

Biodegradation of hexachlorocyclohexane (HCH) by microorganisms

Theresa M. Phillips^{1,*}, Alan G. Seech², Hung Lee¹ & Jack T. Trevors¹

¹*Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1;* ²*Adventus Remediation Technologies, 1345 Fewster Drive, Mississauga, Ontario, Canada, L4W 2A5 (*author for correspondence: e-mail: tphillip@uoguelph.ca)*

Accepted 24 August 2004

Key words: bioremediation, degradation, environmental effects, hexachlorocyclohexane, Lindane, soil microorganisms

Abstract

The organochlorine pesticide Lindane is the γ -isomer of hexachlorocyclohexane (HCH). Technical grade Lindane contains a mixture of HCH isomers which include not only γ -HCH, but also large amounts of predominantly α -, β - and δ -HCH. The physical properties and persistence of each isomer differ because of the different chlorine atom orientations on each molecule (axial or equatorial). However, all four isomers are considered toxic and recalcitrant worldwide pollutants. Biodegradation of HCH has been studied in soil, slurry and culture media but very little information exists on *in situ* bioremediation of the different isomers including Lindane itself, at full scale. Several soil microorganisms capable of degrading, and utilizing HCH as a carbon source, have been reported. In selected bacterial strains, the genes encoding the enzymes involved in the initial degradation of Lindane have been cloned, sequenced, expressed and the gene products characterized. HCH is biodegradable under both oxic and anoxic conditions, although mineralization is generally observed only in oxic systems. As is found for most organic compounds, HCH degradation in soil occurs at moderate temperatures and at near neutral pH. HCH biodegradation in soil has been reported at both low and high (saturated) moisture contents. Soil texture and organic matter appear to influence degradation presumably by sorption mechanisms and impact on moisture retention, bacterial growth and pH. Most studies report on the biodegradation of relatively low (<500 mg/kg) concentrations of HCH in soil. Information on the effects of inorganic nutrients, organic carbon sources or other soil amendments is scattered and inconclusive. More in-depth assessments of amendment effects and evaluation of bioremediation protocols, on a large scale, using soil with high HCH concentrations, are needed.

Abbreviations: CB – chlorobenzene; CHQ – chlorohydroquinone; 2,5-DCHQ – 2,5-dichlorohydroquinone; DCP – dichlorophenol; DDD – 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane; 2,5-DDOL – 2,5-dichloro-2,5-cyclohexadiene-1,4-diol; DDT – 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane; 2,4,5-DNOL – 2,4,5-trichlorocyclohexenol; GC-MS – gas chromatography-mass spectroscopy; GEMS – genetically engineered microorganisms; HCH – hexachlorocyclohexane; HQ – hydroquinone; γ -HMSA – γ -hydroxymuconic semialdehyde; MCB – monochlorobenzene; PCCH – pentachlorocyclohexene; PCP – pentachlorophenol; PVC – polyvinylchloride; TCB – trichlorobenzene; TLC – thin-layer chromatography; TCCH – tetrachlorocyclohexene; 1,4-TCDN – 1,3,4,6-tetrachloro-1,4-cyclohexadiene; TeCB – tetrachlorobenzene

Introduction

The gamma isomer of hexachlorocyclohexane (HCH), commercially known as Lindane, is a

highly chlorinated, recalcitrant pesticide. It has been used worldwide as a general, broad spectrum insecticide for a variety of purposes including fumigation of household and commercial storage

areas, pest control on domestic animals, mosquito control and to kill soil-dwelling and plant-eating insects such as aphids, grasshoppers, flies, boil weevils, mange mites, termites, ants, leafminers, thrips, armyworms and wireworms, on fruits, vegetables and Christmas and ornamental trees. It is widely used for stem borer control in rice paddies and is applied in Canada as a seed treatment for crops, such as canola, to protect from flea beetle infestations.

Lindane is manufactured by photochemical chlorination of benzene under UV light. Commercial production of technical grade Lindane for insecticidal use results in a mixture of isomers. The mixture typically contains 10% to 15% γ -HCH (Lindane), and the remaining fraction consists predominantly of α -, β -, and δ -isomers (Deo et al. 1994; Waliszewski 1993; Walker et al. 1999; Willett et al. 1998). Purified Lindane may still contain traces of the other three isomers.

Although only Lindane is insecticidal, HCHs as a group are toxic and considered potential carcinogens (Walker et al. 1999), and are listed as priority pollutants by the US EPA. Technical Lindane is banned in many developed countries and the use of purified Lindane is currently restricted in the US, proposed for ban in Canada, and has been banned or restricted in many other developed countries. Due to their persistence and recalcitrance, however, HCHs continue to pose a serious toxicological problem at industrial sites where past production of Lindane along with unsound disposal practices has led to serious contamination. In addition, many countries still allow HCH production and use and, despite localized limitations, HCH contamination continues to be a global issue, as these compounds have moderate volatility and can be transported by air to remote locations (Galiulin et al. 2002; Glotfelty & Schomburg 1989; Walker et al. 1999). As a result, HCHs have been found in trace amounts worldwide, in air, soil and water (Wania et al. 1999; Willett et al. 1998). Mean global concentrations of α - and γ -HCH in air and water, obtained from a series of studies from 1980 to 1996, were summarized by Walker et al. (1999). Concentrations in air ranged from 3.6 to 1021 pg m⁻³ α -HCH, and 1 to 580 pg/m⁻³ γ -HCH. Of the regions tested, some of the highest reported concentrations were found over the northwest Pacific Ocean, Central Ontario, Bering Sea and Green Bay (α - and γ -isomers), the Beaufort Sea and northeast

