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REVIEW

Charles S. Wong

Environmental fate processes and biochemical transformations of chiral emerging organic pollutants

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Abstract This review highlights the analytical chemistry, environmental occurrence, and environmental fate of individual stereoisomers of chiral emerging pollutants, which are modern current-use chemicals of growing environmental concern due to their presence in the environment and potential for deleterious effects. Comparatively little is known about individual stereoisomers of pollutants, which can have differential toxicological effects and can be tracers of biochemical weathering in the environment. Stereoisomers are resolved by gas chromatography (GC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE). Separation techniques in environmental analysis are typically coupled to mass spectrometry (MS) and tandem mass spectrometry (MS/MS), as these provide the sensitivity and selectivity needed. The enantiomer composition of phenoxyalkanoic and acetamide herbicides, organophosphorus and pyrethroid pesticides, chiral polychlorinated biphenyl metabolites, synthetic musks, hexabromocyclododecane, and pharmaceuticals in the environment show species-dependent enantioselectivity from biotransformation and other biologically mediated processes affecting enantiomers differentially. These enantiomer compositions are useful in detecting biologically mediated environmental reactions, apportioning sources of pollutants, and gaining insight into the biochemical fate of chiral pollutants in the environment, which are needed for accurate risk assessment of such chemicals.

Keywords Chiral pollutants · Environmental chemistry · Biotransformation · Source apportionment

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Abbreviations CE: capillary electrophoresis · CZE: capillary zone electrophoresis · AHDI: synthetic musk 6-acetyl-1,1,2,3,3,5-hexamethyldihydroindene · AHTN: synthetic musk 7-acetyl-1,1,3,4,4,6-hexamethyltetralin · ATII: synthetic musk 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindene · DDT: dichlorodiphenyltrichloroethane · ECD: electron capture detector · ECNI: electron capture negative ionization · GC: gas chromatography · GC/MS: gas chromatography/mass spectrometry · HBCDD: hexabromocyclododecane · HHCB: synthetic musk 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran · HPLC: high-performance liquid chromatography · HPLC/MS/MS: high-performance liquid chromatography–tandem mass spectrometry · MEKC: micellar electrokinetic chromatography · $MeSO_2$-PCB: polychlorinated biphenyl methyl sulfone · MS: mass spectrometry · MS/MS: tandem mass spectrometry · NP: normal phase · NSAID: non-steroidal anti-inflammatory drugs · PCB: polychlorinated biphenyl · POP: persistent organic pollutant · RP: reversed phase · OPs: organophosphorus pesticides · UV: ultraviolet detection

Introduction

Pollutant enantiomers are a growing area of research in the field of environmental chemistry for several reasons. First, many chemicals of environmental interest and concern are chiral. About 25 % of all agrochemicals are chiral [1], as are many other legacy and current-use chemicals. Surprisingly, contaminant stereoisomerism has received little attention, despite the significantly different toxicology that enantiomers can have compared to each other and to the parent mixture, and also the enhanced insights enantiomer analysis can bring to understanding environmental chemical processes.

A compound's enantiomers have identical physical and chemical properties. Thus, abiotic environmental processes (e.g., air–water exchange, sorption, abiotic transformation) are generally identical for both stereoisomers. However, biochemical processes (e.g., biotransformation) and toxicological effects can be enantioselective because individual stereoisomers can interact differentially with other chiral molecules, such as enzymes and biological receptors. Thus, stereoisomers can have different biological and toxicological effects. These range from beneficial, such as greater herbicidal activity (e.g., *S*-metolachlor is the active ingredient, whereas *R*-metolachlor is inactive [2]) to deleterious, such as the (−)-enantiomer of *o*,*p*′-DDT being a weak estrogen mimic and endocrine disruptor, whereas (+)-*o*,*p*′-DDT is inactive [3]. In addition, enantiomers thus mark biologically mediated processes, as these may change enantiomer distributions in the environment, whereas abiotic processes generally will not, even though they may change concentrations and fluxes equally for both enantiomers. Thus, enantiomers can be used as tracers of environmental biochemical activity affecting pollutants. This feature is particularly useful, as environmental processes are complex and quite variable, so detecting and analyzing biochemical processes such as biotransformation may be confounded by the many physical and chemical processes that also act upon the chemical of interest.

Most studies on chiral chemicals do not explicitly account for individual stereoisomers, even though they have different biological fates and endpoints. As a result, current knowledge of chiral pollutants is often inaccurate, as it implicitly and incorrectly assumes that enantiomers have identical environmental behavior. Standard achiral analyses cannot measure enantiomer compositions, but only the sum total of stereoisomers. Thus, for example, if an enantiomer with more toxic effects is preferentially degraded, then exposure assessed from achiral concentration measurements would overestimate ecotoxicity. Conversely, the preferential elimination of relatively innocuous enantiomers would underestimate toxicity based on achiral measures. This issue is recognized by regulatory agencies, which have requested information on manufacturing processes and analytical enantiomer separation for pesticide registration [4–6], and by the increasing registrations of single-enantiomer agrochemicals [1]. Despite the recognized need for enantiomer-specific fate and toxicity, regulatory bodies still lack such information [7] needed for proper risk assessment and for sound public policy.

This review highlights the current state of knowledge on the environmental occurrence and fate processes of a number of major types of chiral emerging and modern organic pollutants. These are defined as chemicals that are currently being used and released to the environment and are of environmental concern from widespread environmental occurrence and potential for toxic effects. It also includes metabolites of past-use chemicals, which are poorly understood. The specific focus of this review is several classes of current-use pesticides (herbicidal phenoxyalkanoic acids and related compounds, acetamide herbicides, organophosphorus compounds, and pyrethroids), metabolites such as chiral polychlorinated biphenyl (PCB) methyl sulfones, synthetic musks, the brominated flame retardant hexabromocyclododecane, and pharmaceuticals (Table 1). The environmental stereochemistry of these compounds has not been studied as well as that of the legacy persistent organic pollutants (POPs), such as PCBs and organochlorine pesticides. These have been reviewed extensively elsewhere [8–10]. Developments on chiral separations as applied to trace environmental analysis, such as chiral GC, HPLC, and CE, are discussed as well for the target analytes (Table 1). However, general analytical methodologies for chiral measurements are not the focus of this article, as these have been extensively reviewed elsewhere [8, 10–14]. Nor are developments in immunochemical detection (e.g., immunoaffinity stationary phases, immunochemical assays) or molecularly imprinted polymers discussed. These analytical techniques are widely used (see reviews [15–18]) and have environmental applications [16, 19]; however, little effort to date has been made to apply them for enantiomer analysis in natural waters [20], even though they have been used for chiral analysis [21, 22]. This review will also be limited to research that explicitly addresses individual stereoisomers, as the achiral literature on these chemicals is extensive as evidenced by other reviews [12, 23–27].

Environmental chemistry of emerging chiral pollutants

Agrochemicals

Phenoxyalkanoic acids and related herbicidal compounds

The phenoxyalkanoic acid herbicides are the most-studied modern chiral pollutants. These chemicals are heavily used as pre- and post-emergence herbicides in agriculture, forestry, and industrial weed control. They are highly water soluble, and therefore generally mobile in the environment. Substantial quantities leach after application to surface soil to runoff and to groundwater, resulting in contamination of water supplies. Thus, the study of their environmental occurrence and fate is crucial in risk assessment to water resources.

