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MINI-REVIEW

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Chirality of pollutants—effects on metabolism and fate

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Abstract In most cases, enantiomers of chiral compounds behave differently in biochemical processes. Therefore, the effects and the environmental fate of the enantiomers of chiral pollutants need to be investigated separately. In this review, the different fates of the enantiomers of chiral phenoxyalkanoic acid herbicides, acetamides, organochlorines, and linear alkylbenzenesulfonates are discussed. The focus lies on biological degradation, which may be enantioselective, in contrast to non-biotic conversions. The data show that it is difficult to predict which enantiomer may be enriched and that accumulation of an enantiomer is dependent on the environmental system, the species, and the organ. Racemization and enantiomerization processes occur and make interpretation of the data even more complex. Enantioselective degradation implies that the enzymes involved in the conversion of such compounds are able to differentiate between the enantiomers. "Enzyme pairs" have evolved which exhibit almost identical overall folding. Only subtle differences in their active site determine their enantioselectivities. At the other extreme, there are examples of non-homologous "enzyme pairs" that have developed through convergent evolution to enantioselectively turn over the enantiomers of a chiral compound. For a better understanding of enantioselective reactions, more detailed studies of enzymes involved in enantioselective degradation need to be performed.

Introduction

A chiral (Greek *cheir*, hand) object or molecule is not superimposable on its mirror-image, whereas an achiral object can be superimposed on its mirror-image. An alternative definition lies in the lack of symmetrical elements: a chiral object lacks reflectional symmetry (Sheldon 1993). Common known chiral objects are a person's right and left hands, snail shells, and clockwise- or counterclockwise-threaded screws. For chiral molecules, a tetrahedral C-atom bound to four different substituents is most common. The carbon atom is the stereogenic center and the two possible structures behave like the image and mirror-image of each other and are not superimposable. These structures are called enantiomers (Greek *enantios*, opposite). Amazingly, chirality is more the rule than the exception in our living world; and the important building blocks of life, such as DNA, RNA, and proteins, are all composed of chiral molecules. Moreover, they are homochiral—proteins consist of L-amino acids and DNA and RNA consist of the D-enantiomers of deoxyribose and ribose, respectively.

The phenomenon of molecular chirality was first observed in the middle of the nineteenth century. Pasteur prepared sodium ammonium salts of the optically active (+)-tartaric acid and the optically inactive racemic acid. He observed that both crystals comprised hemihedral facets and that the hemihedral facets of the crystals of the optically active (+)-tartaric acid all lay in the same direction, whereas some of the hemihedral facets of the crystals of the optically inactive racemic acid inclined to the left and some to the right. He separated the crystals and found that the solution of crystals with hemihedral facets to the right deviated the plane of polarized light to the right and vice versa. He had discovered that the optical inactivity of racemic tartaric acid is due to the fact that it is a mixture of right- and left-handed tartaric acid. Later, van't Hoff and Le Bel introduced the model of the tetrahedral carbon atom to explain enantiomerism.

Although pure enantiomers of chiral compounds have identical physico-chemical properties—for a more thorough discussion about racemates and their enantiomer compounds, see Eliel and Wilen (1994)—their behavior in biochemical processes might be strikingly different. The

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effects of differently acting stereoisomeric drugs can be categorized basically as follows (Ariëns 1989).

A. The stereoisomers of a chiral drug may have similar modes of action, but may differ in their affinity to a receptor or an enzyme, resulting in different reaction rates.
B. The inactive stereoisomer may act as a competitive antagonist.
C. Enantiomers may have opposite or different effects, as is the case for barbiturates, where the (–)-enantiomer is a sedative and the (+)-enantiomer has convulsive effects.
D. There are many chiral drugs for which one or both enantiomers have the desired effect and only one enantiomer causes unwanted side-effects. Well known is the Contergan tragedy. Contergan was a sedative that contained racemic thalidomide. Both enantiomers had the desired therapeutic effects, whereas only the (*S*)-enantiomer had teratogenic effects and caused severe malformations of human babies (Bentley 1995).
E. Side-effects may be non-stereoselective and both isomers may cause them, but only one isomer may have the desired effect.
F. In contrast to this, the inactive enantiomer may antagonize the side-effects of the active isomer. In such cases, an enantiomerically pure compound is not preferred.

As this list shows, the relationships between the effects of active and inactive stereoisomers in a pharmacological context can get quite complicated and certainly cannot be easily predicted, but need to be empirically established. Therefore, stereoisomers should be treated as separate drugs and developed accordingly (FDA 1992).

It is important to note that the dispositions mentioned above not only hold for pharmacologically active compounds but equally apply to all bioactive compounds that are chiral. Chiral pesticides, for instance, are introduced into the environment in large amounts as racemic mixtures. In many cases, the effects of the enantiomers of herbicides on plants have been investigated, but not the fate of the enantiomers after application. Furthermore, not much is known about the degradation potential of the enantiomers. The application of racemic mixtures leads often to "isomeric ballast", thereby unnecessarily polluting the environment (Ariëns 1983; Williams 1996).

In the environment, abiotic transformations of chiral compounds are mostly non-enantioselective. This is in contrast to biological processes, which usually proceed with high stereo- or enantioselectivity. Therefore, changes in enantiomeric ratio (ER) and enantiomeric fraction (EF) are good indicators of biological degradation. The ER is defined as the ratio of the concentration of one of the enantiomers of a chiral compound divided by the concentration of the other enantiomer; and the EF is defined as the ratio of ER divided by ER+1 (Zipper et al. 1998a; Harner et al. 1999; Kohler 1999; Hegeman and Laane 2002; Williams et al. 2003).

Here, we review the literature on the metabolism and environmental fate of chiral pollutants (Table 1). We focus on differences in the environmental fate of the enantiomers, i.e. on enantioselective biotic conversions. We intend to show how important it is to differentiate between enantiomers and stereoisomers and to treat enantiomers as distinct compounds with respect to their degradation potential. Furthermore, we describe enzymes involved in enantioselective processes. We discuss stereoselectivity and the similarities and differences between such enzyme pairs that act on the enantiomers of chiral substrates.

Chiral pollutants and their fate in the environment

Phenoxypropanoic acid herbicides

Representatives of phenoxyalkanoic acid herbicides are 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4,5-trichlorophenoxyacetic acid, and the chiral compounds mecoprop (2-(*R,S*)-2-methyl-4-chlorophenoxypropanoic acid) and dichlorprop (2-(*R,S*)-2,4-dichlorophenoxypropanoic acid; Fig. 1). They are systemic and post-emergence herbicides and act as synthetic auxins (Åberg 1973; Loos 1975; Ahrens 1994). They were introduced in the 1940s and 1950s to control broadleaf weeds in agriculture, lawn pastures, and

Table 1 Chiral environmental pollutants discussed in this review

Class	Representatives	Main use	Environmental systems discussed in this review
Phenoxypropionic acids	Mecoprop, dichlorprop	Herbicide	Soil, aquatic systems, wastewater treatment plants, sewage sludge, landfill leachates, plants
Acetamide pesticides	Acetochlor, metalaxyl, metolachlor, dimethenamide	Pesticide	Soil, aquatic systems, sewage sludge, plants
Organochlorines	α-Hexachlorocyclohexane, chlordane	Contaminant of technical lindane (insecticide)	Soil, sediment, aquatic systems, sewage sludge, methanogenic conditions, biota
Linear alkylbenzencesulfonates, linear alkylbenzenes		Detergent	Soil

industry. Racemic mecoprop is also used to control the growth of weeds in building materials such as bituminous seals, insulators for flat roofs, and rubber seals (Bucheli et al. 1998a, 1998b). Phenoxyalkanoic acid herbicides, often applied in formulation with other herbicides, are among the most widely used herbicides in the world (Worthing and Hance 1991). In 1999, 12.7–15.0×10^6 kg active ingredient (a.i.) of 2,4-D were used to control broadleaf weeds in agriculture, 7.7–9.0×10^6 kg a.i. in the industrial/commercial/government market and 3.2–4.1×10^6 kg a.i. in the home and garden sector in the United States (Donaldson et al. 2002). Mecoprop was applied in a range of 1.4–2.3×10^6 kg a.i. in the United States (Donaldson et al. 2002).