Pacific Ocean (α -isomer), and south Norway, south Pacific Ocean and Antarctica (γ -isomer). Mean concentrations in surface water, measured during the same time span, ranged from 0.09 to 49 ng m⁻³ and from 0.04 to 61 ng m⁻³, for α - and γ -HCH, respectively. The majority of water bodies tested contained less than 3 ng m⁻³, with the exception of the northeast Atlantic (49 and 61 ng m⁻³ α - and γ -HCH, respectively) and the southeast Atlantic (12.2 and 31.5 ng m⁻³ α - and γ -HCH, respectively). More recent measurements of γ -HCH concentrations in the air at shore locations surrounding the Great Lakes show a range of 19.9–51.4 pg m⁻³ during summer months, and much lower concentrations during autumn and winter months (Ma et al. 2003). Wania et al. (1999) reported α -HCH concentrations for southern Scandinavia ranging from 100 to 1,000 pg m⁻³ in the early 1980s and falling below 500 pg m⁻³, from the mid-1980s to the mid-1990s.

Due to the toxicity and persistence of HCH, soils contaminated with these compounds have been targeted for remediation. The transport of HCHs (and other organochlorine pesticides) in the environment, their toxicity and abiotic degradation have been the subject of several reviews (Deo et al. 1994; El Beit et al. 1981; Galiulin et al. 2002; Walker et al. 1999; Willett et al. 1998) and are not discussed in detail here.

Biodegradation of the most common HCH isomers, α , β , δ and γ , have been extensively studied in the laboratory, and less so in pilot or full-scale *in situ* field settings. The HCH isomers differ in their persistence and the properties (solubility, volatility) that determine their rates of biodegradation. Early studies with Lindane-contaminated soils suggested that degradation was faster under anoxic conditions and that microbial degradation was the primary route of HCH disappearance from soil (MacRae et al. 1967). Microbial degradation of the four common isomers has since been observed under oxic conditions, both in soil (Bachmann et al. 1988b; Doelman et al. 1985; Sahu et al. 1993) and in pure cultures of microorganisms (Bhuyan et al. 1993; Thomas et al. 1996). This paper summarizes the current knowledge on the biodegradability of HCH by microorganisms in laboratory cultures and in soil, and the results of bench, pilot and full-scale bioremediation protocols that have been applied to contaminated soils. The subject of bio-

remediation of HCH-contaminated soils remains inadequately addressed to date, and successful *in situ* bioremediation or mineralization at a highly contaminated field site has not been reported.

Physical properties and persistence of HCHs

There is controversy in the literature over the persistence of HCHs in soil, water and air, probably due to contrasting data resulting from the complex interactions of environmental factors affecting rates of both abiotic and biotic removal. Although Lindane has been shown to be readily biodegradable under appropriate conditions, it has also been reported to persist in soil for many years (MacRae et al. 1969). Published half-lives for Lindane in soil range from 4 to 6 weeks (Johri et al. 1996) or 120 days (Bintein & Devillers 1996), to 260 days (Jury et al. 1983) to 2 years (Johri et al. 1996), depending on the method of application and application rate, as well as initial concentrations, soil differences and environmental conditions. HCH isomers, applied to field plots as a mixture of pesticide, can persist for as long as 15 years after the last application (Stewart & Chisholm 1971).

The structures of the most frequently detected HCH isomers (α , β , δ and γ) are shown in Figure 1. The relative persistence of each isomer is generally attributed to the orientation of the chlorine atoms on the molecule. For example, β -HCH is the most persistent isomer (Bachmann et al. 1988b; Beurskens et al. 1991; Johri et al. 1998; Sahu et al. 1990, 1993), and has all equatorially oriented chlorine atoms ('e e e e e'), resulting in a more structurally stable compound. In the more easily degraded α -, δ - and γ -isomers, the chlorine atoms have 'a a a a e', 'a e e e e' and 'a a a e e' orientations, respectively. The presence of axial chlorine atoms is thought to provide available sites for enzymatic degradation, and it is generally observed that γ - and α -HCH are more easily biodegraded than the δ -isomer, which has more equatorial chlorine atoms (Chessells et al. 1988; Deo et al. 1994; Doelman et al. 1985; Siddaramappa & Sethunathan 1975). In a long-term study of the persistence of HCH isomers from a technical Lindane formulation, applied annually to small field plots, the α -isomer was the least persistent after 15 years, with only 4% remaining in the soil, compared to the most persistent β -isomer, of

