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Environmental Fate of Chiral Pollutants – the Necessity of Considering Stereochemistry

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Abstract. Many organic compounds regulated by environmental laws are chiral and are released into the environment as racemates. 3-Phenylbutanoic acid and mecoprop, two chiral pollutants, were enantioselectively degraded by pure cultures of microorganisms. This indicates the importance of assessing the environmental impact of stereoisomers separately, because selective enrichment of one of the enantiomers may occur in the environment. Field studies on the fate of highly polar, chiral compounds, like sulfophenylcarboxylates, are hampered by the lack of appropriate analytical methods for the separation of the enantiomers. Therefore, a method based on capillary electrophoresis with α -cyclodextrin as chiral selector was developed to separate the enantiomers of such compounds. In a field study at a Swiss waste disposal site, the fate of the chiral herbicide mecoprop was investigated. The enantiomeric ratio of (*R*)-mecoprop to (*S*)-mecoprop altered during groundwater passage of landfill leachate. This is a strong indication for *in situ* biodegradation. Our data imply that not only the enantiomers of a chiral drug or pesticide may exert different effects on the biological targets, but also their biodegradation and environmental fate may differ.

Introduction

Many natural and man-made objects are nonsuperimposable on their mirror images. This property is described with the notion 'chirality'. Organic compounds with at least one stereogenic center are chiral and consist of two enantiomers, which differ in structure only in the left- and right-handedness of their orientations. It is well-known to chemists that many physicochemical properties of enantiomers, e.g. boiling points, vapor pressures, and water solubilities are identical. Therefore, enantiomers generally behave in the same way in physicochemical processes. However, when enantiomers interact with

other chiral objects, e.g. during metabolism in cells (Fig. 1) [1], selective interactions can occur. Ever since the 'contergan-incidence' (on taking the racemic sedative thalidomide during pregnancy, the (*S*)-enantiomer caused severe malformations of the foetus), regulatory agencies ask for data about the fate and the effects of both enantiomers of a chiral pharmaceutical compound in the course of registration. Although a chiral object and its mirror image apparently are different, there is no reason at the outset to think that one should be superior to the other. Nevertheless, in many instances nature seems to favor one of the forms. The macromolecules of life, the proteins and the nucleic acids (DNA and RNA), consist of chiral monomers and nature has used one of the stereoisomers over the other. All proteins consist of L-amino acids and only rarely do polypeptides made of D-amino acids fulfill special biological functions. For DNA, RNA, and the polysaccharides the D-enantiomers of the monomeric sugar backbones are preferred.

A significant number of the organic chemicals regulated by environmental laws are chiral and are released into the environment as racemates [2]. Although several studies concerning the fate of envi-