Table 1 Chiral emerging pollutant analytes discussed in this review and the chiral analytical techniques used to quantify stereoisomer composition in environmental matrices (i.e., studies focusing only on enantiomer separations of standards or technical mixtures not included)

Analyte	Matrix	Analytical methods	References
Phenoxyalkanoic acids			
Dichlorprop	Water	RP HPLC/UV	[29, 30, 32]
	Soil	CE/UV	[34, 36, 44]
	Water	CE/UV	[35]
	Soil	GC/MS	[41]
	Water	GC/MS	[42]
	Activated sludge	GC/MS	[33]
	Activated sludge	RP HPLC/UV	[33]
Mecoprop	Soil	GC/MS	[41]
	Soil	CE/UV	[36, 38]
	Water	GC/MS	[41–43, 45, 47, 49, 61]
	Water	RP HPLC/UV	[31, 50]
	Activated sludge	GC/MS	[33]
	Activated sludge	RP HPLC/UV	[33]
Acetamide pesticides, acetamide degradates			
Acetochlor	Soil, activated sludge	GC/MS	[59]
Dimethenamid	Soil, activated sludge	GC/MS	[59]
Metalaxyl	Activated sludge	GC/MS	[59]
	Soil	GC/MS	[58, 59, 62]
	Soil	RP HPLC/UV	[63]
	Soil, plant tissue	NP HPLC/UV	[66]
	Soil	CE/UV	[57]
Metolachlor	Soil, activated sludge	GC/MS	[59]
	Water	GC/MS	[60, 61]
	Water	CE/UV	[35]
	Soil, water	RP HPLC/MS	[64]
	Soil, water	CE/UV	[64]
	Soil	Immunoassay	[20]
Organophosphorus pesticides			
Fenamiphos	Soil, surface water	NP HPLC/UV	[73]
Fonofos	Soil	CE/UV	[57]
Malathion	Tap water	CE/UV	[72]
Ruelene	Soil	CE/UV	[44, 57]
Pyrethroids			
Bifenthrin	Sediment	GC/ECD	[79]
	Water	GC/ECD	[77, 78]
Cyfluthrin	Water	GC/ECD	[77]
Cypermethrin	Rat tissue	NP HPLC/UV	[80]
	Water	GC/ECD	[77]
Fenvalerate	Soil	NP HPLC/UV	[82]
Permethrin	Sediment	GC/ECD	[79]
	Water	GC/ECD	[77, 78]
Polychlorinated biphenyl method sulfones			
Chiral congeners[a]	Mammalian tissues[b]	GC/MS	[93, 95–99]
Synthetic polycyclic musks			
AHDI	Fish muscles, surface water	GC/MS	[105]
	Wastewater, activated sludge	GC/MS	[106]
AHTN	Fish muscles, surface water	GC/MS	[104, 105]
	Wastewater, activated sludge	GC/MS	[106]
ATII	Fish muscles, surface water	GC/MS	[105]
	Wastewater, activated sludge	GC/MS	[106]

Table 1 (continued)

Analyte	Matrix	Analytical methods	References
HHCB	Fish muscles, surface water	GC/MS	[104, 105]
	Wastewater, activated sludge	GC/MS	[106]
Hexabromocyclododecane			
α-,β-,γ-HBCDD	Fish livers and muscle	RP HPLC/MS/MS	[110]
Pharmaceuticals			
Atenolol	Surface water, wastewater	RP HPLC/MS/MS	[126]
Ibuprofen	Surface water, wastewater	GC/MS	[119]
Metoprolol	Surface water, wastewater	RP HPLC/MS/MS	[126]
Propranolol	Surface water, wastewater	GC/MS	[125]
	Surface water, wastewater	RP HPLC/MS/MS	[126]

[a]Chiral $MeSO_2$-PCB congeners found in mammalian tissues include: *meta*-$MeSO_2$-PCB 91 (3-methylsulfonyl-2,2′,4′,5,6-pentachlorobiphenyl), *para*-$MeSO_2$-PCB 91 (4-methylsulfonyl-2,2′,4′,5,6-pentachlorobiphenyl), *meta*-$MeSO_2$-PCB 132 (3-methylsulfonyl-2,2′,3′,4′,5,6-hexachlorobiphenyl), *para*-$MeSO_2$-PCB 132 (4-methylsulfonyl-2,2′,3′,4′,5,6-hexachlorobiphenyl), *meta*-$MeSO_2$-PCB 149 (3-methylsulfonyl-2,2′,4′,5,5′,6-hexachlorobiphenyl), *para*-$MeSO_2$-PCB 149 (4-methylsulfonyl-2,2′,4′,5,5′,6-hexachlorobiphenyl), *meta*-$MeSO_2$-PCB 174 (3-methylsulfonyl-2,2′,3′,4′,5,5′,6-heptachlorobiphenyl), *para*-$MeSO_2$-PCB 174 (4-methylsulfonyl-2,2′,3′,4′,5,5′,6-heptachlorobiphenyl)

[b]Species: polar bear, ringed seals, grey seals, harbor porpoises, rats, humans. Tissues: blubber, liver, lung, adipose tissue

Among the most important of this class that are chiral are mecoprop and dichlorprop (Fig. 1), of which only the *R*-enantiomers are herbicidal [28]. Since the 1980s, enantiopure products containing only the active enantiomer have been developed and registered to complement and/or replace the original racemic products. This "chiral switch" decreases overall loading of xenobiotic compounds to the environment, as only the active ingredient is applied.

Because of their polarity, phenoxyalkanoic acid enantiomers are usually analyzed by reversed-phase HPLC

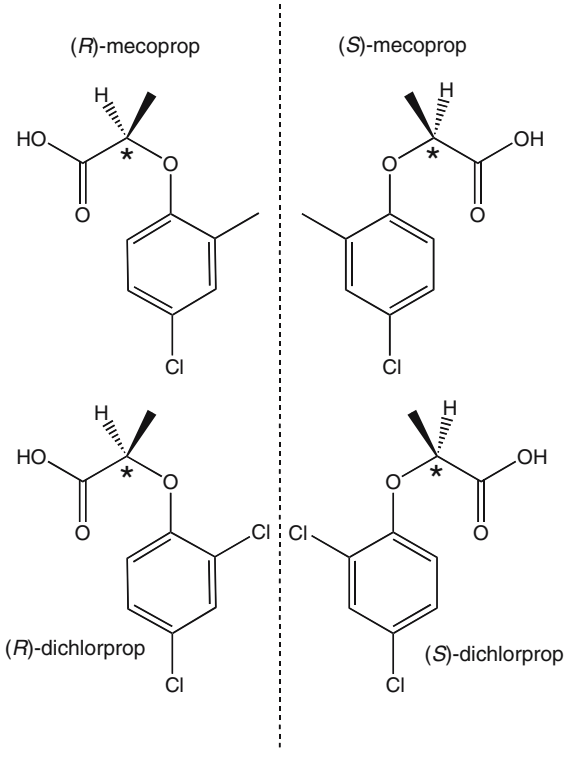

Fig. 1 Structures of the enantiomers of mecoprop and dichlorprop. *Asterisks* denote stereogenic centers

[29–33] or CE in the capillary zone electrophoresis (CZE) mode using added chiral selectors and UV [34–38] or MS detection [39]. Sensitivity can be increased in CE by using partial filling methods to keep the chiral selector from being present during detection [36]. Micellar electrokinetic chromatography (MEKC) with laser-induced fluorescence has also been used to increase chiral CE sensitivity for phenoxyalkanoic herbicide detection if the analytes are derivatized with a fluorescent tag [40]. Some studies have used chiral GC/MS analysis after derivatization to the methyl ester [41–44] or the pentafluorobenzyl ester [33, 45].

