar results were obtained by Díaz et al. and Pourzamani et al. They showed that the degradation of diclofenac and naproxen, respectively, began by generating intermediates of reaction with an aromatic ring, which was further degraded upon cleavage and formed carboxylic acids that were oxidized to carbon dioxide and water [57, 60].

Finally, even though some materials show great removal of pharmaceuticals, it is of utterly importance to mention that some of the research done in electrochemistry is performed in artificial solutions where the concentration of the drug to be removed is much higher than the actual concentration present in wastewater; therefore, diffusion limitations can become greater and limit the efficiency of the process. In consequence, some anodic oxidation has been coupled with other techniques such as the use of ultrasound, UV light, or Fenton's reagents, because they help either in enhancing the mass transfer or producing additional hydroxyl radicals [66].

5 Conclusions and Perspectives

Adsorption, photocatalysis, and electrocatalysis, the most important treatment processes incorporating nanomaterials to remove NSAIDs from wastewater, have been reviewed in this chapter. As it has been shown, the incorporation of nanomaterials in these processes de facto increases their active surface area, resulting in improved adsorption capacity and catalytic efficiency. As it is expected, these materials' performance depends on their chemical nature. Properties such as hydrophilicity, specificity toward certain molecules, stability, and ability to produce specific reactive species when exposed to UV-vis light or after a voltage is applied will always rely on their chemical structure; therefore, different nanotechnologies can be applied to tune these materials' properties.

Physically and chemically activated carbon materials derived from biological wastes have proven to be effective for removing selected NSAIDs in adsorption methods even at very low concentrations and under different processing conditions. Besides, the combination of these materials with magnetic nanoparticles is becoming a simple alternative for the separation of adsorbents/catalysts by means of an external magnet.

On the other hand, the nanostructured morphology of common bulk photocatalysts or electrocatalysts are not only useful for increasing the active surface area but for changing other physical properties such as band gap, electrical conductivity, and stability; in turn, all of these make them more efficient materials in terms

of energy conversion and catalytic degradation of different pollutants including pharmaceuticals.

Wastewater treatment still faces major challenges efficiency-wise; one of the most important is that it needs to perform adequately when applied in the different environments where pollutants are found, and this is not an exception for pharmaceuticals whose different nature and great stability require the use of very powerful techniques to destroy them. Thus, even the current AOPs can be benefitted by using nanotechnology on preparation of their catalysts. In terms of photocatalysis, it cannot be denied that the preparation of semiconductors in the nanosized range has greatly improved the photocatalytic conversion efficiency; however, some other approaches such as the addition of metal nanoparticles or graphene to avoid electron-hole pair recombination still need further research to overcome some drawbacks including stability and hereby reusability of the compound materials. These materials have shown high photocatalytic efficiency. Therefore, efforts to make them more stable and resistant to different environments still represent a challenge in terms of their applications.

In electrocatalysis, nanotechnology is beginning to be explored for the preparation of nanoelectrocatalysts with increased surface area that can produce a larger concentration of highly reactive species. Also, as with photocatalysts, some nanostructured materials have shown improved electron mobility such as the nanotubular structures of metal oxides. This opens the possibility of using the latter as platforms to hold other electroactive oxides, metal nanoparticles, and carbon nanomaterials, which can also be of aid in improving catalyst performance toward oxidation of NSAIDs.

As previously mentioned, not only the combinations of different materials can develop unique functionalities within these processes, but the combination of such procedures could represent an additional strategy for fighting against pollution. Based on current trends, we expect that more mixed technologies will be investigated in the future, aimed at improving removal of NSAIDs, and other pharmaceuticals from wastewaters and nanotechnology will play a key role in their development.

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Biological Technologies Used for the Removal of Nonsteroidal Anti-inflammatory Drugs

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Abstract Chronic pain is one of the most important causes of disability worldwide and represents a major public health challenge. The presence of inflammation is a common underlying mechanism of chronic pain. Nonsteroidal anti-inflammatory drugs (NSAIDs), COX2-selective and non-selective, showing analgesic and anti-inflammatory properties, are useful options for the treatment of chronic pain. Non-metabolized pharmaceutical products and their metabolites are excreted and enter sewage as biologically active substances. The accumulation of emerging pollutants, such as active pharmaceutical ingredients and their metabolites in the aquatic environment, has recently become a serious problem due to their bioaccumulation and ecotoxicity potential that affects living organisms. Pharmaceutical products considered as emerging pollutants are partially removed during the treatment of wastewater that contains them and are detected in groundwater, surface water, and wastewater effluent, as well as in drinking water at concentrations ranging from a few nanograms per liter to 15 μg/L. The elimination of these contaminants is essential due to the toxicity that causes in the organisms. Biological techniques that include microorganisms in their processes could be more effective for the elimination of pharmaceutical contaminants compared to the physicochemical techniques currently used.

Keywords Aquatic ecosystems, Bacterial biodegradation, Biological technologies, Emerging pollutants, Nonsteroidal anti-inflammatory drugs

1 Nonsteroidal Anti-inflammatories

Nonsteroidal anti-inflammatory drugs (NSAIDs) are part of a heterogeneous group of active ingredients with different chemical structures but similar therapeutic and adverse effects [1]. They act as non-selective inhibitors of cyclooxygenase, COX-1 and COX-2 isoforms, which play a crucial role in prostaglandin synthesis [2].

Daily, about 30 million people worldwide use nonsteroidal anti-inflammatory drugs as a treatment for chronic inflammation, with variations in elimination, half-life, routes of administration, and tolerance of these pharmaceuticals [3].

In 2017, among the ten most studied NSAIDs detected in natural environments are ibuprofen, diclofenac, and naproxen. The maximum concentrations of these

pharmaceutical products in wastewater were 20,783 ng/L, 6167 ng/L, and 13,159 ng/L, respectively [4].

The concern about finding NSAIDs in the environment lies in the fact that these pharmaceutical products are designed to have biological activity at low doses, so they could cause undesirable effects on exposed organisms [5].

2 Emerging Pollutants

In recent decades, the consequences of the development of human activity in multiple activities (industry, transport, agriculture, urbanization) have become evident around the world. These activities have given rise to the contamination of different ecosystems with micropollutants [6].

The term "emerging pollutants" refers to components found in low concentrations in the environment, for which no regulations have currently been established [7]. Many of these compounds are pharmaceutical or personal care products, which enter the environment by excretion in human and animal urine and feces, as well as by an inadequate disposition [8]. The main classes of emerging pollutants are pesticides, disinfection products, industrial chemicals, and pharmaceuticals such as nonsteroidal anti-inflammatory drugs [9].

The abundance of emerging pollutants in wastewater, surface water, and groundwater is a problem that is attracting the attention of scientists due to its toxicity in aquatic organisms and as an impact on humans [10].

In the case of pharmaceutical products, consumption has been increasing; different compounds have been identified around the world. A large number of these microcontaminants come from hospitals and are the result of pharmacological treatments, clinical laboratory techniques, and research activities, as well as the excretion of the non-metabolized active substance [11].

The detection, identification, and quantification of emerging pollutants and their transformation products in various ecosystems are essential to know about their frequency and fate [9].

The degradation and effective removal of these types of pollutants are a matter of concern, mainly for biotechnologists and environmental scientists, who must propose viable and effective strategies for their elimination [12].

3 NSAIDs in Aquatic Ecosystems

The accumulation of emerging pollutants, such as active pharmaceutical ingredients and their metabolites in the aquatic environment, has recently become a serious problem. NSAIDs are partially removed during wastewater treatment; they have been detected in groundwater, surface water, and wastewater effluents, as well as in

drinking water, at concentrations ranging from a few nanograms per liter to 15 μg/L according with the latest reports [6].

The removal of these pollutants is necessary due to their toxicity to all living organisms. Physicochemical techniques are currently used for the elimination of NSAIDs; however, biological techniques based on microorganisms could be more effective for the elimination of pharmaceutical contaminants.

4 Toxicological Effects of NSAIDs

Several studies have been conducted where the toxicity of nonsteroidal anti-inflammatory drugs in different animal species exposed to them is demonstrated. The mechanism of action of drugs can explain alterations in organisms, prostaglandins, which are inhibited, not only participate in the inflammatory process but also in neurotransmission and ionic transport for the regulation of the circulatory system and vascular permeability. In addition, there is a reduction in leukotrienes that is related to cell survival signaling [13].

Reported effects of some nonsteroidal anti-inflammatory drugs on aquatic species are mentioned below.

Diclofenac has been reported to be teratogenic during the embryonic development of *Xenopus laevis* (LD_{50} 9.56 mg/L, LD_{50} for malformations 2.74 mg/L) and *Lithobates catesbeianus* (LD_{50} 12.10 mg/L, LD_{50} for malformations 2.88 mg/L) [14]. The adverse effects of this drug were also studied in *Cyprinus carpio* (LD_{50} 70.98 mg/L in 96 h) where it was found to induce oxidative stress, mainly in the liver and gills [15]; this effect was also studied in *Hyalella azteca* (LD_{50} 0.467 mg/kg in 72 h) [16, 17].