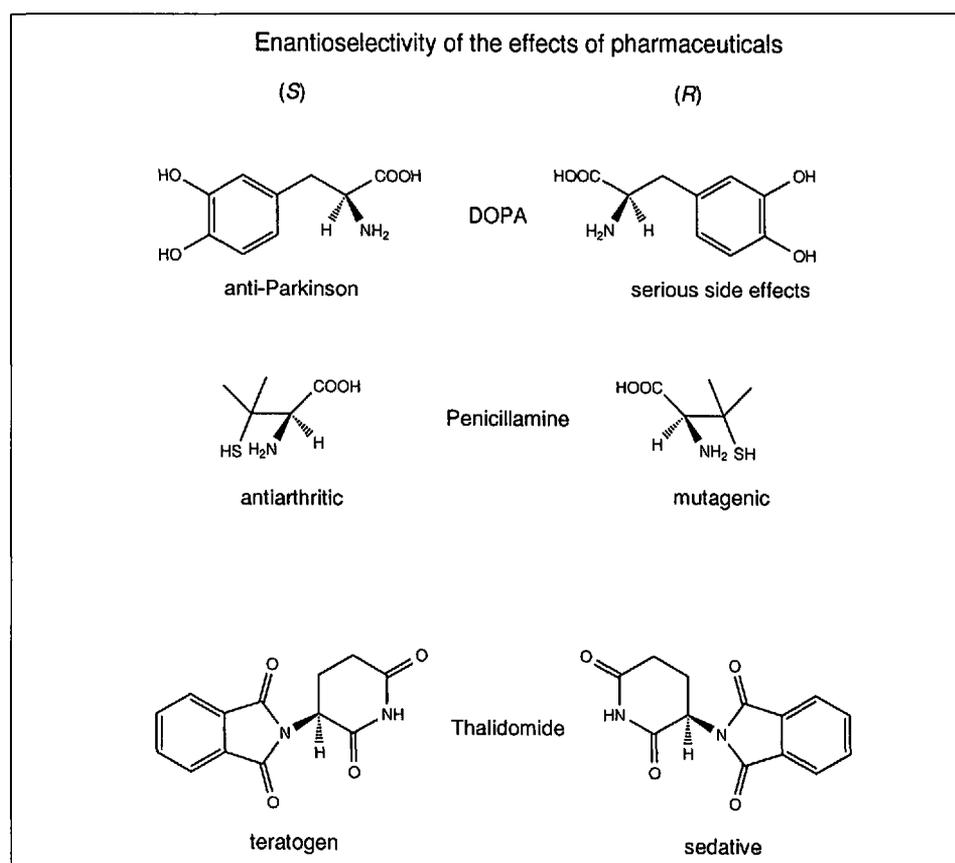


Fig. 1. Examples of chiral pharmaceuticals, whose nonactive enantiomer (distomer) causes undesirable toxic side effects. Modified after [1].

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ronmentally relevant chiral compounds demonstrate the utmost importance of dealing with enantiomers as separate entities [3], most environmental regulations and scientific environmental studies treat racemates as they would only consist of one kind of molecule [4]. At EAWAG, in close collaboration with the Agricultural Research Station in Wädenswil, we investigate different aspects of the environmental fate of chiral compounds. Our goal is to develop new analytical methods for the separation of enantiomers, to study the biochemistry of microbes that enantiose-

lectively degrade chiral chemicals, and to conduct field studies to assess the environmental relevance of the enantiomer-specific behavior of chiral compounds. In this article, it will be shown that microbes may degrade chiral compounds in an enantioselective manner and that alterations in the enantiomeric ratio of a chiral compound in environmental samples allow to distinguish between abiotic and biotic transformations. We intend to emphasize the necessity of dealing with enantiomers as different molecules, the importance of considering stereochemistry when study-

ing the degradation and environmental fate of chiral compounds, and the benefit of reducing environmental loads by preventing 'enantiomeric ballast'.