The phenoxyalkanoic acids have been known for decades to biodegrade by attack at the carbon side chain to form the corresponding phenols, or hydroxylation at the aromatic ring and its subsequent opening [28]. However, biotransformation at the asymmetric carbon atom has been less studied, despite the similarities of these pesticides with well-studied structurally similar non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen [46]. Biological activity in environmental solids and waters typically results in enantioselective microbial degradation, resulting in nonracemic residues of phenoxyalkanoic acids. Ludwig et al. [29] found that marine microorganisms exclusively degraded *R*-dichlorprop and did not transform the *S*-enantiomer. The opposite was observed in soils from a test field [34] and in an incubation with anaerobic sewage sludge that also produced the chlorophenol degradates [33], indicating microbial consortia with different enantiomer preferences in the two matrices. Lewis et al. [44] found that environmental changes in soils (e.g., addition of nutrients) can shift enantiomer preferences of dichlorprop in Brazilian and North American soils towards preferentially degrading the inactive *S*-enantiomer. Changes in soil microbial populations were observed via 16S ribosomal RNA amplification analysis. Such an enantiomer shift could potentially lead to increased phytotoxicity of dichlorprop residues, as the active *R*-enantiomer would be more persistent. The authors suggested that enantios-

electivity of pollutant microbial degradation is controlled by activation of microbial populations or induction of enantiomer-specific enzymes that are activated by environmental changes. In Swiss soils, the *R*-enantiomers of both dichlorprop and mecoprop were enriched in soils due to slower degradation compared to the respective *S*-enantiomers [41]. This enantiomer composition was observed for mecoprop in some Swiss lakes, but the reverse enantiomer preference was found in others suggesting the existence of different enantioselective biotic processes and/or surface water contamination with racemic mecoprop [43, 47, 48]. Significant biologically mediated racemization, of the order of 25–65 % of biotransformation rates, was observed in both soils and surface waters for dichlorprop and mecoprop [41, 43, 47]. This enantiomerization is consistent with chiral inversions observed for structurally similar NSAIDs [46].

A likely source of the reversed enantiomer preference for mecoprop in Swiss lakes was from rooftop runoff. In Switzerland, rooftops treated with bituminous sealing membranes containing Preventol B 2 had concentrations of both mecoprop enantiomers up to several hundred micrograms per liter, orders of magnitude above both Swiss and European drinking water standards of 100 ng L^{-1} and concentrations in rainwater [49]. The sealant contains bi-esters of *R*,*S*-mecoprop, which released mecoprop upon hydrolysis. The major factors controlling mecoprop release from experimentally treated rooftops were the type of treatment and the biological activity on the roof. Microbially mediated hydrolysis on the roof caused preferential release of *S*-mecoprop, and the presence of root tips penetrating the treated membranes might have enhanced leaching. Such processes are difficult to quantify or predict (e.g., the amount of microbially mediated racemization). Wastewater treatment plants were a major source of mecoprop in surface waters, with loadings of the same order of magnitude as agricultural application. This observation suggests that rooftop leaching was a major source to wastewater, and that control of the sealant could substantially reduce mecoprop levels in natural waters.

Chiral analysis has also been used to detect in situ microbial biotransformation of mecoprop that has leached to contaminate aquifers. A landfill in Switzerland had racemic amounts of mecoprop in its leachate, which contaminated groundwater and showed an increasing excess of *R*-mecoprop with distance from the landfill [45]. Sorption to aquifer minerals, an abiotic process, was low and not enantioselective. Thus, the increasing excess of *R*-mecoprop with distance was consistent with biochemical weathering in the aquifer that could not be detected by achiral studies. Similar results were observed in a mecoprop-contaminated UK landfill; however, enantioselectivity reversed between aerobic aquifer zones, in which *R*-mecoprop was in excess, whereas *S*-mecoprop was dominant in nitrate-reducing zones [50]. This observation indicates that redox conditions affect mecoprop biotransformation, as different bacterial consortia are active under aerobic and anaerobic conditions.

Mecoprop and dichlorprop are also well-studied with regards to factors affecting enantioselectivity in microbially mediated biotransformation. Both mecoprop enantiomers were used by *Sphingomonas herbicidovorans* MH as a sole carbon and energy source [30]. This microbial strain can degrade racemic mecoprop to completion, but preferentially transformed the *S*-enantiomer when offered the racemate as a growth substrate. However, resting cells grown on *R*-mecoprop preferentially metabolized this enantiomer, suggesting that specific different enzymes were involved in the degradation of each enantiomer. This hypothesis was confirmed when different protein bands were identified for cells grown on the individual enantiomers [31]. Labeling experiments with $^{18}O_2$ confirmed that these enzymes were dioxygenases. Thus, "enzyme pairs" that are almost identical except for subtle differences in their active sites can determine enantioselectivities [51]. The *S. herbicidovorans* MH strain also completely degraded dichlorprop as well, with similar enantioselectivity [32]. This study also found enantiomer-specific inhibition of transport into cells, providing evidence for enantioselective, inducible active transport of these herbicides into cells of this strain. *R*-Mecoprop was used as a sole carbon source by a strain of *Alcaligenes denitrificans*, which degraded this compound in the same way it degraded 2,4-D, by transformation to 4-chloro-2-methylphenol and then to a substituted catechol [52]. Two other microbial strains, *Rhodoferax* sp. P230 and *Delftia acidovorans* MC1, also preferentially degraded *R*-enantiomers of mecoprop and dichlorprop via dioxygenases; the responsible genes were identified and were similar to those of other bacterial strains that could degrade chloroaromatic compounds [53]. These results are consistent with many of the laboratory and field studies showing enantioselective biodegradation of these herbicides, and with the suggestions of Lewis et al. [44] that enantioselectivity was related to genetic similarity among microbial strains.

Acetamide pesticides

Acetamide pesticides are an important class of agrochemicals, widely used as selective herbicides and fungicides for weed and fungi control in both agriculture and noncrop use (e.g., weed control on lawns). As with the phenoxyalkanoic acids, acetamide pesticides generally have high water solubility and leach after application to surface and ground waters. They are therefore among the most detected compounds in natural waters [54], and are monitored and studied to assess their contamination potential. Many of these pesticides are chiral, and some have more than one stereogenic center (Fig. 2). This results in the existence of diastereomers, which can have different physical and chemical properties from each other and be separated by achiral means. Each diastereomer consists of two or more enantiomers. An example is metolachlor, with an asymmetric carbon atom and axial chirality (atropisomerism) caused by hindered bond rotation at room temperature [55]. As with the phenoxyalkanoic acids, acetamides are undergoing a "chiral switch" as initial products were racemic, while more current registrations include enantiomer-

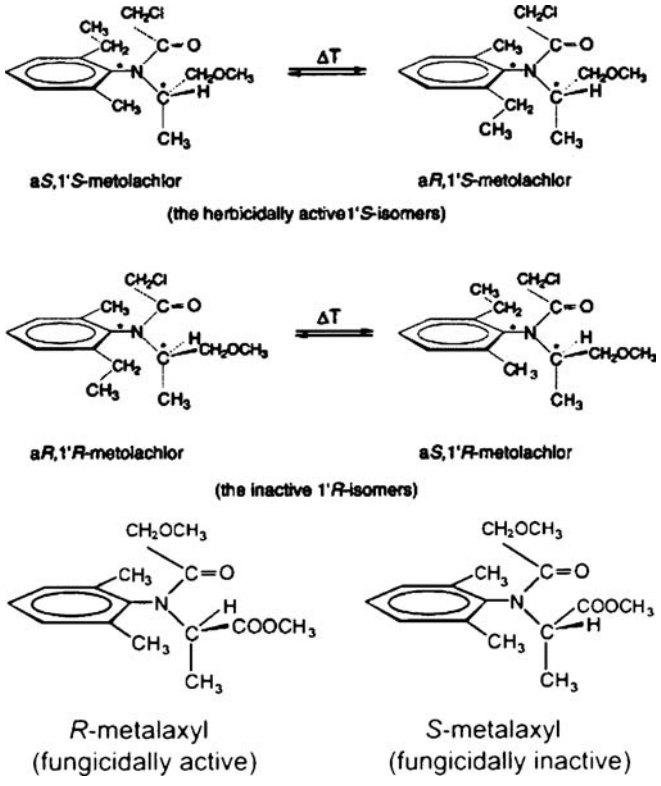

Fig. 2 Structures of the enantiomers of phenoxyalkanoic herbicides metolachlor and metalaxyl. Reprinted with permission from refs. [58] and [60]. Copyright 2000, 2002 American Chemical Society

enriched products containing the active stereoisomers (e.g., *S*-metolachlor, *R*-metalaxyl).