5 Over the Counter NSAIDs

Currently, over-the-counter medications are very popular, especially monocyclic and polycyclic nonsteroidal anti-inflammatory drugs [18]. The free sale of NSAIDs and their easy access for the population represents a problem of accumulation in the environment, because they are not always eliminated in the correct way, in addition to the excretion of these through urine and feces to municipal effluents.

6 Consumption of NSAIDs in the World

Each year, a large number of pharmaceutical compounds are consumed, and after their partial metabolism, they enter the wastewater treatment plants. However, the elimination of these compounds is not efficient, and pharmaceutical products are still

observed in the effluents of wastewater treatment plants, as well as in surface, underground, and even drinking water [19].

In many countries large amounts of nonsteroidal anti-inflammatory drugs are consumed annually. In Germany, for example, approximately 836 tons of acetylsalicylic acid, 622 tons of paracetamol, 345 tons of ibuprofen, and 86 tons of diclofenac were consumed in 2001. In 2000, in England, 35 tons of naproxen were consumed [20]. In Poland, 58 tons of ibuprofen, another important drug in this group, were consumed [21]. Picquet in 2013 reported that Albemarle Company, one of the world's leading chemicals companies, produces about 500 tons of naproxen per year [22].

7 NSAIDs Disposal

Physicochemical techniques for the elimination of microcontaminants are mainly based on filtration and oxidation processes [23] but may have specific disadvantages, such as high costs [24], the formation of toxic by-products [25], and inefficiency against certain compounds [26]. On the other hand, biogenic metals have proven to be useful catalysts for the elimination of recalcitrant organic compounds.

Removal of pharmaceuticals from the environment is possible and can be achieved using different methods, such as adsorption and abiotic removal. However, biodegradation represents the most important method of elimination.

In the following sections, relevant aspects of three examples of NSAIDs, diclofenac, ibuprofen, and naproxen will be discussed as they are the most consumed worldwide.

8 Diclofenac

Diclofenac is a nonsteroidal anti-inflammatory medication administered to reduce inflammation and relieve pain in patients. Recent studies have estimated that, at present, an average of 1,443 tons of diclofenac is consumed worldwide [27], a number that does not take into account that diclofenac sold over the counter (without a prescription) can mask the quantification of its use.

Due to the high resistance to biodegradation and the harmful impact on some environmental species in low concentration ($\leq$1 μg/L) [28], in 2015 the drug was included in the first list of substances monitoring in the EU that require surveillance of the environment in all member states [29]. This type of report is not found in developing countries and underdeveloped countries.

Today, this medicine is ubiquitously present in the aquatic environment [30] due to its continuous release by wastewater treatment plants, being considered a pseudo-persistent pollutant [31]. According to Vieno and Sillanpää, the maximum concentrations of diclofenac in municipal wastewater can vary between 0.44 and 7.1 μg/L

[32]. The disposal efficiency of wastewater treatment plants varies between 0 and 80%, but they are mainly in the range of 21–40% [33].

9 Ibuprofen

Ibuprofen, which belongs to the family of nonsteroidal anti-inflammatory drugs, is among the most consumed pharmaceutical products worldwide and shows analgesic, anti-inflammatory, and antipyretic effects by inhibiting the synthesis of prostaglandin [34, 35]. This pharmaceutical product has been detected in the aquatic environment at minimum concentrations of up to 603 μg/L in untreated wastewater, up to 85 μg/L in treated effluents, and up to 5 μg/L in surface waters [34–36].

Ibuprofen is the third most consumed pharmaceutical product in the world [37]. The sale of ibuprofen in Poland and Germany reached 58 tons in 2000 and 345 tons in 2001, respectively [20]. This medicine is the most frequently detected pharmaceutical product in the aquatic environment [38].

The potentially harmful effect of ibuprofen present in water has led to the search for new methods for its elimination from the environment. Technological and economic solutions include microbiological degradation. The search for new strains capable of degrading ibuprofen could be one of the answers to increase the detection of pharmaceutical products in water.

10 Naproxen

Naproxen is one of the most popular nonsteroidal anti-inflammatory drugs, with antipyretic and analgesic properties. It shows a broad spectrum of work in the treatment of mild to moderate pain. Naproxen inhibits cyclooxygenases I and II, which influence the level of prostaglandins and thromboxanes [39].

Due to its biological activity, naproxen can influence living organisms and reduce the biodiversity of natural environmental communities. Brozinski et al. (2013) [39], observed a seasonal variation in the concentration of naproxen in the water of Lake Haapajarvi, Finland, which ranges between 40 ng/L in November and 210 ng/L in February. At the same time, the concentration of naproxen detected in bile from two species of wild fish, the goldfish (*Abramis brama*) and the cockroach (*Rutilus rutilus*) caught in this lake, was almost 1,000 times higher and varied from 6 to 32 ng/mL and from 11 to 103 ng/mL, respectively [39].

It is known that naproxen can affect mRNA expression and has a negative influence on the gastrointestinal tract and kidneys of the zebrafish (*Danio rerio*) [40]. According to Li et al., the lethal dose 50% (LD_{50}) of naproxen for zebrafish embryos and larvae was 115.2 and 147.6 mg/L, respectively [41].

Because naproxen causes pericardial edema and histopathological liver damage, it can be considered a potential threat to aquatic organisms [41]. It is also suggested that naproxen may induce genotoxicity [42].

The problems generated by the accumulation of NSAIDs and the effects they cause on aquatic microorganisms, as well as their impact on humans, have been considered in this report to address the most relevant examples of biological technologies used in the elimination of NSAIDs.

11 NSAIDs Bioremediation

Biodegradation is the breakdown of a chemical substance in the elements that compose it; it is achieved by the action of biological agents such as plants, animals, and microorganisms, which use these elements in the metabolic processes required in their vital activities [43].

Biological treatments of areas affected by emerging pollutants offer a less expensive and environmentally friendly alternative. Most microorganisms used in this practice are capable of producing oxidoreductases enzymes that can degrade several types of contaminants including NSAIDs [44].

Biodegradation models of emerging pollutants are necessary to evaluate, understand, and predict the main factors that influence the biodegradation of this type of compounds in wastewater [45].

12 Biological Technologies for the Elimination of Diclofenac

12.1 Microbial Consortium

The microbial consortium is a potential technique in the degradation of highly polluting drugs for the environment, specifically for the elimination of NSAIDs [46].

The usefulness of this technology for the elimination of diclofenac has been reported through the capacity of a microbial consortium composed of *Alcaligenes faecalis*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, and *Proteus mirabilis*, to degrade different medications. A percentage of diclofenac degradation of up to 89% has been observed over a period of 120 h at a concentration of 150 mg/L. The maximum specific growth rate of the microorganisms was 0.096 mg/L/h. The maximum specific biodegradation rate was 0.89 mg/L/h [46].

Residual metabolites predicted by spectroscopic analysis were also reported in this study, including hydroxy-sodium diclofenac and acyl glucuronide. The presence of these metabolic products suggests that the activity of the enzymes monooxygenase and glucuronidase catalyzed the degradation reaction [46].

12.2 Biologically Activated Carbon Filter

It is an efficient method used concomitantly with bacterial growth, through a co-metabolism with acetate, glucose, or methanol. The evaluation of the bioremoption potential of a biologically activated carbon filter for the elimination of three NSAIDs, diclofenac, ibuprofen, and naproxen, showed that the biologically activated carbon column effectively eliminates the three NSAIDs (>90%). A bacterial strain isolated from the filter, *Pseudoxanthomonas* sp., was able to simultaneously eliminate the three drugs supplied as the sole source of carbon [47].

In 14 days, 23, 41, and 39% of diclofenac, ibuprofen, and naproxen (50 μg/L), respectively, were removed biologically. *Pseudoxanthomonas* sp. eliminated ibuprofen faster than the other two NSAIDs. By adding a single drug as a single carbon source, the elimination capacity was overestimated by 5.0–27.0%. The results obtained provide a basis for the use of *Pseudoxanthomonas* sp. in the bioremoption of polycyclic environments contaminated with NSAIDs [47].

12.3 Artificial Wetland Systems

Artificial wetland systems are a promising technology for the treatment of wastewater containing microcontaminants, including pharmaceutical residues. Wetland systems are based on the fact that endophytic bacteria may be exposed to secondary metabolites in plant tissues; therefore, they may have the potential to transform or degrade aromatic structures, including pharmaceutical products in particular NSAIDs.

Mycobacterium flavescens MG7, an endophytic strain, obtained from *Phalaris arundinacea* root tissues exposed to NSAIDs, was used to test the ability to eliminate 2 mg/L of diclofenac in monosubstrate cultures and in the presence of phenol as an additional carbon source. The bacterium was able to eliminate approximately 15% of diclofenac present after 20 days of monosubstrate culture. However, a decrease in the optical density of bacterial growth was observed, caused by an insufficient carbon source for adequate growth and proliferation [48].

12.4 Biogenic Compounds

The emergence of a range of recalcitrant organic microcontaminants in the aquatic environment has led to the development of several tertiary wastewater treatment methods.