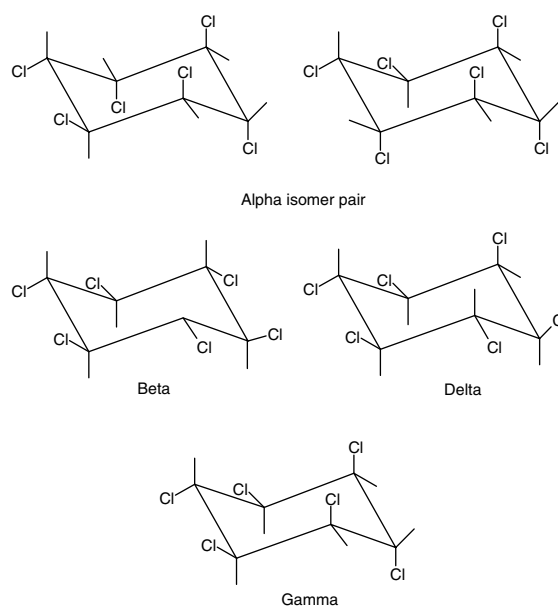


Figure 1. Structures of HCH isomers, including the two enantiomers of α -HCH. The axial and equatorial positions of the chlorine atoms are as follows: α , a a a a e; β , e e e e e; δ , a e e e e; and γ , a a a e e. Adapted from Willett et al. (1998).

which 44% remained in the soil (Stewart & Chisholm 1971).

The α -isomer can exist in two enantiomeric forms as shown in Figure 1. Studies of natural attenuation in air, soil and water bodies (Helm et al. 2000; Padma et al. 2003) have used enantiomer-specific degradation of this isomer to evaluate whether biodegradation was taking place. The enantiomer that is preferentially degraded varies with the site. For example, Helm et al. (2000) reported that the (+) enantiomer was preferentially degraded in three streams found in the Canadian Arctic. Preferential removal of the (+) enantiomer was detected in a mid-latitude estuary (Padma et al. 2003) and the removal rate of (+)- α -HCH exceeded that of the (–) enantiomer in bench-scale studies using anoxic sewage sludge (Buser & Muller 1995). In contrast, groundwater samples from a pesticide reformulating site in Florida showed preferential degradation of the (–) enantiomer (Law et al. 2004).

The chemical structure and polarity of pesticides affect their solubility, volatility and sorption characteristics, factors that contribute to their transport, persistence and biodegradability, and might be used to explain the relative recalcitrance of the four isomers (α , β , γ , δ) found in technical

Table 1. Chemical characteristics of the four most commonly found HCH isomers^a

	Alpha	Beta	Delta	Gamma
Melting point (°C)	159–160 ^{a,b} 158 ^c	314–315 ^a 309–310 ^b	141–142 ^a 138–139 ^b 141.5 ^c	112.5 ^{a,c,d} 112–113 ^b
Boiling point (°C)	288 at 760 mm Hg ^a	60 at 0.5 mm Hg ^a	60 at 0.36 mm Hg ^a	323.4 at 760 mm Hg ^a
Density (g cm ⁻³)	1.87 at 20 °C ^a	1.89 at 19 °C ^{a,c}	no data	1.89 at 19 °C ^a
Solubility in water (mg l ⁻¹)	10 ^a	5 ^a	10 ^a	Insoluble ^e 0 – 17 ^f 7 ^g 7.52 at 25 °C ^d
Solubility in 100 g ethanol (mg)	1.8 ^a	1.1 ^a	24.4 ^a	6.4 ^{a,c}
Log <i>K</i> _{ow}	3.8 ^a 3.9 + /-0.2 ^b	3.78 ^a 3.9 + /-0.1 ^b	4.14 ^a 4.1 + /-0.02 ^b	3.72 ^a 3.7 + /-0.5 ^b 3.85 ^d
Log <i>K</i> _{oc}	3.57 ^a	3.57 ^a	3.8 ^a	3.0, 3.57 ^a 3.04 ^g
Vapor pressure (mm Hg)	4.5 × 10 ⁻⁵ at 25 °C ^a	3.6 × 10 ⁻⁷ at 20 °C ^a	3.5 × 10 ⁻⁵ at 25 °C ^a	4.2 × 10 ⁻⁵ at 20 °C ^a 9.4 × 10 ⁻⁶ at 20 °C ^c 3.1 × 10 ⁻⁵ at 25 °C ^d 3.3 × 10 ⁻⁵ at 20–25 °C ^g

^aSource: ATSDR (1989).^bSource: Willett et al. (1998).^cSource: CRC Handbook of Chemistry and Physics (Lide 2003).^dSource: Bintein Devillers (1996).^eMerck Index.^fAlthough this range of solubilities has been reported in the literature, the consensus value appears to be closer to 7–10 mg l⁻¹.^gSource: Paraiba Spadotto (2002).

