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Analysis, fate and toxicity of chiral non-steroidal anti-inflammatory drugs in wastewaters and the environment: a review.

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Analysis, fate and toxicity of chiral non-steroidal anti-inflammatory drugs in wastewaters and the environment: a review

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Abstract
Non-steroidal anti-inflammatory drugs (NSAIDs) are found in the aquatic environment globally. Such drugs including naproxen, ibuprofen and ketoprofen are chiral molecules. Enantiomers of those drugs have identical physicochemical properties but can behave and interact differently in chiral environments due to differences in their three-dimensional shape. This results in enantiospecific differences in environmental fate and toxicity, which is often overlooked. Therefore, we review the analytical methods, occurrence and fate, and toxicity of chiral non-steroidal anti-inflammatory drugs at the enantiomeric level. The advancement of enantioselective chromatography methods, particularly the use of polysaccharide-based stationary phases, has enabled trace determination of enantiomers in complex environmental matrices. Macrocosm and microcosm studies of engineered and natural environments revealed that such drugs can undergo both enantioselective degradation and chiral inversion. Enantioselectivity has been reported during wastewater treatment, in surface waters and in agricultural soils. The use of microcosms spiked with individual enantiomers over racemates is essential to evaluate these degradation and inversion fate processes. The chiral inversion process whereby one enantiomer converts into its antipode can be significant if the more toxic enantiomers are formed. Existing enantiospecific effect studies report less than an order of magnitude difference in enantiomer toxicity. However, toxicity data for enantiomers are limited and further research is needed to better appreciate the environmental risk at the enantiomeric level.

Keywords Emerging contaminant · Enantionic fraction · Metabolite · River · Soil · LC–MS/MS

Introduction
The presence of pharmaceutical drugs in the environment has been an increasingly active area of research over the last 20 years. NSAIDs are a common therapeutic group of pharmaceuticals used to relieve pain and reduce inflammation. They act via inhibition of the cyclooxygenane isoenzymes (Brune and Patrignani 2015). NSAIDs are mainly excreted in urine as metabolites with a small proportion of the consumed dose being excreted unchanged. For example, 1% or less of naproxen, ibuprofen and ketoprofen is reported to be excreted unchanged in urine (Upton et al. 1980).

The extensive use of NSAIDs as prescription and over-the-counter medication results in the presence of µg L−1 concentrations in untreated wastewater (Camacho-Muñoz et al. 2014; Cizmas et al. 2015; Gardner et al. 2013; Kasprzyk-Hordern et al. 2009; Larssen et al. 2014; Petrie et al. 2015; Roberts and Thomas 2006; Tijani et al. 2016). Furthermore, it is not uncommon for naproxen and ibuprofen concentrations to be greater than 10 µg L−1 in wastewater (Camacho-Muñoz et al. 2014; Kasprzyk-Hordern et al. 2009; Larsson et al. 2014; Petrie et al. 2015; Roberts and Thomas 2006). Incomplete removal during wastewater processing is reported for conventional wastewater treatment plants such as trickling filters and activated sludge (Camacho-Muñoz et al. 2012; Kasprzyk-Hordern et al. 2009; Petrie et al. 2015; Roberts and Thomas 2006; Verlicchi et al. 2012). Despite dilution of wastewater effluent upon discharge to surface waters, NSAIDs are found at ng L−1 to µg L−1 concentrations in the aquatic environment (Kasprzyk-Hordern et al. 2009; Petrie et al. 2015; Roberts and Thomas 2006).

NSAIDs are weakly acidic with acid-dissociation constants (pKa, pH at which 50% of the drug is ionized) in the range 3.7–4.9 (Table 1). Therefore, at typical environmental pH values (6.5–8.0) they will be ionized and negatively
| Drug, formula and CAS number | Structure (* denotes chiral carbon) | \( pK_a \) | Log \( K_{ow} \) | Log \( D_{ow} \) | Solubility (mg L\(^{-1}\)) | Log \( K_{oc} \) | Prescription | Pharmacological activity | Notes |
|-----------------------------|-----------------------------------|---------|-------------|-------------|----------------|-------------|----------------|-----------------------|-------|
| \( R/S(\pm)-ibuprofen \)  | ![ibuprofen structure]           | 4.85    | 3.79        | 1.64        | 41.05         | 2.60        | \( R/S(\pm)-ibuprofen \) | \( S(\pm)-ibuprofen inhibits COX-1 (IC\(_{50}\)=2.6 \mu M) and COX-2 (IC\(_{50}\)=1.53 \mu M). S(\pm)-ibuprofen inhibits COX activity, thromboxane formation, activation and platelet aggregation than \( R(\pm)-ibuprofen \). Chiral inversion in vivo in human from \( R \) to \( S \) is limited to approx. 10%. 90% of the dose is eliminated via urine as metabolites or their conjugates. | 50–60% of \( R(\pm)-ibuprofen \) undergoes metabolic inversion. |
| \( C_{13}H_{18}O_{2} \)  | ![ibuprofen structure]           | 3.97    | 1.97        | -1.06       | 1453          | 3.08        | --            | --                    | --    |
| \( R/S(\pm)-2-hydroxyibuprofen \) | ![2-hydroxyibuprofen structure] | 4.63    | 2.29        | -0.08       | 2974          | 1.23        | --            | --                    | --    |
| \( C_{13}H_{18}O_{3} \)  | ![2-hydroxyibuprofen structure] | 4.34    | 1.85        | -1.01       | 4987          | 1.65        | --            | --                    | --    |
| \( R/S(\pm)-2-phenylpropionic acid \) | ![2-phenylpropionic acid structure] | 3.88    | 3.12        | -0.66       | 120.4         | 2.46        | \( R/S(\pm) \) or as \( S(\pm)-keto-profen \) | \( S(\pm)-ketoprofen \) is a potent inhibitor of COX-1 and COX-2 (IC\(_{50}\)=1.9 and 27 nM, respectively). \( R(\pm)-ketoprofen \) is 100 to 1000 times less potent. Chiral inversion in vivo in human from \( R \) to \( S \) is limited to approx. 10%. 80% of the dose is eliminated via urine, primarily as the glucuronide metabolite. | -- |
| \( C_{9}H_{10}O_{2} \)  | ![2-phenylpropionic acid structure] | 4.30    | 2.56        | -0.51       | 1136          | 2.19        | --            | --                    | --    |
| \( R/S(\pm)-naproxen \) | ![naproxen structure]           | 4.19    | 3.10        | -0.27       | 144.9         | 2.54        | \( S(\pm)-naproxen \) | \( S(\pm)-naproxen \) is a non-selective COX inhibitor. The IC\(_{50}\) values for human recombinant COX-1 and COX-2 are 0.6–4.8 \mu M and 2.0–28.4 \mu M, respectively. 95% of naproxen and its metabolites can be recovered in the urine with 66–92% recovered as conjugated metabolite and less than 1% recovered as naproxen and desmethyl-naproxen. Less than 5% of naproxen in excreted in the faeces. | 95% of naproxen and its metabolites can be recovered in the urine with 66–92% recovered as conjugated metabolite and less than 1% recovered as naproxen and desmethyl-naproxen. Less than 5% of naproxen in excreted in the faeces. |
### Table 1 (continued)

| Drug, formula and CAS number | Structure (* denotes chiral carbon) | $pK_a^a$ | Log $K_{ow}^b$ | Log $D_{ow}^c$ | Solubility (mg L$^{-1})^b$ | Log $K_{oc}^b$ | Prescription | Pharmacological activity | Notes |
|-----------------------------|-----------------------------------|---------|---------------|--------------|------------------------|---------------|--------------|------------------------|-------|
| *R/S*(±)-O-desmethylnaproxen C$_{13}$H$_{12}$O$_3$ S(+) : 52079-10-4 R(−) : 123050-98-6 | ![Structure](image) | 4.34 | 2.54 | 0.24 | 2296 | 2.90 | – | – | – |
| *R/S*(±)-indoprofen C$_{17}$H$_{15}$NO$_3$ R/S(±) : 31842-01-0 | ![Structure](image) | 3.74 | 2.32 | −1.13 | 67.9 | 2.13 | *R/S*(±)-indoprofen | – | n.a |
| *R/S*(±)-carprofen C$_{17}$H$_{12}$ClNO$_2$ R/S(±) : 53716-49-7 S(+) : 52263-84-0 | ![Structure](image) | 4.42 | 3.73 | 1.11 | 2.20 | 3.69 | *R/S*(±)-carprofen | S(+) - carprofen IC$_{50}$ 176 µM for COX-1 and 7 µM for COX-2. *R(−)-carprofen IC$_{50}$ 380 µM for COX-1 and 161 µM for COX-2 in dog.$^j$ | n.a |
| *R/S*(±)-flurbiprofen C$_{15}$H$_{13}$FO$_2$ R/S(±) : 5104–49-4 | ![Structure](image) | 4.42 | 3.81 | 0.86 | 17.7 | 3.44 | *R/S*(±)-flurbiprofen | S(+) - flurbiprofen is the COX-active enantiomer of the non-selective COX inhibitor flurbiprofen with IC$_{50}$ values of 0.48 and 0.47 µM for COX-1 and COX-2, respectively, in guinea pig whole blood.$^k$ | 70% of the dose eliminated in the urine as parent drug and metabolites.$^n$ |
| *R/S*(±)-fenoprofen C$_{15}$H$_{14}$O$_3$ R/S(±) : 29679-58-1 | ![Structure](image) | 3.96 | 3.90 | −0.20 | 30.1 | 2.84 | *R/S*(±)-fenoprofen | S(+) - fenoprofen IC$_{50}$ 149 µM for COX-1 and 59 µM for COX-2. No data on *R(−)-fenoprofen.$^l$ | 90% of a dose is eliminated as glucuronide and 4′-hydroxyfenoprofen glucuronide.$^o$ |

Key: *R/S*(±), racemic mixture; $pK_a$, acid dissociation constant; $K_{ow}$, n-octanol/water partition coefficient; $D_{ow}$, n-octanol/water distribution coefficient; $K_{oc}$, organic/carbon partition coefficient; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; IC$_{50}$, half maximal inhibitory concentration; n.a., not available

$^a$ChemAxon; $^b$ChemSpider database; $^c$Dow was calculated as a function of pH using $D_{ow} = K_{ow}/(1 + 10^{pH-pK_a});^d$Barnett et al. (1994); $^e$Evans et al. (1991); $^f$Villanueva et al. (1993); $^g$Palomer et al. (2000); $^h$Ghezzi et al. (1998); $^i$Laneuville et al. (1994); $^j$Riviere and Papich (2009); $^k$Carabaza et al. (1996); $^l$Poggi et al. (2006); $^m$Evans (2001); $^n$DrugBank; $^o$Rudy et al. (1998)
charged. Their pH-dependent octanol–water partition coefficients (log $D_{OW}$) range from −1.13 to 1.64 at pH 7 (Table 1). Being charged enables electrostatic attractions with positively charged ions and surfaces in the environment. Other than the aqueous phase of environmental matrices, NSAIDs have been reported in the particulate phases of wastewater and activated sludge (Martín et al. 2012; Radjenović et al. 2009b) as well as being found in solid matrices including digested sludge, sediments and soils (Albero et al. 2014; Kumirska et al. 2015; Martín et al. 2012; Radjenović et al. 2009a).

Pharmaceutical drugs as a group of environmental pollutants remain termed emerging contaminants as their behaviour in the environment is not fully understood. Drug stereoreactivity plays a significant role on the fate and effects of chiral pharmaceuticals in the environment (Kasprzyk-Hordern 2010; Sanganyado et al. 2017). However, NSAID stereoreactivity in environmental settings is often overlooked. Therefore, the purpose of this review is to provide an up-to-date appraisal on the analysis, fate and toxicity of chiral NSAIDs, and to discuss recommendations for future research in this area.

**Chiral non-steroidal anti-inflammatory drugs**

Several NSAIDs are chiral including ibuprofen, naproxen, ketoprofen, indoprofen, flurbiprofen, carprofen and fenoprofen as well as several of their metabolites (Table 1). Enantiomers of chiral pharmaceuticals have identical chemical structures but different spatial arrangements of atoms around a stereogenic centre, e.g. see ketoprofen in Fig. 1a. Pairs of enantiomers have identical physicochemical properties but behave and interact differently in chiral environments due to differences in their three-dimensional shape. Consequently, chiral pharmaceuticals can exhibit enantioselectivity in environmental occurrence, fate and toxicity (Eaglesham et al. 2020; Kasprzyk-Hordern 2010; Ribeiro et al. 2012a, b, 2013; Sanganyado et al. 2017; Wong 2006). Pharmaceuticals with two stereogenic centres have two pairs of enantiomers and so on. Diastereomers are those stereoisomers that are not mirror images and can have different physicochemical properties, e.g. see the metabolite dihydroketoprofen in Fig. 1b.

Differences that exist in the way chiral NSAIDs are dispensed. For example, ibuprofen is dispensed as the racemate.
i.e. equal dose of both enantiomers, and naproxen is dispensed in enantiopure form as $S(+)$-naproxen only. Naproxen is dispensed in this way because $R(-)$-naproxen causes hepatic toxicity, and the majority of the desired pharmacological activity resides with $S(+)$-naproxen (Harrington and Lodewijk 1997). Ketoprofen is dispensed as the racemate and as enantiomerically pure $S(+)$-ketoprofen (Table 1). As enantiomers of the same drug can behave differently in biologically mediated environments, enantioselective changes during human metabolism can occur.