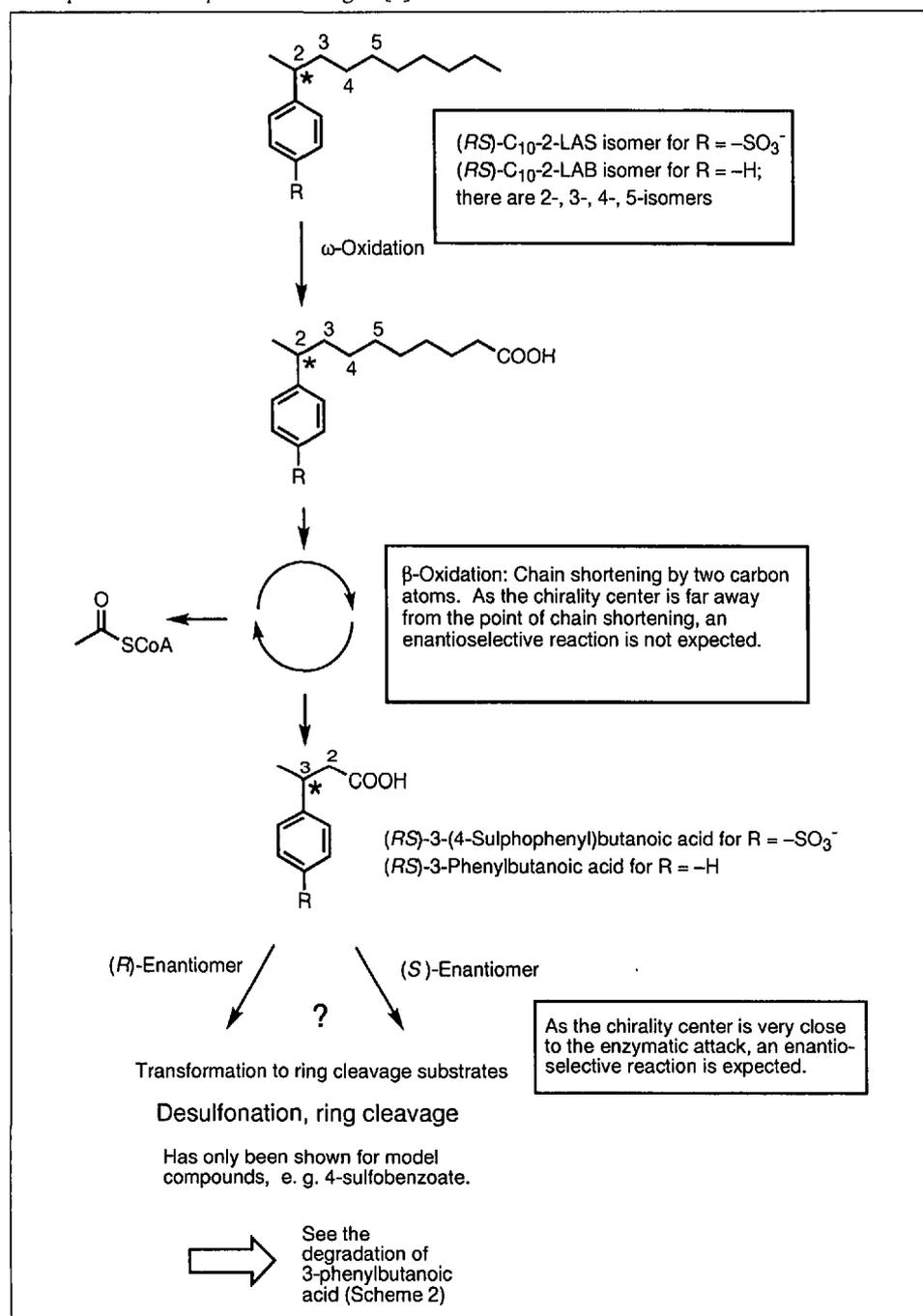
Microbial Degradation of Linear Alkylbenzenes and Linear Alkylbenzene Sulfonates

Linear alkylbenzenes (LAB) are hydrophobic compounds that are used as intermediates for the synthesis of linear alkylbenzene sulfonates (LAS), which are the most important anionic surfactants in laundry and cleaning products. LAS are amphiphilic chemicals consisting of a hydrophobic and a hydrophilic part. Commercial LAB and LAS are not pure compounds but mixtures of homologs and isomers. The compounds in the mixture may differ in the length of the alkyl side chain (homologs), in the position of attachment of the phenyl or sulfophenyl substituent (isomers), and in the topology (stereoisomers) (Scheme 1) [5].

The microbial degradation of LAB and LAS has been studied extensively since the early sixties [6]. Released into the environment, they biodegrade under aerobic conditions. For complete mineralization many different enzyme systems are required. Enzymes have to cope with alkyl chains and with sulfonated (LAS only) aromatic ring structures that are distributed along the alkyl chain. Therefore, several species of microorganisms are generally responsible for the transformation of even one single isomer. The different isomers and homologs probably are degraded with different rates and through different mechanisms. Studies with mixed cultures have shown that within 13 days only ca. 29% of the radioactively labeled LAS is mineralized [7].