The use of biogenic manganese oxides, biogenic silver nanoparticles, and ionic silver for the oxidative elimination of the drug diclofenac and its dechlorinated form, 2-anilinophenylacetate, has been evaluated.

Diclofenac was rapidly degraded during the ongoing manganese oxidation by *Pseudomonas putida*. The study of the biogenic silver and ionic silver nanoparticles separately showed no ability to eliminate diclofenac. Improved elimination occurred when biogenic manganese oxides and silver species were combined [49].

Similar results were obtained for the dechlorinated form, 2-anilinophenylacetate. Finally, a slow elimination of diclofenac was observed, but there was faster degradation of the dechlorinated 2-anilinophenylacetate form when silver was added to the biomass of *P. putida* free of manganese [49].

This study demonstrates the use of *P. putida* for water treatment purposes. It is the first report of the application of silver combined with biogenic manganese for the removal of organic pollutants from water [49].

12.5 Bacterial Biodegradation

The bacterial strain *Labrys portucalensis* is capable of biotransforming 70% of diclofenac (1.7–34 μM), as the only source of carbon, in 30 days. Complete degradation was achieved by co-metabolism with acetate, over a period of 6 days for 1.7 μM and 25 days for 34 μM of diclofenac [50]. This study concluded that complete degradation of diclofenac can be achieved by the action of a single bacterial strain isolated from the environment.

On the other hand, *Brevibacterium* sp. D4, bacteria recovered and isolated from a wastewater treatment plant, demonstrated the ability to degrade diclofenac in 35% of 10 mg/L diclofenac as the sole source of carbon and 90% of the same amount when periodically fed with acetate as supplement [50].

12.6 Metabolic and Co-metabolic Biodegradation

Enterobacter hormaechei, isolated from an activated sludge, can metabolize diclofenac at an elimination rate of 52.8%. In the presence of an external carbon source (glucose), the removal rate increased to approximately 82%. GC-MS analysis detected and identified a metabolite as 1-(2,6-dichlorophenyl)-1,3-dihydro-2H-indole-2-one, which occurred as a result of dehydration and lactam formation reactions [51].

12.7 Bioremediation by Laccase Enzymes

Laccases are polyphenol oxidases that catalyze the oxidation of various aromatic compounds, particularly those with electron donor groups such as phenols (−OH) and anilines ($-NH_2$), using molecular oxygen as an electron receptor [52]. A

potential means to reduce the amounts of NSAIDs released into the environment is to improve their biodegradation in a post-treatment step using microorganisms that produce oxidative enzymes such as laccases [53, 54].

To analyze this method, four strains of the bacterial genus *Streptomyces* (*S. cyaneus, S. ipomoea*, *S. griseus*, and *S. psammoticus*) and the white rot fungus *Trametes versicolor* were studied, for their ability to produce extracellular active laccase in wastewater biologically treated with different carbon sources [55].

The evaluation of the five organisms showed that *T. versicolor* was the most promising strain. This fungus produced more than 20 times more laccase activity than *S. cyaneus*, the best candidate of the *Streptomyces* strains evaluated, and this especially in wastewater treated with forest wastes as the sole substrate, a cheap and widely available product. Laccase of *T. versicolor* was also more active than that of *S. cyaneus* at almost neutral pH and between 10 and 25°C, conditions generally found in municipal wastewater [55].

13 Biological Technologies for the Elimination of Ibuprofen

13.1 Artificial Wetlands

Constructed wetlands are ecological and economical, they have also aroused a growing interest in their application to treat pharmaceutical contaminants in wastewater [56, 57]. In a study evaluating the dynamics of ibuprofen degradation, diversity, and bacterial uniformity, and the structure of the bacterial community in a bed planted with *Typha angustifolia* [56], it was shown that plants promote microbial degradation of ibuprofen, especially in the downstream areas of the wetland [56].

In the area upstream of a third of the wetland, the presence of plants did not significantly improve the degradation of ibuprofen, probably due to the much greater contribution of the co-metabolic behaviors of certain microorganisms that do not degrade the ibuprofen of plants [56].

When analyzing the bacterial characteristics, it was found that the aerobic species of the *Flavobacteriaceae* family, the *Methylococcaceae* family, and the *Methylocystis* genus and the anaerobic species of the *Spirochaetaceae* family and the *Clostridium* genus were the most relevant bacteria for the ibuprofen co-metabolic degradation. The *Rhodocyclaceae* family and the genus *Ignavibacterium* closely related to plants appear to be associated with the metabolic degradation of ibuprofen [56].

The family *Rhodocyclaceae* and the genus *Ignavibacterium* closely related to the plants appeared to be associated with the metabolic degradation of ibuprofen [56].

13.2 Biodegradation in Biological Filter

Serratia marcescens, isolated from the activated sludge in a sewage treatment plant, is capable of degrading ibuprofen. The degradation of ibuprofen required the presence of primary substrate. After a 5-day culture with baking powder at 30°C and pH 7, the highest degradation was achieved (93.47 ± 2.37%). The bacterium was applied to a small biological aerated filter device to form a biofilm with activated sludge. The elimination of ibuprofen was 32.01–44.04% higher than for a biological aerated filter without a bacterial component. The indigenous bacterial community was able to effectively eliminate COD Mn (permanganate index) and ammoniacal nitrogen in the presence of *Serratia marcescens* [58].

13.3 Bacterial Biodegradation

Raoultella sp., obtained after chemical mutagenesis of contaminated soil isolates, effectively eliminated diclofenac (92% removal) over a period of 72 h at 28°C. The degradation of the analgesic was investigated in detail by means of a cellular catalyst. With this method, a maximum degradation of diclofenac of 91% was achieved at pH 7 (1 g/L of diclofenac). The specific elimination rate at high concentrations of diclofenac increased to 16.5 mg/h [59].

On the other hand, the bacterial strain *Bacillus thuringiensis* isolated from the soil of the chemical factory "Organika-Azot" in Jaworzno, Poland, grown in monosubstrates and co-metabolic systems with 1, 3, 5, 7, and 9 mg of ibuprofen and 1 g of glucose as a source of carbon, eliminated ibuprofen up to 9 mg in 232 h in the monosubstrate culture, while in the co-metabolic culture, the elimination of the drug was six times faster [60].

In the co-metabolic system, the maximum specific growth rate of the bacterial strain was 0.07 ± 0.01 mg/mL/h and the substrate concentration K sμ 0.27 ± 0.15 mg/L. The maximum specific ibuprofen elimination rate and the value of the medium saturation constant were q max = 0.24 ± 0.02 mg/mL/h and the half-saturation constant K s = 2.12 ± 0.56 mg/L, respectively [61].

B. thuringiensis can degrade ibuprofen in both monosubstrates and co-metabolic systems. However, ibuprofen is not a sufficient carbon source for this strain. The effective degradation of this drug occurs in the presence of glucose. Toxicity studies showed that ibuprofen has a mean value of the microbial toxic concentration EC_{50} of 809.3 mg/L and is higher than the toxic microbial concentration 545.50 ± 7.78 mg/L [62].

This indicates that the strain examined is resistant to ibuprofen [61]. However, a decrease in the optical density of bacterial cultures was also reported since these compounds are not a sufficient carbon source. An additional carbon source can improve the degradability of the strain by increasing biomass [63].

The above characteristics of *B. thuringiensis* suggest the possibility of its use as a powerful and useful tool in the bioremediation of environments contaminated with nonsteroidal anti-inflammatory drugs [61].

On the other hand, the bacteria *Comamonas aquatica* and *Bacillus* sp. obtained from water samples tested the biodegradation of ibuprofen. In batch trials, they were able to degrade 100 mg/L of ibuprofen in 33 h, with a specific growth rate (μ) of 0.21 h^{-1}. The removal of the compound, as determined by high-performance liquid chromatography (HPLC), exceeded 99% of the initial concentration, with a removal of 92.3% of the chemical oxygen demand [64].

Similarly, Murdoch and Hay [37], described the bacterial strains *Sphingomonas* Ibu-2 and *Variovorax* Ibu-1 capable of degrading ibuprofen to high concentrations.

14 Biological Technologies for the Elimination of Naproxen

14.1 Bacterial Biodegradation

For the biodegradation of naproxen, it has been reported that the bacterium *Bacillus thuringiensis* as a good candidate. It is the first bacterial strain in which the key metabolites of the degradation of naproxen are detected: O-desmethylnaproxen and salicylic acid; in addition it was found that the presence of aromatic compounds in the reaction environment does not inhibit or only slightly decrease the degradation of naproxen and that the biodegradation of naproxen decreases in the presence of Cd (II) and Co (II) while the addition of Cr (VI) and Cu (II) has no negative effect on this process [65].

On the other hand, the bacteria *Planococcus* sp. is capable of removing approximately 30% of naproxen after 35 days of incubation in monosubstrate culture. Under co-metabolic conditions, with glucose or phenol as a growth substrate, degradation efficiency increased. During 35 days of incubation, 75.14 ± 1.71% and 86.27 ± 2.09% of naproxen were degraded in the presence of glucose and phenol, respectively [66].