Chiral NSAIDs are unlike many other chiral pharmaceuticals in that they can undergo chiral inversion whereby one enantiomer can convert into its antipode (Wsol et al. 2004). For example, unidirectional inversion of the biologically less active enantiomer $R(-)$-ibuprofen to the active enantiomer $S(+)$-ibuprofen has been reported in in vivo mammalian studies (Hao et al. 2005). The inversion process proceeds with an enzyme mediated reaction to form an activated Coenzyme A derivative of $R(-)$-ibuprofen (Fig. 2). This initial biotransformation step is considered to be enantioselective. The derivative is then racemized by an epimerase and cleaved by a hydrolase to release $S(+)$-ibuprofen (Kato et al. 2004, 2003). Racemization could result from the enzymatic deprotonation of the Coenzyme A derivative forming an enol-type intermediate (Khan et al. 2014). Enzymatic protonation can then take place either side of the planar double bond system.

Further enantioselectivity of chiral NSAIDs can also be observed during wastewater treatment and in the environment itself (Caballo et al. 2015a; Camacho-Muñoz et al. 2019; Hashim and Khan 2011; Kasprzyk-Hordern 2010; Khan et al. 2014; Matamoros et al. 2009; Sanganyado et al. 2017). This results in enantiomeric compositions in the environment being considerably different to that of the dispensed medication. Significantly, laboratory-based ecotoxicity testing of pharmaceuticals to assess environmental risk is typically undertaken using ‘off-the-shelf’ analytical reference standards. This can lead to the underestimation or overestimation of the associated environmental risk if enantiospecific toxicity exists. Nevertheless, an improved understanding on the importance of drug stereochemistry has resulted in more exposure and effect-driven studies of NSAIDs undertaken at the enantiomeric level (Camacho-Muñoz et al. 2019; Yuan et al. 2018). This has been possible by the improvements in analytical methodologies capable of enantioselective determination at the ng L$^{-1}$ concentrations found in the environment.

**Enantioselective analytical methods for non-steroidal anti-inflammatory drugs in environmental matrices**

The role of the analytical method is to achieve adequate separation of enantiomers such that their concentrations can be measured accurately. Traditionally, analytical methods capable of enantioselective analysis were used for quality control purposes in the manufacturing industry. Such methods facilitate the analysis of single drugs only, commonly making use of ultraviolet detection. These methods utilized chiral stationary phases for direct enantiomer separations using liquid chromatography (LC) which avoids the need for derivatization. These LC methods were typically operated in normal phase with mobile phases not compatible with mass spectrometry (MS). However, the use of MS is essential for environmental analysis due to the complexity of environmental matrices and the comparatively low concentrations of pharmaceutical drugs present (Kasprzyk-Hordern 2010; Ribeiro et al. 2012b, 2014; Sanganyado et al. 2017; Wong 2006). Therefore, new enantioselective methods have been developed to enable the coupling of the chromatographic system to MS specifically for environmental analysis. This
also helps facilitate the simultaneous determination of several drugs in the same analytical run.

Enantioresolution ($R_S$) is a critical parameter to optimize during enantioselective method development. Ideally, $R_S$ greater than 1.5 ensures baseline separation; however, $R_S$ of 1.0 is considered adequate for quantitative purposes as it represents a maximum 2% peak overlap (Bagnall et al. 2012).

To date, enantioselective methodologies for NSAIDs have been developed for wastewater, river water, drinking water, sediments, sludge and fish tissues (Table 2). LC, gas chromatography (GC) and more recently, supercritical fluid chromatography (SFC) have been utilized as the separation method for environmental analysis. Prior to instrumental analysis, such methodologies require appropriate sample extraction and clean-up methods such as solid-phase extraction (SPE) and pressurized liquid extraction, depending on the matrix under investigation. Extraction methods are not considered enantioselective in nature and not discussed in this review. These have been reviewed extensively elsewhere, e.g. see (Boyaci et al. 2015; Buchberger 2011; Evans and Kasprzyk-Hordern 2014; Farré et al. 2012; Llompart et al. 2019; Madikizela et al. 2018; Plotka-Wasylka et al. 2016; Zuloaga et al. 2012).

**Liquid chromatography–mass spectrometry**

Liquid chromatography (LC) is the most popular method of enantioselective analysis due to availability of chiral columns. Direct enantiomer separations typically rely on different stereospecific interactions with the chiral stationary phase. Chiral additives can also be added to the mobile phase to achieve direct separations; however, they are often not compatible with MS (Sanganyado et al. 2017). The direct approach relies on formation of transient diastereomeric complexes between the chiral selector and drug enantiomers in thermodynamic equilibria. The formation of these transient diastereomeric complexes for enantioseparation is driven by hydrogen bonds or ionic, ion–dipole, dipole–dipole, van der Waals and π–π interactions (Scriba 2016). However, the precise mechanisms of enantioseparation remain poorly understood. Nevertheless, several tools are available to study the mechanism of chiral recognition including spectroscopic techniques, especially nuclear magnetic resonance spectroscopy. Molecular modelling is also proposed to visualize and analyze the dynamics of the process (Scriba 2016). Traditionally, the three-point interaction model was often used to describe the chiral recognition process (Bao et al. 2013; Pirkle and Pochapsky 1989). Here, three functional groups around the stereogenic centre of one of the enantiomers undergo molecular interactions with the chiral stationary phase (Berthod 2006). A variety of chiral stationary phases have been utilized for enantioseparation of NSAIDs including derivatized polysaccharides (Camacho-Muñoz et al. 2016; Li et al. 2020; Ma et al. 2019; Wang et al. 2018; Yuan et al. 2018), glycopeptides (Camacho-Muñoz and Kasprzyk-Hordern 2017), glycoproteins (Camacho-Muñoz and Kasprzyk-Hordern 2015) and Pirkle-type columns (Caballo et al. 2015a; Coelho et al. 2019).

**Chiral selector: polysaccharide phases**

The most popular phases for chiral NSAIDs are the derivatized polysaccharides (Table 2). Amylose tris-(3,5-dimethylphenylcarbamate), amyllose tris-(3-chlorophenylcarbamate), amyllose tris-(3-chloro-5-methylphenylcarbamate) and cellulose tris-(4-methylbenzoate) have all been achieved (Table 2). Such phases have a high number of chiral centres in the ordered polysaccharide backbone and substituents. Separation of carprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen, loprofen, naproxen and pranoprofen has all been achieved by polysaccharide phases (Table 2). Separation of NSAIDs is achieved in reversed phase mode under isocratic (Li et al. 2020; Ma et al. 2019; Yuan et al. 2018) and gradient elution mode (Camacho-Muñoz et al. 2016; Wang et al. 2018). Mobile phases typically consist of MS compatible, i.e. thermally labile, aqueous ammonium buffers such as formate or acetate, and organic modifiers such as acetonitrile or methanol. The type and concentration of buffer as well as the organic modifier influence enantioseparations (Camacho-Muñoz et al. 2016; Li et al. 2020; Ma et al. 2019; Wang et al. 2018; Yuan et al. 2018). For example, Li et al. (2020) reported a reduction in organic modifier of acetonitrile from 50 to 35% approximately doubled the flurbiprofen $R_S$ on an amyllose tris-(3-chlorophenylcarbamate) phase. Less significant changes to $R_S$ were observed by varying the ammonium formate concentration from 20 to 30 mM (Li et al. 2020).

Mobile phase pH also plays an important role in the separation. For example, when the analyte is fully ionized, it has a greater affinity with the aqueous mobile phase than the polysaccharide stationary phase. Polysaccharide phases such as amyllose tris-(3,5-dimethylphenylcarbamate) do not have ionic sites within their structure for interaction with charged analytes (Ma et al. 2019). As the $pK_a$ of the NSAIDs is 3.7–4.9 (Table 1), methods typically report the use of mobile phases with pH less than 5 (Table 2). However, care is needed as pH will also have a significant influence on MS sensitivity. Too low a pH will result in the neutral form of the drug predominating and poor MS response. Therefore, development of such methods for environmental analysis requires trade-offs between enantiomer separation and MS response. This could be overcome by post-column infusion of acidic/basic modifier which has been applied in LC–MS/MS and SFC–MS/MS (Borges et al. 2011; Svan et al. 2015). Nevertheless, existing methods using polysaccharide-based phases (and appropriate sample preparation) report method detection limits (MDLs) at low ng L$^{-1}$ for aqueous matrices.
| Drug | Matrix | Sample collection and storage | Extraction and clean-up | Stationary phase and column dimensions | Mobile phase conditions and run time | Analysis | Calibration method | $R_s$ | Recovery (%) | MDL (ng L$^{-1}$ or ng g$^{-1}$) | References |
|------|--------|------------------------------|-------------------------|----------------------------------------|--------------------------------------|---------|-------------------|------|---------------|------------------------|-----------|
| Carprofen, flurbiprofen, indoprofen and naproxen | Fish tissue (5 g) | $-20^\circ\text{C}$ USE-SPE | Amylose tris-(3-chlorophenylcarbamate); 250×4.6 mm, 5 μm | 20 mM NH$_4$HCO$_3$/ACN pH 3.5 (4:6, v/v) @ 0.6 mL/min; 25 °C; 10 μL injection; 35 min | LC–ESI–MS/MS | Matrix-matched | 0.66–7.17 | 82.6–106.7 | 1–8$^a$ | A |
| Flurbiprofen, ibuprofen and naproxen | River water (500 mL) | Stored in dark glass bottles with 0.02% (w/v) NaN$_3$ @ 4 °C; filtered SPE | Amylose tris-(3,5-dimethylphenylcarbamate); 150×4.6 mm, 5 μm | 10 mM NH$_4$OAc (pH 5): ACN (65:35, v/v) @ 0.4 mL/min; 25 °C; 20 μL injection; ≥ 17 min | LC–ESI–MS/MS | Matrix-matched | ≥ 1.0 | 89.3–100.5 | 0.35–11.1 | B |
| Flurbiprofen, ibuprofen, ketoprofen and naproxen | River water (500 mL) | Amber glass bottles @ 4 °C; filtered 0.45 μm; acidified to pH 2 SPE | 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene; 250×4.6 mm, 5 μm | MeOH/ H$_2$O + 0.1% AcOH (6:4); 1 mL/min; room T°; 10 μL injection; 90 min | LC–ESI–MS/MS | Internal standard (deuterated surrogates) | 0.68–28.1 | 96.0–107.0 | 0.36–2.66 | C |
| Flurbiprofen, ibuprofen and ketoprofen | River water, lake water and wastewater (100 mL), sediment and sludge (1 g) | Liquid samples: air-dried, ground, sieved, desiccator @ room T° Liquid samples: MSPE-DLLME; Solid samples: USE-MSPE-DLLME | Amylose tris-(3-chlorophenylcarbamate) 250×4.6 mm, 5 μm | H$_2$O+0.1%FA/ACN (4:6, v/v); 0.4 mL/min; 20 °C; 10 μL injection; ≥ 25 min | LC–ESI–MS/MS | Matrix-matched | > 1.0 | 75.8–92.1 | 1.85–3.73$^b$ | D |