The chiral herbicides mecoprop and dichlorprop each have one stereogenic center and, therefore, two enantiomers exist (Fig. 1). Since 1953, it has been known that only the (*R*)-enantiomers show herbicidal activity (Matell 1953). Nevertheless, the racemic mixtures were and still are applied, thereby introducing large amounts of isomeric ballast into the environment. In many countries, mecoprop and dichlorprop are nowadays also sold as enantiomerically pure compounds (named mecoprop-P and dichlorprop-P, respectively; Williams 1996).

As outlined above, the enantiomers of chiral compounds behave differently in biochemical processes. Therefore, to study the fate of chiral herbicides, it is important to differentiate between the enantiomers and to monitor the different degradation patterns. Today, GC, GC-MS, and HPLC methods are available to separate and quantitate enantiomers and to selectively investigate the environmental fate of the enantiomers of chiral pollutants (Müller and Buser 1997).

The environmental fate of phenoxyalkanoic acid herbicides is determined by their physico-chemical properties and by their biodegradability. They are water soluble (up to 620 mg/l) and are strong acids (Worthing and Hance 1991). Since they are mostly present in the dissociated (anionic) form in the environment, they do not adsorb onto soil and have a low tendency to accumulate in organic matter (Felding 1995; Zipper et al. 1998b). The half-life in soil after application is from one to several weeks (Table 2) and, due to their mobility, there is a risk of contaminating aquatic systems (Heron and Christensen 1992). Indeed, phenoxyalkanoic acid herbicides are often found in subsurface and groundwater samples (Gintautas et al. 1992; Felding 1995). Concentrations in surface soils were in the range of milligrams per kilogram of soil, whereas concentrations were lower in groundwater samples, i.e., 10–250 µg/l downstream of landfills (Lyngkilde and Christensen 1992; Zipper et al. 1998b) and less than 1 µg/l in groundwater aquifers polluted due to agricultural use (Scheidleder et al. 1999).

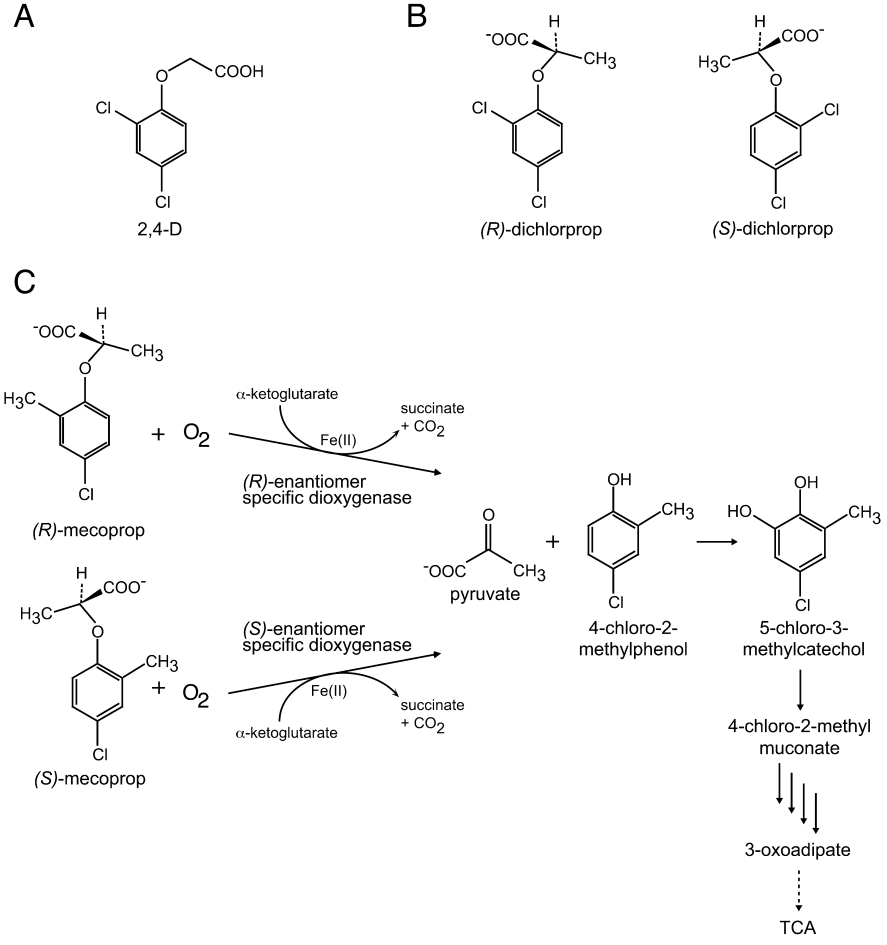

Fig. 1 Chemical structure of the phenoxyalkanoic acid herbicides 2,4-dichlorophenoxyacetic acid (*2,4-D*; **A**), which is achiral, and the two enantiomers of dichlorprop (**B**), which is chiral. **C** Proposed degradation pathway of (*R*)- and (*S*)-mecoprop by *Alcaligenes denitrificans* and *Sphingomonas herbicidovorans* MH. Note that *A. denitrificans* exclusively degrades (*R*)-mecoprop, whereas *S. herbicidovorans* MH degrades both enantiomers (modified from Nickel et al. 1997; Tett et al. 1997; Müller et al. in preparation)

Table 2 Degradation rates for dichlorprop and mecoprop found in environmental samples

Compound	System	Sample	Rate constants for enantiomers ($t_{1/2}$; days)			Degradation time (days)[a], (lag phase; days)	Reference
			R, S	*R*	*S*		
Dichlorprop	Brazilian soil	Forest	(11)[b,c]				Lewis et al. (1999)
		Pasture	(16)[b,d]				Lewis et al. (1999)
	Calcareous soils	Silt loam	55 (13)[e]	77 (9)[e], 155 (5)[f]	41 (17)[e]		Romero et al. (2001)
		Sandy loam	53 (13)[e]	59 (12)[e]	47 (15)[e]		
		Clay loam	11 (62)[e]	7 (98)[e], 38 (18)[f]	18 (38)[e]		
	Garden soil	Sandy loam		32 (21.9), 150 (4.6)[e,g]	98 (7.1), 176 (3.9)[e,g]		Müller and Buser (1997)
	Soil		(6.6)[b]	(8.7)[b]	(4.4)[b]		Garrison et al. (1996)
	Aquatic system	Aerobic aquifer (in situ)				120 (60)[h]	Rügge et al. (2002)
	Sewage sludge	Aerobic		213±41, 352±92[f,j]	225±79, 188±3[f,j]	(4.33–6.20)	Zipper et al. (1999)
Mecoprop	Calcareous soils	Silt loam	43 (15)[e]	58 (12)[e], 157 (4)[f]	33 (21)[e]		Romero et al. (2001)
		Sandy loam	63(11)[e]	86 (8)[e], 97 (7)[f]	56 (12)[e]		
		Clay loam	14[e] (50)	9 (77)[e], 17 (40)[f]	22 (32)[e]		
	Soil	Sandy loam	(9±1)[e]				Smith and Hayden (1981)
		Heavy loam	(8±2)[e]				Smith and Hayden (1981)
		Clay loam	(7±2)[e]				Smith and Hayden (1981)
	Garden soil	Sandy loam		69 (10),174 (4)[e,g]	102 (6.8), 189 (3.7)[e,g]		Müller and Buser (1997)
	Aquatic system	Aerobic aquifer (laboratory)				200 (20–110)[k]	Heron and Christensen (1992)
		Aerobic aquifer (in situ)				106–132 (60–80)[h]	Rügge et al. (2002)
		Limestone aquifer: aerobic		1.32[l]	1.9[l]		Williams et al. (2003)
		limestone aquifer: nitrate-reducing		0.65[l]	Not degraded		Williams et al. (2003)
		Limestone aquifer: Fe-reducing (NO_3^- added)				225 (21)[m]	Williams et al. (2003)
		Limestone aquifer: methanogen/sulfate-reducing	Not degraded				Williams et al. (2003)
	Sewage sludge	Aerobic		176±28, 304±88[f,j]	174±52, 273±47[f,j]	(4.33–6.20)	Zipper et al. (1999)

[a] Degradation time is defined as the time until dichlorprop or mecoprop was degraded to a concentration below the detection limit

[b] First-order kinetic

[c] In 38% of the samples, the *(S)*-enantiomer was degraded faster, in 13% the *(R)*-enantiomer