HCH. These characteristics are also influenced by the orientation of the chlorine atoms on each HCH isomer. A list of chemical properties, such as solubility, vapor pressure and octanol/water partition coefficient, of the four most commonly found HCH isomers, is provided in Table 1. Many of these properties play key roles in the transport of HCHs, not only by affecting volatilization from soil surfaces into the atmosphere, but also within soil itself (Spencer & Cliath 1973). The volatilization of Lindane can be influenced by soil moisture content and may be facilitated by a proposed wicking or capillary effect, through which more soluble compounds are brought to the soil surface more quickly and more volatile compounds disappear from the surface more rapidly (Spencer & Cliath 1973). The rate of disappearance of the various HCH isomers could be explained, in part, by this effect (Chessells et al. 1988). Although soil moisture content is critical (Glottfelty & Schomburg 1989), volatilization of pesticides in soil is also dependent on tem-

perature, soil organic matter (influencing sorption), ambient relative humidity and other interacting factors. For example, it has been observed that adsorption of Lindane decreases as temperature increases (El Beit et al. 1981). Soil moisture content has been identified as a key factor influencing Lindane transport within soil and, along with temperature, was incorporated into a model for volatilization from surface soil (Cohen & Ryan 1989). Paraiba and Spadotto (2002) reported a relationship between soil temperature and the retardation factor, a calculated number that represents the delay of pesticide leaching in soil as it is affected by sorption, volatilization and solubility. Oxic bioremediation practices for contaminated soil normally involve tillage to promote aeration. It is important to consider that tillage not only promotes aeration and aerobic biodegradation processes, but affects the exposed surface area and soil moisture content, with consequences with respect to volatilization (Glottfelty & Schomburg 1989).

As with many chlorinated pesticides, HCHs dissolved in water will degrade abiotically if exposed to sunlight. The extent of photodegradation depends on light intensity and temperature (Malaiyandi et al. 1982), or the presence of photooxidizers (Malaiyandi et al. 1982; Prakash et al. 1994). There is also evidence that HCHs are chemically decomposed under alkaline conditions (El Beit et al. 1981). Yule et al. (1967) demonstrated the breakdown of γ -HCH in buffered water at pH 8 but not at pH 6 to 7.

Isomerization plays a role in the stability and persistence of HCH isomers in a contaminated soil by changing the relative concentrations of certain isomers without resulting in overall concentration reductions. As a result, isomerization might either enhance or inhibit successful bioremediation of a contaminated site, depending on the stability of the isomers that are converted and those that are produced. Isomerization of certain HCHs has been observed by both biotic or abiotic processes. Abiotic isomerization of γ -HCH to α -HCH has been reported in aqueous solutions exposed to sunlight (Malaiyandi et al. 1982) while isomerization of β -HCH to δ -, α - and γ -HCH was observed in sterile deionized water (Deo et al. 1980). The topic of biotic isomerization is relevant to any discussion of biodegradation processes, and it is included in this section as it pertains to the persistence of HCH contamination. Evidence of biotic isomerization of HCHs has been reported for bench-scale studies of sediment and soil slurries, and bacterial cultures. However, enzymes responsible for biotic isomerization have not been identified. Isomerization of γ -HCH to α -HCH, in 600 ml bottles of sediment and seawater under anoxic conditions, was observed by Benezet and Matsumura (1973). Vonk and Quirijns (1979) found detectable amounts of α -HCH in submerged (anoxic) soil slurries that had been spiked with γ -HCH, but not in moist (oxic) soils or sterile soils. Kohnen et al. (1975) reported that 1.1% of ^{14}C - γ -HCH isomerized to α -HCH in microcosms containing 100 g submerged soil and spiked with 10–20 mg kg⁻¹ ^{14}C - γ -HCH. Only 0.2% of the spike was isomerized in autoclaved soil. An enrichment culture of marine microorganisms has also been found to convert γ -HCH to α -HCH (Huhnerfuss et al. 1992).

Isomerization by *Pseudomonas putida* was investigated by Benezet and Matsumura (1973)

who reported elevated α -HCH concentrations in pure cultures that had been spiked with γ -HCH, and in inoculated microcosms of sediment spiked with γ -HCH and incubated under simulated natural conditions for 30 days. *Escherichia coli* cells were also found to isomerize α -HCH to γ -HCH in rich medium incubated under anoxic conditions but not in the presence of oxygen, lending further evidence of bioisomerization by microorganisms (Vonk & Quirijns 1979).

Wu et al. (1997) demonstrated isomerization of α -HCH to γ -, δ -, and predominantly β -HCH, under both oxic and anoxic conditions in a bench-scale sediment/water system. Organic nutrient addition enhanced the rate of isomerization, which was concluded to be primarily a biotic process. The significance of these findings lies in the relative persistence of the different isomers of HCH. Predominant formation of significant amounts of β -HCH from other isomers could result in increased stability of the overall contamination, and a reduced bioremediation rate. In contrast, formation of more, or equally, readily biodegraded isomers might merely be considered a preliminary step towards biodegradation. The contribution of isomerization to overall bioremediation rates is also dependent upon the quantities of HCH being converted. A conversion of 1% as detected by Kohnen et al. (1975) might result in significant changes to residual HCH concentrations following bioremediation treatment depending on which isomers are involved and the initial concentration of each. In a 4-year field study on the disappearance of γ -HCH applied twice each spring and twice each autumn, at 1.7 kg per hectare, Waliszewski (1993) concluded that isomerization of γ -HCH to β -HCH was possible under field conditions in sandy soils, but is not likely a major route of γ -HCH removal or introduction of other isomers of HCH into the soil environment.