$^a$ Carothers et al. (2018) 
$^b$ Carothers et al. (2019) 
$^c$ Carothers et al. (2020)
| Drug Matrix Sample collection and storage | Extraction and clean-up | Stationary phase and column dimensions | Mobile phase conditions and run time | Analysis Calibration method | $R_S$ | Recovery (%) | MDL (ng L$^{-1}$ or ng g$^{-1}$) | References |
|----------------------------------------|------------------------|----------------------------------------|----------------------------------|---------------------------|-------|-------------|-----------------|------------|
| Flurbiprofen, ibuprofen, indoprofen, laxoprofen and prano-profen | River water, influent and effluent wastewater (200 mL) | MSPE | Cellulose tris-(4-methylbenzoate) 150 x 4.6 mm, 5 μm | (A) 5 mM NH$_4$OAc (pH5) and (B) MeOH; Gradient: 0 min 30% A; 23 min, 30% A; 25 min, 20% A; 35 min, 20% A @ 0.4 mL/min; 20 °C; 10 μL injection ≥ 35 min | LC-ESI-MS/MS | Matrix-matched | > 1.5 | 76–91 | 9.40–26.3 | E |
| Ibuprofen and naproxen | Influent and effluent wastewater (500 mL) | Amber glass bottles @ 4 °C | Dimethyl-β-cyclodextrin 20 m, 0.25 mm i.d. 0.12 μm | He; Oven: 100 °C for 2 min, increased by 2 °C/min to 200 °C, held at 200 °C for 5 min; 2 μL injection | GC-EI-MS/MS | n.a | n.a | n.a | 100$^4$ | F |
| Carboxy-ibuprofen, 2-hydroxy-ibuprofen, ibuprofen, indoprofen, ketoprofen and naproxen | Influent and effluent wastewater (100 mL), river water (200 mL) | High-density polyethylene bottles @ 4 °C; filtered 0.7 μm | Glycopeptide teicoplanin 250 x 2.1 mm, 5 μm | 10 mM NH$_4$OAc (pH 4.2)/MeOH (7:3, v/v) @ 0.08 mL/min; 15 μL injection; min | LC-ESI-MS/MS | Internal standard (deuterated surrogates) | > 0.4 | 55.5–121.2 | 1.32–1319 | G |
| Drug Matrix Sample collection and storage | Extraction and clean-up | Stationary phase and column dimensions | Mobile phase conditions and run time | Analysis | Calibration method R<sub>s</sub> | Recovery (%) MDL (ng L<sup>−1</sup> or ng g<sup>−1</sup>) | References |
|------------------------------------------|------------------------|----------------------------------------|-------------------------------------|----------|-------------------------------|-------------------------------------------------|-----------|
| Carprofen, flurbiprofen, 2-hydroxyibuprofen, ibuprofen, indoprofen and naproxen | Influent and effluent wastewater (50 mL) | High-density polyethylene bottles @ −20 °C | SPE Amylose tris-(3,5-dimethylphenylcarbamate) 150×2.1 mm, 2.5 μm | CO<sub>2</sub> (A)/MeOH + 0.2% NH<sub>4</sub>OH (B); Gradient: 0 min—95% A, 3.5 min—95% A, 10 min—40% A, 13.5 min—40% A, 13.8 min—95% A, 16 min—95% A @ 1.5 mL/min, 30 °C, 5 μL injection; 16 min | UHSFC-ESI–MS/MS Internal standard (deuterated surrogates) | >0.7 47–127 2.38–2173 | H |
| Dihydromethylpropan, 2-hydroxyibuprofen, ibuprofen, indoprofen, ketoprofen and naproxen | River water (250 mL) and effluent wastewater (500 mL) | High-density polyethylene bottles @ 4 °C | SPE α1-acid glycoprotein 100×2 mm, 5 μm | 10 mM NH<sub>4</sub>OAc (pH 6.2)/ACN (99:1, v/v)/MeOH (7:3, v/v) @ 0.08 mL/min; 25 °C; 15 μL injection; <20 min | LC–ESI–MS/MS Internal standard (deuterated surrogates) | >0.7 73.7–158.2 0.1–23.2 | I |
| Ibuprofen, ketoprofen and naproxen | Freshwater fish (200 mg) | n.a | SUPRAS (R)-1-naphthylglycine and 3,5-dinitrobenzoic acid 250×4.6 mm, 5 μm | THF/50 mM NH<sub>4</sub>OAc in MeOH (9:1) @ 0.5–1.2 mL/min, 25 °C; 10 μL injection | LC–TIS–MS/MS Internal standard (deuterated surrogates) | >1.4 97–103 0.5–1<sup>a</sup> | J |
| Ibuprofen, ketoprofen and naproxen | Influent and effluent wastewater (72.2 mL) | Dark glass containers | SUPRAS (R)-1-naphthylglycine and 3,5-dinitrobenzoic acid 250×4.6 mm, 5 μm | THF/50 mM NH<sub>4</sub>OAc in MeOH (9:1) @ 0.5–1.2 mL/min, 25 °C; 10 μL injection | LC–TIS–MS/MS Internal standard (deuterated surrogates) | >1.4 97–103 0.5–1.2 | K |
| Drug                          | Matrix                                  | Sample collection and storage                                      | Extraction and clean-up | Stationary phase and column dimensions | Mobile phase conditions and run time | Analysis                  | Calibration method | $R_S$ | Recovery (%) | MDL (ng L$^{-1}$ or ng g$^{-1}$) | References |
|-------------------------------|-----------------------------------------|---------------------------------------------------------------------|-------------------------|----------------------------------------|-------------------------------------|---------------------------|-------------------|-----|-------------|-------------------------------|------------|
| Ibuprofen, ketoprofen and naproxen | Ultra-pure water, drinking water, synthetic effluent wastewater and river water (500 mL) | Amber glass bottles; filtered at 0.45 µm; acidified to pH 2.5 | SPE | Derivatization with (R)-1-phenylethylamine; HP5-MS fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 mm film thickness) | He @ 0.8 mL/min; 270–280 °C; Oven: 120 °C for 1 min, increased by 40 °C/min to 240 °C, increased by 5 °C/min to 300 °C, maintained for 4 min; 1 µL injection; 18 min | GC–EI–MS/MS | Internal standard (deuterated surrogates) | 2.87–4.02 | n.a | 0.2–3.3 | L |
| Ibuprofen                     | River water (1 L), influent and effluent wastewater (250 mL) | – 20 °C | SPE | 16-m OV1701-DMPen heptakis(2,6-O-dimethyl-3-O-n-pentyl)-â-cyclodextrin; 1:1 diluted with OV1701) fused silica column (0.25 mm i.d) | He; Oven: 70 °C for 2 min, increased by 20 °C/min to 120 °C, increased by 2.5 °C/min to 152 °C increased by 20 °C/min to 230 °C, maintained isocratically; 1 µL injection; 8 min | GC–EI–MS/MS | n.a | n.a | 1$^a$ | M |

Key: ACN, acetonitrile; AcOH, acetic acid; CO$_2$, carbon dioxide; DLLME, dispersive liquid–liquid microextraction; FA, formic acid; GC–EI–MS/MS, gas chromatography–electron impact–tandem mass spectrometry; H$_2$O, water; He, helium; LC–ESI–MS/MS, Liquid chromatography-electrospray ionization-tandem mass spectrometry; LC–TIS–MS/MS, Liquid chromatography-turbo ion spray-tandem mass spectrometry; MDL, method detection limit; MeOH, methanol; MSPE, magnetic solid-phase extraction; n.a., not available; NaN$_3$, sodium azide; NH$_4$OAc, ammonium acetate; NH$_4$HCO$_3$, ammonium formate; NH$_4$OH, ammonium hydroxide; $R_S$, resolution; SPE, solid-phase extraction; SUPRAS, supramolecular solvents; THF, tetrahydrofuran; UHSFC–ESI–MS/MS, ultra-high supercritical fluid chromatography-electrospray ionization-tandem mass spectrometry; USE, ultrasonic extraction

$^a$Limit of detection; $^b$liquid phase; $^c$particulate phase; $^d$Li et al. (2020); $^e$Ma et al. (2019); $^f$Coelho et al. (2019); $^g$Yuan et al. (2018); $^h$Wang et al. (2018); $^i$Matamoros et al. (2009); $^j$Camacho-Muñoz and Kasprzyk-Hordern (2017); $^k$Camacho-Muñoz et al. (2016); $^l$Camacho-Muñoz and Kasprzyk-Hordern (2015); $^m$Caballo et al. (2015b); $^n$Caballo et al. (2015a); $^o$Hashim et al. (2011); $^p$Buser et al. (1999)
or ng g⁻¹ concentrations for solid matrices. Chromatographic run times range from 16 to 35 min (Table 2).

**Chiral selector: glycopeptide and glycoprotein phases**

Glycopeptide-based stationary phases have been widely applied for the separation of pharmaceutical drugs (Bagnall et al. 2012; Camacho-Muñoz and Kasprzyk-Hordern 2017; Evans et al. 2015; Petrie et al. 2018). Vancomycin is more suited to those basic drugs, e.g. beta-blockers, and teicoplanin for acidic drugs, e.g. NSAIDs. Teicoplanin has an isoelectric point of ~3.5, and electrostatic interactions are considered one of the dominant interactions occurring between the analytes and stationary phase. As the chiral selector itself is ionizable, changes to pH affects its degree of ionization as well as the analyte (Ilisz et al. 2013). Teicoplanin has been successfully applied for the simultaneous enantioseparation of indoprofen, naproxen, ketoprofen and ibuprofen as well as its metabolites carboxyibuprofen and 2-hydroxyibuprofen (Camacho-Muñoz and Kasprzyk-Hordern 2017). Separation was achieved in reversed phase mode with a mobile phase consisting of 70:30 (v/v) 10 mM ammonium acetate at pH 4.2/methanol (Table 2). However, the chromatographic run time for the separation of all studied NSAIDs was 60 min. A contributing factor to the comparatively long run time was the inclusion of additional drugs from different therapeutic groups including anthelmintic drugs, anti-cancer drugs, antibacterial drugs, central nervous system drugs and anti-fungal drugs within the same method.

The protein-based phase α₁-acid glycoprotein (AGP) has also been successfully applied for the enantioseparation of NSAIDs (Table 2). This phase consists of a single peptide chain with 181 amino acids and five heteropolysaccharide units, containing 14 residues of sialic acid (Hermansson 1983). Due to the complexity of proteins, hydrophobic, ionic, π–π and steric interactions, and hydrogen bonding are assumed to be the main retention mechanisms (Haginaka 2001). However, a limitation of AGP is the maximum content of organic modifier in the mobile phase cannot exceed 20%. Furthermore, the operating pH range is limited to pH 4–7. Nature and content of the organic modifier as well as the mobile phase pH are considered to have the greatest influence on enantioseparation using AGP phases (Hermansson and Hermansson 1994; Michishita et al. 2010). Successful separation has been achieved for ibuprofen, naproxen, ketoprofen and dihydroketoprofen using a mobile phase comprising 99:1 (v/v) 10 mM ammonium acetate (pH 6.2):acetonitrile (Camacho-Muñoz and Kasprzyk-Hordern 2015). Separation of all NSAIDs was achieved in 20 min (Table 2).

**Chiral selector: Pirkle-type phases**

Pirkle-type stationary phases comprise a small chiral molecule bonded to a chromatographic support via a spacer (Fernandes et al. 2013; Pirkle and Pochapsky 1989). A number of different phases have been developed with the most popular being 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene. It was initially used for the separation of naproxen enantiomers (Welch 1994) and separates analytes with an aromatic system with a hydrogen-bond acceptor group near the stereogenic centre (Fernandes et al. 2013). Coelho et al. (2019) successfully developed a separation method for flurbiprofen, ibuprofen, ketoprofen and naproxen using a 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene stationary phase. The optimum mobile phase of 60:40 (v/v) methanol/0.1% acetic acid required a chromatographic run time of 90 min (Table 2). On the other hand, Caballo et al. (2015a) used a Pirkle-brush column with a (R)-1-naphthylglycine and 3,5-dinitrobenzoic acid chiral selector for enantioseparation of ibuprofen, ketoprofen and naproxen. In this study, the mobile phase comprised 90:10 (v/v) tetrahydrofuran/50 mM ammonium acetate in methanol operated under a variable flow rate (Table 2). Under such conditions, the ammonium acetate concentration was reported to have the greatest influence on Rₜ using this stationary phase. The chromatographic run time was 36 min (Caballo et al. 2015a).

**Gas chromatography–mass spectrometry**

Chiral GC methods for the environmental analysis of NSAIDs and pharmaceuticals in general are limited (Buser et al. 1999; Hashim and Khan 2011). This is due to the non-volatile nature of drugs requiring chemical derivatization to make them GC amenable. Hashim and Khan (2011) reported the derivatization of ibuprofen, ketoprofen and naproxen using (R)-1-phenylethylamine. This resulted in the conversion of the enantiomers to amide diastereomers facilitating indirect separation using a (5%-phenyl)-methylpolysiloxane phase (Hashim and Khan 2011). The derivatization process was complete within 5 min; however, an additional SPE treatment was required to separate the diastereomers from the derivatizing reagent. Nevertheless, chromatographic separation was achieved within 18 min and MDLs were in the range 0.2–3.3 ng L⁻¹ and commensurate with those more sensitive LC methods (Table 1). An alternative methodology was utilized by Matamoros et al. (2009) whereby methylation of the carboxylic acid groups of ibuprofen and naproxen was performed using trimethylsulphonium hydroxide. Enantiomers were then separated directly on a chiral dimethyl-b-cyclodextrin column. In this case, the run time was 57 min (Table 2).
**Supercritical fluid chromatography–mass spectrometry**

SFC–MS has also shown applicability for enantioselective analysis of pharmaceuticals (Camacho-Muñoz et al. 2016; Garzotti and Hamdan 2002; Hamman et al. 2011; Plotka et al. 2014). Supercritical fluids have the advantages of both gas states and liquid states (Plotka et al. 2014). SFC is normally operated similar to normal phase mode with carbon dioxide as the main mobile phase component enabling MS compatibility. Addition of organic modifiers to the mobile phase increases solvent strength. The viscosity and diffusivity of carbon dioxide make enantioselective separation possible in comparatively short run times, i.e. less than 10 min (Hamman et al. 2011; Plotka et al. 2014). However, the application of enantioselective SFC to environmental analysis is limited. Camacho-Muñoz et al. (2016) utilized an amylose tris-(3,5-dimethylphenylcarbamate) phase for the enantioseparation of carprofen, flurbiprofen, naproxen, ibuprofen and its metabolite 2-hydroxyibuprofen (Table 2). The mobile phase consisted of a gradient between carbon dioxide and 0.2% (v/v) ammonium hydroxide in methanol. Simultaneous enantioseparations were achieved within 16 min.

**Occurrence and fate of non-steroidal anti-inflammatory drugs at the enantiomeric level in wastewater and the environment**

The development of robust analytical methodologies has facilitated the enantioselective study of chiral NSAIDs in complex environmental matrices. This includes wastewaters, receiving surface waters and soil systems where the occurrence and fate of NSAIDs have been investigated at the enantiomeric level.

**Wastewater**

**Influent wastewater**

Influent or untreated wastewater typically contains ibuprofen and naproxen at µg L⁻¹ concentrations and flurbiprofen and ketoprofen at sub-µg L⁻¹ concentrations (Camacho-Muñoz et al. 2014; Gardner et al. 2013; Kasprzyk-Hordern et al. 2009; Larsson et al. 2014; Petrie et al. 2015; Roberts and Thomas 2006). The enantiomeric composition of chiral drugs is typically reported as enantiomeric fraction (Sanganyado et al. 2020). The enantiomeric fraction of chiral NSAIDs can be calculated using Eq. (1):

\[
\text{Enantiomeric fraction} = \frac{S(+) }{ [S(+)+ R(-)] } \tag{1}
\]

where \( S(+) \) is the \( S(+) \)-enantiomer and \( R(-) \) is the \( R(-) \)-enantiomer. Therefore, a racemate, i.e. 50:50 mixture of two enantiomers, has an enantiomeric fraction of 0.5, whereas an enantiomerically pure substance has an enantiomeric fraction of 0.0 or 1.0. There are several ways to calculate and report enantiomeric fraction (Tiritan et al. 2018). Therefore, to ensure consistency in presentation and data interpretation, all enantiomeric fraction data reported in this review are calculated using the above equation.