[d] In 93% of the samples, the *(S)*-enantiomer was degraded faster, in 6.7% the *(R)*-enantiomer

[e] Data obtained from experiments with the racemate. First-order kinetic, unit of the rate constant: 10^{-3} day^{-1}

[f] Data obtained from experiments with the single pure enantiomer. First-order kinetic, unit of the rate constant: 10^{-3} day^{-1}

[g] The two rate constants were determined during two degradation phases: slow phase 0–16 days, fast phase 16–35 days

[h] Non-enantioselective degradation

[j] Zero-order kinetic; units expressed as *x* μmol h^{-1} g^{-1} dry weight

[k] The concentrations were too low to measure enantioselective degradation. However, a second lag phase after removal of 50% of the initial concentration strongly indicated enantioselective degradation

[l] Data obtained from experiments with the racemate. Zero-order kinetic; units expressed as *x* mg l^{-1} day^{-1}

[m] No degradation was observed for 119 days. Nitrate was then added to the microcosm and, after a lag-phase of 21 days, mecoprop degraded, suggesting that nitrate acted as an electron-acceptor

The effect of environmental conditions on the photodecomposition of dichlorprop and mecoprop in soil surfaces was investigated by Romero et al. (1998). They found that, on dry soil surfaces, photolysis may dominate other transformation pathways. However, in moist soils, photodecomposition played an important role only during the first 2 days of exposure. Afterwards, when microbial degradation became dominant, photodecomposition was much less important. In a study using different types of calcareous soils, mecoprop and dichlorprop dissipation was investigated (Romero et al. 2001). Generally, degradation was slower in clay loam soils than in silt and sandy loam soils (Table 2). Dissipation was enantioselective, indicating biological degradation, and was dependent on the soil type. The (*S*)-enantiomer persisted for a longer time in silt and sandy loam soils than in clay loam soils. The addition of peat had intriguing effects, as the persistence of mecoprop and dichlorprop increased in silt and sandy loam soils, but decreased in clay loam soils. Lewis et al. (1999) studied the effect of environmental changes on the enantioselective degradation of mecoprop and dichlorprop in Brazilian forest and pasture soils. The pasture samples preferentially degraded (*S*)-dichlorprop, whereas both enantiomers were equally transformed in forest samples. Enrichments in organic nutrients shifted the enantioselectivity for methyl-dichlorprop towards the preferential degradation of the (*S*)-enantiomer. Other studies showed that the (*S*)-enantiomer was degraded significantly faster in soil than the herbicidally active (*R*)-enantiomer (Garrison et al. 1996; Müller and Buser 1997). Only two studies reported enantiomerization and/or racemization (Buser and Müller 1997; Müller and Buser 1997). Both studies showed that enantiomerization was biologically mediated. The authors drew the important conclusion that, due to enantiomerization, the measured enantiomeric composition of residues does not reflect that of the applied products. Additionally, two-phase degradation kinetics with initially slower rates was observed in one of these studies (Müller and Buser 1997). The authors suggested that two or more different enzyme systems are involved in the degradation.

In marine systems polluted with racemic dichlorprop, (*R*)-dichlorprop is preferentially degraded by microorganisms (Ludwig et al. 1992a, 1992b). In aerobic aquifer samples incubated with different concentrations of mecoprop in laboratory experiments, mecoprop was degraded within 200 days (Heron and Christensen 1992). (*R*)- and (*S*)-mecoprop was found in equal concentrations in the landfill leachate of Kölliken (Switzerland), indicating a racemic mixture of mecoprop in the landfill itself. But in groundwater samples downstream of the landfill, the enantiomeric ratio of mecoprop increased, i.e., (*R*)-mecoprop was in excess. As sorption to organic material was of minor importance and should be non-enantioselective, this finding indicates in situ biodegradation (Zipper et al. 1998b). Williams et al. (2003) showed in a recent study that degradation of mecoprop in a limestone aquifer downstream of a landfill depended on the redox conditions. Under methanogenic, sulfate-reducing, or iron-reducing conditions, mecoprop was not degraded. In nitrate-reducing microcosms, (*R*)-mecoprop was degraded, whereas the (*S*)-enantiomer was stable. In contrast, (*S*)-mecoprop degraded faster than (*R*)-mecoprop under aerobic conditions. In a field experiment in Denmark, mecoprop and dichlorprop were degraded in an aerobic aquifer within a distance of 1 m from the source within a period of 120 days. But in contrast to the reports mentioned beforehand, no enantioselective degradation was observed (Rügge et al. 2002).

Experiments with activated and digested sludge showed that phenoxypropanoic acid herbicides are degraded aerobically but not anaerobically. Mecoprop and dichlorprop (10–40 mg/l) were degraded aerobically within 7 days, the (*S*)-enantiomers being preferentially degraded, while degradation was not observed during 49 days of incubation under anaerobic conditions (Zipper et al. 1999). An experiment with 1 mM mecoprop in a simulated wastewater treatment plant showed 100% removal after 40 days (Nitschke et al. 1999).

All these studies showed that biological degradation is the most important process by which these herbicides are eliminated from the environment. Furthermore, biological degradation was enantioselective in most cases, emphasizing the importance of investigating the environmental fate of each enantiomer separately. In these cases, the observed changes in enantiomeric fractions or ratios gave conclusive evidence for natural attenuation of the herbicides (Zipper et al. 1998b; Williams et al. 2003). However, racemization may occur and therefore, careful experiments and measurements are needed for solid interpretations.

In most cases, microorganisms enantioselectively degrade mecoprop and dichlorprop. A particular strain might degrade only one enantiomer or it might sequentially degrade the two enantiomers. Degradation was well investigated for the achiral phenoxyalkanoic acid herbicide 2,4-D in *Ralstonia eutropha* JMP134(pJP4). 2,4-D is degraded by an α-ketoglutarate-dependent dioxygenase (TfdA) by ether-bond cleavage to 2,4-dichlorophenol (Streber et al. 1987; Fukumori and Hausinger 1993a, 1993b). Then, 2,4-dichlorophenol is hydroxylated to 3,5-dichlorocatechol by a phenol-hydroxylase (TfdB; Liu and Chapman 1984; Perkins et al. 1990; Farhana and New 1997). The catechol undergoes *ortho*-ring fission catalyzed by a chlorocatechol 1,2-dioxygenase (TfdC) yielding 2,4-dichloro-*cis,cis*-muconate (Perkins et al. 1990; Bhat et al. 1993), which is then metabolized to *cis*-2-chlorodienelactone and further to 2-chloromaleylacetate by a muconate cycloisomerase and a dienelactone hydrolase (TfdD and TfdE, respectively; Kuhm et al. 1990; Perkins et al. 1990). It was suggested that the chiral phenoxyalkanoic acid herbicides are degraded accordingly. Table 3 lists all known bacterial strains in pure culture that are able to grow with mecoprop and dichlorprop as the sole carbon and energy source. The earliest reported investigations were made with *Alcaligenes denitrificans* (Table 3), which was isolated from a consortium and exclusively degrades the (*R*)-enantiomer, using it as sole carbon and energy source (Tett et al. 1994,

1997). (*R*)-Mecoprop degradation was shown to proceed in a manner analogous to 2,4-D degradation, i.e., degradation to the achiral 4-chloro-2-methylphenol and then to 5-chloro-3-methylcatechol (Fig. 1C). Subsequently, 5-chloro-3-methylcatechol is degraded through the modified *ortho*-cleavage pathway, yielding 2-methyllactone, 2-methylmaleylacetate, and finally 5-methyl-3-oxoadipate. In a further study, the question was addressed whether the genes encoding the enzymes involved in mecoprop degradation were similar to those encoding the enzymes for 2,4-D degradation. A fragment of a *tfdA*-like gene was amplified by PCR from *A. denitrificans* and sequenced. It was 86% identical to the corresponding region of *tfdA* from *R. eutropha* JMP134(pJP4) (Saari et al. 1999). However, the α-ketoglutarate-dependent dioxygenase and the other enzymes involved in degradation have not been further characterized in this strain.