Microbial degradation of LAB and LAS happens in three phases (Schemes 1 and 2) [8]. In the first phase, the terminal methyl groups of the linear alkyl chains are oxidized to acids (ω -oxidation) in analogy to the oxidation of linear alkanes [6]. Subsequently, the long-chain phenyl- and sulfophenylcarboxylates are shortened by enzymes of the fatty-acid metabolism (β -oxidation) stepwise by two C-atoms. Chain shortening by β -oxidation seems to become hindered when the side chains are cut back to four or five C-atoms from the point of attachment to the benzene ring, a phenomenon known as the 'distance principle' [9]. The products of ω - and β -oxidation, the short-chain phenyl- and sulfophenylcarboxylates (SPC), are usually excreted into the medium by the strains ox-

Scheme 1. Pathway Proposed for the Degradation of Linear Alkylbenzenesulfonates (LAS) and Linear Alkylbenzenes (LAB) with Respect to One Specific Isomer. The enzymes catalyzing ω - and β -oxidation probably do not discriminate between the enantiomers. Chiral phenyl- and sulfophenylcarboxylates are the products of these oxidations; here, 2-phenylbutanoic acid and 2-(4-sulfophenyl)butanoic acid are depicted as examples. According to [5].



idizing the side chain. All the carboxylates, except the symmetrical dicarboxylates, are chiral. They are the substrates for the second phase of degradation.

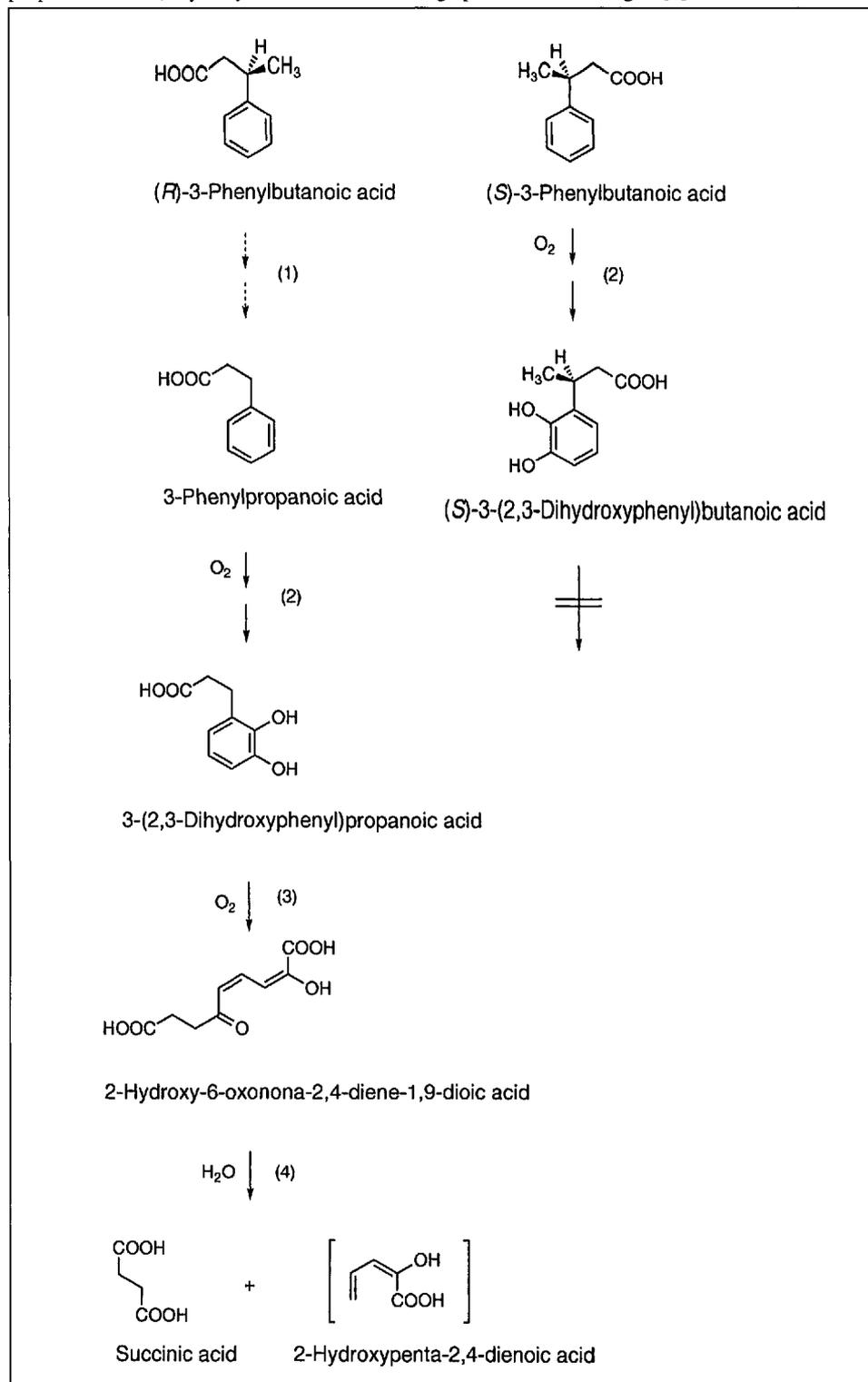
In the second phase the carboxylates are transformed to compounds that can undergo ring cleavage. Regarding ring fission of such intermediates, Cain [6] suggested that once the side chain is oxidized, the compounds (even short chain, e.g. C₄-phenylalkanoates) undergo further side-chain shortening before ring attack begins, or simply accumulate. He further pointed out, that direct fission of aromatic rings carrying a carboxylated side chain longer than C₃ is extremely rare. We have found at EAWAG that degradation of 3-phenylbutanoic acid in *Rhodococcus rhodochrous* PB1 proceeds in an enantioselective manner [8]. Only the (*R*)-enantiomer is degraded. The (*S*)-enantiomer is cometabolically transformed to (*S*)-3-(2,3-dihydroxyphenyl)butanoic acid, a compound that is not further degraded but undergoes abiotic transformations in aqueous solutions to reactive and potentially toxic quinones [8]. The question to what extent these findings are valid for SPC isomers is currently being clarified. An analytical tool based on high-performance capillary electrophoresis with α -cyclodextrin as the chiral selector was developed to separate the enantiomers of *p*-sulfophenyl-2- and *p*-sulfophenyl-3-butanoic acid (Fig. 2; [10]). Future studies will be aimed at measuring enantiomeric ratios in environmental samples.