The ibuprofen enantiomeric fraction has been found to range from 0.63 to 0.94 (\( n = 11 \) studies) in Australia, China, Germany, Spain and UK influent wastewaters (Table 3). The enrichment of the pharmacologically active enantiomer \( S(+)\)-ibuprofen following its consumption as the racemate can be explained by the unidirectional inversion of \( R(-)\)-ibuprofen to \( S(+)\)-ibuprofen in the body (Buser et al. 1999). The consumption of enantiomerically pure \( S(+)\)-naproxen resulted in enantiomeric fractions equal to or greater than 0.88 (\( n = 13 \) studies) in influent wastewater (Table 3). Mammalian studies have shown \( S(+)\)-naproxen is not inverted into \( R(-)\)-naproxen (Sugawara et al. 1978; Wsol et al. 2004). The presence of comparatively low levels of \( R(-)\)-naproxen in influent wastewater could be a result of inversion of \( S(+)\)-naproxen during wastewater transport in the sewer network.

Wastewater typically takes between 0.5 and 24 h to arrive at the wastewater treatment plant. Sewer pipes have a biofilm that can result in drug biotransformation (Choi et al. 2020; Gao et al. 2019; Li et al. 2019). Naproxen and ibuprofen have been found to be stable in pilot scale gravity and rising main pipes over 8 h (Gao et al. 2019). However, enantioselective changes of other drugs have been observed in influent wastewater previously (Castrignanò et al. 2017). However, further work is needed to ascertain the enantioselectivity of NSAIDs under sewer transport conditions. Ketoprofen has been reported in influent wastewaters with enantiomeric fractions ranging from 0.54 to 0.68 (\( n = 5 \) studies) in the UK, Spain and Australia (Table 3). The enrichment of \( S(+)\)-ketoprofen is likely to be a result of it being prescribed as both the racemate and enantiomerically pure \( S(+)\)-ketoprofen (Table 1). Only about 10% of \( R(-)\)-ketoprofen is inverted to \( S(+)\)-ketoprofen in the body (Rudy et al. 1998). A single study reported the enantiomeric composition of flurbiprofen in influent wastewater with an enantiomeric fraction of 0.54 (Wang et al. 2018).

**Enantioselectivity during wastewater treatment and effluent wastewater composition**

Conventional biological wastewater treatment plants such as trickling filters and activated sludge are designed for carbonaceous material removal and can be adapted for nutrient removal. Removal of many pharmaceutical drugs is also
| Drug     | Country | Analysis method | Sampled matrix                  | $R(-)$-enantiomer (µg L$^{-1}$) | $S(+)$.enantiomer (µg L$^{-1}$) | EF  | References |
|----------|---------|----------------|---------------------------------|--------------------------------|--------------------------------|-----|------------|
| Ibuprofen| UK      | LC–MS/MS       | Influent wastewater$^{TF}$       | n.a                            | n.a                            | –   | A          |
|          |         |                | Effluent wastewater$^{TF}$       | 0.244                          | 0.572                          | 0.71|            |
|          |         |                | River water                      | <0.009                         | <0.009                         | –   |            |
|          | China   | LC–MS/MS       | River water                      | <0.009–0.101                   | <0.011–0.325                   | 0.70| B          |
|          | China   | LC–MS/MS       | Influent wastewater              | 0.228                          | 0.390                          | 0.63| C          |
|          |         |                | Effluent wastewater              | 0.036                          | 0.075                          | 0.68|            |
|          |         |                | River water                      | 0.040                          | 0.075                          | 0.62|            |
|          | Europe  | UHSFC–MS/MS    | Influent wastewater              | < 1.41                         | 5.24                           | >0.79| D          |
|          | Spain   | LC–MS/MS       | Influent wastewater              | 0.256–0.415                    | 0.957–1.643                    | 0.79–0.86| E          |
|          |         |                | Effluent wastewater              | 0.042–0.098                    | 0.072–0.210                    | 0.63–0.68|            |
|          | UK      | UPLC–MS/MS     | Effluent wastewater$^{TF}$       | 0.24                           | 0.46                           | 0.66| F          |
|          |         |                | River water                      | <0.263                         | <0.114                         | –   |            |
|          | Australia| GC–MS/MS      | Influent wastewater$^{FASOS}$     | 0.065                          | 0.179                          | 0.73| G          |
|          |         |                | Effluent wastewater$^{MBR}$       | 0.007                          | 0.007                          | 0.50|            |
|          |         |                | Effluent wastewater$^{AS}$       | 0.005                          | 0.005                          | 0.50|            |
|          |         |                | Effluent wastewater$^{AS}$–$^{BNR}$| <0.001                         | <0.001                         | –   |            |
|          |         |                | Creek upstream of wastewater     | 0.049                          | 0.121                          | 0.71|            |
|          |         |                | discharge                        | 0.015–0.026                    | 0.023–0.086                    | 0.60–0.77|            |
|          | Australia| GC–EI–MS/MS   | Influent wastewater$^{MBR}$       | 0.444–2.92                     | 5.27–39.5                      | 0.88–0.94| H          |
|          |         |                | Effluent wastewater$^{MBR}$       | 0.006–0.017                    | 0.004–0.011                    | 0.38–0.40|            |
|          | Spain   | GC–EI–MS       | Influent wastewater$^{Deep}$      | n.a                            | n.a                            | 0.73| I          |
|          |         |                | wastewater$^{Shallow}$            | n.a                            | n.a                            | 0.76|            |
|          |         |                | wastewater$^{VF}$                 | n.a                            | n.a                            | 0.90|            |
|          |         |                | wastewater$^{VF}$                 | n.a                            | n.a                            | 0.72|            |
|          | Switzerland| GC–EI–MS/MS | Influent wastewater$^{biological}$| 0.990–3.300$^a$                | 0.85–0.89                      | 0.47–0.67| J          |
|          |         |                | Effluent wastewater$^{biological}$| 0.002–0.081$^a$                | 0.41–0.81                      |            |            |
|          |         |                | River/Lake water                 | <0.0002–0.008$^a$              | 0.41–0.81                      |            |            |
| Ketoprofen| UK      | LC–MS/MS       | Effluent wastewater$^{TF}$       | 0.032                          | 0.039                          | 0.54| A          |
|          |         |                | Effluent wastewater$^{TF}$       | 0.005                          | 0.005                          | 0.65|            |
|          |         |                | Effluent wastewater$^{AS}$       | 0.012                          | 0.016                          | 0.58|            |
|          |          |                | Effluent wastewater$^{AS}$–$^{BNR}$| <0.0005                        | <0.0005                        | –   |            |
|          | Spain   | LC–MS/MS       | Effluent wastewater$^{MBR}$       | 0.008–0.240                    | 0.121–0.510                    | 0.54–0.68| E          |
|          |         |                | Effluent wastewater$^{AS}$       | 0.024–0.086                    | 0.038–0.177                    | 0.61–0.68|            |
|          | Australia| GC–EI–MS/MS   | Effluent wastewater$^{MBR}$       | 0.065–12.0                     | 0.084–15.3                     | 0.56–0.60| H          |
|          |          |                | Effluent wastewater$^{MBR}$       | 0.001–0.005                    | 0.002–0.006                    | 0.54–0.68|            |
| Naproxen  | UK      | LC–MS/MS       | Effluent wastewater$^{TF}$       | 0.311                          | 24.3                           | 0.99| A          |
|          |         |                | Effluent wastewater$^{TF}$       | 0.391                          | 4.12                           | 0.92|            |
|          |          |                | Effluent wastewater$^{AS}$       | <0.010                         | 32.9                           | >0.99|            |
|          |          |                | Effluent wastewater$^{AS}$       | <0.004                         | 0.116                          | >0.97|            |
|          |          |                | Effluent wastewater$^{SR}$       | <0.010                         | 17.0                           | >0.99|            |
|          |          |                | Effluent wastewater$^{SR}$       | <0.004                         | 0.579                          | >0.99|            |
Table 3 (continued)

| Drug          | Country    | Analysis method | Sampled matrix | R(-)-enantiomer (µg L⁻¹) | S(+) -enantiomer (µg L⁻¹) | EF  | References |
|---------------|------------|-----------------|----------------|--------------------------|---------------------------|-----|------------|
|               |            |                 | River upstream of wastewater discharge | 0.09 | 0.35 | 0.92 |
|               |            |                 | River downstream of wastewater discharge | 0.01 | 0.35 | 0.97 |
| Flurbiprofen  | China      | LC–MS/MS        | River water    | <0.0004–0.002           | <0.0004–0.043             | 0.93 | B          |
|               | UK         | LC–MS/MS        | Influent wastewaterAS | <0.011 | 0.37 | >0.99 | K          |
|               |            |                 | Effluent wastewaterAS | <0.014 | <0.013 | -          |
|               |            |                 | River water    | <0.008 | <0.007 | -          |
|               | Europe     | UHSFC–MS/MS     | Influent wastewater | <0.233 | 4.75 | >0.95 | D          |
|               | UK         | UPLC–MS/MS      | Effluent wastewaterTF | 0.09 | 1.33 | 0.94 | F          |
|               |            |                 | River water    | <0.008 | 0.136 | >0.98 |
|               | Spain      | LC–MS/MS        | Influent wastewaterAS | 0.018–0.030 | 1.049–3.172 | 0.98–0.99 | E          |
|               |            |                 | Effluent wastewaterAS | 0.009–0.022 | 0.175–0.481 | 0.93–0.96 |
|               |            |                 | Effluent wastewaterAS | 0.03–0.43 | >0.99 |
|               |            |                 | River water    | 0.08 | <0.008 | 0.025 | >0.96 |
|               | Australia  | GC–MS/MS        | Influent wastewaterFASOS discharge | <0.001 | 0.024 | >0.96 | G          |
|               |            |                 | Effluent wastewaterMBR | 0.001 | 0.008 | 0.90 |
|               |            |                 | Effluent wastewaterAS | 0.003 | 0.033 | 0.92 |
|               |            |                 | Effluent wastewaterAS–BNR | 0.007 | 0.013 | 0.65 |
|               |            |                 | Creek upstream of wastewater discharge | <0.001 | 0.025 | >0.96 |
|               | Australia  | GC–EI–MS/MS     | Influent wastewaterMBR | 0.010–0.032 | 0.827–67.6 | 0.99 | H          |
|               |            |                 | Effluent wastewaterMBR | 0.002–0.014 | 0.025–0.161 | 0.86–0.94 |
|               | Spain      | GC–EI–MS        | Influent wastewaterDeep and shallow HFCW | n.a | n.a | 0.89 |
|               |            |                 | Effluent wastewaterDeep HFCW | n.a | n.a | 0.82 |
|               |            |                 | Effluent wastewaterShallow HFCW | n.a | n.a | 0.72 |
|               |            |                 | Effluent wastewaterVFCW(saturated) | n.a | n.a | 0.90 |
|               |            |                 | Effluent wastewaterVFCW(un saturated) | n.a | n.a | 0.71 |
|               | Australia  | GC–EI–MS/MS     | Influent wastewater | 0.079 | 0.088 | 0.54 | C          |
|               |            |                 | Effluent wastewater | <0.013 | <0.014 | -          |
|               |            |                 | River water    | 0.032 | 0.04 | 0.55 |

Key: AS, activated sludge; AS−BNR, activated sludge-biological nitrogen removal; EF, enantiomeric fraction; FASOS, fuller avenue sewage overflow structure; GC–EI–MS, gas chromatography–electron impact–mass spectrometry; GC–EI–MS/MS, gas chromatography–electron impact–tandem mass spectrometry; GC–MS/MS, gas chromatography–tandem mass spectrometry; HFCW, horizontal subsurface-flow constructed wetlands; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MBR, membrane bioreactor; n.a., not available; SF, sand filter; TF, trickling filter beds; UHSFC–MS/MS, ultra-high supercritical fluid chromatography-tandem mass spectrometry; UPLC–MS/MS, ultra-performance liquid chromatography-tandem mass spectrometry; VFCW, vertical-flow constructed wetlands

*Total enantiomeric concentration; aCamacho-Muñoz et al. (2019); bMa et al. (2019); cWang et al. (2018); dCamacho-Muñoz et al. (2016); eCaballo et al. (2015a); fCamacho-Muñoz and Kasprzyk-Hordern (2015); gKhan et al. (2014); hHashim et al. (2013); iMatamoros et al. (2009); jBuser et al. (1999); kCamacho-Muñoz and Kasprzyk-Hordern (2017); lSuzuki et al. (2014)
observed including chiral NSAIDs (Camacho-Muñoz et al. 2012; Kasprzyk-Hordern et al. 2009; Petrie et al. 2015; Roberts and Thomas 2006; Verlicchi et al. 2012). Removal of NSAIDs can be variable between wastewater treatment plants and individual drugs. For example, higher removal of naproxen has been reported by activated sludge over trickling filters (Kasprzyk-Hordern et al. 2009). Furthermore, five full-scale activated sludge wastewater treatment plants in Japan showed ibuprofen removals were greater than 90%, whereas average removals of both naproxen and ketoprofen were 45% (Nakada et al. 2006). The removal of NSAIDs is mainly attributed to biodegradation (Matamoros et al. 2009; Nakada et al. 2006; Onesios et al. 2009; Patel et al. 2019; Petrie et al. 2014; Verlicchi et al. 2012), whereby the parent drugs are transformed into a metabolite or degradation product, or is mineralized (Patel et al. 2019). Importantly, microbial processes during wastewater treatment can result in enantioselectivity.

During activated sludge, trickling filter and membrane bioreactor treatment, removal favours $S(+)$-ibuprofen causing a reduction of enantiomeric fraction (Buser et al. 1999; Caballo et al. 2015a; Hashim et al. 2013; Khan et al. 2014; Matamoros et al. 2009) (Table 3). Ibuprofen enantiomeric fractions in effluent wastewater are in the range 0.50–0.71 with concentrations less than 1 µg L$^{-1}$ (Table 3). Matamoros et al. (2009) also investigated the enantiomeric behaviour of ibuprofen during treatment by horizontal sub-surface and vertical flow constructed wetlands and sand filters. Under aerobic conditions, preferential removal of $S(+)$-ibuprofen was also observed. However, such systems operated under prevailing anaerobic conditions resulted in ibuprofen removal not being enantioselective (Matamoros et al. 2009). Therefore, redox conditions are important as different bacterial consortia are active under aerobic and anaerobic conditions.