Environmental factors affecting biodegradation of HCHs in pure cultures, soil microcosms and field plots

HCH degradation under different redox conditions

HCH biodegradation was initially thought to be an anaerobic process (Castro & Yoshida 1974; MacRae et al. 1967, 1969, 1984; Sethunathan et al.

1983) and dechlorination of all four commonly found HCH isomers (α , β , δ , γ) has been observed under anoxic conditions in soil microcosms (Jagnow et al. 1977; MacRae et al. 1967), soil slurries (MacRae et al. 1984; Siddaramappa & Sethunathan 1975), field studies involving flooded soils (Chessells et al. 1988), pure cultures (Jagnow et al. 1977), and *in situ* in groundwater plumes contaminated with HCHs (Langenhoff et al. 2002). Chessells et al. (1988) reported a correlation between soil moisture content and removal rates of all four isomers in field agricultural soils treated with an HCH pesticide formulation. Enhanced HCH removal in soils with higher moisture contents was explained by the likely resultant anoxic state during flooding. Flooded soils are generally assumed to be anoxic, containing microorganisms capable of predominantly anaerobic metabolism. Based on early studies with flooded soil microcosms, and the assumption that HCH degradation was anaerobic, MacRae et al. (1967) suggested flood fallowing as a way to reduce soil HCH concentrations. However, oxic conditions may exist at the water-air interface, and in certain saturated or nearly saturated soils (Siddaramappa & Sethunathan 1975), therefore confirmation of the soil redox potential is necessary to conclusively state that HCH biodegradation is an anaerobic process occurring under anoxic conditions.

Definitive assessment of the redox potential during a bioremediation study involving soil or soil slurries is important since it is known that certain HCHs (α - and γ -isomers) are also biodegraded aerobically in soil and soil slurries in microcosms (Bachmann et al. 1988a,b; Bhuyan et al. 1992; Francis et al. 1975; MacRae et al. 1984; Wada et al. 1989), field plots of soil (Doelman et al. 1990), and in pure cultures (Johri et al. 1998). In a field study by Doelman et al. (1990), α -HCH was degraded under oxic conditions in small plots of either moist soil or soil slurries. Bachmann et al. (1988b) studied the degradation of α -HCH under oxic, denitrifying, sulfate-reducing and methanogenic conditions in soil slurries for a 100 day period. Degradation was most rapid under oxic conditions (23 mg kg⁻¹ per day). Significant reductions were also observed under methanogenic conditions (13 mg kg⁻¹ per day), although the metabolites detected in each redox environment differed. In this study, α -HCH did not appear to degrade significantly under denitri-

fying or sulfate-reducing conditions, however, Van Eekert et al. (1998) reported removal of α -HCH from a sandy soil containing a low concentration of the isomer (37 mg kg⁻¹) in slurries where lactate or sulfide had been added to reduce the redox potential. Middeldorp et al. (1996) reported that α -HCH was degraded in glass columns packed with contaminated sediments, and held under methanogenic conditions, although the degrading population of microorganisms appeared not to be methanogens.

Middeldorp et al. (1996) also observed degradation of β -, δ -, and γ -isomers in the methanogenic glass columns described above, providing evidence of the removal of these isomers under anoxic conditions. MacRae et al. (1984) found that although biodegradation of α -, β -, δ -, and γ -isomers was generally greater in soil suspensions incubated under anoxic conditions, aerobic removal of all four isomers could be detected in the presence of no amendment (α , β , δ), amino acids (α , β , γ), yeast extract (α , δ , γ), urea (β , γ , δ) or inositol (α , γ). In the same study, δ -HCH did not degrade anaerobically without the addition of amendments. In another study, γ -HCH degraded more quickly in flooded soils that had lower negative redox potentials than in those with higher negative or positive potentials (Siddaramappa & Sethunathan 1975). Evidence of aerobic γ -HCH degradation in flooded soil samples was reported by Bhuyan et al. (1992). Yule et al. (1967) described γ -HCH degradation under oxic conditions. The extent of γ -HCH degradation was greater in moist soil than in dry soil, presumably because the microorganisms involved in the degradation process were more active in the moist soil.

The β -HCH isomer is indisputably the most recalcitrant isomer, and does not undergo biodegradation easily. β -HCH concentrations did not decrease noticeably in a field study by Doelman et al. (1990) under any treatment conditions (moist soil and oxic soil slurries in small plots). This isomer was also reported by Bachmann et al. (1988b) to be recalcitrant under the four redox conditions tested in soil slurry at bench scale (oxic, methanogenic, sulfate-reducing and nitrate-reducing). Several studies have suggested that significant β -HCH degradation does not occur under oxic conditions. For example, Siddaramappa & Sethunathan (1975) reported that a lower redox potential was needed for β -HCH removal, than for

γ -HCH, and that both isomers were degraded rapidly when redox potentials were between -40 and -100 mV. Van Eerkert et al. (1998) also showed that a strongly negative redox potential is required for β -HCH biodegradation. In contrast, MacRae et al. (1984) reported 14% removal of β -HCH from a soil slurry incubated under oxic conditions, compared to 11% removal under anoxic conditions and degradation of β -HCH has also been demonstrated in solid phase, oxic soil microcosms (Phillips et al. 2000c). However, removal of this isomer in pilot or field-scale plots, under either oxic or anoxic conditions, remains a challenge.