14.2 Biodegradation and Enzymes

Little is known about the degradation of naproxen by bacteria. So far, only a few bacterial strains, mainly of the genera *Pseudomonas*, *Sphingomonas*, *Patulibacter*, *Nocardia*, *Rhodococcus*, and *Stenotrophomonas*, have been described as capable of degrading nonsteroidal anti-inflammatory drugs [37, 67–69].

In the case of *Stenotrophomonas maltophilia*, it transformed naproxen in 35 days with a degradation efficiency of approximately 28%. Under co-metabolic conditions with glucose or phenol as a carbon source, the degradation efficiency was 78% and 40%, respectively. In addition, in the presence of naproxen phenol monooxygenase, naphthalene dioxygenase, hydroxyquinol 1,2-dioxygenase and gentisate 1,2-dioxygenase was induced. This suggests that the degradation of naproxen is

produced by its hydroxylation to 5,7,8-trihydroxynaproxen, an intermediate that can be cleaved by hydroxyquinoline 1,2-dioxygenase [70].

The cleavage product is probably oxidatively cleaved further by 1,2-dioxygenase gentisate. The results obtained provide the basis for the use of co-metabolic systems in the bioremediation of environments contaminated with polycyclic NSAIDs [70]. This is the first report on the biotransformation of naproxen, a polycyclic NSAID, by a bacterial strain.

14.3 Use of Bacterial Enzymes

Planococcus sp. has the ability to efficiently degrade naproxen in the presence of 4-hydroxybenzoate as a carbon source. In this condition, the activity of monooxygenase, hydroxyquinoline 1,2-dioxygenase, and two different dioxygenase protocatecate are observed. The presence of various metabolic pathways and the induction of different oxygenases involved in the degradation of aromatic compounds allow the use of *Planococcus* sp. in the degradation of various aromatic contaminants, including nonsteroidal anti-inflammatory drugs [71].

14.4 Biodegradation by Fungi and Lacquer Action

So far, only a few microorganisms, mainly fungi (*Penicillium* sp., *Trametes versicolor, Cunninghamella elegans, C. echinulata, C. blakesleeana, Beauveria bassiana, Phanerochaete chrysosporium, P. sordida, Bjerkandera sp., B. adusta, Irpex lacteus* and *Ganoderma lucidum*) and the actinomycete *Actinoplanes* sp., have been identified to transform or degrade nonsteroidal anti-inflammatory drugs [72–75].

In the transformation of naproxen to 2-(6-hydroxy-naphthalen-2-yl) propionic and 1-(6-methoxynaphthalen-2-yl) ethanone by the fungus *T. versicolor*, cytochrome P450 and laccase were probably hired [73, 76]. They also demonstrated the degradation of naproxen by means of the commercial laccase from *Myceliophthora thermophila* [72]. They observed a 100% degradation of this pharmaceutical product in the presence of the redox mediator [72].

Table 1 includes the bacteria reported useful in different processes used in the degradation of the nonsteroidal anti-inflammatory drugs of greater consumption.

15 Conclusion

In this chapter we have addressed relevant aspects of the nonsteroidal anti-inflammatory drugs of higher consumption, we have mentioned that being considered emerging pollutants require the necessary attention to be eliminated from the

Table 1 Microorganisms used in different processes of biological technologies for the degradation of NSAIDs

NSAIDs	Biological technologies	Microorganisms	Reference
Diclofenac	Microbial consortium	*Alcaligenes faecalis*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Proteus mirabilis*	[46]
	Biologically activated carbon filter	*Pseudoxanthomonas* sp.	[47]
	Artificial wetland systems	*Mycobacterium flavescens*	[48]
	Biogenic compounds	*Pseudomonas putida*	[49]
	Bacterial biodegradation	*Labrys portucalensis*, *Brevibacterium* sp.	[50]
	Metabolic and co-metabolic biodegradation	*Enterobacter hormaechei*	[51]
	Bioremediation by laccase enzymes	*Streptomyces cyaneus*, *Streptomyces ipomoea*, *Streptomyces griseus*, *Streptomyces psammoticus*, *Trametes versicolor*	[52–55]
Ibuprofen	Artificial wetland systems	Family: *Flavobacteriaceae*, *Methylococcaceae*, *Spirochaetaceae*, *Rhodocyclaceae* Genus: *Methylocystis* sp., *Clostridium* sp., *Ignavibacterium* sp.	[56, 57]
	Biodegradation in biological filter	*Serratia marcescens*	[58]
	Bacterial biodegradation	*Raoultella* sp. *Bacillus thuringiensis*, *Comamonas aquatica*, *Bacillus* sp. *Sphingomonas* sp. *Variovorax* sp.	[37, 59–64]
Naproxen	Bacterial biodegradation	*Bacillus thuringiensis*, *Planococcus* sp.	[65, 66]
	Biodegradation and enzymes	*Pseudomonas* sp., *Sphingomonas* sp., *Patulibacter* sp., *Nocardia* sp., *Rhodococcus* sp., *Stenotrophomonas maltophilia*	[37, 68–70]
	Use of bacterial enzymes	*Planococcus* sp.	[71]
	Biodegradation by fungi and lacquer action	*Penicillium* sp., *Trametes versicolor*, *Cunninghamella elegans*, *Cunninghamella echinulata*, *Cunninghamella blakesleeana*, *Beauveria bassiana*, *Phanerochaete chrysosporium*, *Phanerochaete sórdida*, *Actinoplanes* sp., *Bjerkandera* sp., *Bjerkandera adusta*, *Irpex lacteus*, *Ganoderma lucidum*, *Myceliophthora thermophila*	[72–76]

environment using biological technologies that in addition to being less expensive generate a lower number of toxic products. There are several technologies; the most commonly used are those that include bacterial strains. There are currently several prokaryotic species that have been successfully tested in the processes for the degradation of NSAIDs. It is important to mention that current studies are focused on continuing to search for more bacteria to be used in these bioremediation processes, without ruling out that fungi also play an important role in biodegradation processes of pharmaceutical contaminants.

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Risk Evaluation and Legal Framework of the Nonsteroidal Anti-inflammatory Drugs Around the World

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Contents

Abstract Ecological risk assessment is generally carried out to assess the dose that may be harmful to particular species found in the freshwater. The ecological risk assessment is generally expressed through RQ or hazardous quotient, RQ < 0.1 suggests no risk, $0.1 \leq$ RQ ≤ 1.0 suggests medium risk, and RQ > 1.0 suggests high risk to that particular species. Regarding this, the risk of nonsteroidal anti-inflammatory drugs (NSAIDs) has been particularly studied by several researchers in many countries because they are pharmaceuticals of high consumption by the population due to their analgesic, anti-inflammatory, and antipyretic properties, as well as their occurrence in different water bodies at different concentrations and the toxic effects reported in organisms that are exposed to them. Due to the results obtained, they have also been considered for future regulations, and in particular, diclofenac was added to the list of priority substances of the European Commission which should be monitored as water pollution agents in the European Union. In this chapter we will discuss some of these risk assessment studies as well as the legislation proposed by some countries for their regulation.

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1 Introduction

Pharmaceutical products are present in the environment as a result of patient use, production, and formulation of the drug and improper disposal [1]. Based on this knowledge, the European Medicines Agency (EMA) issued a Guide for Environmental Risk Assessment (ERA) of medicines for human use in 2006 [2]. This guideline consists of two phases. In Phase I, the predicted environmental concentration (PEC) for surface water is calculated, and the octanol-water partition coefficient (K_{ow}) is measured. If the PEC is above 0.01 μg L^{-1}, a Phase II evaluation is performed; if logK_{ow} 4.5, persistence, bioaccumulation potential, and toxicity should be evaluated. Pharmaceuticals that are known to have toxic activity in concentrations below 0.01 μg L^{-1} must also enter Phase II, following a personalized risk assessment strategy that addresses their specific mechanism of action. Phase II is divided into two levels (A and B). Level A involves a basic set of aquatic toxicity and target tests to determine the predicted no-effect concentration (PNEC) for three trophic levels (algae, daphnia, and fish). Level B consists of an extended evaluation that uses refined values for PEC and PNEC calculations. At this stage, both an analysis of the destination and effect studies can be performed. The pharmaceutical product is evaluated by generating a risk quotient (RQ), evaluating the relationship between the PEC and the PNEC; when the ratio is less than 1, no risk is expected from the pharmaceutical environment for the aquatic environment.

In recent years, the level of health care is increasing, so various pharmaceutical products are frequently used to cure diseases such as muscle pain, headache, and some inflammatory conditions. Nonsteroidal anti-inflammatory drugs (NSAIDs) are generally used to cure any type of pain. NSAIDs are mainly derivatives of carboxylic acid that act by inhibiting the synthesis of prostaglandins produced by the cyclooxygenase enzyme that is present in various tissues and that is responsible for the mediation of various physiological signals. Among the most consumed NSAIDs are diclofenac, naproxen, ketoprofen, ibuprofen acetylsalicylic acid, and paracetamol [3]. Among the main reasons that this pharmacological class is among the most consumed worldwide is that its sale does not require a prescription; they are inexpensive and usually have fewer side effects [4].