In 1990, *Sphingomonas herbicidovorans* MH (formerly *Flavobacterium* sp.) was isolated from soil samples polluted with dichlorprop (Horvath et al. 1990; Zipper et al. 1996). In contrast to *A. denitrificans*, *S. herbicidovorans* MH is able to degrade both enantiomers of dichlorprop and mecoprop to completion (Zipper et al. 1996). Growth experiments with either the racemate or single isomers showed that *S. herbicidovorans* MH degraded the substrates enantioselectively. The (*S*)-enantiomer was degraded before the (*R*)-enantiomer in all growth experiments performed so far. When *S. herbicidovorans* MH was incubated with the single enantiomer, a lag phase of about 3 days for (*S*)- and one of about 7 days for (*R*)-mecoprop was observed. However, when grown on the racemate, degradation was sequential, the (*S*)-enantiomer being used first (Zipper et al. 1996). Enantioselectivity was also found for substrate uptake. When *S. herbicidovorans* MH grew on the single enantiomers, only the enantiomer that served as the substrate was taken up. These data and further experiments indicated that *S. herbicidovorans* MH harbors two inducible transport systems involved in enantioselective uptake (Nickel et al. 1997; Zipper et al. 1998a).

Two strains, *Alcaligenes* sp. CS1 and *Ralstonia* sp. CS2, were isolated from agricultural soils. They are able to degrade racemic mecoprop and dichlorprop in addition to the achiral herbicides 2,4-D and MCPA (Smejkal et al. 2001). The genomes of both isolates were screened under low-stringency conditions with *tfd*-gene probes in hybridization experiments. The experiments demonstrated that both strains harbored *tfdABC*-like genes on plasmids. *Rhodoferax* strain P230, another strain that is able to degrade chiral phenoxyalkanoic acid herbicides, was isolated from contaminated building material. According to preliminary PCR experiments, this strain also harbors *tfdA*-like genes (Ehrig et al. 1997). *Delftia acidovorans* MC1 (formerly *Comamonas acidovorans* MC1) was isolated from a herbicide-contaminated building site (Müller et al. 1999, 2001). The strain was able to degrade racemic dichlorprop but, unfortunately, enantiospecific degradation was not investigated. To isolate the genes coding for the enzymes, PCR experiments were carried out

Table 3 Isolated mecoprop- and dichlorprop-degraders. *2,4-D* 2,4-Dichlorophenoxyacetic acid, *MCPA* 2-methyl-4-chlorophenoxyacetic acid

Strain	Phenoxyalkanoic acid substrates	Isolation source	References
Sphingomonas herbicidovorans MH	(*R,S*)-Mecoprop, (*R,S*)-dichlorprop, 2,4-D, MCPA	Soil (dichlorprop)	Horvath et al. (1990); Zipper et al. (1996); Kohler (1999)
Delftia (formerly *Comamonas*) *acidovorans* MC1	(*R,S*)-Mecoprop, (*R,S*)-dichlorprop, 2,4-D, MCPA	Herbicide contaminated building rubble (2,4-D)	Müller et al. (1999, 2001)
Rhodoferax sp. strain P230	(*R,S*)-Mecoprop, (*R,S*)-dichlorprop, 2,4-D, MCPA	Concrete rubble of a demolished herbicide production plant (2,4-dichlorophenol)	Ehrig et al. (1997)
Alcaligenes denitrificans	(*R*)-Mecoprop	Garden soil	Tett et al. (1997)
Alcaligenes sp. CS1	(*R,S*)-Mecoprop, (*R,S*)-dichlorprop, 2,4-D, MCPA	Agricultural soil (mecoprop)	Smejkal et al. (2001)
Ralstonia sp. CS2	(*R,S*)-Mecoprop, (*R,S*)-dichlorprop, 2,4-D, MCPA	Agricultural soil (mecoprop)	Smejkal et al. (2001)

with primers for *tfdA*, *tfdB*, and *tfdC* (Vallaeys et al. 1996; Kleinsteuber et al. 1998), revealing the presence of *tfdBC* genes. In contrast to these genes, a *tfdA*-like gene could not be amplified with the applied primers, although enzyme activities dependent on ferrous ions and α-ketoglutarate could be detected in cell-free extracts.

Acetamide pesticides

The group of acetamide pesticides comprises a large number of herbicides and fungicides (Fig. 2). The activity is dependent on the acyl moiety. In the case of herbicides, the substituent is often $–CH_2Cl$, whereas in the case of fungicides the substituent is often $–CH_2OCH_3$. Acetamide pesticides are used to control annual grasses and certain broadleaf weeds in corn, soybeans, and peanuts. They are also used to control phytopathogenic fungi, such as *Peronosporales* in potatoes, sugar beets, and other crops (LeBaron et al. 1988; Sharp 1988; Worthing and Hance 1991). Acetamide pesticides act as protein synthesis inhibitors (Chesters et al. 1989) and RNA-polymerase I inhibitors (Buchenauer 1990). The compounds metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide], metolachlor-s, and alachlor [2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl) acetamide] are among the most commonly used pesticides in the world and, in 1999, $11.8–13.6\times10^6$ kg, $7.3–8.6\times10^6$ kg , and $3.2–4.5\times10^6$ kg a.i., respectively, were applied in the United States (Donaldson et al. 2002). Other commonly applied acetamide pesticides are acetochlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(ethoxymethyl)acetamide], metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine methyl ester], and dimethenamid [2-chloro-*N*-(2,4-dimethylthien-3-yl)-N-(2-methoxyl-1-methylethyl) acetamide]. Alachlor is achiral, acetochlor is axial-chiral, and metalaxyl is C-chiral, giving two enantiomers. Dimethenamid and metolachlor are axial and C-chiral and, therefore, four stereoisomers exist. For metolachlor, herbicidal activity is exclusively associated with the 1′(*S*)-isomers. In the case of metalaxyl, the (*R*)-enantiomer is about 3–10 times more fungicidally active than the (*S*)-enantiomer (Fisher and Hayes 1985).

Degradation of different acetamides in soil and sewage sludge proceeds in the order alachlor >acetochlor >dimethenamid >metolachlor >metalaxyl, with half-lives ranging over 4–32 days in soil and 10–86 h in sewage sludge (Buser and Müller 1995a; Müller and Buser 1995). Dimethenamid and metolachlor degradation showed low to moderate enantio- and/or stereoselectivity, whereas metalaxyl was degraded highly enantioselectively. Enantioselectivity was dependent on the environmental system: in soil, 1′(*R*)-(–)-metalaxyl was degraded faster than the (*S*)-enantiomer, whereas reversed enantioselectivity was found in sewage sludge (Buser and Müller 1995a; Müller and Buser 1995). Different rates and opposite enantioselectivity were also found in a study in which degradation in soil was compared to that in plants. The (*R*)-enantiomer was degraded faster in soil, the (*S*)-enantiomer faster in plants (Marucchini and Zadra 2002). In a recent study (Buser et al. 2002), the faster degradation of the herbicidally active (*R*)-enantiomer in soil was confirmed. It was also found, that only 40–50% of metalaxyl was degraded to the chiral intermediate metalaxyl carboxylic acid {MX-acid; 2-[(2,6-dimethylphenyl)methoxy-acetylamino]propanoic acid}. In this step, the configuration of the chiral C-atom was retained. Further degradation of MX-acid is also enantioselective, the (*S*)-enantiomer being converted more rapidly. The remaining metalaxyl is metabolized through other pathways. Both enantiomers of metalaxyl and MX-acid are configurationally stable and no indications of biological mediated racemization or enantiomerization have been found (Buser et al. 2002; Marucchini and Zadra 2002). Monkiedje and coworkers (2003) investigated the degradation and persistence of racemic and enantiopure forms of metalaxyl in temperate and tropical soils. All forms of metalaxyl had lower degradation rates in Cameroonian soils than in German soils. Interestingly, an opposite enantioselectivity was observed in the two regions. The (*R*)-enantiomer was degraded faster than the (*S*)-enantiomer in German soils, but slower than the (*S*)-enantiomer in Cameroonian soils. This finding suggests that different microbial populations are involved in the degradation process. In another recent study, the enantioselectivity correlated with the soil pH (Buerge et al. 2003). In aerobic soils with pH >5, the (*R*)-enantiomer was degraded faster than the (*S*)-enantiomer. In aerobic soils with pH 4–5, both enantiomers were degraded similarly, whereas in aerobic soils with pH <4 and in anaerobic soils, the enantioselectivity was reversed. The authors reevaluated published kinetic data from dichlorprop and mecoprop studies and found indications of similar correlations. However, no correlation between enantioselectivity and soil pH was observed for MX-acid, the chiral intermediate.