Complete mineralization of HCHs is generally only reported under oxic conditions (Bachmann

et al. 1988b; Huntjens et al. 1988; Nagasawa et al. 1993; Sahu et al. 1995). A summary of published mineralization data is provided in Table 2. Aerobic mineralization of ^{14}C - α -HCH has been reported in a soil slurry (Bachmann et al. 1988b) and a pure culture of *Pseudomonas vesicularis* (Huntjens et al. 1988). Aerobic mineralization of ^{14}C - γ -HCH was measured in a pure culture of *S. paucimobilis* UT26 (Nagasawa et al. 1993) and aerobic mineralization of ^{14}C - α -, β - and γ -HCH by a *Pseudomonas* sp. has been reported (Sahu et al. 1995). Examples of ^{14}C - γ -HCH mineralization in submerged soils were reported (Kohnen et al. 1975; MacRae et al. 1967) with the assumption that anoxic conditions prevailed in the microcosms. However, anoxic conditions were not confirmed by measurement of redox

Table 2. Mineralization of radiolabelled HCH isomers in soils, soil slurries and by pure cultures

HCH isomer	Microorganisms and media	Initial amount of ^{14}C added (specific activity)	Percentage Recovery as $^{14}\text{CO}_2$ (%)	Duration	Reference
γ	Pure culture <i>Sphingomonas paucimobilis</i> UT26 (10 ml)	4.7×10^{10} dpm	9.3	4 d	Nagasawa et al. (1993)
α	Pure culture <i>Pseudomonas vesicularis</i> (4 ml)	2.05×10^7 dpm	50	50 h	Huntjens et al. (1988)
α	Pure culture <i>Pseudomonas</i> sp. (10 ml)	1.0×10^5 dpm	10	48 h	Sahu et al. (1995)
β	Pure culture <i>Pseudomonas</i> sp. (10 ml)	1.2×10^5 dpm	<5	240 h	Sahu et al. (1995)
γ	Pure culture <i>Pseudomonas</i> sp. (10 ml)	1.1×10^5 dpm	11.5	48 h	Sahu et al. (1995)
γ	<i>Phanerochaete chrysosporium</i> BKM-F-1767				
	(1) Soil/corn cob mix	(1) 5.6×10^5 dpm	(1) 22.8	(1) 60 d	(1) Kennedy et al. (1990)
	(2) 10 ml media	(2) 1.5×10^5 dpm	(2) 15.3	(2) 30 d	(2) Bumpus et al. (1985)
	(3) 25 g soil	(3) 2.0×10^5 dpm	(3) 49.1	(3) 9 wk	(3) Mougin et al. (1997)
α	Aerobic soil slurry	NA ^a	20–40	NA	Bachmann et al. (1988b)
γ	Three different submerged soils (20 g)	2.2×10^5 dpm	16.4–39.3 ^b	60 d	MacRae et al. (1967)
γ	(1) Submerged soil, (2) Aerated moist soil, (3) Aerated moist soil + organic amendment (50 or 100 g dry wt)	10–20 ppm (NA)	(1) 17.8 ^c , (2) 3, (3) 16.7	(1) 140 d, (2) 105 d, (3) 105 d	Kohnen et al. (1975)
γ	(1) Nearly saturated, (2) Aerated moist soil (95 g dry wt)	1×10^6 dpm	(1) < 10 (2) 47	(1) 275 d (2) 80 d	Phillips et al. (2004)

^aNA = information not available.

^b4.4% recovery in 15 d and 27.9% recovery in 30 d in the most rapidly mineralizing soil.

^cAuthors report 47.8% recovery from submerged soil in 130 d in preceding unpublished experiment.

potential, nor was anaerobic degradation confirmed. Phillips et al. (2004) reported that negligible mineralization of ^{14}C - γ -HCH occurred in soil microcosms where highly anoxic conditions were alternated with aeration and oxic conditions. In contrast, considerable mineralization (47% recovery of $^{14}\text{CO}_2$) was detected in strictly oxic microcosms after 80 days incubation (Table 2). These results suggest that although HCH removal is observed under both oxic and anoxic bioremediation treatments (Phillips et al. 2000c), oxic treatment most frequently results in the most complete removal via mineralization.