In particular, diclofenac has been used as a NSAID for human health purposes since the 1970s, and in some countries, it has been used as an all-purpose veterinary medicine for domestic livestock. Regarding human health purposes, the global annual consumption averaged 1,443 ± 58 t [5]. It is due to this that it has been habitually detected in the environment and that the study of the risk that it could have when being in contact with the organisms becomes relevant. Table 1 presents some of the studies that have been carried out regarding this pharmaceutical.

Table 1 Reported studies on the risk of diclofenac in the environment

Reference	Country	Conclusion
Li et al. [6]	China	They provide a ranking of ten pharmaceuticals in terms of their occurrence, persistence, bioaccumulation, and toxicity based on the predicted environmental concentration; the pharmaceuticals that ranked at the highest level in this study include ibuprofen, erythromycin, and diclofenac, which are also ranked at the highest priority level in other countries. Ibuprofen were classified as high risk because they have large consumption and is detected frequently in water environment in China
Ashfaq et al. [7]	Pakistan	NSAIDs were predominated in the wastewater; acetaminophen showed the highest concentration with a median concentration of 13,880 ng L^{-1} and 12,120 ng L^{-1} in Cantonment Drain and Shama Drain. Other NSAIDs, which showed their median concentration >100 ng L^{-1}, included mefenamic acid (1952–3,240 ng L^{-1}), ibuprofen (1728–2,300 ng L^{-1}), naproxen (1386–1970 ng L^{-1}), diclofenac (260–470 ng L^{-1}), antipyrine (109–206 ng L^{-1}), and ketoprofen (81–132 ng L^{-1}). In the other hand, they carried out a risk assessment study in the wastewater and the surface water and report that acetaminophen showed high risk in wastewater against *S. proboscideus* and *D. magna* and medium risk in surface water against *Daphnia*, *while* other NSAIDs (included ibuprofen and diclofenac) showed high risk against one or more aquatic species
Kötke et al. [8]	Germany	Diclofenac have a medium risk for freshwater organisms with a RQmax of 0.271
Peña-Guzmán et al. [9]	Latin America review	According to the review carried out, it was found that the most measured emergent contaminants are pharmaceuticals, followed by personal care products and endocrine disruptors. Diclofenac was detected in surface water in concentrations of 0.04–500 ng L^{-1}, in groundwater in 3.19–2000 ng L^{-1}, and in drinking water treatment plants of Brazil in concentrations of 11–74 ng L^{-1}. They conclude that the true impacts of these pollutants on the environment and public health should be taken into further consideration
Offiong et al. [10]	South Korea	The occurrence and removal of four NSAIDs (ibuprofen, diclofenac, acetaminophen, and ketoprofen) in influent and effluent samples from three wastewater treatment plants were evaluated and all, except diclofenac, were detected in wastewater influents and effluents; diclofenac was detected in only one influent sample at a concentration of 7.8 ng L^{-1}; on the other hand, they calculated that the potential risk level of acetaminophen was low to fish and green algae since RQ values were far less than unity, ketoprofen posed significantly high risk to fish and low-medium risk to green algae, and ibuprofen posed a high risk to algae and low-medium risk to fish and invertebrates
Gworek et al. [11]	Global review	Based on their review, they suggest that phototransformation was the main process eliminating diclofenac from the lake

(continued)

Table 1 (continued)

Reference	Country	Conclusion
		waters. Ibuprofen, which is a drug with a relatively high coefficient of sorption to particles, can be eliminated by the sedimentation process. And ketoprofen and naproxen can be removed by the biodegradation and phototransformation processes. On the other hand, diclofenac posed an environmental risk (RQ 23.1)
Cesen et al. [12]	Slovenia	They assessed an ERA using maximal and average concentrations in wastewater effluents and surface water before and after discharge and obtained toxicity data (lowest values for worst-case scenario) from the literature or by using the ECOSAR software (SI-XIV), they concluded that the environmental risk of diclofenac was not confirmed in their study due to lower mass loads in Slovene effluents (2.09–48.5 ng L^{-1}), and similarly, the degradation products of diclofenac revealed negligible risk, though their toxicity was assessed using the ECOSAR software (SI-XIV). The RQs for these degradation compounds will be re-evaluated when experimentally derived toxicity data becomes available
Subari et al. [13]	Malaysia	They study the sources of pharmaceuticals in surface water samples and determine their presence in influent and effluent wastewater samples from six extended aeration wastewater treatment plants (WWTPs) located on Klang River and report that the lower removal rate of diclofenac (38.7%) resulted in high contribution of this compound from treated wastewater into the river; then, results for the RQ value were calculated from acute and chronic toxicity data for the three trophic levels (fish, invertebrates, and algae) and found that salicylic acid showed high risk for acute toxicity in fish, while diclofenac displayed high risk for chronic toxicity in fish (RQ $>$ 1). However, the levels of these pharmaceuticals were not toxic for invertebrates and algae
Acuña et al. [5]	Global review	Diclofenac has been detected in many freshwater ecosystems worldwide; they review 142 scientific publications and reported diclofenac occurrence data in freshwater, covering 38 countries with a median concentration of 21 $\pm$ 722 ng L^{-1}. Also their review suggests that diclofenac might pose harmful effects on the environment, but some of the phototransformation products of diclofenac carry a higher toxicity than the parent compound, as has been reported by several studies, and they suggest that more research is needed before robust conclusions about the ecotoxicology of diclofenac can be made
Kummerová et al. [14]	Czech Republic	They report that the exposure to environmentally relevant concentrations of diclofenac and paracetamol affects biochemical processes of duckweed plants via formation of ROS and RNS that results in enhanced lipid peroxidation, loss of the plasma membrane integrity, and changes in antioxidant systems prior to the appearance of any physiological and growth responses

(continued)

Table 1 (continued)

Reference	Country	Conclusion
Lolic et al. [15]	Portugal	Assess the spatial distribution of seven pharmaceuticals and two metabolites belonging to NSAIDs (ibuprofen, 1-hydroxyibuprofen, carboxyibuprofen, acetaminophen, acetylsalicylic acid, naproxen, nimesulide, ketoprofen, and diclofenac) among north Portuguese coast. And report that acetaminophen, ketoprofen, and the metabolite hydroxyibuprofen were detected in all the seawater samples at maximum concentrations of 584, 89.7, and 287 ng L^{-1}, respectively, while carboxyibuprofen had the highest seawater concentration (1,227 ng L^{-1}). The temporal distribution of the selected pharmaceuticals during the bathing season showed that, in general, higher concentrations were detected in August and September. They also assessed the environmental risk posed by the pharmaceuticals detected in seawaters toward different trophic levels (fish, daphnids, and algae), and only diclofenac showed hazard quotients above one for fish, representing a potential risk for aquatic organisms

As can be seen, NSAIDs, like other pharmaceutical products, have the ability to cause toxicity in the environment, even at very low concentrations in the order of ng L^{-1} to μg L^{-1} [16], so that the presence of NSAIDs in the environment has become a topic of great concern due to their potential ecotoxicity, as there is evidence that they can negatively affect aquatic and terrestrial organisms at different trophic levels and may cause damage to function of the ecosystem and the services that depend on it [5, 17, 18]. Regarding this, there are several studies that have reported risk assessments for this group of pharmaceuticals, and some of them are found below:

In Hernando et al. [19]'s study, a general description of the environmental occurrence and the ecological risk assessment of pharmaceutical waste is presented from the literature data. The risk quotient method (RQ) was applied to estimate the environmental risk of pharmaceutical products that are most frequently detected in wastewater, surface water, and sediment effluents.

Comparing the concentrations present in the effluents of the wastewater treatment plants and in surface waters against toxicity data, in this study, in both cases the nonsteroidal anti-inflammatory ibuprofen, naproxen, diclofenac, and ketoprofen present a high ecological risk for representative species of the food chain that are typically used in acute toxicity tests (bacteria, algae, and invertebrates) in these circumstances.

Yamamoto et al. [20] selected eight pharmaceutical products based on their internal consumption in Japan, the proportion of excretion of the original compound, and the frequency of detection in the aquatic environment or in the wastewater treatment plants. Toxicity tests were performed using Japanese medaka (*Oryzias latipes*), *Daphnia* (*Daphnia magna*), and green algae (*Pseudokirchneriella subcapitata*). The predicted no-effect concentration (PNEC) was calculated using values of lethal or effective concentration 50 (LC_{50} or EC_{50}), and the concentration

without effect (NOEC) was obtained from toxicity tests performed in the study for pharmaceutical compounds. The expected environmental concentration (PEC) was also calculated based on annual consumption, excretion rate, and disposal rate in the preliminary treatment of activated sludge. In the study, they found a PEC/PNEC ratio greater than 0.01 for acetaminophen (0.036) and for ibuprofen (0.066). This assessment does not suggest an urgent or serious concern about the ecological risk of these compounds; however, the authors suggest further studies using chronic toxicity tests, including evaluations of endocrine disruption and reproduction.