Only limited data exist on the enantioselective behaviour of ketoprofen during wastewater treatment (Table 3). No significant changes to ketoprofen enantiomeric fractions were reported by activated sludge or membrane bioreactor treatment despite considerable removal (Caballo et al. 2015a; Hashim et al. 2013). However, Camacho-Muñoz et al. (2019) reported an enrichment of $S(+)$-ketoprofen during trickling filter treatment. Influent and effluent enantiomeric fractions were 0.54 and 0.65, respectively (Table 3). Differences in enantioselectivity during activated sludge and trickling filter treatment has been observed for other drugs such as 3,4-methylenedioxymethamphetamine due to different consortia of microorganisms between process types (Kasprzyk-Hordern and Baker 2012).

Enantioselectivity has been reported for naproxen during wastewater treatment by trickling filters, activated sludge, membrane bioreactors, constructed wetlands and sand filters (Caballo et al. 2015a; Camacho-Muñoz et al. 2019; Khan et al. 2014; Matamoros et al. 2009; Suzuki et al. 2014). An increase in the relative concentration of $R(-)$-naproxen resulted in enantiomeric fractions within wastewater effluents being in the range 0.65–0.98 (Table 3). Khan et al. (2014) reported $R(-)$-naproxen concentrations in wastewater effluents up to 0.007 µg L$^{-1}$, whereas no measurable concentration was present in influent wastewater. Due to this increased concentration, it was postulated that chiral inversion of $S(+)$-naproxen took place (Khan et al. 2014). The enantioselectivity of naproxen is reported to be similar under both prevailing aerobic and anaerobic conditions (Matamoros et al. 2009). Therefore, it was also suggested that the change in enantiomeric fraction observed for naproxen during wastewater treatment could be used to distinguish between treated and untreated sources of wastewater in the environment (Khan et al. 2014). Similar observations have been made for the beta-blocker propranolol (Fono and Sedlak 2005) and the stimulant amphetamine (Ramage et al. 2019).

**Spiked wastewater microcosms for fate evaluation**

Understanding the fate processes responsible for enantioselectivity, i.e. degradation or inversion, during full-scale wastewater treatment is challenging. Batch and flow through microcosm studies using spiked enantiomer concentrations enable a greater understanding of these processes whereby operational variables can be closely controlled. Several studies have reported limited or no enantioselectivity of ibuprofen in batch studies of diluted activated sludge (Escuder-Gilabert et al. 2018) or laccase enzyme obtained from a culture of the white rot fungus *Pleurotus ostreatus* (Nguyen et al. 2017). Furthermore, no enantioselectivity was observed in constructed wetlands treating synthetic or real wastewater under predominantly anaerobic conditions at dissolved oxygen concentrations less than 0.5 mg L$^{-1}$ (Matamoros et al. 2009).

In a continuous flow membrane bioreactor system treating synthetic wastewater containing $R(S(\pm))$-ibuprofen, an enrichment of $R(-)$-ibuprofen was observed (Hashim et al. 2011). In this study, the enantiomeric fraction reduced from 0.50–0.54 to 0.31–0.44 (Table 4). However, as ibuprofen was used as the racemate, it was not possible to establish the process(es) responsible for enantioselectivity, i.e. degradation or inversion. Nguyen et al. (2017) investigated the behaviour of individual ibuprofen enantiomers separately in a continuous flow enzymatic membrane bioreactor dosed with laccase. The enantiomeric fraction increased from 0.01 to 0.18 in studies of $R(-)$-ibuprofen only and reduced from 0.99 to 0.75 in studies of $S(+)$-ibuprofen only (Table 4). These observations confirmed that ibuprofen enantiomers can undergo bidirectional inversion during wastewater treatment (Nguyen et al. 2017).

The mechanism(s) of inversion or microbes responsible for inversion during wastewater treatment remain poorly understood. Fungi including *Verticillium lecanii* are...
Table 4 | Bench and pilot-scale microcosm investigations on the enantioselectivity of non-steroidal anti-inflammatory drugs using spiked drug concentrations

| Microcosm | Incubation time | Incubation conditions | Drug                  | Initial concentration | EF (initial) | EF (final) | Observations                                                                                     | References |
|-----------|-----------------|-----------------------|-----------------------|-----------------------|--------------|------------|-----------------------------------------------------------------------------------------------|------------|
| Activated sludge (2 L) | 24 h          | Dark conditions       | \(R/S(\pm)\)-ketoprofen | 1 µg L\(^{-1}\)                  | 0.54         | 0.50       | No significant degradation or enantioselectivity observed.                                     | A          |
|           |                 | Continually stirred   |                       |                        |              |            | No change in abiotic controls                                                                 |            |
|           |                 | DO 8–18 mg L\(^{-1}\) |                       |                        |              |            |                                                                                                |            |
|           |                 | pH 7–8.2              |                       |                        |              |            |                                                                                                |            |
|           |                 | 10–20 °C              |                       |                        |              |            |                                                                                                |            |
|           |                 | Abiotic controls      | (1 g L\(^{-1}\) sodium azide) under same conditions |                       |              |            |                                                                                                |            |
|           |                 | Sampling times: 0, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 24 h |                       |                        |              |            |                                                                                                |            |
|           |                 | Duplicate bioreactors |                       |                        |              |            |                                                                                                |            |
| Activated sludge (0.1 mL diluted to 3.1 mL using water) | 28 days | Natural light | \(R/S(\pm)\)-ibuprofen | 15.6 mg L\(^{-1}\) | 0.5         | 0.6 (28 days) | Slight enrichment of \(S(+)\)-ibuprofen. \(t_{1/2}\) values of 18 and 25 days for \(R(-)\)-ibuprofen and \(S(+)\)-ibuprofen, respectively | B          |
|           |                 | 20 °C                 |                       |                        |              |            |                                                                                                |            |
|           |                 | Mixed at 150 rpm      |                       |                        |              |            |                                                                                                |            |
|           |                 | Sampling times: 0, 3, 5, 11, 18, 21, 25 and 28 days |                       |                        |              |            |                                                                                                |            |
|           |                 | Sacrificial reactors  | prepared in duplicate |                       |              |            |                                                                                                |            |
|           |                 | Controls using 0.1 mL water instead of activated sludge |                       |                        |              |            |                                                                                                |            |
| Activated sludge (2 L) | 24 h          | Dark conditions       | \(R/S(\pm)\)-naproxen | 1 µg L\(^{-1}\)                  | 0.76         | 0.84 (after 2 h) | Initial EF > 0.5 due to background levels of \(S(+)\)-naproxen. Moderate enrichment of \(S(+)\)-naproxen during incubation. Both enantiomers removed to < MQL within 3 h. Under abiotic conditions \(S(+)\)-naproxen removed by 50% during 24 h. Increase of \(R(-)\)-naproxen due to inversion | A          |
|           |                 | Continually stirred   |                       |                        |              |            |                                                                                                |            |
|           |                 | DO 8–18 mg L\(^{-1}\) |                       |                        |              |            |                                                                                                |            |
|           |                 | pH 7–8.2              |                       |                        |              |            |                                                                                                |            |
|           |                 | 10–20 °C              |                       |                        |              |            |                                                                                                |            |
|           |                 | Abiotic controls      | (1 g L\(^{-1}\) sodium azide) under same conditions |                       |              |            |                                                                                                |            |
|           |                 | Sampling times: 0, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 24 h |                       |                        |              |            |                                                                                                |            |
|           |                 | Duplicate bioreactors |                       |                        |              |            |                                                                                                |            |
| Activated sludge (0.1 mL diluted to 3.1 mL using water) | 28 days | Natural light | \(R/S(\pm)\)-ibuprofen | 15.6 mg L\(^{-1}\) | 0.5         | 0.6 (28 days) | Slight enrichment of \(S(+)\)-ibuprofen. \(t_{1/2}\) values of 18 and 25 days for \(R(-)\)-ibuprofen and \(S(+)\)-ibuprofen, respectively | B          |
|           |                 | 20 °C                 |                       |                        |              |            |                                                                                                |            |
|           |                 | Mixed at 150 rpm      |                       |                        |              |            |                                                                                                |            |
|           |                 | Sampling times: 0, 3, 5, 11, 18, 21, 25 and 28 days |                       |                        |              |            |                                                                                                |            |
|           |                 | Sacrificial reactors  | prepared in duplicate |                       |              |            |                                                                                                |            |
|           |                 | Controls using 0.1 mL water instead of activated sludge |                       |                        |              |            |                                                                                                |            |
| Activated sludge (2 L) | 24 h          | Dark conditions       | \(R/S(\pm)\)-naproxen | 1 µg L\(^{-1}\)                  | 0.76         | 0.84 (after 2 h) | Initial EF > 0.5 due to background levels of \(S(+)\)-naproxen. Moderate enrichment of \(S(+)\)-naproxen during incubation. Both enantiomers removed to < MQL within 3 h. Under abiotic conditions \(S(+)\)-naproxen removed by 50% during 24 h. Increase of \(R(-)\)-naproxen due to inversion | A          |
|           |                 | Continually stirred   |                       |                        |              |            |                                                                                                |            |
|           |                 | DO 8–18 mg L\(^{-1}\) |                       |                        |              |            |                                                                                                |            |
|           |                 | pH 7–8.2              |                       |                        |              |            |                                                                                                |            |
|           |                 | 10–20 °C              |                       |                        |              |            |                                                                                                |            |
|           |                 | Abiotic controls      | (1 g L\(^{-1}\) sodium azide) under same conditions |                       |              |            |                                                                                                |            |
|           |                 | Sampling times: 0, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 24 h |                       |                        |              |            |                                                                                                |            |
|           |                 | Duplicate bioreactors |                       |                        |              |            |                                                                                                |            |
| Activated sludge (0.1 mL diluted to 3.1 mL using water) | 28 days | Natural light | \(R/S(\pm)\)-ibuprofen | 15.6 mg L\(^{-1}\) | 0.5         | 0.6 (28 days) | Slight enrichment of \(S(+)\)-ibuprofen. \(t_{1/2}\) values of 18 and 25 days for \(R(-)\)-ibuprofen and \(S(+)\)-ibuprofen, respectively | B          |
|           |                 | 20 °C                 |                       |                        |              |            |                                                                                                |            |
|           |                 | Mixed at 150 rpm      |                       |                        |              |            |                                                                                                |            |
|           |                 | Sampling times: 0, 3, 5, 11, 18, 21, 25 and 28 days |                       |                        |              |            |                                                                                                |            |
|           |                 | Sacrificial reactors  | prepared in duplicate |                       |              |            |                                                                                                |            |
|           |                 | Controls using 0.1 mL water instead of activated sludge |                       |                        |              |            |                                                                                                |            |