Environmental analysis of acetamide pesticides have typically employed either chiral GC or chiral HPLC, although CE methods with chiral selectors have been used in CZE [56] or MEKC modes [57]. However, chiral GC is unsuitable for the atropisomeric compounds in this class (e.g., metolachlor, acetochlor, dimethenamid), because their *ortho*-substituents do not provide significant hindrance to rotation around the axial center of chirality (energy barriers of 30–105 kJ mol^{-1}) to prevent them from rotating under the elevated temperatures of GC [55]. Chiral GC can still be used to separate enantiomers of diastereomers arising from an asymmetric carbon atom [58–62]. Proton nuclear magnetic resonance has been used to study possible hindered rotation around the amide bond (Fig. 3), but the atropisomers around this bond are not stable at room temperature [56]. Although MS is typically used for detection, particularly for samples from field studies with greater amounts of potential coextracted interferences, UV detection can be used for some laboratory experiments, such as soil slurries for degradation studies [57, 63]. The separation techniques can also separate major degradates of these pesticides at the same time, such as the ethanesulfonic and oxanilic acid metabolites of acetochlor [56] and metolachlor [64] by CZE, and the carboxylic acid metabolite of metalaxyl by chiral GC after derivatization [58] and chiral HPLC [63]. Chiral HPLC has also been used to isolate individual enantiomers for further measurement of optical rotation and absolute configuration [65], and in experimental incubations [58]. Stereoselective immunoassays have also been developed to distinguish between the 1′*S*- and 1′*R*-diastereomers of metolachlor in soil and water samples [20]; this is one of the few examples of chiral immunoassay for environmental applications to date.

The first study on environmental stereochemistry of acetamide pesticides [59] found stereoselective biodegradation of metolachlor, dimethenamid, and metalaxyl in soil and sewage sludge incubations, with the last of these compounds having more pronounced enantioselectivity in the more rapid degradation of *S*-(+)-metalaxyl compared to its more fungicidal antipode. Surface waters and rain contained detectable quantities of some of these pesticides at racemic compositions, suggesting little enantioselective biological degradation post-application. The enantiomer composition in two Swiss lakes rapidly changed as a result of the introduction of *S*-metolachlor to the Swiss market in 1997, followed by expiration of the registration for the racemate [60]. Within a year of the switch, the fraction of metolachlor present attributed to *S*-metolachlor use was estimated to be 55 % through stereoisomer measurement backed by mass balance modeling. This fraction increased to 90 % within two years [60] and was essentially complete after three years [61], indicating a rapid environmental response to changed inputs and consequently limited persistence of this chemical in these waters. In contrast, biodegradation of metolachlor was not enantioselective in soils and activated sludge spiked with racemic and *S*-enriched residues in a different study, likely from microbial consortia with different enantiomer preferences [64].

Metalaxyl has been the focus of several studies to elucidate factors affecting enantiomer fate. *R*-(−)-Metalaxyl degraded faster than *S*-(+)-metalaxyl in soil slurries [57, 58], with about 50 % of each enantiomer transformed to the carboxylic acid metabolite and the rest degraded via other pathways [58]. This enantiomer preference is opposite to that observed in sewage sludge [59], indicating

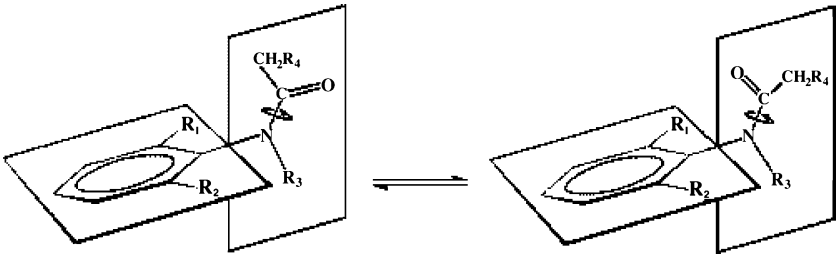

Fig. 3 Atropisomeric amide bonds in acetamide compounds. Reprinted with permission from ref. [56]. Copyright 1999 American Chemical Society

that the microbial consortia involved have different enantiomer preferences. No enantiomerization was observed, in contrast to that noted for phenoxypropionic acids [41, 43]. Soils from Germany had much faster degradation rates than Cameroonian soils [63], indicating site-specific differences in microbial populations responsible for transformation. Soil pH had a significant effect on degradation, as the *R*-enantiomer degraded faster in aerobic soils with pH >5, but the *S*-enantiomer degraded faster when pH<5 [62]. Both enantiomers were biotransformed at similar rates at pH 4–5. For soils that did not degrade metalaxyl enantioselectively, pH adjustment changed enantiomer preferences accordingly, suggesting that different microorganisms and enzymes (which have pH-dependent reaction kinetics) were involved. This pH dependence was also observed in the more acidic Cameroonian soil (pH 4.8) compared to the German soil (pH 7.2) [63]. Another study found that *R*-metalaxyl degraded faster than its antipode in near-neutral pH soils, consistent with the previous results, but that sunflower plants grown in that soil degraded the *S*-enantiomer preferentially [66].

Organophosphorus pesticides

Organophosphorus pesticides (OPs) are acetylcholinesterase inhibitors. As such, they are powerful neurotoxins with high toxicity to invertebrates. Although the mammalian toxicity of OPs is lower, the same mode of action exists [26]. Thus, these compounds can pose substantial environmental risk if exposure to non-target biota occurs. For these reasons, substitutes for OPs are being deployed, such as the pyrethroids. Some OPs are chiral about the phosphorus atom (e.g., ruelene, leptofos, fonofos), whereas others are chiral about an asymmetric carbon atom (e.g., malathion) (Fig. 4). As with other chiral pesticides, chiral OPs have differential insecticidal activities and toxicities between enantiomers [67, 68].

Chiral OPs are generally separated by chiral HPLC and CE techniques. Fourteen chiral OPs were separated by reversed-phase chiral HPLC on a Pirkle-type stationary phase by one group [69], whereas twelve OPs were separated on Chiralpak stationary phases by a different group [70]. Capillary electrophoresis in the MEKC mode was employed to resolve stereoisomers of malathion, ruelene, dialifos [37], and fensulfothion [71]. Chiral CE was used with offline solid-phase extraction to measure malathion in tap water samples [72].

The environmental chemistry of OP enantiomers is less well studied than that of the previously discussed pesticides. The (+)-enantiomer of fenamiphos, which is more toxic to daphnids, is also dissipated from soils faster than its antipode with no dependence on soil texture, organic carbon content, or pH [73]. Fonofos was lost from soils nonstereoselectively [57]. Racemic ruelene transformation rates in soils were not affected by temperature changes or deforestation, but enantioselectivity was changed [44]. Deforestation caused microbial consortia in Brazilian soils to switch from preferentially degrading the less toxic (−)-enantiomer of ruelene to degrading exclusively the (+)-enantiomer, while warming of Norwegian soils caused about a quarter of the samples studied to switch from removing (+)-ruelene to removing (−)-ruelene. This observation suggests that climate change (e.g., temperature shifts, changes in land use/land cover patterns) can affect pollutant degradation by shifting the composition of the involved microbial populations.

Pyrethroid pesticides

Pyrethroids are a widely used class of agricultural and urban insecticides that are expected to be even more important in the future given increasing restrictions on organophosphate pesticides. These chemicals have low toxicity to mammals, but are acutely toxic to aquatic organisms at low concentrations [74]. Unlike the other pesticide classes discussed, they are nonpolar and sorb to particles. However, this is not likely to render them immobile post-application, because they can be moved in runoff with soil particles to which they are attached, and end up in sediments. Once in sediments, they can be bioavailable to the aquatic food web [75]. Thus, there are significant drawbacks to the use of pyrethroid pesticides, which spurs studies into their environmental behavior and effects for risk assessment. All pyrethroids are chiral; more than one asymmetric center is common, resulting in several diastereomers (Fig. 5). As with other chiral chemicals, differential biological activity among enantiomers is common. For example, only two of the eight stereoisomers of cypermethrin have insecticidal activity [76]. However, the environmental behavior of pyrethroid enantiomers is not well understood at present.