In the case of metolachlor, there is currently a chiral switch from racemic metolachlor to (*S*)-metolachlor, which is highly enriched in the herbicidally active enantiomer. Most likely, this results in lower overall concentrations and a changed enantiomeric ratio in

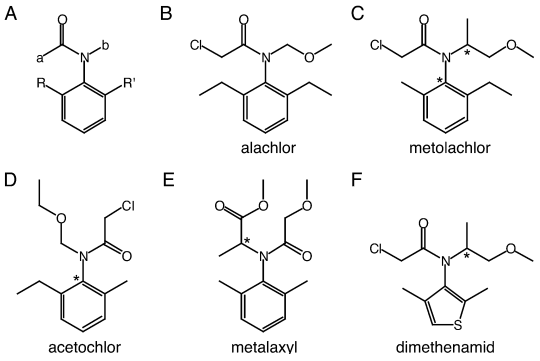

Fig. 2A–F Chemical structures of acetamide pesticides. **A** General structure. *R* and *R′* represent CH_3- and/or C_2H_5-. **B–F** Structures of the five acetamide pesticide alachlor (achiral), metolachlor, acetochlor, metalaxyl, and dimethenamid. The chiral centers and the axial-chiral elements are indicated by asterisks. Note that, in dimethenamid, phenyl is replaced by 2,4-dimethylthien-3-yl

environmental samples. Indeed, in Switzerland, a rapid response in terms of the enantiomeric composition was observed in surface waters after the replacement of the racemic metolachlor by the highly enriched enantiomer (Buser et al. 2000).

Organochlorines: hexachlorocyclohexane and chlordane

Hexachlorocyclohexane (HCH) belongs to the organochlorine compounds and used to be one of the most widely applied broad-spectrum insecticides. It was introduced during world war II (Müller et al. 1992) and was used mainly in forestry, agriculture, and as a wood preservative. HCH comprises eight isomers, the chiral α-, and the achiral β-, γ-, δ-, ε-, η-, υ-, and ι-isomers, of which only the γ-HCH has insecticidal properties. Technical grade lindane (Fig. 3A) consists typically of 60–70% α-HCH, 5–12% β-HCH, 10–15% γ-HCH, and 6–10% δ-HCH (Iwata et al. 1993; Buser and Müller 1995b), whereas the commercial insecticide marketed today comprises 99% of the γ-isomer. In Europe, the use of technical grade lindane was banned in the 1970s and, in 2000, lindane was completely banned for all agricultural and gardening applications. Although the total amount of applied HCH was reduced globally, it is still a contaminant of great concern in many countries (e.g., in India). HCH is semivolatile and has a low tendency to accumulate in soils. It is transported by water and air and, today, HCH has accumulated in regions where HCH was never used, such as the Arctic and the Baltic Sea (e.g., see Harner et al. 1999; Meharg et al. 1999; Wiberg et al. 2001). Chlordane is another representative of the organochlorine pesticides and was widely applied in the United States from 1945 until it was banned in 1988 (Dearth and Hites 1991; Hayes and Laws 1991). Technical chlordane comprises 140 compounds and was used to control pests in lawn, garden, and crops and as a termiticide (Hayes and Laws 1991). The organochlorine representatives *cis*- and *trans*-chlordane (Fig. 3B) and heptachlor epoxide, a metabolite of heptachlor, are chiral and each enantiomer has different biological properties and environmental fate (Pfaffenberger et al. 1994; for a review, see Hegeman and Laane 2002).

Today, the Arctic Ocean is a source of HCH. HCH is eliminated from the Arctic Ocean by water outflow, volatilization, and degradation (LeBaron et al. 1988). A study of the removal of α- and γ-HCH in the eastern Arctic Ocean found that the rate constants for microbial degradation were about 3–10 times higher than those for hydrolysis (Harner et al. 1999). Enantioselective degradation was observed, the (+)-α-HCH being converted preferentially. The calculated half-lives for (+)-α-HCH, (–)-α-HCH, and γ-HCH were 5.9, 23.1, and 18.8 years, respectively. However, reversed enantioselectivity was found in other marine environments, such as the Bering Sea and the Chukchi Sea (Jantunen and Bidleman 1998). Enantioselective degradation was also investigated in

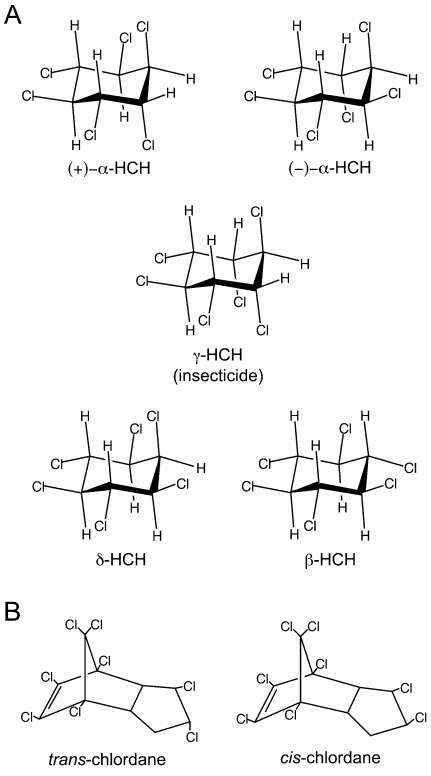

Fig. 3A, B Chemical structures of pesticides. **A** Chemical structures of five hexachlorocyclohexane (HCH) stereoisomers in technical lindane. Note that only α-HCH is chiral and that only γ-HCH has insecticidal properties. **B** Chemical structures of the organochlorine pesticides *cis*- and *trans*-chlordane

other aquatic environments. It was found that enantioselective degradation was greatest in small Arctic lakes with enantiomeric ratios between 0.3 and 0.7, although the nutrient availability in such lakes is very low (Falconer et al. 1995; Law et al. 2001). Law et al (2001) suggested this was due to biofilm formation and environmental conditions in which the contact between α-HCH and the microbial population was maximized by either the path length, as in streams, or the long water-residence time, as in lakes. In the food web, no preference for the enrichment of one enantiomer in higher trophic levels could be observed. Cetaceans, dolphin species, showed preferential accumulation of (+)-α-HCH (Hummert et al. 1995). (+)-α-HCH was also more abundant than the (–)-enantiomer in the blubber of harbor seals, grey seals, and harp seals and in the liver, kidney, and muscle tissues of ducks (Hummert et al. 1995). In contrast to this, an ER of <1 was found in hooded seals (Hummert et al. 1995). In invertebrates, mussels, and fishes, the ratio reflected that of the surrounding water, indicating no preferential accumulation (Hühnerfuss et al. 1993; Moisey et al. 2001). Also, for chlordane compounds, no uniform trend for ER changes and increasing trophic levels could be found (Wiberg et al. 2000). These findings suggest that it will be difficult to predict which enantiomer may be enriched and that accumulation is dependent on both the organ and the species.

In soils, organochlorines are also enantioselectively degraded. In soils from the United States corn belt, (+)-*trans*-chlordane was preferentially degraded, whereas for *cis*-chlordane, the (−)-enantiomer was converted faster except in four soils, in which non-enantioselective degradation was found (Aigner et al. 1998). α-HCH was enantioselectively degraded in three muck soils, but not in silt loam soils from British Columbia (Falconer et al. 1997). In soils from near a former HCH factory, only low enantioselectivity was found (ER=1.099; Müller et al. 1992). In archived UK soils, statistically significant enantioselective degradation was not observed for *cis*- or *trans*-chlordane, nor for α-HCH (Meijer et al. 2001). The reported half-lives for α-HCH and other organochlorines in soils were from ~7 years (α-HCH) to 25 years (dieldrin; Meijer et al. 2001).

In anaerobic sewage sludge, HCH is degraded with half-lives of 20–178 h, in the order γ-HCH >(+)-α-HCH > (–)-α-HCH >δ-HCH >β-HCH. Indications are given that degradation is 80–95% biologically mediated, although abiotic degradation in sterilized sewage sludge is much higher than hydrolysis in water (Ngabe et al. 1993; Buser and Müller 1995b). α-HCH is degraded enantioselectively, whereby the (+)-enantiomer is degraded faster. This leads to enrichment of (−)-α-HCH in sewage sludge (Buser and Müller 1995b). Middeldorp et al. (1996) described a bacterial consortium which was able to degrade both β-, α-, γ-, and δ-HCH under methanogenic conditions.