Kim et al. [21] studied four of the most widely used pharmaceutical products in Korea, including acetaminophen. For toxicity tests, they used a marine bacterium (*Vibrio fischeri*), a freshwater invertebrate (*Daphnia magna*), and the Japanese medaka fish (*Oryzias latipes*). In general, *Daphnia* was the most susceptible among the test organisms. The expected environmental concentrations (PEC) obtained for pharmaceutical products ranged from 0.14 to 16.5 μg L^{-1}, the latter for acetaminophen. The calculated risk ratio for acetaminophen was 1.8, which suggests possible environmental concerns and the need for further research, according to the authors.

In Santos et al. [22]'s study, the presence of four anti-inflammatories (diclofenac, ibuprofen, ketoprofen and naproxen) was evaluated in samples from four wastewater treatment plants in Seville, Spain. In addition, elimination rates in treatment plants and risk assessment of pharmaceutical compounds were studied. Ibuprofen had the highest concentrations in the range of 12.13–373.11 μg L^{-1} and 0.78–48.24 μg L^{-1} in influent and effluent wastewater, respectively; followed by naproxen (1.10–27.40 μg L^{-1} and 0.22–4.28 μg L^{-1}) and ketoprofen (0–3.59 μg L^{-1} and 0–1.50 μg L^{-1}). The estimation of the average environmental concentration (MEC) in influent and effluent samples, the expected concentrations without effect (PNEC), and the risk ratios (MEC/PNEC) for aquatic organisms was made. The risk quotient for ketoprofen was less than one in both influent (RQ = 0.23) and effluent (RQ = 0.10) wastewater; therefore, no ecological risk is expected to occur. The risk ratios for ibuprofen were 41.18 and 5.32 for influent and effluent wastewater, respectively. This means that despite the significant decrease in the concentration of ibuprofen due to wastewater treatments, there is still an ecological risk in effluent wastewater. Finally, the risk quotient of naproxen was greater than one (RQ = 1.28) in influent wastewater but less than one (RQ = 0.20) in effluent wastewater, which indicates that any ecological risk due to naproxen has been considerably reduced after the treatment of wastewater.

In Martín et al. [23]'s study, an evaluation was carried out of the presence of 16 pharmaceutical compounds present in wastewater (influents and effluents) and in primary, secondary, and digested sludge, in the city of Seville, in southern Spain, over a period of 1 year. In addition, the ecotoxicological risk for aquatic and terrestrial ecosystems was evaluated, due to wastewater discharges to receiving currents and the application of digested sludge as fertilizers in soils. All compounds found in wastewater were also found in sewage sludge at average concentrations of 8.1–2,206 μg kg^{-1}, except diclofenac. Among the nonsteroidal anti-inflammatory drugs, ibuprofen and salicylic acid were the compounds that had the highest

concentrations (50.6 and 27.8 μg L^{-1}). To estimate the RQ values in the effluent wastewater and digested sludge, the highest concentrations found were taken and used as MEC; as for the RQ values in the receiving streams and in the soils modified with digested sludge, they were applied the highest PEC values. The greatest ecotoxicological risk in wastewater and in digested sludge was ibuprofen (RQ: 3.2 and 4.4, respectively).

Bouissou-Schurtz et al. [24] focused on the list of 33 chemicals that was established through a French national prioritization strategy. The assessment of the potential risks to the environment was a gradual procedure: (1) the expected environmental concentration (PEC) of all the molecules evaluated in the national survey was determined based on the highest recommended dose used and, (2) the concentration used Average Measured Environmental Concentration (MEC) and the predicted no-effect concentration (PNEC) to establish the risk quotient (RQ) based on the PEC/PNEC (estimated risk) or MEC/PNEC (real risk). The risk assessment was performed using a binary ecological classification that suggests that an appreciable risk is likely (RQ 1). In this study, the environmental risk was estimated likely for the following nonsteroidal anti-inflammatory drugs: acetaminophen (RQ = 1.6), ibuprofen (RQ = 600), and diclofenac (RQ = 15). Only ibuprofen was identified with a real environmental risk based on its MEC (RQ = 1.9).

Lolić et al. [15] conducted a study where the presence of seven drugs and two metabolites belonging to the therapeutic classes of nonsteroidal anti-inflammatory drugs and analgesics were evaluated. A total of 101 samples covering 14 beaches and 5 cities on the north coast of Portugal were evaluated. Acetaminophen, ketoprofen, and the hydroxyibuprofen metabolite were detected in all seawater samples at maximum concentrations of 584, 89.7, and 287 ng L^{-1}, respectively. Carboxyibuprofen had the highest concentration in seawater (1,227 ng L^{-1}). The temporal distribution of the selected pharmaceutical products showed that, in general, higher concentrations were detected in August and September. The environmental risk represented by these pharmaceutical products detected in marine waters at different trophic levels (fish, daphnids, and algae) was also evaluated, with respect to their hazard quotient (HQ), which was calculated according to the guideline of the European Union. The result was obtained from the quotient between the mean environmental concentration (MEC) which was obtained from the different beaches evaluated and the expected concentration without effect (PNEC), which was estimated using the acute ecotoxicological data of lesser magnitude reported in the literature (EC_{50} or LC_{50}) for acute toxicity studies at the three trophic levels and applying an evaluation factor (usually 1,000), to establish the "worst-case" scenario. If HQ is equal to or greater than 1, there is a possible environmental risk situation, while when it is less than 1, no risk is expected. The fish were the species that showed the highest HQ values and were the only species that had HQ 1 values, while the daphnia and the algae showed a similar sensitivity to the detected pharmaceutical products and never exceeded the threshold value of one. Diclofenac was the pharmaceutical product that recorded the highest HQs for fish at two of the sampling points. Ibuprofen also showed a high HQ for fish, although the threshold limit was never exceeded. For daphnids, the highest HQs for acetaminophen were obtained,

while algae showed a similar sensitivity to ketoprofen, ibuprofen, and naproxen pharmaceuticals.

Gamarra et al. [25] conducted a study which had as its main objective the environmental risk assessment of the anti-inflammatory diclofenac and ibuprofen in cities of the state of Paraná, Brazil (319 cities for Diclofenac and 104 for ibuprofen), over the course of 3 years, using the available data of the public health system of Brazil. The environmental risk (ER) was evaluated using the approach of the European Medicines Agency, and the environmentally planned concentrations (PEC) were calculated considering the metabolism of the drug, excretion data, biological filter removal rates and treatment processes of wastewater with activated sludge to define environmental scenarios. The predicted no-effect concentration (PNEC) for these drugs was obtained from the literature, and the risk coefficient (RQ) of the PEC/PNEC ratio was calculated; RQ 1 values suggested an environmental risk. RQ 1 values for diclofenac were found in 12 cities, while for ibuprofen these values were found in 51 cities in the study area. It is important to keep in mind that almost all cities reach ER values in the third year. These results suggest an environmental alert status for some cities due to the current usage and consumption patterns of both pharmaceutical products. The authors suggest the monitoring of these patterns by health and environmental authorities to establish public policies, in order to minimize possible environmental impacts.

In Mendoza et al. [26]'s work, the presence of 25 pharmaceutical compounds belonging to 7 different therapeutic groups (including nonsteroidal anti-inflammatory drugs) and an iodinated contrast medium were analyzed in a medium-sized Spanish hospital located in the community of Valencia. The analysis of the compounds in the hospital wastewater was performed, and a risk assessment of the detection levels was applied. Further the environmental hazard associated with the various measured compounds was assessed by calculating the persistence, bioaccumulation, and toxicity index (PBT), which classifies the compounds according to their harmful characteristics for the environment. It was used in risk ratio (HQ) to assess the level of toxicological risk. The HQs for each individual compound were calculated according to the guidelines of the European Union as the ratio between the measured environmental concentration (MEC) and the predicted no-effect concentration (PNEC). The maximum individual concentration quantified for each pharmaceutical product in the various samples of hospital effluents was taken as MEC. PNEC values were obtained from the available aquatic toxicity data using three species of different trophic levels, representing the aquatic ecosystem (algae, crustaceans, and fish). If the HQ values are below 0.1, no adverse effects are expected, being classified as negligible risk. If the HQ values are between 0.1 and 1, the risk is low but should be considered as potential to generate adverse effects. If the HQ values are between 1.0 and 10, some adverse effect or moderate risk is likely. Finally, if the calculated HQ values are above 10, a high risk is anticipated. The anti-inflammatory that had the highest concentration was acetaminophen (44.3 $\mu g\ L^{-1}$), followed by ketoprofen, naproxen, ibuprofen, and diclofenac. The anti-inflammatory drugs acetaminophen (21.7), diclofenac (13.5), naproxen (21.5), and ibuprofen (219.6) showed an HQ greater than 10, which means a high risk for aquatic

organisms, while for ketoprofen the value obtained was between 1 and 10, which means a moderate risk for aquatic organisms. Regarding the environmental hazard index (PBT), diclofenac and ibuprofen obtained the maximum PBT Index value of 9, indicating a great potential to cause damage to the environment, ketoprofen and naproxen showed a PBT index of 6, indicative from an average danger to the environment, and, finally, acetaminophen obtained a PBT index of 3, which indicates a low hazard.