Pyrethroids are semivolatile nonpolar compounds, so they are typically analyzed by chiral GC with either

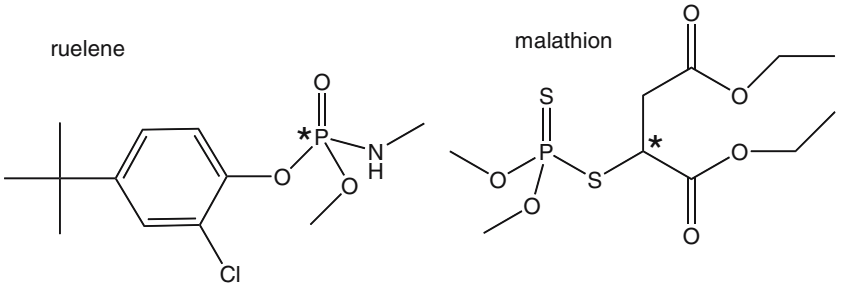

Fig. 4 Structures of representative chiral organophosphorus pesticides. *Asterisks* denote stereogenic centers

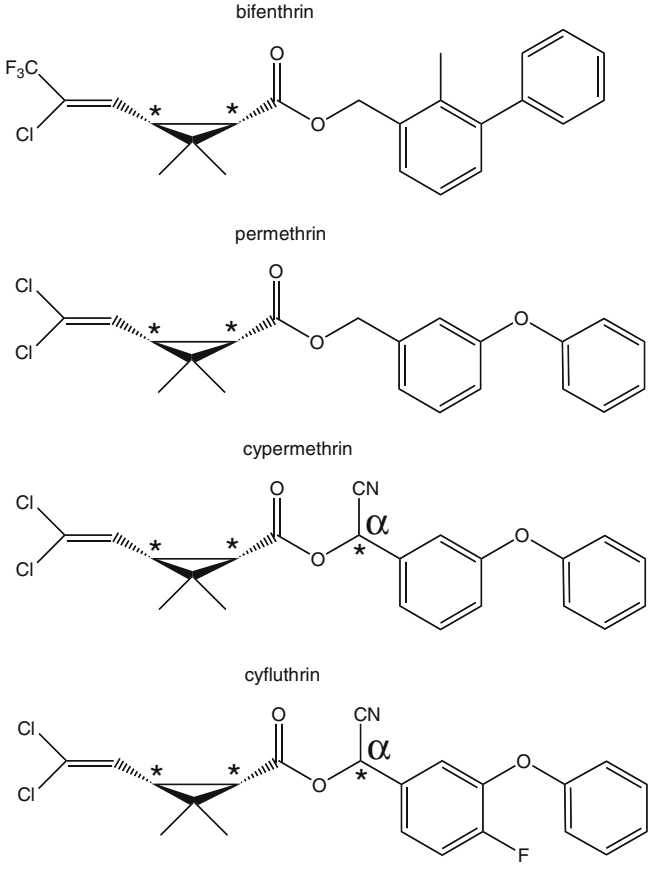

Fig. 5 Structures of representative pyrethroid pesticides. *Asterisks* denote stereogenic centers, α denotes α-carbon

electron capture (ECD) or MS detection. Normal-phase chiral HPLC has been used, mostly to isolate individual enantiomers for further characterization [77–79], but also for measurements in biological tissues [80]. Reversed-phase chiral HPLC and capillary electrophoresis techniques have also been used [81], as well as two-dimensional HPLC through coupling of achiral and chiral columns [82].

One potential drawback of GC analysis is the possibility of isomerization for pyrethroids with cyano substituents at the asymmetric α-carbon atom, such as cypermethrin and cyfluthrin (Fig. 5). This isomerization can take place in the presence of heat, polar solvents, or light. About 9 % conversion of these two compounds was observed at GC split/splitless inlet temperatures of 260 °C, but isomerization was relatively insignificant when the inlet temperature was lowered to 180 °C or when on-column injection was used [77]. No evidence for isomerization was observed for bifenthrin and permethrin, both of which lack cyano substituents. Cypermethrin [77] and cyfluthrin [77, 83] also isomerized slowly at the asymmetric α-carbon atom (Fig. 5) in methanol and in sterile water. Bifenthrin and permethrin, on the other hand, were stable in all solvents tested [77]. These results are consistent with reports of epimerization of other pyrethroids with cyano substitents at the asymmetric α-carbon atom in polar solvents (e.g., deltamethrin [84, 85]), and in the presence of light (e.g., deltamethrin [84, 86] and cyhalothrin [87]). Consequently, caution should be applied in the analysis of such pyrethroids to avoid use of incompatible solvents, and in interpretation of enantiomer data from natural waters to account for abiotic isomerization.

The environmental fate of bifenthrin and permethrin was assessed in laboratory incubations with soil slurries and individual microbial strains, and in field studies [78, 79]. In addition, these studies also examined enantiomer-specific acute toxicity, which is rare for most studies on environmental fate. The authors found large differences of up to 40-fold for lethal acute concentrations of pyrethroid enantiomers on daphnids, suggesting that aquatic toxicity was primarily due to a specific enantiomer in the racemate. *trans*-Permethrin was selectively degraded over its diastereomer *cis*-permethrin by the six bacterial strains tested, which also preferentially degraded the 1*S*-enantiomers of *cis*-bifenthrin and *cis*-permethrin over the corresponding 1*R*-*cis*-enantiomers. Enantioselectivity was more pronounced for *cis*-permethrin than for *cis*-bifenthrin, and also varied by strain, suggesting that isomer selectivity and toxicity are common in bacteria that can degrade pollutants. The (−)-enantiomer of both pyrethroids was preferentially degraded in field sediments adjacent to a plant nursery, indicating that natural attenuation by bacteria is enantioselective. Another study found enantioselective degradation of fenvalerate in soil slurries, consistent with these results [82].

Stereoselective toxicokinetics was also observed for pyrethroids at the other end of the food web, in mammals. Wang et al. [80] found that in rats injected with a racemic dose of θ-cypermethrin, the (+)-*S*-enantiomer was much less prevalent than the (−)-*R*-enantiomer in plasma, heart, liver, kidney, and fat tissues. In addition, the (+)-enantiomer was rapidly converted to the (−)-enantiomer in plasma, but the reverse conversion of (−)- to (+)-enantiomer did not occur. These results indicate significant enantioselectivity in the processing of cypermethrin by rats in vivo, but it was not clear if this processing was due to chiral inversion or another type of biotransformation. Some stereoselective redistribution of cypermethrin among organs and tissues was suggested, consistent with the highly enantioselective screening by the rat blood–brain barrier for the legacy pesticide α-hexachlorocyclohexane [88]. The extent of redistribution is not known for cypermethrin.

Chiral metabolites of polychlorinated biphenyls

Metabolites of environmental pollutants are generally less well studied than parent compounds, for a number of reasons. They are generally present at much lower concentrations, and often require more extensive and rigorous cleanup and processing techniques in order to analyze them reliably from environmental matrices. However, some metabolites may exhibit greater toxicity than the parent compounds. Thus, metabolites can be considered as emerging pollutants, even those of legacy past-use POPs. In addition, metabolic products can be chiral due to

formation from a parent compound that is either chiral or achiral (i.e., prochiral). In this section, the environmental occurrence and chemistry of one major class of chiral metabolites, PCB methyl sulfones, is discussed.