A microbial community able to degrade HCH was isolated from marine environments (Hühnerfuss et al. 1992). It degrades (+)-α-HCH and the corresponding β-pentachlorocyclohexene (β-PCCH) faster than the respective enantiomers. Another consortium comprising eight bacterial strains and a fungus was isolated from soil and sewage. It preferentially degrades α-HCH, but nothing is reported about enantioselectivity (Manonmani et al. 2000). Two *Bacillus* strains, *B. circulans* and *B. brevis*, isolated from contaminated soil, are able to degrade α-, β-, γ-, and δ-HCH at high rates (Gupta et al. 2001); and two *Pseudomonas* strains isolated from agricultural soils are able to degrade γ-HCH (Nawab et al. 2003). *S. paucimobilis* UT26 is able to grow on γ-HCH as sole carbon and energy source (Imai et al. 1991; Nagasawa et al. 1993; Nagata et al. 1999). The *lin* genes were sequenced and shown to code for the enzymes involved in HCH metabolism (Nagata et al. 1993a, 1993b, 1999).

Linear alkylbenzenesulfonates and linear alkylbenzenes

Linear alkylbenzenesulfonates (LAS) and linear alkylbenzenes (LAB) and their degradation products are environmentally relevant chiral substances (Fig. 4). LAS are used as detergents and LAB are the precursors in LAS synthesis and are found in low amounts in commercial LAS (Holt and Bernstein 1992; De Almeida et al. 1994). LAS and LAB are biodegradable under aerobic conditions (Swisher 1987). It was suggested that degradation starts with ω-oxidation of the alkyl side-chain (Painter 1992) and chain-shortening proceeds with β-oxidation, which is hindered when the side-chain is cut back to four or five carbon atoms from the point of attachment to the benzene ring (Huddleston and Allred 1963; Douros and Frankenfeld 1968; White and Russell 1994). *Rhodococcus rhodochrous* PB1 enantioselectively metabolizes the LAB intermediate 3-phenylbutyric acid. *R. rhodochrous* is able to grow on (*R*)-3-phenylbutyric acid, whereas the (*S*)-enantiomer is only cometabolically transformed to presumably (*S*)-3-(2,3-dihydroxyphenyl)butyric acid (Simoni et al. 1996). This compound is then abiotically transformed to reactive and potentially toxic quinones (Simoni et al. 1996; Kohler et al. 1997). Recently, *D. acidovorans* SPB1 was isolated from an enrichment culture (Schulz et al. 2000) which degrades 2-(4-sulfophenyl)butyrate (SPB) sequentially (Fig. 4B). The (*R*)-enantiomer is degraded first and only when it is exhausted does the (*S*)-enantiomer start degrading. Metabolism converges at the achiral 4-sulfocatechol. 4-Sulfocatechol undergoes *ortho*-cleavage via 3-sulfo-*cis, cis*-muconate.

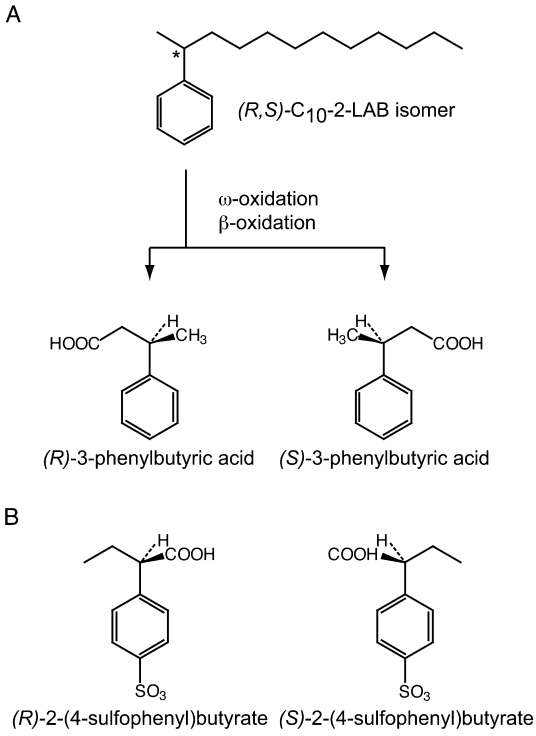

Fig. 4A, B Linear alkyl benzenes (LAB) and derivatives. **A** Chemical structure of a LAB isomer and the metabolite 3-phenylbutyric acid. Chain-shortening is supposed to be non-enantioselective, as the stereogenic center is far away from the reaction point. **B** Chemical structure of 2-(4-sulfophenyl)butyrate

Enantioselective enzymes

Enantioselective degradation of chiral pollutants by microorganisms is rather the rule than the exception. For enantioselective metabolism, one or more enzyme reac-

tions involved in the uptake or in different degradation steps must be enantioselective. The three-point model (Easson and Stedman 1933; Ogston 1948) is often used to explain the phenomenon of stereoselectivity in enzymatic reactions. As shown in Fig. 5, the model postulates that the active enantiomer binds more tightly to the active site of the enzyme because the sequence of the three groups around the asymmetric carbon atom, ABC, forms the triangular face of a tetrahedron that matches the complementary triad on the chiral binding site, A′B′C′, of the active site. The less active enantiomer binds ineffectively, since it has a mirror-image sequence of the three groups, CBA, which leads to a mismatch with the active site (Kohler et al. 2000). In some cases, this model needs to be expanded to a so-called four-location model (Mesecar and Koshland Jr. 2000; Fig. 5, III). When isocitrate dehydrogenase is provided with the substrate racemate, L-isocitrate is exclusively bound to the protein crystals in the absence of Mg^{+} but, in the presence of Mg^{+}, the D-isomer binds. The crystal structure revealed that three of the four groups of the C2-atom of isocitrate bind to the same three residues, but not the fourth group. In other words, the protein needs not three but four locations in the active site to differentiate between the two enantiomers. In general, the three-point model works as long as it is assumed that the binding site can be approached only from one direction. But if the active site is in a cleft or on protruding residues, only binding or direction to the fourth group enables the protein to distinguish between the enantiomers.

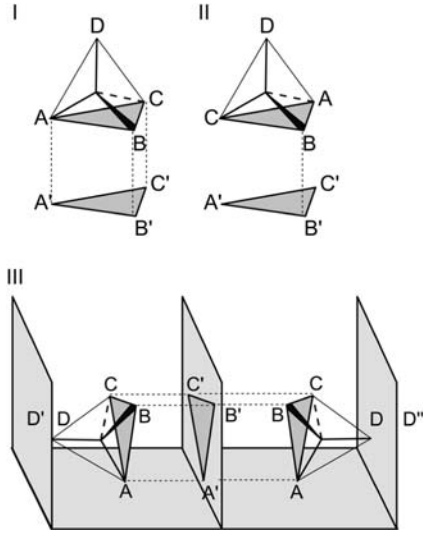

Fig. 5 Three-point attachment model modified from Easson and Stedman and Ogston (1933; Ogston 1948; *diagrams I, II*) and the four-location model by Mesecar and Koshland Jr. (2000; *diagram III*). *A′, B′, C′* Binding sites in the active site of the enzyme. For one enantiomer of the chiral substrate, the three ligands (*A, B, C*) are oriented counterclockwise and coincide with the binding sites of the enzyme. It can be seen that the ligands of the other enantiomer (*diagram II*) bind ineffectively to the enzyme. If the active site can be approached from both sides (*diagram III*), both enantiomer can bind. In such a case, an additional binding site—a fourth location (*D′* , respectively)—is necessary for the selective recognition of an enantiomer

Degradation of the enantiomers of chiral pollutants may proceed along different avenues:

A. Two enantioselective enzymes exist, each converting only one substrate enantiomer.
B. Both enantiomers are simultaneously converted by one enzyme, but at different rates.
C. Sequential conversion of the substrate enantiomers by one enzyme, i.e., the enzyme preferentially degrades one enantiomer. The other enantiomer is eventually also degraded, but only when the former one has been (completely) degraded.
D. Enantioselective conversion of one enantiomer by one enzyme and isomerization of the other enantiomer by an isomerase.