An exhaustive study was conducted in which the seasonal occurrence, elimination, and environmental risk assessment of 55 pharmaceutical products and personal care products were evaluated; the study was conducted over a year, collecting samples from the treatment plants of wastewater located in Volos, Greece. The risk coefficient (RQ) of each pharmaceutical product was evaluated by calculating the relationship between the average environmental concentration (highest concentration found in the water samples) and the predicted no-effect concentration (PNEC). Acute and chronic toxicity values for fish, invertebrates, and algae were obtained. According to the results obtained for acute toxicity in fish, diclofenac presents a high risk (RQ $>$ 1), while salicylic acid presents a medium risk. Regarding chronic toxicity problems, diclofenac also showed a high risk in fish. No compound appears to be toxic to invertebrates and algae. Finally, according to the environmental risk assessment data, diclofenac proves to be potentially dangerous for the aquatic ecosystem; the authors recommend that this compound should be included in specific monitoring campaigns on a regular basis to provide additional information on possible risks (Papageorgiou et al. [27]).

The presence of 48 emerging compounds of concern in wastewater and Slovenian surface waters was determined. The environmental risk was assessed by risk quotients (RQ) by calculating it as follows: RQ = MEC/PNEC. No anti-inflammatory evaluated in this study presented a high risk to the environment, having an RQ of 0.0163, 0.0280, 0.0844, and 0.00398, for diclofenac, ibuprofen, naproxen, and ketoprofen, respectively (Česen et al. [12]).

This study conducted in Cuernavaca, Morelos (Mexico), is presented as the first study known to date on the presence of pharmaceutical products in surface and wastewater of this Mexican state. In general, the most abundant pharmaceuticals in surface waters were the anti-inflammatory drugs naproxen (732–4,880 ng L^{-1}), acetaminophen (354–4,460 ng L^{-1}), and diclofenac (258–1,398 ng L^{-1}). Even though some of the most abundant compounds showed a good disposal (97%) during the wastewater treatment, the concentrations downstream of the wastewater treatment plant were slightly lower than upstream. The authors suggest the existence of additional untreated sewage inlets in the river. The risk that pharmaceutical products may represent for the aquatic environment was estimated through their HQ at three trophic levels, representative of the aquatic ecosystem (algae, daphidae, and fish). HQs were calculated as the ratio between the pharmaceutical MEC (highest concentration found in the analyzed samples) and its PNEC (calculated by dividing the lowest acute toxicity value reported in the literature reviewed for the three trophic levels selected by the relevant evaluation factor (usually 1,000)). Based on the HQ values obtained, the concentrations of ibuprofen (HQ = 111), diclofenac

(HQ = 28), and naproxen (HQ = 14.8) present in the river could present a high risk of toxicity to the aquatic ecosystem. The authors suggest the need to continue monitoring this type of compounds to adopt appropriate measures focused on safeguarding the ecosystem and, finally, human health (Rivera-Jaimes et al. [28]).

In Biel-Maeso et al. [29]'s study, the presence and distribution of 78 pharmaceutical products in different aquatic marine environments of the Gulf of Cádiz (Spain), including acetaminophen, diclofenac, ibuprofen, ketoprofen, naproxen, and salicylic acid, was evaluated for the first time. The results obtained revealed that pharmaceutical products were present in seawater at concentrations ranging from 16 to 2,133 ng L^{-1}. Critical points of potential marine pollution were observed in closed and semi-enclosed bodies of water (Bahía de Cádiz), which show concentrations that were one or two orders of magnitude higher than in the open ocean. The presence of these compounds in local wastewater treatment plants, one of the main sources of contamination, was also evaluated. The pharmaceutical products with the highest frequencies and detection concentrations in the sampling region were nonsteroidal anti-inflammatory drugs in the case of the Bay of Cadiz samples. The potential environmental risk for the target pharmaceutical products was evaluated based on the "wors-case scenario" according to the Technical Guidance Document on the European Union's risk assessment [30]. The risk quotients (RQ) for aquatic organisms were calculated from the measurable environmental concentrations (MEC) and the predicted no-effect concentration (PNEC) of each individual chemical considering the most sensitive species among those for which there was ecotoxicological information available. Most species are freshwater, and only two species *Artemia* (an invertebrate crustacean) and *Synechococcus leopoliensis* (unicellular cyanobacteria) are marine. This approach is limited but is often used due to the limited data on toxicity tests in marine aquatic species, especially for emerging pollutants. The maximum concentrations of acetaminophen, ibuprofen, naproxen, and salicylic acid measured in influential residual water samples were higher than their PNEC, obtaining RQ values of 3.74, 6.86, 1.12, and 28.20, respectively. However, after the wastewater treatment, all these compounds reduced their risk levels by showing RQ 0.1 in the effluent of the treatment plant. Finally, for the Bay water samples, ibuprofen and salicylic acid presented RQ = 0.30 and 0.48, respectively. The authors of this study encourage further research, since the distribution of pharmaceutical products and other emerging pollutants in marine environments is still relatively unknown, as well as the long-term synergistic effects of mixtures of these compounds toward marine biota. Performing specific toxicity tests with marine species would lead to an improvement in the environmental risk assessment of these compounds in the ocean.

Na et al. [31] examined the distribution of pharmaceutical products in the Yeongsan River (Republic of Korea) and in specific sources in the associated water system, conducting a risk assessment based on the findings. The samples included effluents collected from 3 wastewater treatment plants and 2 industrial complexes, as well as surface waters collected from 7 main streams and 11 river tributaries. Among the pharmaceutical products studied were acetylsalicylic acid and naproxen, which showed surface water concentrations of 44.7 and 51.6 ng L^{-1},

respectively. For the risk assessment of pharmaceutical products, the expected concentration without effect (PNEC) was estimated, based on the ecotoxicity values reported in various studies and then the hazard quotient (HQ). The HQ were less than one for the eight pharmaceutical products evaluated, including acetylsalicylic acid (HQ = 0.0014) and naproxen (0.0028). These results indicate that the probability of toxicity in the aquatic ecosystem is low and, therefore, there may not be a considerable overall risk.

The importance of this study is that, due to the lack of wastewater treatment facilities in Pakistan, wastewater is discharged directly through urban drains, which can cause a large load of contaminants of emerging concern and possible risks environmental. So, this study focused on the evaluation of the occurrence and risk of 52 pharmaceutical and personal care products of various kinds, in water and sediment samples from urban drainage and Lahore canals (the second city with the highest population density), Pakistan. Nonsteroidal anti-inflammatories were the pharmaceutical products predominantly detected in sewage from urban drains and surface water from canals. The highest concentration was observed for acetaminophen, with an average concentration of 13,880 ng L^{-1}, followed by ibuprofen (2,300 ng L^{-1}), naproxen (1970 ng L^{-1}), diclofenac (470 ng L^{-1}), and ketoprofen (132 ng L^{-1}). Another relevant aspect that the study showed was that population density has a significant correlation with the level of pharmaceutical and personal care products in surface waters, in addition to one of the drainage systems affected by the direct discharge of wastewater from the nearby industrial area. Regarding the environmental risk assessment, acetaminophen showed the highest RQ value of 18.0 over *Daphnia*, which indicates a high risk in wastewater. The other anti-inflammatories that showed an RQ 1 and therefore a high ecological risk were ibuprofen and diclofenac, while naproxen and ketoprofen showed minimal risk in surface waters, with a high or medium environmental risk in wastewater (Ashfaq et al. [7]).

The occurrence of 24 pharmaceutical products, including ibuprofen and diclofenac, was evaluated in 75 water samples collected in 4 bays in Uganda, in the Lake Victoria region. Ibuprofen was detected in all water samples (6–780 ng L^{-1}), while diclofenac was only present in water samples from Murchison Bay (2–160 ng L^{-1}). The ecotoxicological risk assessment showed that diclofenac presents a high toxic risk (RQ = 3.2) for aquatic organisms in the lake, while ibuprofen presents a medium risk (RQ = 0.39). This study is the first of its kind to report the levels and ecotoxicological risks of pharmaceutical compounds in the waters of Lake Victoria, Uganda, and East Africa as a whole (Nantaba et al. [32]).