The metabolites of PCBs can be chiral, as Phase I conjugation of PCBs produces hydroxylated PCBs through either direct hydroxide insertion or via arene oxide intermediates [12]. The arene oxides can also form glutathione conjugates, which can then undergo a series of reactions through the mercapturic acid pathway [89] to form methylthio-PCBs and then PCB methyl sulfones ($MeSO_2$-PCBs). As with the parent compounds, PCB metabolites can be atropisomeric from asymmetric substitution about the molecule's long axis and restricted rotation around the biphenyl bond (Fig. 6), with much more stability (rotational energy barriers >200 kJ mol^{-1} [90]) than that of the acetamide pesticides. Of the 837 possible $MeSO_2$-PCBs, 456 are chiral, and 170 with tri- or tetra-*ortho* substitution are environmentally stable [90]. However, only about 60 $MeSO_2$-PCB congeners have been observed in the environment [12], and of those, ten are chiral at ambient temperatures. The environmental chemistry and toxicology of hydroxylated and $MeSO_2$-PCBs were recently reviewed [12]. Our discussion of chiral PCB metabolites is limited to the methyl sulfones, as nothing is known about the environmental occurrence and fate of enantiomers of the other PCB metabolites.

Chiral PCB metabolites have been quantified using chiral chromatography. Chiral HPLC has been used to obtain $MeSO_2$-PCB stereoisomers for further characterization of properties, including absolute configuration by vibrational circular dichroism [91, 92] and enantiomer-specific biological studies. A custom GC column resolved enantiomers of eight $MeSO_2$-PCBs [93], whereas a different group used a partially ethylated γ-cyclodextrin column to separate *meta*-substituted $MeSO_2$-PCBs 95, 132, 149, and 174 but not the corresponding *para*-substituted congeners [94]. The commercially available BGB-172 column, coupled to ion trap GC/MS/MS or GC/ECNI-MS for detection, was used to separate enantiomers of *meta*- and *para*-$MeSO_2$-PCBs 132 and 174, *para*-$MeSO_2$-PCB 91, and *meta*-$MeSO_2$-PCB 149 [95]. Chirasil-Dex could also separate enantiomers of *meta*- and *para*-$MeSO_2$-PCBs 149 and 174, and *meta*-$MeSO_2$-PCB 132 [96], but suffered from long run times (>300 min), broad peaks, and poor detection limits because of the low maximum temperature limit of the column (225 °C).

Despite analytical issues with $MeSO_2$-PCBs, chiral GC/MS analysis has shown that these metabolites are present in mammalian species with extreme stereoselectivity. Wiberg et al. [95] found that ringed seals formed highly nonracemic *meta*-$MeSO_2$-PCB 149 which was not present in arctic cod, their major prey. This observation indicated that the presence of the metabolite in seals was from enantioselective formation and/or clearance. This metabolite was present as almost a single enantiomer in polar bears (*Ursa maritimus*) [95]. Based on enantiomer analysis, the authors concluded that at least some of the *meta*- and *para*-$MeSO_2$-PCBs 91 and 149 in polar bears was from in vivo metabolism, whereas *meta*- and *para*-$MeSO_2$-PCB 132 were from bioaccumulation of these contaminants from seals. Similar results were also found in grey seals (*Halichoerus grypus*) of the Baltic Sea. Liver tissues of these seals had much higher $MeSO_2$-PCB concentrations than lung and blubber tissues; nearly pure enantiomers occurred in all three tissues [97]. In harbor porpoises, *meta*- and *para*-$MeSO_2$-PCBs 149 and 174 and *meta*-$MeSO_2$-PCB 132 were also highly nonracemic, suggesting that these metabolites were preferentially formed, or were retained in a highly stereoselective manner [96]. The

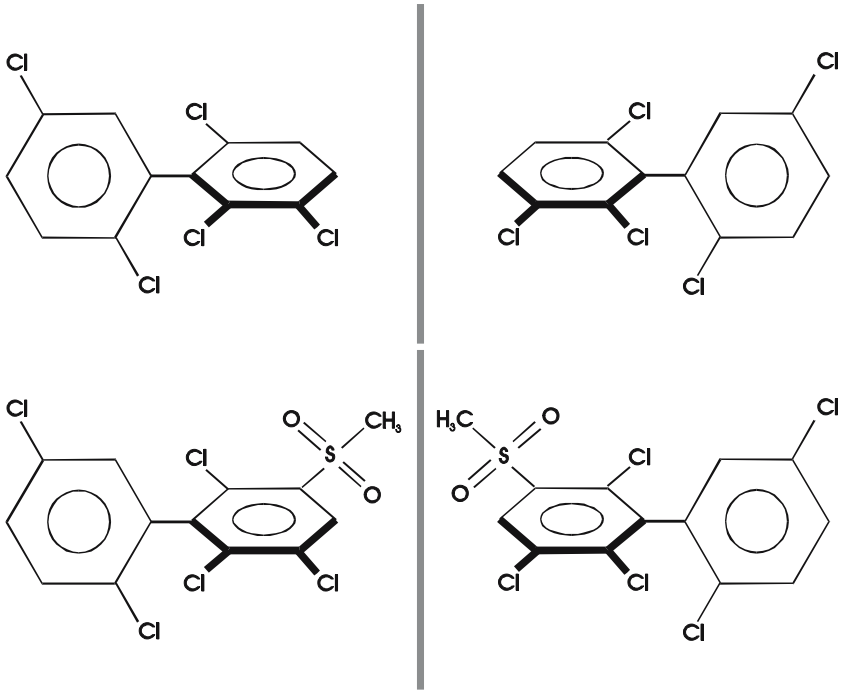

Fig. 6 Structures of atropisomeric 2,2′,3,5′,6-pentachlorobiphenyl (PCB 95, *top*) and metabolite 3-methylsulfonyl-2,2′,5,5′6-pentachlorobiphenyl (*meta*-$MeSO_2$-PCB 95, *bottom*)

observations of species-specific, highly enantioselective signatures in field studies are consistent with results from laboratory experiments. Rats dosed with Clophen A50, a technical PCB mixture, produced PCB methyl sulfones stereoselectively [98]. Tissue retention was enantioselective and organ-specific, with lung tissues having reversed enantiomer preferences for *para*-$MeSO_2$-PCB 149 compared to adipose and liver tissue. Parent PCB compounds were not analyzed in this study. Rat liver hepatocytes transformed PCB 149 nonstereoselectively in vitro, but degraded *meta*-$MeSO_2$-PCB 149 in a highly stereoselective manner [99] that was consistent with the previously reported in vivo results. Although $MeSO_2$-PCBs have been observed in nonmammalian species, such as sculpin fish [100, 101], these metabolite stereoisomers have not yet been studied in such biota.

Synthetic polycyclic musks

Synthetic musk compounds have been heavily used as fragrances in perfumes, soaps, lotions, detergents, air fresheners, and other scented personal products at concentrations up to several milligrams per gram of product [102]. More than 6,000 tonnes of polycyclic musks have been produced worldwide since 1996 [103]. As with POPs, synthetic musks are hydrophobic and persistent. Thus, they bioaccumulate in food webs, and are widespread in surface waters, sediments, fish, and human adipose tissues and milk [103]. Because of the persistence and bioaccumulation of synthetic musks and their reported toxic effects, which have been recently reviewed [103], these chemicals are considered emerging pollutants.