Not many of the enzymes involved in the degradation of chiral pollutants are well studied in terms of their stereoselectivity. Recently, two genes, *rdpA* and *sdpa*, were isolated and sequenced from *S. herbicidovorans* MH (Müller et al. 2003). The genes encode two distinct α-ketoglutarate-dependent dioxygenases, are involved in the enantioselective degradation of dichlorprop and mecoprop in this strain and cleave the ether bond to the corresponding phenol, with the concomitant release of pyruvate and succinate. α-Ketoglutarate and oxygen are required as cosubstrates, iron(II) as a cofactor, and ascorbate as a reducing agent. It was shown that SdpA is constitutively expressed whereas RdpA is induced during growth on the (*R*)-enantiomer or the racemate. Interestingly, SdpA was repressed when *S. herbicidovorans* MH was grown on (*R*)-mecoprop (Nickel et al. 1997). *rdpA* and *sdpA* genes were also isolated and sequenced from the dichlorprop degrader *D. acidovorans* MC1. RdpA and SdpA were partially purified and it was found that RdpA converts (*R*)-dichlorprop, (*R*)- and (*S*)-mecoprop, but not (*S*)-dichlorprop (Westendorf et al. 2002, 2003). SdpA from this strain is enantioselective and converts only the (*S*)-enantiomer of the two phenoxypropanoic acid herbicides. The amino acid residues, which are involved in substrate- and cofactor-binding, have not yet been determined. However, from alignments with other α-ketoglutarate-dependent dioxygenase and inhibitor studies, it is likely that there are two histidines and one aspartate involved in Fe(II)-binding (the 2-His-1-carboxy-facial triad; Hegg and Que 1997). At the moment, nothing is known about the stereospecificity of the substrate-binding sites and whether the substrate-binding sites of the two enzymes are similar or not. α-Ketoglutarate-dependent dioxygenases involved in 2,4-D degradation were also able to utilize phenoxypropanoic acids as substrates. Interestingly, they are highly enantioselective and convert only one enantiomer. TfdA from *R. eutropha* JMP134(JP4) and *Burkholderia cepacia* RASC oxidize only (*S*)-dichlorprop, whereas TfdA from *A. denitrificans* exclusively converts the (*R*)-enantiomer (Saari et al. 1999). This indicates that closely related enzymes may exhibit different enantioselectivities.

Another example of an enzyme that enantioselectively catalyzes a key reaction in the degradation of a recalcitrant pollutant is γ-HCH dehydrochlorinase (LinA) from *S.*

paucimobilis UT26 (Nagata et al. 1993a, 1993b). LinA catalyzes two reactions. The first reaction is the dechlorination of γ-HCH to γ-PCCH and the second reaction is the dechlorination of γ-PCCH to 1,3(*R*),4,6-(*R*)-tetrachlorocyclo-hexa-1,4-diene, a compound that is presumed to spontaneously rearrange to 1,2,4-trichlorobenzene (TCB). For these reactions, no cofactors are needed. Beside γ-HCH, LinA also converts α- and δ-HCH, but not β-HCH, which indicates that the enzyme requires a biaxial HCl pair on the substrate molecule. δ-HCH is only converted to δ-PCCH. Interestingly, LinA differentiates between 1,3(*S*),4(*R*),5(*R*),6(*S*)-PCCH and 1,3(*R*),4(*S*),5(*S*),6(*R*)-PCCH, whereby only the former enantiomer is a metabolite in the degradation of γ-HCH. When provided with the racemate obtained by chemical alkaline dehydrochlorination, LinA converts the former enantiomer to 1,2,4-TCB and the latter to 1,2,3-TCB. Therefore, the first step in the degradation of γ-HCH is highly enantioselective and gives rise to only one product enantiomer. Site-directed mutagenesis experiments allowed a closer look at the active site and it was found that the catalytic dyad, His-73 and Asp-25, is involved in stereoselectivity. It was suggested that the topological differentiation is caused by this catalytic dyad, whereas the enantiomeric differentiation is due to noncovalent interaction of the double-bond substituents with noncatalytic residues in the active site (Trantírek et al. 2001).

Ring-hydroxylating dioxygenases are important enzymes in the degradation of many aromatic pollutants. In most cases, they convert their achiral substrate to chiral products (*cis*-dihydrodiols), a process which is regio- and enantioselective; and many conversions lead to enantiomerically pure products (Hudlicky et al. 1999). Naphthalene dioxygenase (NDO) belongs to this class of enzymes. Because the structure of NDO was solved (Kauppi et al. 1998; Karlsson et al. 2003), it is the model enzyme for studying molecular aspects of enantioselectivity in such reactions. NDO is a multicomponent enzyme and catalyzes a wide range of reactions, such as *cis*-hydroxylations, monooxygenations, and desaturations. It consists of three components: an iron-sulfur-flavoprotein reductase, an iron-sulfur-ferredoxin, and the oxygenase itself, which is built of a small α- and a large β-subunit with the overall structure $\alpha_3\beta_3$ (Ensley and Gibson 1983; Kauppi et al. 1998). NDO is NAD(P)H dependent and the reductase and ferredoxin component transfer the electron from NAD(P)H to the oxygenase (Ensley et al. 1982; Ensley and Gibson 1983; Haigler and Gibson 1990). Each subunit of the oxygenase component contains a Rieske-type [2Fe-2S] center and a mononuclear nonheme iron. Electrons are transferred from the Rieske center to the mononuclear iron of an adjacent α-subunit, which is the site of oxygen activation and catalysis. Phenylalanine residue 253 (F253) has been shown to play an important role in controlling regio- and enantioselectivity in phenanthrene, biphenyl, and naphthalene oxidation. By site-directed mutagenesis, F352 was altered into different amino acids. NDO variant F352W, in which phenylalanine was replaced by tryptophan, exhibited the most pronounced changes in stereochemistry (Parales et al. 2000a, 2000b). Other variants of F352 also showed altered regioselectivity with biphenyl and phenanthrene. The combination of a F352V variant of NDO and the enantioselective toluene *cis*-dihydrodiol dehydrogenase from *P. putida* F1 was used to produce the enantiomerically pure (−)-biphenyl *cis*-(*3S*,*4R*)-dihydrodiol and (−)-phenanthrene *cis*-(*1S*,*2R*)-dihydrodiol from biphenyl and phenanthrene, respectively (Parales et al. 2000b). The enantioselective oxidation of dihydrodiols was also investigated for chlorobenzene *cis*-dihydrodiol dehydrogenase (TcbB) from *Pseudomonas* sp. strain P51 and it was found that the enantioselectivity of the conversion is highly dependent on the substrate (Raschke et al. 1999). TcbB exclusively oxidizes (+)-*cis*-(*1R*,*2S*)-indandiol, whereas both enantiomers of *cis*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene are converted.

Racemases (isomerases) catalyze racemization of their substrates and are thereby involved in enantioselective degradation pathways. One well studied example is mandelic acid racemase (MR). MR converts D- to L-mandelic acid and vice versa. *P. putida* biotype A degrades both enantiomers of mandelic acid by means of a racemase. L-Mandelic acid is the substrate of a L-(+)-mandelic acid dehydrogenase, which is highly enantioselective. Further metabolism proceeds through the so-called mandelate pathway to benzoic acid (Kenyon and Hegeman 1979), which is further metabolized via catechol and through the *ortho*-cleavage pathway. D-Mandelic acid is metabolized by the same enzymes, except that it is initially converted to the L-enantiomer by MR. Interestingly, MR itself is non-enantioselective, i.e., D-mandelic acid is converted to the L-enantiomer at the same rate as the L-enantiomer is converted to the D-enantiomer (Whitman et al. 1985). The racemization proceeds by a two-base mechanism (Powers et al. 1991). This means that two basic amino acid residues, which are juxtaposed on either side of the chiral substrate carbon, are involved. One base abstracts the proton of the substrate whereas the conjugate acid of the other base protonates the intermediate from the other side. For the opposite reaction, the roles are reversed. For MR from *P. putida* F1, the two basic residues were identified as lysine residue 166 (K166) and histidine residue 297 (H297; Landro et al. 1991, 1994; Neidhart et al. 1991; Kallarakal et al. 1995). K166 abstracts the α-proton from the (*S*)-enantiomer, whereas H297 deprotonates the (*R*)-enantiomer.