The third phase of degradation consists of desulfonation (LAS only) and ring cleavage. Although there is not a complete agreement in the literature, desulfonation and ring fission seem to coincide in most cases. Desulfonation can occur either concomitant with dihydroxylation of the sulfophenyl compounds by dioxygenases or concomitant with ring cleavage of the sulfocatechols by ring fission dioxygenases. However, it has not been shown to date that the results obtained with the model compounds *p*-toluenesulfonic acid, *p*-aminobenzenesulfonic acid, and *p*-sulfobenzoic acid [11] can be applied to the degradation of SPC. With respect to this point, LAS degradation schemes are based on mere analogy.

Microbial Degradation and Environmental Fate of the Chiral Herbicide Mecoprop

Chiral (*RS*)-2-(4-chloro-2-methylphenoxy)propanoic acid (mecoprop) is a systemic, postemergence herbicide developed

Scheme 2. Pathway Proposed for the Metabolism of (*R*)- and (*S*)-3-Phenylbutanoic Acid by *Rhodococcus rhodochrous* PB1. 1) Reactions that convert (*R*)-3-phenylbutanoic acid to 3-phenylpropanoic acid in a yet unknown but enantioselective manner. 2) Dioxygenation of 3-phenylpropanoic acid and (*S*)-3-phenylbutanoic acid (cometabolically). 3) *meta* Cleavage of 3-(2,3-dihydroxyphenyl)propanoic acid. 4) Hydrolysis of the *meta* cleavage product. According to [8].



in the fifties for use in the control of broad-leaved weeds in cereal crops and lawns, and is often commercially used in formulations together with other herbicides. Already in 1953, studies about the stereospecificity of plant growth regulators revealed that the herbicidal activity was associated only with the (*R*)-enantiomer of mecoprop [12]. Our investigations of the degrada-

tion of mecoprop by *Sphingomonas herbidovorans* MH demonstrated that both enantiomers are completely degraded by this pure culture, albeit in an enantioselective manner. The (*S*)-enantiomer is preferentially degraded. Degradation of the (*R*)-enantiomer only starts after a considerable lag period (Fig. 3, [12]). Biochemical studies with crude cell extract of strain MH