Most of the literature on these chemicals does not address the stereochemistry of musks. Several of these chemicals are chiral (Fig. 7), such as HHCB (Galaxolide), AHTN (Tonalide), ATII (Traseolide), and AHDI (Phantolide). Two asymmetric centers are present in HHCB and ATII, so each of these compounds can exist as a pair of diastereomers. However, over 95 % of technical ATII consists of the *trans*-isomer, so this is the diastereomer that is found in the environment. Only a handful of studies have investigated environmental stereochemistry of musks. The first such study found significantly nonracemic amounts of HHCB and AHTN, separated and quantified by chiral GC/MS, in some fish species (rudd and carp for HHCB, these species and tench and eel for AHTN) in ponds filled by wastewater effluent discharge [104]. Water samples, obtained via semipermeable membrane devices, were racemic or near-racemic. There was no correlation between enantiomer composition in fish and lipid levels. The highest deviation from a racemic mixture was observed for *trans*-HHCB and *trans*-ATII in crucian carp [105]. The enantiomer patterns of the musk compounds studied were species-specific, likely due to biotransformation when combined with other data, such as lower concentrations in carp compared to tench [105]. Musks in raw wastewater were mostly racemic, suggesting (but not proving) little stereoselective degradation prior to environmental release,

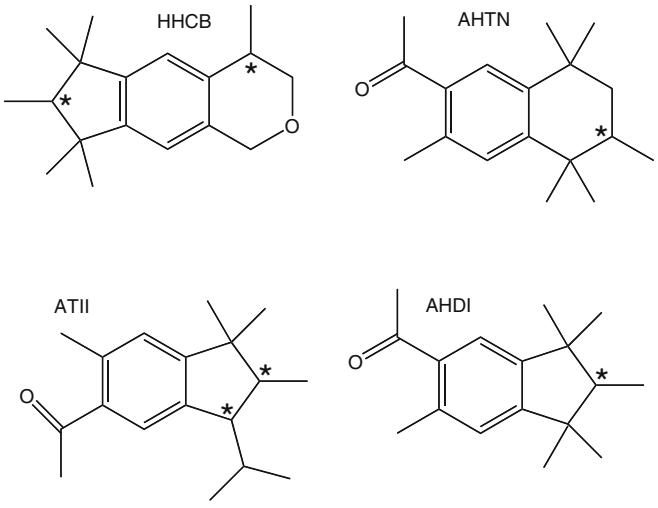

Fig. 7 Structures of representative chiral synthetic musks *HHCB* (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-γ-2-benzopyran), *AHTN* (7-acetyl-1,1,3,4,4,6-hexamethyltetralin), *ATII* 5-acetyl-1,1,2,6-tetramethyl-3-isopropylindene), and *AHDI* (6-acetyl-1,1,2,3,3,5-hexamethyldihydroindene). *Asterisks* denote stereogenic centers

whereas wastewater effluent was nonracemic for ATII [106]. Sludge stabilized aerobically or anaerobically was racemic for some musks (*cis*- and *trans*-HHCB) but was significantly nonracemic for others (ATII, AHTN), indicating some stereoselective removal of these compounds consistent with their degradation in experiments in batch reactors [106].

Hexabromocyclododecane (HBCDD)

Hexabromocyclododecane (HBCDD) is commonly used as a flame retardant in polystyrene foams produced for insulation in buildings and in upholstered furniture, with an estimated production of 16,700 tonnes in 2001 [107, 108]. Although HBCDD has six chiral centers and four *meso*-forms for a total of 16 possible stereoisomers [108], technical products are a mixture of three sets of diastereomers (Fig. 8): α- (10–13 %), β- (0.5–12 %) and the dominant γ-isomer (75–89 %) [109]. Minor amounts of the *meso*-forms (the δ- and ε-isomers) are also present. Hexabromocyclododecane is an emerging pollutant; it is found worldwide [110, 111], bioaccumulates [112], and may have chronic neurotoxic and endocrine-disrupting effects even though its acute toxicity is low [27].

Because HBCDD diastereomers can interconvert at temperatures above 160 °C and do not elute from GC columns below this temperature [109], early measurements of HBCDD done by GC are not reliable, and more accurate analyses require HPLC/MS/MS [113, 114]. Studies on HCBDDs have focused on the three major sets of diastereomers, which have sufficiently different physical and chemical properties from each other to show differences in bioaccumulation and biotransformation [111, 112]. The α-isomer is preferentially bioaccumulated in biota and

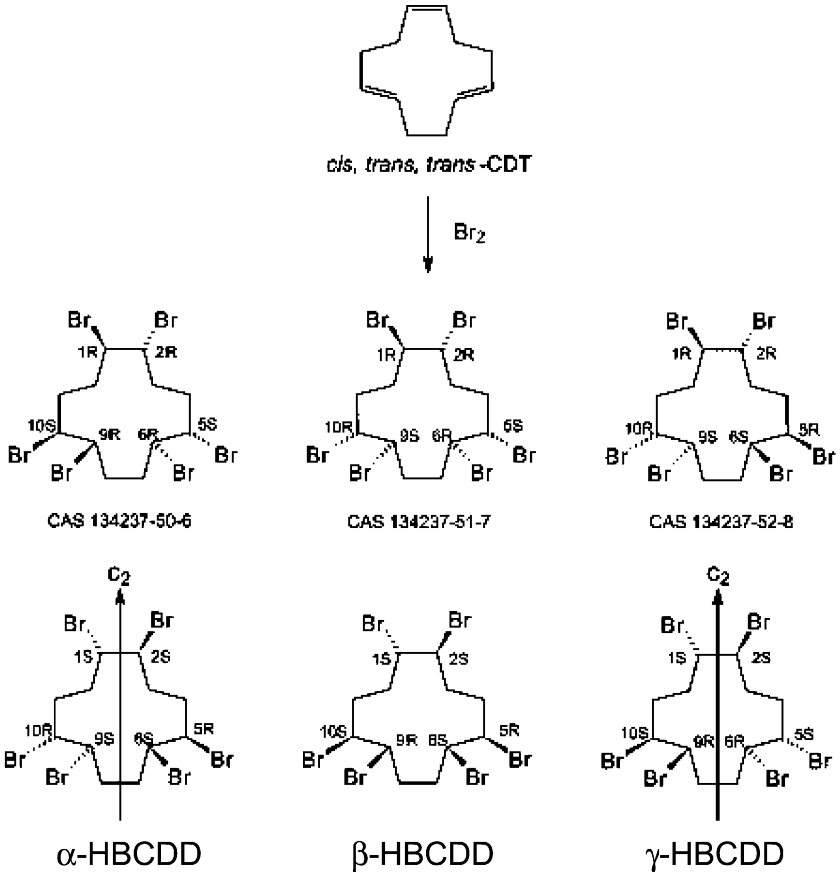

Fig. 8 Structures of the enantiomers of α-, β-, and γ-hexabromocyclododecane (HBCDD). Reprinted with permission from ref. [107]. Copyright 2005 Elsevier

increases in concentration at higher levels in food webs [112]. This may be caused by differences in hydrophobicity of the isomers [115], from in vivo biotransformation (e.g., Phase I conjugation by cytochrome P450 [111]) and/or possible biotic isomerization [27].

Achiral environmental analysis of HBCDD is nontrivial, given the variability of HBCDD in technical mixtures and the many environmental processes that can differentially affect isomers. Enantiomer analysis is even more challenging, but can provide additional insights into environmental behavior of HBCDD. The individual enantiomers of α-, β-, and γ-HBCDD were resolved by chiral HPLC/MS/MS [110] on a β-cyclodextrin Nucleodex column. However, unequal areas of the two peaks for racemic α-HBCDD purified from technical mixtures were observed, in contrast with most chiral chromatography, where the detector has identical responses to enantiomers present at equal concentrations. Because similar results resulted from analysis of purified racemic HBCDD diastereomers from different sources, the authors ruled out enantioselectivity in purification processing, and attributed this observation to differential matrix effects during electrospray ionization. A similar problem, in that the HBCDD ion signal was dependent on extract volume (i.e., on amount of coextracted endogenous material), was also observed in achiral HPLC/MS/MS [114], which can be addressed by use of isotopically labeled HBCDD internal standards. These are potential issues in any electrospray-based analysis, particularly for environmental samples that often have many coextracted interferences present, that should be addressed in method development for environmental applications. Janák et al. [110] did note that the observed deviation from the expected racemic composition was not significant, and that HBCDD enantiomer measurements from fish livers and muscles were significantly different from these values in any event. For α-HBCDD, the most abundant isomer in fish [110], enrichment of the (+)-enantiomer was observed in bib and whiting liver, but the opposite was true for (−)-α-HBCDD in sole liver and muscle. Eel muscles contained racemic α-HBCDD. Fewer measurements were possible in fish for the less abundant β- and γ-HBCDD, which had larger associated uncertainties, but (−)-β-HBCDD and (+)-γ-HBCDD were preferentially enriched over their antipodes. These species-dependent preferences are not observable by achiral measurements. Another study found that although brominated flame retardants, including HBCDD, were reductively dehalogenated in laboratory anaerobic microcosms of sewage sludge, this degradation was not enantioselective for any of the three major HBCDD isomers [116], at least in that particular study.