An interesting example of an enzyme pair which metabolizes the enantiomers of a chiral compound is the paired tropinone reductases (TR-I, TR-II) from *Datura stramonium*. Many plants produce tropane alkaloids and, in this synthesis, the TRs reduce a carbonyl group of an alkaloid intermediate, tropinone, to hydroxyl groups with different diastereometric configurations: tropine (3α-hydroxytropane) and *P*-tropine (3β-hydroxytropane). The crystal structures of both TRs were determined (Nakajima et al. 1998). The two enzymes share 64% amino acid identity and the overall folding is almost identical. The binding sites for the cofactor NADH and the

positions of the active site residues are well conserved. The active sites are composed mainly of hydrophobic amino acids, but differently charged amino acids cause different electrostatic environments. In TR-I, there is a positively charged histidine (H112), whereas in TR-II a polar tyrosine (Y100) occupies this position. In TR-II, the hydrophobic valine residue 168 of TR-I is replaced by the negatively charged glutamate residue 156. These differences are of importance for the positioning of tropinone. The nitrogen atom of tropinone is negatively charged under physiological pH conditions and, thereby, tropinone is oriented in different ways in the active site by the different electrostatic environments.

These examples demonstrate how single amino acid residues are responsible for highly enantioselective reactions and enable the enzymes to differentiate between enantiomers. Just by changing a few amino acid residues involved in regio- and enantioselective substrate recognition, the stereoselectivity of an enzymatic reaction can be changed or even reversed.

Convergent evolution is another approach nature has chosen to metabolize enantiomers of chiral compounds. Many enzyme pairs, each turning over one enantiomer of a chiral compound, have evolved independently and are not related to each other. Examples are the D- and L-lactate dehydrogenases (D-LDH, L-LDH), which belong to the D- and L-ketoacid dehydrogenase families, respectively. Sequence comparisons show that these two enzyme families are not related to each other evolutionarily. L- and D-LDH catalyze the reduction of pyruvate to lactate, with the concomitant consumption of NADH, producing enantiomerically pure products. By crystal structure analysis, it was found that the overall folding of the D-LDH is completely different from that of L-LDH. It was hypothesized that, despite the different folding, the active sites are mirror-images of each other (Goldberg et al. 1994; Lamzin et al. 1994). This is true insofar as the same amino acid residues are involved in substrate-binding and catalysis and they are in structurally equivalent positions. However, their exact roles in binding and catalysis may not be the same (Stoll et al. 1996). Convergent evolution was also found for the D-amino acid transferases (D-AAT) and L-aspartate aminotransferases (L-Asp-AT). D-AAT and L-Asp-AT do not share any identity at the sequence level and their overall folding is different, but the enzymatic mechanism is similar. Both enzymes contain a pyridoxal phosphate (PLP) and catalyze a transamination of the respective amino acid enantiomer. Sugio et al. (1995) showed that there are striking similarities between the active sites of the two enzymes, especially concerning the binding of PLP and its intermediates. The α-amino- and α-carboxyl groups of the substrate amino acid are bound in the same orientation in relation to the pyridoxal phosphate ring and the protein. The side-chain is therefore, due to the inverse chirality, oriented in the opposite direction.

Other interesting enzymes in terms of their enantioselectivity are the 2-haloacid dehalogenases (DEXs) which convert chiral 2-haloacids into 2-hydroxyacids. DEXs are involved in the degradation of halogenated organic compounds and are classified into four groups based on their substrate and stereochemical specificities (Soda et al. 1996). L-DEXs convert L-haloacids into D-hydroxyacids with inversion of the configuration at the C2-carbon atom of the substrate. D-DEX acts specifically on the D-enantiomer to produce L-hydroxyacids. DL-DEX_i dehalogenates both enantiomers with inversion of the configuration at the C2-carbon atom; and DL-2–DEX_r converts both enantiomers to the corresponding hydroxyacids, with retention of the configuration at the C2-carbon atom. L-DEXs are well studied, both in terms of reaction mechanisms and in terms of substrate- and stereospecificity (Smith et al. 1990; Kurihara et al. 1995; Liu et al. 1995; Li et al. 1998). Additionally, the crystal structures of two representative enzymes, L-DEX from *Pseudomonas* sp. YL and L-DEX from *Xanthobacter autotrophicus* GJ10 have been solved (Hisano et al. 1996; Ridder et al. 1997; Li et al. 1998). The normal reaction mechanism proceeds via an ester intermediate. First, Asp-10 acts as a nucleophile and attacks the C2-carbon atom of the substrate, forming an ester-intermediate and a halide ion. A water molecule is activated and hydrolyzes the intermediate from the back, giving the D-hydroxyacid. From mutagenesis studies and the crystal structure of L-DEX YL, several amino acid residues (Tyr-12, Leu-45, Phe-60, Trp-179, Gln-44, Lys-151, Asn-177) were identified which built a hydrophobic pocket. While the carboxylic moiety of the substrate is bound to Asp-10, the alkyl group is located in the hydrophobic pocket. This hydrophobic pocket is responsible for the stereo- and substrate-specificity of the enzyme. It is not possible to accommodate an alkyl group within the place for a hydrogen atom, due to steric hindrance by the main chain and the side-chain atoms of Leu-11, Tyr-12, and their neighbors in this pocket. This determines the enantioselectivity (and substrate specificity) of the enzyme. In contrast to L-DEX, DL-DEX_r converts both enantiomers. DL-DEX from *Pseudomonas* sp. strain 113 (DL-DEX 113) has a significant sequence homology with D-DEXs, e.g., 23% with D-DEX from *P. putida* AJ1, but little with L-DEXs (Nardi-Dei et al. 1997). To study the reactive site, several polar and charged amino acid residues conserved among DL- and D-DEX were mutated. When the enzymatic activity was lower than in the wild type, the effect was always equal for both enantiomers. The results suggest that DL-DEX 113 has one single active site for D- and L-2-haloacids. In other words, DL-DEX 113 does not—in contrast to L-DEX—discriminate between the alkyl group and the hydrogen atom at the C2-atom of 2-haloacids (Nardi-Dei et al. 1997).

L-DEXs are examples of enzymes that discriminate between enantiomers by the spatial arrangement of the active site rather than by specific interactions of single amino acid residues with the substrate. It would be interesting to know how substrates are bound to the active sites in D- and DL-DEX in order to compare the mechanisms, but crystallographic data are not yet available.

We have described enzymes with overall conserved folding, where just a few amino acid residues determine stereospecificity, together with examples of enzymes that express opposite stereospecificity and have completely different folds and surprisingly similar active sites that are mirror-images. Theoretically, two enzymes with identical sequences but built from enantiomeric amino acid monomers, i.e., one built from L-amino acids and the other from D-amino acids, should have opposite stereospecificities for chiral substances. Such an enzyme pair was studied by Milton et al. (1992). They synthesized the HIV-1 protease completely with D-amino acids. The D-HIV-1 protease indeed showed the opposite substrate specificity. Inverse specificity was also observed with enantiomeric inhibitors. These data imply that the L- and the D-forms of the enzyme are the exact image and mirror-image of each other, resulting in opposite substrate specificity.

Conclusion

The environmental fate of the enantiomers of chiral compounds differs not only with regard to unwanted side-effects but also with regard to degradation. As the examples show, there is no rule to decide which enantiomer is preferably degraded. It depends on the specific compounds, the environmental compartment, the environmental conditions, and the microbial community. As other authors have emphasized, it is important to consider both stereochemistry and chirality when studying the effects and degradation potential of chiral compounds (e.g. (Ariëns 1989, 1993; Kohler et al. 1997, 2000). This is also true when enantiomerically pure compounds are used and therefore only one enantiomer is introduced into the environment. As was pointed out, enantiomers may undergo racemization or enantiomerization processes and therefore the environmental fate of each enantiomer always needs to be investigated.

Studies on enantioselective enzymes have helped and will help to broaden our understanding of the effects of chirality on the living world. It will be important to study such enzymes more intensively to learn how nature deals with chiral objects. The following questions need to be addressed. Does an enzyme change its stereospecificity easily? How do enzymes which are evolutionarily not related to each other adapt to convert enantiomers of a chiral compound? Evolution has not preferred one particular mechanism, as far as we know, but further studies need to be carried out to answer such questions in more detail.

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