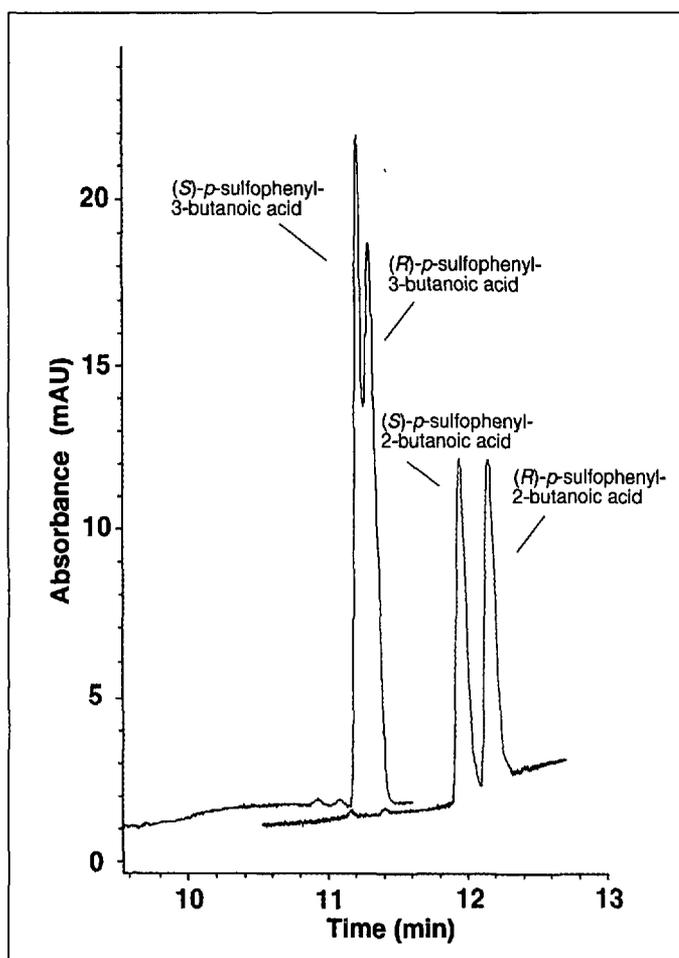


Fig. 2. Electropherograms of solutions containing 0.1 mM of racemic *p*-sulfophenyl-2-butanoic acid and *p*-sulfophenyl-3-butanoic acid. According to [10].