Chiral pharmaceuticals

Many drugs are chiral. Some are used as racemates (e.g., ibuprofen), whereas others are used as single enantiomers (e.g., *S*-(+)-naproxen). The stereochemistry of drugs in the body is well studied [46, 117]. However, pharmaceuticals

can be released to the environment by excretion from humans and animals (in the case of veterinary drugs), and by disposal (e.g., flushing expired prescriptions down the drain). Although wastewater treatment can reduce the concentration of drugs, it was not designed to eliminate these chemicals from waste streams. Thus, pharmaceuticals are found in wastewater effluents and receiving waters such as rivers and lakes [118–124], and even in the open ocean [43]. Because of this presence and the fact that drugs are specifically designed to have profound biological effects, pharmaceuticals have become a class of emerging pollutants. These chemicals can potentially have significant biological and toxicological effects towards non-target organisms [24, 25]. As a result, environmental pharmaceuticals have received increasing attention in recent years [24, 25] in order to understand their environmental behavior and effects.

Despite this growing body of research, the environmental occurrence, fate, and effects of pharmaceuticals are still poorly understood. Even less understood is the environmental behavior of chiral drugs. Part of the reason for this lack of understanding is the difficulty in measuring stereoisomers of drugs in environmental matrices. Although chiral separations of drugs by CE [13, 14] and HPLC [14] are commonplace, most of these methods are not applicable to environmental analysis at trace levels (micrograms per liter and below) in extremely complex samples: natural waters and wastewaters with a plethora of interferences that are often more complex than biological fluids, such as blood plasma and urine, for which many methods are intended. Thus, high sensitivity and selectivity are necessary, usually by MS and MS/MS. Most existing chiral separation methods use detection methods that do not have sufficient sensitivity and selectivity (e.g., UV absorbance). In addition, such methods often use mobile phases that are incompatible with MS (e.g., normal-phase solvents, involatile buffers such as phosphates). Chiral GC separations are often not feasible without derivatization, as many drugs are too polar to be amenable to GC analysis.

As a result, our current understanding of environmental biochemical weathering of chiral drugs (Fig. 9) is extremely limited. The first study that specifically analyzed drug enantiomers [119] measured ibuprofen stereoisomers in wastewaters and surface waters in Switzerland by chiral GC after derivatization. An excess of pharmacologically active *S*-(+)-ibuprofen over the inactive *R*-(−)-enantiomer was observed in wastewater influent, along with the major hydroxy- and carboxy-metabolites at higher concentrations than the parent compound. These metabolites are also chiral, but enantiomer compositions were not measured. Ibuprofen was rapidly degraded in wastewaters and surface waters in both field observations and laboratory incubations, with the *S*-enantiomer being biotransformed faster than its antipode by waterborne microbes. Biochemical transformations led to a reversal of enantiomer composition in surface waters (i.e., $R > S$). However, it is not clear to what extent this change in enantiomer composition was due to biodegradation versus enantiomerization, as happens with the structurally similar phenoxyalkanoic herbicides [41, 43]. Nonetheless, this observation suggests that drug enantiomers can be used as a marker for the presence of wastewater. Such an approach was evaluated by Fono and Sedlak [125], who found racemic amounts of the β-blocker propranolol in raw wastewater in seven US wastewater treatment plants and in surface waters known to be contaminated with raw wastewater, but nonracemic compositions in wastewater effluent caused by stereoselective degradation during treatment. These results suggest that the stereoisomer composition of this drug could be used to assess the presence of raw wastewater discharge into natural waters. However, the authors warn that much additional work had to be done to understand and characterize enantiomer compositions of drugs in wastewater before, during, and after treatment. A different study used chiral HPLC/MS/MS for the first time to quantify

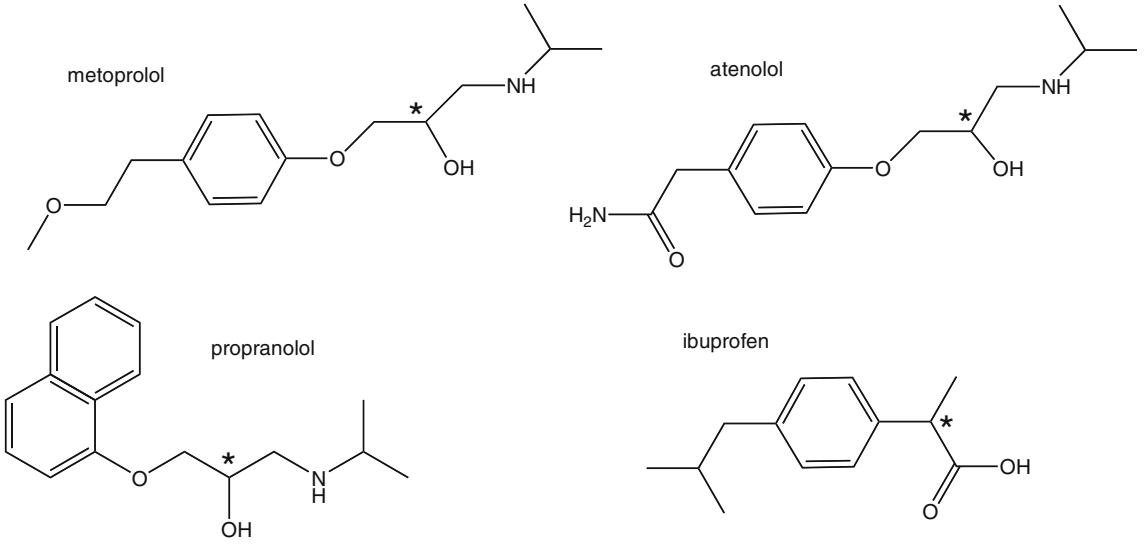

Fig. 9 Structures of representative chiral drugs studied in the environment. *Asterisks* denote stereogenic centers

stereoisomers of three β-blocker drugs (atenolol, metoprolol, and propranolol) in raw and treated wastewaters in Canada [126]. While the enantiomer composition of propranolol and atenolol were consistent with that found by Fono and Sedlak [125], metoprolol was different in that it was nonracemic in raw wastewaters but racemic in effluent at the outfall [126]. Moreover, one metoprolol enantiomer was prevalent in one treatment plant, but the other enantiomer was prevalent in another plant. Thus, further characterization of enantiomer composition of drugs is necessary to assess environmental processes affecting the levels of these chemicals in natural waters.

Conclusions

Chiral analysis is a powerful tool for detecting and gaining insight into biochemical environmental fate processes affecting pollutants, which otherwise may remain unknown based purely on achiral means. The limited research done to characterize enantioselectivity in emerging modern pollutants shows that biotransformation and toxicity can be heavily affected by stereochemistry. Future directions entail understanding the role of stereochemistry in ecotoxicity, which remains poorly understood to date. In addition, elucidation of factors controlling environmental fate of pollutant enantiomers is vital, as there is currently no clear way to predict enantioselectivity of pollutants a priori. Such work would also include linking observed stereoselectivity in laboratory and field studies with biochemical processes in the organisms involved, and ultimately to their metabolomic, proteomic, and genetic profiles that may indicate the source of the enantioselectivity. A predictive capability for chiral pollutants would enable chemical manufacturers to produce chiral chemicals with enantiomer compositions that provide maximum benefit with minimum environmental impact [127].

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