Mixed chlorobenzenes (CBs) and benzene are anaerobic degradation products of γ -HCH by marine microorganisms, cyanobacteria (Kuritz & Wolk 1995), sulfate-reducing bacteria (Boyle et al. 1999) and other unidentified anaerobes (Middel-dorp et al. 1996), and in soil slurries under different redox conditions (Bachmann et al. 1988a,b). Monochlorobenzene (MCB) and benzene also appeared to be the dominant metabolites produced during biodegradation of β -HCH under anoxic conditions (Middel-dorp et al. 1996; Van Eekert et al. 1998). Their formation was proposed to occur either spontaneously (MCB) or enzymatically (benzene) from tetrachlorocyclohexane (TCCH) via the putative unstable intermediate dichlorocyclohexadiene. Certain microorganisms can aerobically mineralize MCB (Mars et al. 1997) and benzene (Deeb & Alvarez-Cohen 2000; Fairlee et al. 1997), and benzene can also be degraded or mineralized anaerobically (Edwards & Grbic-Galic 1992; Rooney-Varga et al. 1999; Wilson & Bouwer 1997). Therefore, a bioremediation strategy that applies a combination of anoxic conditions to initiate HCH dechlorination, followed by oxic conditions to facilitate complete removal of the subsequent biodegradation products, might result in complete mineralization of the HCH contaminants (Langenhoff et al. 2002).

Bioavailability

The persistence of organic contaminants is often mediated, in part, by the extent of partitioning between liquid and solid phases of the soil. Compounds with high solubility are more mobile and susceptible to leaching, but are also in closer proximity to microorganisms in the liquid phase. Sorption to the surface of soil particles reduces

mobility but increases the proximity of contaminants to surface-bound microorganisms. Thus, the same factors that affect solubility and sorption of HCHs influence their movement within the soil matrix, thereby affecting their bioavailability and biodegradation. Factors that affect the volatility of HCHs (temperature, humidity, vapor pressure, soil organic matter and moisture) can influence biodegradation rates, in that the extent to which they volatilize through air pockets of the soil, or escape from the surface, affect their concentrations in the solid and liquid phases of the soil and, as a consequence, their bioavailability (El Beit et al. 1981).

It is generally assumed, for organic contaminants, that increased soil organic matter results in increased adsorption (El Beit et al. 1981), thus reducing bioavailability. However, a higher organic matter content might also increase microbial activity thus enhancing biodegradation rates. High clay content in a soil can also increase sorption of γ -HCH and data has been reported that suggests calcareous materials and other soil constituents influence α -HCH bioavailability through sorption (Rijnaarts et al. 1990). Minerals such as iron and magnesium in soil affect HCH degradation by increasing retention of the pesticide, or by influencing the soil pH (El Beit et al. 1981). In addition to affecting sorption and soil pH, soil composition can also affect bulk density and water retention, all of which, in turn, affect aeration, nutrient availability, bioavailability and biodegradability.

In a demonstration of the effects of different soil characteristics on HCH degradation, Siddaramappa & Sethunathan (1975) added ^{14}C -labelled β - and γ -HCH to tubes containing five different rice soils from India. Removal of these isomers was negligible in the sandy soil that contained only 0.02% organic matter and had a pH of 6.1, and in the soil designated 'kari' with 27.8% organic matter and a pH of 3.0. In soils with pH values ranging from 4.2 to 6.2 and moderate organic carbon concentrations ranging from 1.61% to 8.21%, γ -HCH removal rates ranged from 95% to 98%, and removal rates for β -HCH ranged from 67% to 97%, in 41 days. The kari soil had a high electrical conductivity ($15.0 \text{ mmhos cm}^{-1}$; a measure of the dissolved salts in the soil aqueous phase), relative to the other soils which ranged from 0.2 to $8.5 \text{ mmhos cm}^{-1}$. Soil redox potentials remained positive throughout the study in the kari

and sandy soils, in contrast to the negative values obtained for the other soils. These data demonstrate how different soil types can affect redox potential under the same treatment conditions, and how this and differences in pH and organic matter might contribute to differences in HCH removal rates.

Kalsch et al. (1998) used partitioning and sorption of a ^{14}C - γ -HCH spike in columns of sediment and water to demonstrate the impact that particle size distribution in the sediment might have on HCH degradation. More rapid mineralization of the radiolabelled pesticide to $^{14}\text{CO}_2$ was observed in coarse than in fine sediments. Enzymatic (dehydrogenase and esterase) activities of microorganisms in the columns were inversely correlated with HCH degradation rate and could not be used to predict degradation potential of a sediment. Regression analysis between isomer concentrations and soil properties in grid sections of a field site treated annually with HCHs has indicated that a relationship might exist between percent silt and γ -HCH concentrations (Chessells et al. 1988). The higher silt content in a soil results in higher moisture retention, which, it was suggested, increases bioavailability by inhibiting sorption of γ -HCH. Correlations between α -, δ - and γ -isomer concentrations and the organic matter and moisture contents indicated that these parameters were also important. In particular, isomer concentrations were consistently lower in soils with moisture contents higher than the mean of the study plus 1/2 standard deviation. A high correlation coefficient between δ -HCH concentration and percent organic carbon suggested that degradation of this isomer was strongly influenced by sorption to organic matter in soil. The authors proposed that the relatively high octanol : water partition coefficient of this isomer ($\log K_{ow} = 4.14$) rendered it even more susceptible to sorption to organic matter in the soil than the other isomers.