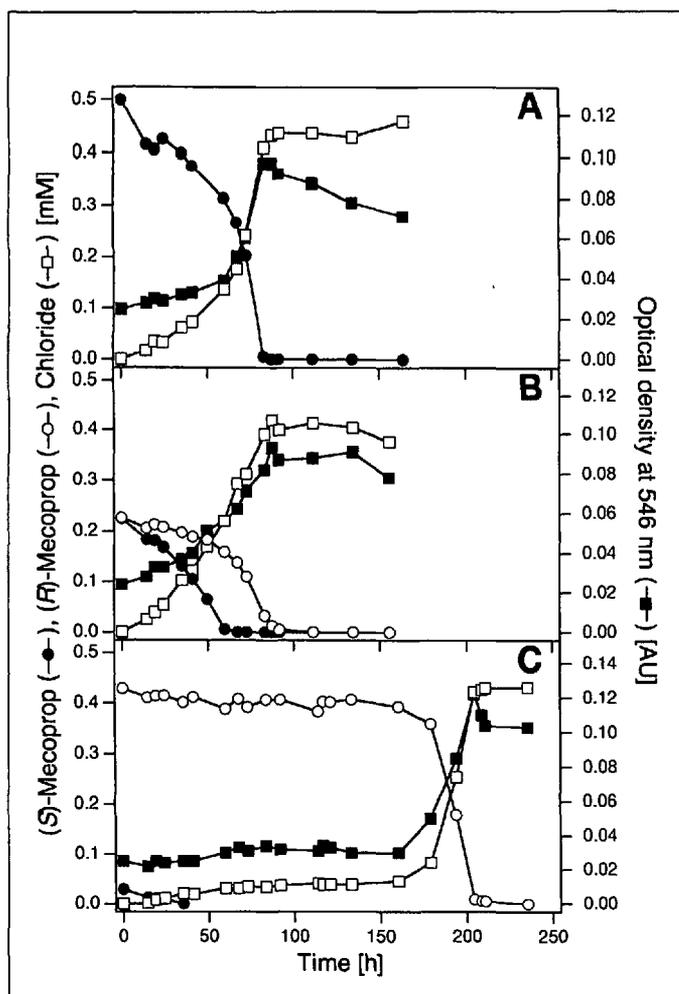


Fig. 3. Enantioselective degradation of mecoprop by *Sphingomonas herbicidovorans* MH. A) Growth on the pure (*S*)-enantiomer. B) Growth on the racemic mixture. C) Growth on the pure (*R*)-enantiomer. According to [10].

revealed that cell extract of *Sphingomonas herbicidovorans* MH grown on (*R*)-mecoprop contains an enzyme activity that selectively converts (*R*)-mecoprop to 4-chloro-2-methylphenol, whereas the enzyme activity in cell extracts of (*S*)-mecoprop-grown cells is selective for the (*S*)-enantiomer. Both reactions are dependent on α -ketoglutarate and ferrous ions. Besides 4-chloro-2-methylphenol, pyruvate and succinate are detected as products of the reactions. Labeling experiments with $^{18}\text{O}_2$ show that both enzyme activities catalyze a dioxygenation reaction (Scheme 3; [13]). These results agree with those obtained by Tett [14], who showed that *Alcaligenes denitrificans* only degrades the (*R*)-enantiomer of mecoprop probably because an (*S*)-enantiomer-specific enzyme system is missing in this strain.

In a recent field study, we determined enantiomeric ratios of (*R*)-mecoprop to (*S*)-mecoprop in leachates from the waste disposal site in K lliken as well as in groundwater from locations 20–50 meters downstream of this landfill. Alterations in the ratio unequivocally indicate the occurrence of biological degradation processes, as physicochemical processes do not lead to discrimination of enantiomers. We successfully applied a sensitive enantiomer-specific analytical method (based on gas chromatography-mass spectrometry [15]) to the leachate as well as to the groundwater samples in order to determine the enantiomeric ratios of (*R*)- to (*S*)-mecoprop. The enantiomeric ratios in the leachate samples from within the landfill turned out to be one. In contrast, the concentrations of (*R*)-mecoprop were elevated up to sevenfold as compared to those of (*S*)-mecoprop in some of the groundwater samples taken from locations downstream. This shows that *in situ* enantioselective microbial degradation of mecoprop occurred during groundwater passage [16]. These findings agree well with those of the laboratory experiments with pure cultures discussed above. There, also the (*S*)-enantiomer is preferentially degraded, which leads to a relative enrichment of the (*R*)-enantiomer [12].