Degradation of ketoprofen enantiomers \(t_{1/2}\) values = 12 days) to < MQL within 25 days. No enantioselectivity observed.
| Microcosm | Incubation time | Incubation conditions | Drug | Initial concentration | EF (initial) | EF (final) | Observations | References |
|-----------|----------------|-----------------------|------|-----------------------|-------------|------------|-------------|------------|
| Laccase enzyme solution (activity = 50–60 µM(DMP) min⁻¹) | 48 h | Dark conditions 28 °C Mixed at 70 rpm Sampling times: 4, 8, 12, 24 and 48 h Controls using water | $R/S$(±)-ibuprofen | 1–3 µg L⁻¹ | ~0.5 | ~0.5 | Little removal after 48 h. No enantioselectivity | C |
| | | | $S$(+)-ibuprofen | 1–3 µg L⁻¹ | ~1.0 | ~1.0 | No chiral inversion. Removal < 30% after 48 h | |
| | | | $R$(−)-ibuprofen | 1–3 µg L⁻¹ | ~0.0 | ~0.0 | No chiral inversion. Removal < 30% after 48 h | |
| | | | $R/S$(±)-naproxen | 1–3 µg L⁻¹ | ~0.5 | ~1.0 | Enrichment of $S$(+)-naproxen due to enantioselective degradation. However, not reflected in individual enantiomer studies | |
| | | | $S$(+)-naproxen | 1–3 µg L⁻¹ | ~1.0 | ~1.0 | No chiral inversion. Removal < 30% after 48 h | |
| | | | $R$(−)-naproxen | 1–3 µg L⁻¹ | ~0.0 | ~0.0 | No chiral inversion. Removal < 30% after 48 h | |
| | | | $R/S$(±)-ketoprofen | 1–3 µg L⁻¹ | ~0.5 | ~0.5 | Little removal after 48 h. No enantioselectivity | |
| | | | $S$(+)-ketoprofen | 1–3 µg L⁻¹ | ~1.0 | ~1.0 | No chiral inversion. Removal < 30% after 48 h | |
| | | | $R$(−)-ketoprofen | 1–3 µg L⁻¹ | ~0.0 | ~0.0 | No chiral inversion. Removal < 30% after 48 h | |
| Microcosm                                      | Incubation time | Incubation conditions | Drug          | Initial concentration | EF (initial) | EF (final) | Observations                                      | References |
|-----------------------------------------------|-----------------|-----------------------|---------------|-----------------------|--------------|------------|--------------------------------------------------|------------|
| **Bench-scale EMBR**                          | 8 h HRT         | 0.8 L reactor volume  | S(+)-ibuprofen| 2.4 µg L\(^{-1}\)    | 0.99         | 0.75       | 93% removal. Chiral inversion                     |            |
| (laccase enzyme solution (activity = 50–60 µM(DMP\(_{\text{min}^{-1}}\))) |                 | Hollow fibre UF       | R(−)-ibuprofen| 2.1 µg L\(^{-1}\)    | 0.01         | 0.18       | 90% removal. Chiral inversion                     |            |
|                                               |                 | membrane              | S(+)-naproxen | 2.4 µg L\(^{-1}\)    | 0.99         | 0.92       | 46% removal. Chiral inversion                     |            |
|                                               |                 | 3 kDa molecular       | R(−)-naproxen | 2.2 µg L\(^{-1}\)    | 0.01         | 0.26       | 46% removal. Chiral inversion                     |            |
|                                               |                 | weight cut off        | S(+)-ketoprofen| 2.7 µg L\(^{-1}\)    | 0.99         | 0.99       | 48% removal. Minimal chiral inversion             |            |
|                                               |                 | 0.2 m\(^2\) surface  | R(−)-ketoprofen| 2.8 µg L\(^{-1}\)    | 0.01         | 0.01       | 48% removal. Minimal chiral inversion             |            |
|                                               |                 | area                  |               |                       |              |           |                                                  |            |
| **HFCW (synthetic wastewater)**               | 26 mm d\(^{-1}\)HLR | Operated for 20 days  | R/S(±)-ibuprofen| 25 µg L\(^{-1}\)    | 0.50         | 0.50       | 52% removal. No enantioselectivity                | D          |
|                                               |                 | 0.55 m\(^2\) surface area |               |                       |              |           |                                                  |            |
|                                               |                 | DO 0.05 mg L\(^{-1}\) |               |                       |              |           |                                                  |            |
| **HFCW (real wastewater)**                    | 36 mm d\(^{-1}\)HLR | Operated for 9 days   | R/S(±)-ibuprofen| 25 µg L\(^{-1}\)    | 0.50         | 0.50       | 80% removal. No enantioselectivity                |            |
|                                               |                 | 55 m\(^2\) surface area |               |                       |              |           |                                                  |            |
|                                               |                 | DO 0.5 mg L\(^{-1}\)  |               |                       |              |           |                                                  |            |
| **MBR (synthetic wastewater)**                | 24 h HRT        | 9 L reactor volume    | R/S(±)-ibuprofen| 1.7–1.9 µg L\(^{-1}\) | 0.50–0.54    | 0.31–0.44 | > 99% removal of both enantiomers. Considerable | E          |
|                                               |                 | 0.04 µm membrane pore size |               |                       |              |           | enrichment of R(−)-ibuprofen                     |            |
|                                               |                 | 0.047 m\(^2\) surface area |               |                       |              |           | 74–78% R(−)-ketoprofen removal and 58–64%       |            |
|                                               |                 | DO 2 mg L\(^{-1}\)    | R/S(±)-ketoprofen| 1.4–1.7 µg L\(^{-1}\) | 0.51–0.53    | 0.60–0.66 | S(+)-ketoprofen removal. Considerable enrichment |            |
|                                               |                 | 70 day sludge age     | S(+)-naproxen  | 2.0–2.4 µg L\(^{-1}\) | > 0.99       | 0.65–0.66 | 38–47% total removal. Chiral inversion            |            |
|                                               |                 | pH 7.4–7.6             |               |                       |              |           |                                                  |            |
|                                               |                 | 8.6–10 g L\(^{-1}\) mixed liquor suspended solids |               |                       |              |           |                                                  |            |
|                                               |                 | 20 °C                  |               |                       |              |           |                                                  |            |
| **Activated sludge (10 g wet weight added to 1L influent wastewater)** | 24 h            | Dark conditions       | S(+)-naproxen | 10 µg L\(^{-1}\)    | > 0.99       | 0.91 (after 24 h) | 95% removal of S(+)-naproxen in 24 h. t\(_{1/2}\) values of 14 h. Chiral inversion. No removal in abiotic controls | F          |
|                                               |                 | 20 °C                  |               |                       |              |           |                                                  |            |
|                                               |                 | Air bubbling           |               |                       |              |           |                                                  |            |
|                                               |                 | Sampling times: 0, 2, 4, 8 and 24 h |               |                       |              |           |                                                  |            |
|                                               |                 | Abiotic controls (autoclaved) |               |                       |              |           |                                                  |            |
| Microcosm                  | Incubation time | Incubation conditions | Drug        | Initial concentration | EF (initial) | EF (final) | Observations                                                                 | References |
|---------------------------|-----------------|-----------------------|-------------|-----------------------|--------------|------------|--------------------------------------------------------------------------------|------------|
| River water (2 L)         | 30 days         | Dark and light conditions | \(R/S(\pm)-\text{ketoprofen}\) 1 \(\mu g\) \(L^{-1}\) | 0.50 (light) | 0.59 (after 5 days) | Moderate enrichment of \(S(+)\)-ketoprofen during incubation. Both enantiomers removed to < MQL within 7 days. Removal to < MQL within 7 days under abiotic conditions (no enantioselectivity) | A          |
|                           |                 | Continually stirred DO 5–15 \(mg\) \(L^{-1}\) pH 7.7–9.0 10–35 °C Abiotic controls (1 g \(L^{-1}\) sodium azide) under same conditions Sampling times: 1, 2, 3, 5, 7, 19 12, 15, 22, 30 days Duplicate bioreactors | | | | | |
|                           |                 |                       | \(R/S(\pm)-\text{naproxen}\) 1 \(\mu g\) \(L^{-1}\) | 0.50 (dark) | >0.99 (after 30 days) | Significant enrichment of \(S(+)\)-naproxen during incubation. \(R(-)\)-ketoprofen removed to < MQL and \(S(+)\)-ketoprofen by 96% within 30 days. No significant degradation or enantioselectivity observed under abiotic conditions | | |
|                           |                 |                       |             | 0.50 (light) | 0.59 (after 30 days) | Moderate enrichment of \(S(+)\)-naproxen during incubation. ≥ 80% removal of naproxen enantiomers within 30 days. Under abiotic conditions moderate enrichment of \(S(+)\)-naproxen observed. Enantiomer removals were ≥ 68% within 30 days | | |
| Microcosm       | Incubation time | Incubation conditions | Drug          | Initial concentration | EF (initial) | EF (final) | Observations                                                                 |
|-----------------|-----------------|-----------------------|---------------|-----------------------|--------------|------------|--------------------------------------------------------------------------------|
| River water (1 L)| 30 days         | Dark conditions 20 °C Air bubbling Sampling times: 0, 1, 3, 5, 7, 14 and 30 days Abiotic controls (autoclaved) | S(+)naproxen | 10 µg L⁻¹                                    | >0.99        | 0.99       | Significant enrichment of S(+)naproxen during incubation. S(+)naproxen removed by 98% and R(−)naproxen removed < MQL within 30 days. Under abiotic conditions no significant degradation or enantioselectivity observed | F |
| River water (15 L) | 10 days         | Sampling times: 0, 0.2, 6.9 and 10 days Abiotic controls (autoclaved) 19 °C Stirred | R/S(±)-ibuprofen | 100 µg L⁻¹                                   | 0.51         | 0.69       | Considerable enantioselectivity. Enrichment of S(+)ibuprofen. No removal in abiotic controls | G |
| Microcosm         | Incubation time | Incubation conditions                                                                 | Drug            | Initial concentration | EF (initial) | EF (final) | Observations                                                                 | References |
|------------------|-----------------|---------------------------------------------------------------------------------------|-----------------|-----------------------|-------------|-----------|------------------------------------------------------------------------------|------------|
| Lake water (2.5 L) | 37 days         | Room temperature<br>Dark and light conditions<br>Sampling times: 0, 4, 10, 21 and 37 days<br>Abiotic controls (autoclaved) | R/S(±)-ibuprofen | 0.2 µg L⁻¹ | ~0.50 (light) | 0.09      | Significant enantioselectivity. Enrichment of R(−)-ibuprofen. $t_{1/2}$ value of ~20 days. No removal under abiotic conditions | H          |
|                  |                 |                                                                                       |                 |                       | ~0.50 (dark) | 0.38      | Moderate enantioselectivity. Enrichment of R(−)-ibuprofen. $t_{1/2}$ value of ~20 days. No removal under abiotic conditions |            |
| Soil (5 g)       | 56 days         | Dark conditions<br>pH 6.6<br>Incubation temperatures of 4 °C and 18 °C<br>Abiotic controls (autoclaved and 200 µg g⁻¹ sodium azide) under same conditions<br>Sampling times: 0, 1, 3, 7, 14, 28, 42 and 56 days<br>Moisture content maintained at 26%<br>Sacrificial microcosms prepared in triplicate | R/S(±)-naproxen | 10 and 1 µg g⁻¹ | 0.52 (10 µg g⁻¹ at 18 °C) | 0.67 (after 56 days) | Considerable enrichment of S(+)naproxen. $t_{1/2}$ values of 9.7 and 11.8 days for R(−)-naproxen and S(+)naproxen, respectively. Faster enantiomer degradation at 1 µg g⁻¹. Enantiomer degradation reduced by 6–9 times at 4 °C. No degradation or enantioselectivity under abiotic conditions | I          |
|                  |                 |                                                                                       | R/S(±)-ibuprofen | 10 and 1 µg g⁻¹ | 0.50 (10 µg g⁻¹ at 18 °C) | 0.38 (after 3 days) | Considerable enrichment of R(−)-ibuprofen. $t_{1/2}$ values of 2.2 and 2.3 days for R(−)-ibuprofen and S(+)ibuprofen, respectively. Faster enantiomer degradation at 1 µg g⁻¹. Reduced enantiomer degradation at 4 °C. No degradation or enantioselectivity under abiotic conditions |            |
Table 4 (continued)

| Microcosm | Incubation time | Incubation conditions | Drug       | Initial concentration | EF (initial) | EF (final) | Observations                                                                 | References |
|-----------|-----------------|-----------------------|------------|-----------------------|--------------|-----------|--------------------------------------------------------------------------------|------------|
| Soil (5 g) | 56 days         | Dark conditions       | \(S^+\)-naproxen | 5 \(\mu g \cdot g^{-1}\) | > 0.99 (18 °C) | 0.78 (after 28 days) | Inversion of \(S^+\)-naproxen to \(R^-\)-naproxen. Very limited inversion observed at 4 °C | A Camacho-Muñoz et al. (2019); BEscuder-Gilabert et al. (2018); CNguyen et al. (2017); DMatamoros et al. (2009); EHashim et al. (2011); FSuzuki et al. (2014); GWinkler et al. (2001); HBuser et al. (1999); IBertin et al. (2020) |
|           |                 | \(pH 6.6\)           | \(R^-\)-naproxen | 5 \(\mu g \cdot g^{-1}\) | < 0.01 (18 °C) | 0.54 (after 28 days) | Inversion of \(R^-\)-naproxen to \(S^+\)-naproxen. Very limited inversion observed at 4 °C |            |
|           |                 | Incubation temperatures of 4 °C and 18 °C | \(S^+\)-ibuprofen | 5 \(\mu g \cdot g^{-1}\) | > 0.99 (18 °C) | 0.57 (after 7 days) | Inversion of \(S^+\)-ibuprofen to \(R^-\)-ibuprofen. Significant inversion observed at 4 °C |            |
|           |                 | Sampling times: 0, 1, 3, 7, 14, 28, 42 and 56 days | \(R^-\)-ibuprofen | 5 \(\mu g \cdot g^{-1}\) | < 0.01 (18 °C) | 0.39 (after 7 days) | Inversion of \(R^-\)-ibuprofen to \(S^+\)-ibuprofen. Significant inversion observed at 4 °C |            |
|           |                 | Moisture content maintained at 26% |                |                       |              |           |                                                                                  |            |
|           |                 | Sacrificial microcosms prepared in triplicate |                |                       |              |           |                                                                                  |            |
|           |                 | Soil stored for 60 days at 4 °C prior to start of the incubation period |                |                       |              |           |                                                                                  |            |

DO, dissolved oxygen; DMP, 2,6-dimethoxyphenol; EF, enantiomeric fraction; EMBR, enzymatic membrane bioreactor; HFCW, horizontal subsurface-flow constructed wetlands; HLR, hydraulic loading rate; HRT, hydraulic retention time; MBR, membrane bioreactor; MQL, method quantitation limit; \(t_{1/2}\), half-life; UF, ultra-filtration

\(^a\)Camacho-Muñoz et al. (2019); \(^b\)Escuder-Gilabert et al. (2018); \(^c\)Nguyen et al. (2017); \(^d\)Matamoros et al. (2009); \(^e\)Hashim et al. (2011); \(^f\)Suzuki et al. (2014); \(^g\)Winkler et al. (2001); \(^h\)Buser et al. (1999); \(^i\)Bertin et al. (2020)
suggested to invert ibuprofen enantiomers in both directions, favouring the formation of $S(\pm)$-ibuprofen (Thomason et al. 1997). Nocardi a bacteria can invert $S(\pm)$-ibuprofen to $R(\pm)$-ibuprofen (Mitsukura et al. 2002). Inversion in this instance is thought to involve several enzymes via a similar mechanism observed in rat liver (Kato et al. 2004, 2003) (Fig. 2). However, considering the diversity in the microbial community present in wastewater, several inversion pathways are possible.

No enantioselectivity in the degradation of $R/S(\pm)$-ketoprofen has been reported in activated sludge or laccase enzyme batch studies (Camacho-Muñoz et al. 2019; Escuder-Gilabert et al. 2018; Nguyen et al. 2017). However, minimal bidirectional chiral inversion was observed in a continuous flow enzymatic membrane bioreactor treating synthetic wastewater containing individual ketoprofen enantiomers (Nguyen et al. 2017). In a further membrane bioreactor study, $R/S(\pm)$-ketoprofen was enriched with $S(\pm)$-ketoprofen whereby a racemic enantiomeric fraction was increased to 0.60–0.66 (Hashim and Khan 2011) (Table 4).