2 Legal Framework

Pharmaceuticals are a subset of micropollutants, present in the environment in trace concentrations that, due to their persistent nature, are of particular concern since little is known about how mixtures of pharmaceutical residues, found in different

effluents, and affect the environment or public health; until now numerous studies show negative outcomes for both aquatic and terrestrial organisms, suggesting that they are given both to bioaccumulation and uptake in plants [33]. Given the volume of prescriptions, toxicity, and their presence in the environment, NSAIDs are one of the most studied pharmaceutical groups [34]. According to Miarov et al. [33], monitoring or treating pharmaceutical substances after their release into the environment is not a common practice internationally; however, there are several regulatory initiatives in some countries that have paid attention to this problem and that can be classified into three levels according to the intensity of the measures taken to regulate pharmaceutical pollutants in the environment:

(a) Level 1. Advanced regulatory framework: where the country/state has taken systematic legal measures to regulate pharmaceutical residues in order to reduce their volume in the environment. An example is California, where *Recycled Water Policy* requires treatment plants which discharge recycled water for the purposes of groundwater recharge to monitor groundwater recharge reuse (surface or subsurface application) for 17β-estradiol, gemfibrozil, and iopromide [35]. Although only these three pharmaceuticals are recommended to be monitored, in practice, the Groundwater Replenishment System (GWRS) voluntarily monitors a wider range of pharmaceutical substances on a quarterly basis in the purified recycled water: 17a-estradiol, 17a-ethynylestradiol, 17b-estradiol, atenolol, diclofenac, diethylstilbestrol, dilantin, epitestosterone, equilin, estriol, estrone, fluoxetine, iohexol, iopromide, meprobamate, naproxen, progesterone, testosterone, trimethoprim, acetaminophen, azithromycin, carbamazepine, erythromycin, gemfibrozil, ibuprofen, sulfamethoxazole, and triclosan; and the reports suggest that California's GWRS effectively eliminates these pharmaceutical contaminants from water supply [36, 37]. On the other hand, another country that is making great efforts to regulate the presence of pharmaceuticals in the environment is Switzerland, since it has a more comprehensive environmental program that includes monitoring pharmaceuticals in several media of the aquatic environment, for which the *National Surface Water Quality Monitoring Program* (NAWA) conducted their first pilot study in 2013 and measured only a small group of pharmaceuticals [38]; however, in 2018, the original NAWA testing program grew, adding 13 pharmaceuticals to regular measurements (atenolol, azithromycin, bezafibrate, carbamazepine, clarithromycin, diclofenac, mefenamic acid, metoprolol, naproxen, sotalol, sulfamethazine, sulfamethoxazole, and trimethoprim). But this country does not conform to monitoring only; since it has begun to act to reduce its presence and from 2016, hundreds of the country's municipal wastewater treatment plants will be improved over the next 20 years to remove micropollutants, such as pharmaceuticals [39].

(b) Level 2. Basic monitoring guidelines: where monitoring of pharmaceuticals is conducted on a regular basis, without a formal statutory requirement. Like Australia, monitoring and reporting contaminants associated with production of recycled effluents are the responsibilities of the state or territorial government, and officially the Australian Department don't regulate pharmaceuticals in

effluents, explaining that it is not a common practice internationally. Nonetheless, the *Australian National Guidelines for Water Recycling (Phase 2)* provides guidelines about concentrations that are applicable to potable water supplies [33]; in the other hand, *Drinking Water Guideline* provides a recommended drinking water concentration for 86 pharmaceuticals, with agricultural and veterinary applications [40], and in two programs, in Western Australia and Queensland, three pharmaceutical indicators are monitored regularly: carbamazepine, estrone, and diclofenac. Carbamazepine and diclofenac are monitored monthly and estrone quarterly, with testing frequency determined by the Department of Health based on estimated risk levels; also, 13 hormones and pharmaceuticals (including estriol, 17 a-estradiol, 17 b-estradiol, testosterone, androstenedione, etiocholanolone, equilenin, ethinyl estradiol, estrone, equilin, mestranol, norethindrone, and progesterone) are measured once a year in line with the standard water quality testing [41–43]. Another example is Singapore, where the National Water Agency has a *Water Monitoring Program* which includes a range of pharmaceuticals in the treated wastewater effluent such as ibuprofen, naproxen, ketoprofen, triclocarban, gemfibrozil, and diclofenac since 2007, and nowadays the capacity for monitoring has expanded and includes carbamazepine, trimethoprim, salicylic acid, and paracetamol [44].

(c) Level 3. Sporadic monitoring and small-scale mechanisms: where pharmaceutical residues are monitored intermittently, with little or no formal statutory foundation. There are many examples of studies of the occurrence of pharmaceuticals in different countries and in different bodies of water; however there are still more studies that can lead us to generate a regulation of the concentrations that do not produce toxic effects in the environment.

As can see, some NSAIDs have already been considered in levels 1 and 2 of the regulations, but it is worth mentioning the particular case of diclofenac, which in 2013, in the Directive 2013/39/EU that talks about priority substances in the field of water policy, in the article 16(4) in which is cited: "The Commission shall review the adopted list of priority substances at the latest four years after the date of entry into force of this Directive and at least every six years thereafter, and come forward with proposals as appropriate," and in accordance with this article, the commission carried out a review of the list of priority substances and concluded that the list should be amended in the light of scientific progress and the establishment of environmental quality standards for biota substances (with 11 additional substances added), it should be noted that this context was very useful, since it allow the inclusion of emerging pollutants to the list of priority substances based on the results obtained by various investigations, and in the case of pharmaceuticals specifically, for the first time are mention as contaminants of emerging concern; in section 15 of the Directive 2013/13/EU refers to them textually: "The contamination of water and soil with pharmaceutical residues is an emerging environmental concern. In evaluating and controlling the risk to, or via, the aquatic environment from medicinal products, adequate attention should be paid to Union environmental objectives. In order to address that concern, the Commission should study the risks of

environmental effects from medicinal products and provide an analysis of the relevance and effectiveness of the current legislative framework in protecting the aquatic environment and human health via the aquatic environment"; and from this, three pharmaceuticals were added to the watch list, including diclofenac.

3 Conclusions

Since 1990, the concern about the presence of trace concentrations of pharmaceuticals in different bodies of water and the risk they represent for the health of both organisms and humans has led to the so-called emerging pollutants being more found in different parts of the world, and between them, one of the most studied groups due to their high consumption is that of NSAIDs, of which high concentrations in effluents as well as various toxic effects have been reported, in addition to several of them being considered as high risk for aquatic organisms, which has led to the emergence of efforts in various countries to regulate their presence in the environment; however, it remains insufficient, and a global action is required to ensure the decrease in concentrations in the environment and their correct use.

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Conclusions

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Contents

Abstract The book Non-Steroidal Anti-Inflammatory Drugs in Water: Emerging Contaminants and Ecological Impact includes knowledge about the problem of the presence of NSAIDs in aquatic ecosystems, their effects on aquatic organisms, and useful methods to reduce their impact on the environment. This chapter summarizes the main conclusions about the history of NSAIDs as emerging pollutants, their presence in water bodies around the world, toxic effects of these compounds on aquatic organisms, effective chemical and biological methods for their removal, and the legal framework for presence of these compounds in water bodies in the world.

Keywords Legal framework, NSAIDs, Occurrence, Removal methods, Toxic effects

1 Conclusions and Study Trends

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been frequently detected in water bodies around the world at concentrations ranging from ng/L to μg/L. This group of medicines has been identified in influents and effluents from wastewater treatment plants but also in lakes, rivers, industrial and hospital effluents, surface and groundwaters, and drinking water and sludge.

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Due to their physicochemical properties and pharmacokinetic behavior, stability, and half-life, these drugs tend to remain in the aqueous phase and are able to resist wastewater treatments. For this reason NSAIDs are not totally eliminated in water treatment plants, and therefore, they can be detected frequently in surface waters causing a potential risk in the water supply.

This group of medicines has a main characteristic that they are not necessarily persistent in the environment to cause negative effects on it, but its continuous introduction into the environment can generate deleterious effects on aquatic organisms.

Most NSAIDs can undergo abiotic transformations such as photodegradation generating degradation products that are more toxic than the original compounds. These compounds also undergo biotransformation processes in aquatic organisms generating metabolites.

Many studies have been conducted in aquatic organisms to evaluate the toxicity of NSAIDs in freshwater environments. The effects that have been identified by exposure to NSAIDs are diverse, including overexpression of cyclooxygenases, increase in CYP1A1 activity, oxidative stress, genotoxicity, cytotoxicity, early developmental alterations, teratogenesis, histological effects, and hematological effects between others. These toxic effects have been identified in species of algae, crustaceans, amphipods, rotifers, amphibians, and fish, among other species.

Various methods have also been used to try to remove NSAIDs from different types of effluents and waters, with regular and good removal rates, among these technologies: photolysis, photocatalysis, Fenton reactions, modified Fenton, ozonolysis, and nanotechnologies.

Several studies have confirmed the high and ubiquitous occurrence of NSAIDs in the freshwater. However, no information has been reported on the occurrence of these pollutants in the marine environments. It is recommended to assess in depth the occurrence, behavior and fate of these pollutants in these water bodies, as their consumption is likely to increase.

There is a need to investigate new alternative post-treatment techniques for NSAIDs removal from wastewater.

As numerous studies have shown NSAIDs-induced harmful toxic effects on non-targeted organisms, we conclude these drugs are a potential threat to the environment. However, further research is needed to better understand the neurotoxicity and endocrine disruption effects of these pollutants. It is also important to harmonize study methodologies to assess toxicological effects.