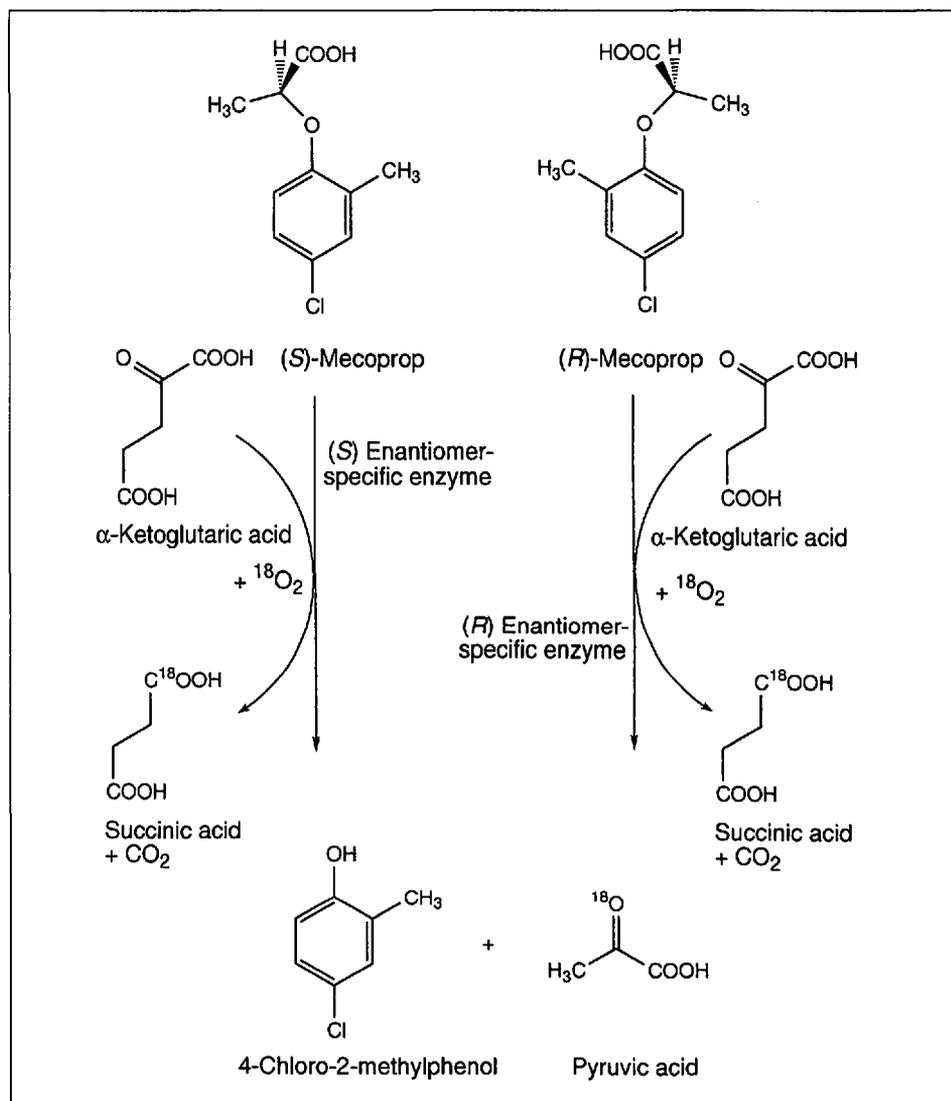
Furthermore, we investigated the fate of herbicides in rainwater and during stormwater infiltration into the subsurface. During these field investigations we located certain bituminous sealing layers of flat roofs as a source of racemic mecoprop. Such layers were found to be equipped with a polyethylene glycol diester of mecoprop that serves as a root protection agent [17]. The quantification of the enantiomers in these studies allowed to evaluate their fate in roof runoff waters, to estimate the

age of mecoprop in rainwater, and to investigate the biological activity on roofs as well as during infiltration into groundwater.

Concluding Remarks and Outlook

Our findings show that it is of outmost importance to treat enantiomers separately when studying the degradation and the environmental fate of chiral pollutants. We believe that the microbial metabolism and environmental fate of many chiral compounds must be reevaluated by using enantiomer-specific techniques and analytical tools. Not only may the enantiomers of a chiral drug or pesticide exert different effects on the biological targets, but their biodegradation and environmental fate may also differ. In the future, more and more bioactive compounds that are currently marketed as racemates will be replaced by enantiomerically pure products that only contain the active ingredient. Such trends will allow the manufacturers to reduce production costs and will keep the 'ballast enantiomers' from the environment. Field studies will be helpful tools for assessing the environmental impact of such measures. Furthermore, there will be a need for the development of additional enantiomer-specific analytical methods that can be applied to environmental matrices.

Scheme 3. Proposed Scheme for the Initial Steps in the Degradation of the Enantiomers of Mecoprop in *Sphingomonas herbicidovorans* MH. According to [13].



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