Studies using enantiomerically pure $S(\pm)$-naproxen have reported inversion to $R(\pm)$-naproxen in activated sludge and membrane bioreactor microcosms (Hashim et al. 2011; Suzuki et al. 2014). Suzuki et al. (2014) found no inversion in autoclaved abiotic controls confirming the inversion process is driven biologically. Activated sludge microcosms spiked with $R/S(\pm)$-naproxen provided evidence of $S(\pm)$-naproxen inversion (Camacho-Muñoz et al. 2019). Nguyen et al. (2017) postulated enantioselective degradation of $R/S(\pm)$-naproxen occurred in laccase enzyme batch studies. Within 4 h, $R(\pm)$-naproxen was removed to below the MDL, whereas $S(\pm)$-naproxen remained largely unchanged for 24 h (Nguyen et al. 2017). Enantioselective degradation was proposed to be responsible because no inversion was observed in single enantiomer microcosms incubated in the same way. However, in these single enantiomer studies limited degradation was observed (Table 4). Therefore, further studies are needed to confirm enantioselective degradation as the main driver to the changes in enantiomeric fraction observed in the racemic microcosm. Nguyen et al. (2017) also demonstrated that naproxen underwent bidirectional inversion in a continuous flow enzymatic membrane bioreactor dosed with laccase. In single enantiomer studies, the formation of $S(\pm)$-naproxen was favoured over $R(\pm)$-naproxen (Table 4). However, it is challenging to identify whether this is a result of the inversion process(es) being enantioselective, i.e. inversion rates of enantiomers are different, or it is a result of enantioselective degradation, i.e. degradation rates of enantiomers are different, or it is a combination of both processes being enantioselective.

Occurrence and behaviour in receiving environments

Aquatic environment

The $R/S(\pm)$-ibuprofen concentration in surface waters is less than 1 $\mu$g L$^{-1}$ (Table 3). The enantiomeric fraction is typically in the range 0.60–0.81 (Buser et al. 1999; Khan et al. 2014; Ma et al. 2019; Wang et al. 2018), similar to those enantiomeric fractions observed in effluent wastewater (Table 3). However, an enrichment of $R(\pm)$-ibuprofen with a corresponding enantiomeric fraction of 0.41 has been reported (Buser et al. 1999). In river water microcosms, considerable enantioselectivity was observed for $R/S(\pm)$-ibuprofen over 10 days (Winkler et al. 2001). Enrichment of $S(\pm)$-ibuprofen was observed with a change in enantiomeric fraction from 0.51 to 0.69 (Table 4). No degradation or enantioselectivity was observed in abiotic controls. On the other hand, Buser et al. (1999) noted an enrichment of $R(\pm)$-ibuprofen in lake water microcosms incubated for 37 days (Table 4). These studies demonstrate the diverse enantiospecific behaviour of ibuprofen in the environment. In both studies, ibuprofen was spiked as the racemate and it was not possible to establish whether or not chiral inversion took place.

Currently, no data exist on the enantiomeric composition of ketoprofen in surface waters. $R/S(\pm)$-ketoprofen has been found to degrade rapidly in river water microcosms (Camacho-Muñoz et al. 2019). Both biodegradation and photolysis contributed to ketoprofen removal from river water microcosms. In biotic and abiotic microcosms under light exposure, the total ketoprofen removal after 5 days was 98% (Camacho-Muñoz et al. 2019). Under biotic conditions (both light and dark exposure), an enrichment of the pharmacologically active enantiomer $S(\pm)$-ketoprofen was observed (Table 4).

Naproxen has been reported in surface waters up to 0.36 $\mu$g L$^{-1}$ with enantiomeric fractions in the range 0.84–0.98 (Table 3). Most studies report the presence of $R(-)$-naproxen due to the inversion observed during wastewater treatment. River water microcosm studies spiked with $R/S(\pm)$-naproxen revealed an enrichment of $S(\pm)$-naproxen during incubation (Camacho-Muñoz et al. 2019). Suzuki et al. (2014) studied the enantiospecific behaviour of naproxen in river water microcosms spiked with individual enantiomers. Very little inversion was observed for both enantiomers during 30-day incubation. However, removal was considerably different between $S(\pm)$-naproxen and $R(\pm)$-naproxen microcosms (Table 4). The half-lives of $S(\pm)$-naproxen and $R(-)$-naproxen were 37 and 99 days, respectively (Suzuki et al. 2014). The lack of inversion and considerably different removal rates between enantiomers demonstrates enantioselective degradation of naproxen in river water.
Terrestrial environment

The application of biosolids as fertilizer or irrigation with reclaimed wastewater can result in the introduction of chiral NSAIDs to agricultural soils (Albero et al. 2014; Radjenović et al. 2009a). Both naproxen and ibuprofen have been reported in amended soils (Biel-Maeso et al. 2018; Gibson et al. 2010). However, no data exist on the enantiomeric composition of these drugs in soil. Nevertheless, spiked microcosm studies have revealed important information on the behaviour of ibuprofen and naproxen here. Individual enantiomer microcosms revealed both naproxen and ibuprofen are subject to bidirectional chiral inversion in soil (Bertin et al. 2020). \(S(+)\)-naproxen and \(R(-)\)-ibuprofen were found to be the more persistent enantiomers. For example, soil microcosms maintained at 18 °C and spiked with \(R(-)\)-naproxen with an initial enantiomeric fraction less than 0.01 increased to 0.54 after 28 days. Microcosms spiked with \(S(+)-\)naproxen, and an initial enantiomeric fraction greater than 0.99 reduced to 0.78 after 28 days (Fig. 3). However, it was not possible to conclude that this was due to different rates of inversion or degradation between enantiomers as these processes can take place simultaneously. Naproxen enantiomers were found to persist in soil for at least 56 days, whereas ibuprofen degraded completely within 28 days (Bertin et al. 2020). Enantioselectivity and enantiomer removal were significantly reduced in soils incubated at 4 °C (Fig. 3), which is an important consideration for cooler and temperate climates. No enantiomer removal or enantioselectivity was observed in abiotic controls.

Enantiospecific toxicity

The effects of drug enantiomers on target organisms such as humans are well understood. Yet, studies on the enantiospecific effects of drugs to non-target organisms are limited, particularly for the NSAIDs. Mennillo et al. (2018) reported the toxicity of \(R/S(\pm)\)-ketoprofen and the pharmacologically active \(S(+)-\)ketoprofen to bacteria, algae and zooplankton. \(S(+)\)-ketoprofen was more potent to algae than the racemate at the same concentration. To demonstrate, median effect concentrations (EC\textsubscript{50}—concentration that the toxicological response is halfway between a normal and maximum response) towards the microalgae \textit{Pseudokirchneriella subcapitata} (growth inhibition after 96 h) were 240 and

![Graph](image-url)

\(\text{EC}_{50}\) values for 
- \(R(-)-\)naproxen
- \(S(+)-\)naproxen
- \(R(-)-\)ibuprofen
- \(S(+)-\)ibuprofen

\(18 \degree\) microcosm; BH18+, biotic high spike level of \(S(+)-\)naproxen
\(18 \degree\) microcosm; BH4+, biotic high spike level of \(R(-)-\)naproxen
\(4 \degree\) microcosm; BH4-, biotic high spike level of \(S(+)-\)naproxen
\(4 \degree\) microcosm; BH4-, biotic high spike level of \(S(+)-\)ibuprofen

\(\text{EC}_{50}\) values were significantly lower for \(S(+)-\)naproxen compared to \(R(-)-\)naproxen in the \(18 \degree\) microcosm. However, in the \(4 \degree\) microcosm, \(R(-)-\)naproxen was more toxic than \(S(+)-\)naproxen. This indicates that temperature plays a significant role in the toxicity of NSAIDs to algae.
66 µg L⁻¹ for R/S(±)-ketoprofen and S(+)ketoprofen, respectively (Mennillo et al. 2018) (Table 5). Furthermore, no observed effect concentration (NOEC) of 4 µg L⁻¹ has been reported for S(+)ketoprofen. Great-enantiospecificity (EC50 0.93 µg L⁻¹) for both enantiomers was reported for S(+)ketoprofen, albeit with only slight enantiospecificity (EC50 2.5 times more active than R(−)-ketoprofen) was undertaken on the pharmacologically less active R(−)-ketoprofen.

Neale et al. (2019) investigated the toxicity of S(+)ketoprofen and R(−)-ketoprofen using a battery of ecotoxicity bioassays including bacteria, algae and fish cells. The photosystem inhibition of P. subcapitata (EC10, 24 h) was greater than 16.7 mg L⁻¹ for both enantiomers. Greatest enantiospecific differences were observed towards ethoxyresorufin-O-deethylase activity in fish cells (induction of cytochrome P450 1A enzymes which are important for xenobiotic metabolism (Fent 2001). The effect concentration causing an induction ratio of 1.5 (EC10₁,₅, 6 h) was 4.3 mg L⁻¹ for R(−)-ketoprofen and greater than 27.2 mg L⁻¹ for S(+)ketoprofen demonstrating at least 6.3 times potency difference between enantiomers (Neale et al. 2019). Here, the more potent enantiomer R(−)-ketoprofen was the less prevalent reported in the environment (Table 5).

Neale et al. (2019) also investigated the toxicity of individual flurbiprofen, ibuprofen and naproxen enantiomers in their study. Of the studied organisms, ibuprofen enantiomers exhibited toxicity towards the bacteria Photobacterium leiognathi. However, toxicity was not enantiospecific with EC50 values (% inhibition, 30 min) of 3.1 and 2.8 mg L⁻¹ for S(−)-ibuprofen and R(+)ibuprofen, respectively (Neale et al. 2019). However, studies on zebra fish (Danio rerio) have reported significant differences on lipid metabolites in brain tissue induced by S(−)-ibuprofen and R(+)ibuprofen (Zhang et al. 2020). In this study, the test fish were exposed to environmentally relevant enantiomer concentrations (5 µg L⁻¹) for 28 days. The authors concluded that ibuprofen can induce enantiospecific toxicity to aquatic organisms (Zhang et al. 2020).

Flurbiprofen showed greatest enantiospecific toxicity towards P. leiognathi albeit only 1.7 times difference between S(+)flurbiprofen and R(−)-flurbiprofen (EC50 1.2 vs 2.1 mg L⁻¹) (Neale et al. 2019). S(+)naproxen was 2.5 times more active than R(−)naproxen in the ethoxyresorufin-O-deethylase assay. However, the lowest EC50 values were reported for P. leiognathi albeit with only slight enantiospecificity (EC50 0.93 vs 0.75 mg L⁻¹) (Table 5) (Neale et al. 2019). Considerably more toxicity data are available on S(+)naproxen (without comparative data for R(−)naproxen) than the ‘off-the-shelf’ standard available (Table 5). This includes chronic toxicity data whereby the lowest NOEC to the crustacean Moina macrocopa (7 days, reproduction) was 0.3 mg L⁻¹ (Kwak et al. 2018) (Table 5). Although S(+)naproxen is the dominant enantiomer in the environment with enantiomeric fractions 0.84–0.98, notable R(−)naproxen concentrations are also present (Table 4). Therefore, toxicity assays also need undertaken on R(−)-naproxen.

Recommendations for enantioselective studies and future perspectives

In recent years, considerable improvements have been made on understanding the enantiospecific behaviour and effects of NSAIDs in the environment. Nevertheless, further research is needed to further our understanding and better appreciate the environmental risks posed by chiral NSAIDs. Therefore, future perspectives and recommendations for research in this area are outlined.

Analytical strategies

Significant progress has been made in analytical methods for enantioselective analysis of NSAIDs in environmental matrices over the past 10 years (Table 2). However, further developments are now needed to improve and ensure the quality of quantitative data, and to shorten analysis times. Although sample extraction processes, e.g. SPE, are not considered enantioselective in nature, sample storage can be. For example, enantioselective degradation of the stimulant amphetamine has been observed in surface water during storage at 4 °C for 48 h (Ramage et al. 2019). Many authors report the storage of water samples for NSAIDs analysis at 4 °C to mitigate any analyte losses or enantiospecific changes (Table 2). However, the suitability of this approach needs investigated for NSAIDs considering chiral inversion and degradation could take place.

A further consideration needed to ensure accurate data are collected is the method of quantitation used. A well-known issue of environmental analysis using electrospray ionization in LC–MS applications is the effect of co-extracted matrix components on analyte signal strength (Cappiello et al. 2008; Furey et al. 2013; Petrović et al. 2005). This can lead to enhancement or suppression of signal strength, which can also be enantioselective (Camacho-Muñoz and Kasprzyk-Hordern 2015, 2017; Camacho-Muñoz et al. 2016; Castrignanò et al. 2018; López-Serna et al. 2013). Such effects can be easily corrected using deuterated surrogates in the analysis process. However, several methods report the use of matrix matched calibrations without deuterated surrogates/internal standards to account for these matrix effects (Li et al. 2019; Ma et al. 2019; Wang et al. 2018; Yuan et al. 2018) (Table 2). This quantification approach needs care, particularly in monitoring studies where the composition of
| Drug          | Testing organism group | Testing organism | Endpoint            | Toxicity measure | Exposure time | $R(\text{−})$-enantiomer (mg L$^{-1}$) | $S(\text{+})$-enantiomer (mg L$^{-1}$) | Enantiospecific difference$^a$ | References |
|--------------|------------------------|------------------|---------------------|------------------|--------------|--------------------------------------|--------------------------------------|----------------------------------|------------|
| Naproxen     | Bacteria               | P. leiognathi    | Bacterial toxicity  | EC$_{50}$        | 30 min       | 0.75                                 | 0.93                                 | 1.2                              | A          |
|             | Algae                  | P. subcapitata   | PSII Inhibition     | EC$_{10}$        | 2 h          | 20.1                                 | > 18.8                               | –                                | A          |
|             | Algae                  | P. subcapitata   | PSII Inhibition     | EC$_{10}$        | 24 h         | 19.8                                 | 23.8                                 | 1.2                              | A          |
|             | Fish liver cells       | PLHC-1           | EROD activity       | EC$_{IR,1.5}$    | 6 h          | 12.6                                 | 5.1                                  | 2.5                              | A          |
|             | Fish liver cells       | PLHC-1           | Cell viability      | EC$_{50}$        | 48 h         | > 18.8                               | > 13.2                               | –                                | A          |
| Invertebrate| D. magna               | Immobility       | EC$_{50}$           | 48 h             | –            | 85.3                                 | –                                    | –                                | B          |
| Invertebrate| M. macrocopa           | Immobility       | EC$_{50}$           | 48 h             | –            | 74.1                                 | –                                    | –                                | B          |
| Invertebrate| D. magna               | Survival         | NOEC                | 21 days          | –            | 30                                   | –                                    | –                                | B          |
| Invertebrate| D. magna               | Reproduction     | NOEC                | 21 days          | –            | 10                                   | –                                    | –                                | B          |
| Invertebrate| D. magna               | Growth           | NOEC                | 21 days          | –            | 10                                   | –                                    | –                                | B          |
| Invertebrate| M. macrocopa           | Survival         | NOEC                | 7 days           | –            | 30                                   | –                                    | –                                | B          |
| Invertebrate| M. macrocopa           | Reproduction     | NOEC                | 7 days           | –            | 0.3                                  | –                                    | –                                | B          |
| Fish         | O. latipes             | Survival         | NOEC                | 40 days          | –            | 0.5                                  | –                                    | –                                | B          |
| Fish         | O. latipes             | Growth           | NOEC                | 40 days          | –            | 50                                   | –                                    | –                                | B          |
| Fish         | D. rerio (embryo)      | Immobility/Death | LC$_{50}$           | 96 h             | –            | 115.2                                | –                                    | –                                | C          |
| Fish         | D. rerio (larvae)      | Immobility/Death | LC$_{50}$           | 96 h             | –            | 147.6                                | –                                    | –                                | C          |
| Fish         | D. rerio (embryo)      | Malformation     | EC$_{50}$           | 96 h             | –            | 98.3                                 | –                                    | –                                | C          |
| Fish         | D. rerio (larvae)      | Malformation     | EC$_{50}$           | 96 h             | –            | 149                                  | –                                    | –                                | C          |
| Invertebrate| D. magna               | Immobility       | EC$_{50}$           | 48 h             | –            | 46.7                                 | –                                    | –                                | D          |
| Fish         | C. carpio              | Immobility       | LC$_{50}$           | 96 h             | –            | 269.2                                | –                                    | –                                | D          |
| Algae        | S. subspicatus         | Growth           | EC$_{50}$           | 72 h             | –            | 625.5                                | –                                    | –                                | E          |
| Invertebrate| D. magna               | Immobility       | EC$_{50}$           | 24–48 h          | –            | 166.3                                | –                                    | –                                | E          |
| Flurbiprofen | Bacteria               | P. leiognathi    | Bacterial toxicity  | EC$_{50}$        | 30 min       | 2.13                                 | 1.22                                 | 1.7                              | A          |
|             | Algae                  | P. subcapitata   | PSII Inhibition     | EC$_{10}$        | 2 h          | 6.92                                 | 9.79                                 | 1.4                              | A          |
|             | Algae                  | P. subcapitata   | PSII Inhibition     | EC$_{10}$        | 24 h         | 5.47                                 | 9.07                                 | 1.7                              | A          |
|             | Fish liver cells       | PLHC-1           | EROD activity       | EC$_{IR,1.5}$    | 6 h          | 8.39                                 | > 12.5                               | > 1.5                            | A          |
|             | Fish liver cells       | PLHC-1           | Cell viability      | EC$_{50}$        | 48 h         | > 13.8                               | > 13.7                               | –                                | A          |
| Ibuprofen    | Algae                  | C. pyrenoidosa   | Growth              | IC$_{50}$        | 48 h         | 66.7                                 | 65.5                                 | –                                | F          |
|             | Algae                  | C. pyrenoidosa   | Growth              | IC$_{50}$        | 72 h         | 64.4                                 | 53.8                                 | 1.2                              | F          |
|             | Algae                  | C. pyrenoidosa   | Growth              | IC$_{50}$        | 96 h         | 61.0                                 | 54.5                                 | 1.1                              | F          |
collected samples can change between samples. For example, the signal suppression of the pesticide aldicarb for five different apple varieties varied by 42% (Kruve et al. 2008).

Considering LC is the preferred method of analysis and is likely to continue being so, it is important to note that existing methods use enantioselective columns with 5 µm stationary phase particle sizes (Table 2). Such columns are restricted to use in high-performance liquid chromatography operation. Commercially available columns particularly for the polysaccharide phases are now available with sub-2 µm particle sizes. The ability to operate at ultra-performance liquid chromatography pressures facilitates higher plate numbers, improved resolution and shorter analysis times.

**Occurrence and fate studies**

Several studies have reported enantioselective changes to NSAIDs during wastewater treatment and in the environment (Tables 3, 4). Both chiral inversion and enantioselective degradation have been demonstrated (Bertin et al. 2020; Hashim and Khan 2011; Nguyen et al. 2017; Suzuki et al. 2014). These studies have shown that it is essential to conduct microcosm studies using individual enantiomers over the racemate where possible to better evaluate enantioselective fate processes. However, studies evidencing the mechanisms of inversion and degradation in an environmental context are lacking. This is challenging for complex environmental compartments including those encountered in engineered systems such as wastewater treatment plants. Nevertheless, studies need to be undertaken on individual microbial species found in wastewater treatment plants to better our understanding of NSAIDs fate during wastewater treatment. This will also help explain the differences in enantioselectivity observed between the same process types, as well as different processes (Tables 3, 4).

### Table 5 (continued)

| Drug          | Testing organism group | Testing organism | Endpoint          | Toxicity measure | Exposure time | $R(-)$-enantiomer (mg L$^{-1}$) | $S(+)\text{-enantiomer}$ (mg L$^{-1}$) | Enantiospecific difference | References |
|---------------|------------------------|------------------|-------------------|------------------|--------------|---------------------------------|--------------------------------------|--------------------------|------------|
| Ketoprofen    | Bacteria               | *P. leiognathi*  | Bacterial toxicity | EC$_{50}$        | 30 min       | 2.84                            | 3.13                                 | 1.1                       | A          |
| Algae         | *P. subcapitata*       |                  | PSII Inhibition   | EC$_{10}$        | 2 h          | $>$ 18.5                        | $>$ 29.5                             | –                         | A          |
| Algae         | *P. subcapitata*       |                  | PSII Inhibition   | EC$_{10}$        | 24 h         | $>$ 18.5                        | $>$ 29.5                             | –                         | A          |
| Fish liver cells | PLHC-1               |                  | EROD activity     | EC$_{IR1.5}$     | 6 h          | $>$ 11.9                        | $>$ 19.0                             | –                         | A          |
| Fish liver cells | PLHC-1               |                  | Cell viability    | EC$_{50}$        | 24 h         | $>$ 13.0                        | $>$ 20.7                             | –                         | A          |
| Ketoprofen    | Bacteria               | *P. leiognathi*  | Bacterial toxicity | EC$_{50}$        | 30 min       | 4.23                            | 4.56                                 | –                         | A          |
| Algae         | *P. subcapitata*       |                  | PSII Inhibition   | EC$_{10}$        | 2 h          | $>$ 16.7                        | $>$ 42.3                             | –                         | A          |
| Algae         | *P. subcapitata*       |                  | PSII Inhibition   | EC$_{10}$        | 24 h         | $>$ 16.7                        | $>$ 42.3                             | –                         | A          |
| Fish liver cells | PLHC-1               |                  | EROD activity     | EC$_{IR1.5}$     | 6 h          | 4.34                            | $>$ 27.2                             | $>$ 6.3                   | A          |
| Fish liver cells | PLHC-1               |                  | Cell viability    | EC$_{50}$        | 24 h         | $>$ 11.7                        | $>$ 29.6                             | –                         | A          |
| Algae         | *P. subcapitata*       |                  | Growth            | EC$_{50}$        | 96 h         | –                               | 0.066                                | –                         | G          |
| Algae         | *P. subcapitata*       |                  | Growth            | NOEC             | 96 h         | –                               | 0.004                                | –                         | G          |
| Algae         | *P. subcapitata*       |                  | Growth            | LOEC             | 96 h         | –                               | 0.008                                | –                         | G          |

Key: EC$_{10}$, effective concentration, 10%; EC$_{50}$, half maximal effective concentration; EC$_{IR1.5}$, concentration that induces an induction ratio of 1.5; EROD, ethoxyresorufin-O-deethylase; LC$_{50}$, lethal concentration, 50%; LOEC, lowest observed effect concentration; NOEC, no observed effect concentration; PSII, photosystem II

$a$ Enantiospecific difference = \( \frac{\text{Higher concentration}}{\text{Lower concentration}} \)

References: aNeale et al. (2019); bKwak et al. (2018); cLi et al. (2016); dGheorghe et al. (2016); eCleveurs (2004); fWang et al. (2020); gMennillo et al. (2018)
The majority of enantioselective occurrence and fate investigations on NSAIDs are focused on aqueous environmental matrices (Tables 3, 4). Chiral NSAIDs have been reported in particulate matrices such as treated and untreated sludge, soils and sediments, albeit not at the enantiomeric level (Albero et al. 2014; Kumirska et al. 2015; Martín et al. 2012; Radjenović et al. 2009a). Although there is some enantiomeric information for ibuprofen and naproxen in soils (Bertin et al. 2020), little enantiospecific data exist for sediments or sludges. Yuan et al. (2018) reported enantimetric data for ibuprofen in sediment and sludge. However, the order of enantiomer elution is unknown and chromatographic peaks could not be assigned S(+)-ibuprofen and R(−)-ibuprofen. Nevertheless, the data showed ibuprofen to be present in sludge in non-racemic form (Yuan et al. 2018). Therefore, further studies on the enantiospecific occurrence and behaviour of NSAIDs in particulate environmental matrices are recommended. Methods which can support such studies are already available or can be adapted to do so (Bertin et al. 2020; Yuan et al. 2018).

Investigations on the enantiomeric occurrence and fate of metabolites during wastewater treatment and in the environment can help better understand the fate of the parent compounds. Several methods exist which facilitate enantioseparation of metabolites including carboxyibuprofen, 2-hydroxyibuprofen and dihydroketoprofen (Camacho-Muñoz and Kasprzyk-Hordern 2015; 2017; Camacho-Muñoz et al. 2016) (Table 2). In such methods, the order of enantiomer elution is not known. Without the use of optical rotation detection to determine enantiomer elution order, the commercial availability of enantiomerically pure reference standards is essential. Such standards are also needed to conduct single enantiomer microcosm studies. However, at present such reference standards are not available for most NSAID metabolites expect for S(+)-O-desmethylnaproxen and R(−)-O-desmethylnaproxen. The availability of enantiopure metabolite standards would also facilitate enantiospecific toxicity studies. Many metabolites are themselves pharmacologically active and have been reported in river waters (Paiga et al. 2016; Zha et al. 2017; Zojaji et al. 2019).

Ecotoxicity studies

Existing data show that although enantiospecific differences exist, they were less than one order or magnitude (Neale et al. 2019). Nevertheless, there is need for additional enantiospecific investigation for a greater range of organisms and endpoints considering the enantiomer enrichment observed in the environment (Table 3). Toxicity testing also needs to investigate chronic exposure as the majority of research undertaken to date has focussed on acute effects (Table 5). It is recommended that these are undertaken at environmentally relevant concentrations as the majority of acute exposures use enantiomer concentrations considerably greater than those encountered in the environment (Table 5). This will improve the accuracy of environmental risk assessments of chiral NSAIDs and establish the significance of considering stereochemistry for the environmental effects of such compounds. Metabolomic approaches are also beneficial to provide information on organism function and health at the molecular level (Bundy et al. 2008). This will provide additional information which would otherwise be missed by traditional bioassays and endpoints. Once a greater body of knowledge is available on the enantiospecific effects of chiral NSAIDs and their modes of action, mixture effects can be considered.

It is suggested that effect studies are supported with appropriate enantioselective analysis to report actual concentrations over nominal concentrations in exposure media over the duration of studies. This analysis is important to undertake throughout the exposure period. Mennillo et al. (2018) reported no change to the concentration of S(+)-ketoprofen in P. subcapitata test media after 72 h. Zhang et al. (2020) also reported drug stability during exposure of D. rerio with ibuprofen enantiomers. Although they confirmed the ‘total’ ibuprofen concentration did not vary substantially, enantioselective analysis was not used to confirm the enantiomeric composition and confirm inversion did not take place. On the other hand, the requirement to confirm drug enantiomeric composition is less critical for studies conducted on cells over whole organisms. For example, Neale et al. (2019) who undertook toxicity assays using fish cells stated that any inversion that takes place is part of the cell’s response to the enantiomer and is still a relevant measure of its biological activity.

Conclusion

Investigating the stereochemistry of NSAIDs in wastewaters and the environment poses several challenges. This review demonstrates that considerable progress has been made in this research area. However, to fully assess the environmental risk posed by NSAIDs at the enantiomeric level, further work is needed as outlined in this review, e.g. fate studies using enantiopure standards, investigation of metabolites and further enantiospecific toxicity studies. Once the risk is established, appropriate mitigation steps can then be undertaken, if needed. The essential use of NSAIDs by growing and aging populations is unlikely to result in any reduction of their entry into wastewater in the future. However, modifying existing wastewater treatment plant operation to target the removal of specific enantiomers is an opportunity to reduce any identified risks. This could be achievable whereby the microbial species that preferentially degrade or invert those more toxic enantiomers are known, and wastewater treatment plants can be operated to facilitate
their prevalence. For example, the microbial community in suspended biomass systems changes with sludge age which can be manipulated through process operation (Pala-Ozkok et al. 2013).

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