Survival, growth and habitats of indigenous HCH-assimilating *Sphingomonas paucimobilis* and inoculum of *S. paucimobilis* strain SS86 were studied in soil to which γ -HCH had been added (Nishiyama et al. 1992). Long-term survival of the microorganisms appeared to be associated with localization on soil aggregates larger than 0.025 mm. Growth of these microorganisms was detected primarily in inter-aggregate pores just after application of γ -HCH, but these organisms

died within 12 weeks, once the supply of HCH was exhausted. A high density of indigenous *S. paucimobilis* was found in the plant debris fraction of the soil and the authors postulated that organic (plant) matter integrated into the soil aggregates served as a micro-habitat rich in growth substrates. This is the underlying principle for application of plant-derived organic amendments to soil as a bioremediation strategy (see below).

A *Pandoraea* species, obtained by enrichment culture (Okeke et al. 2002), was used to study the effects of pH and temperature on HCH degradation in water and soil slurries (Siddique et al. 2002). Although the organism degraded HCHs over a pH range of 4–9, the optimum pH for growth and biodegradation of the α - and γ -isomers in soil slurries was 9. The initial dechlorination reactions caused the pH of the media to fall to as low as 3.3. This effect needs to be considered when extrapolating data to pilot and field-scale studies, as a reduction in pH may hinder further biodegradation of HCH residues left in soil. In another mixed consortium, dechlorination of α -HCH was optimal at more neutral pH (6–8) (Manonmani et al. 2000). A strain of *Clostridium rectum* was isolated that degraded Lindane optimally at pH 7–8 in anaerobic pure culture studies (Ohisa & Yamaguchi 1978).

The optimal temperature for HCH degradation by the above consortium and *Pandoraea* sp. was approximately 30 °C (Manonmani et al. 2000; Siddique et al. 2002). In general, the optimum temperature for HCH biodegradation ranges from 25 to 30 °C in soil (Bhuyan et al. 1993), soil slurry (Bachmann et al. 1988a; Siddique et al. 2002), and bacterial cultures (Manonmani et al. 2000; Siddique et al. 2002). The *Clostridium rectum* strain that dechlorinated Lindane did so at an optimum temperature of 37 to 38 °C (Ohisa & Yamaguchi 1978). Biodegradation appears to slow down at temperatures as high as 40 °C (Manonmani et al. 2000) or as low as 5 °C (Kalsch et al. 1998). The optimum temperature for degradation may vary in field environments depending on the bacterial population. An increase in temperature might enhance HCH removal simply by increasing biological activity (within limits), or might also affect biodegradation by enhancing bioavailability through reduced sorption (El Beit et al. 1981). Bacteria capable of degrading HCHs at extreme temperatures (<5 °C or >40 °C) have not been

reported. Some studies suggest that the influence of temperature is not as significant as that of other soil conditions and characteristics (i.e., redox conditions, moisture content) (Doelman et al. 1988a,b) that affect volatility, sorption and bioavailability. Doelman et al. (1990) compared HCH degradation rates in field plots of soil that were heated to plots that were not. The temperature of the heated plot, at 5 and 40 cm depths ranged from 20 to 32 °C and 35 to 45 °C, respectively, over the course of four months, while untreated soil temperatures ranged from 15 to 35 °C and 8 to 24 °C, at 5 and 40 cm depths, respectively. The degradation data suggested that temperature was not a significant factor in the aerobic degradation of α -HCH in small field plots of moist soil or soil slurries.

As previously noted, physical characteristics such as soil moisture content and composition can affect the efficacy of a bioremediation treatment protocol by changing the bioavailability of contaminants to indigenous soil microorganisms. Increasing moisture contents to create slurry conditions, combined with regular stirring, can improve mass transport and thus bioavailability of HCHs to bacteria (Doelman et al. 1990). Increased soil water may also enhance bioavailability if the degradation rate is limited by dissolved pesticide concentrations or desorption velocities (Rijnaarts et al. 1990), unless the pesticide is so hydrophobic that dissolution depends on biosurfactants, in which case, lower moisture contents may be more desirable. The effects of natural or synthetic emulsifiers on HCH degradation have not been addressed in the literature.

The results of Rijnaarts et al. (1990) indicated that mass transfer rates for desorbed material (facilitated by mixing and diffusion) influenced the rate of biodegradation, particularly of α - and γ -HCH. These authors suggested that microorganisms might be able to penetrate soil aggregates, thereby reducing the distance required for desorbed contaminants to travel to reach the microorganisms. This would increase desorption rates, as they are dependent on the contaminant concentration in the aqueous phase. These data support the postulate that microorganisms increase diffusion rates, when situated close to porous soil constituents on which contaminants are sorbed, by two means: (1) removing contaminants through biodegradation, thereby

increasing the concentration gradient, and (2) decreasing the diffusion distance. The moisture content of a soil/water slurry may affect degradation by individual bacterial strains (Siddique et al. 2002) and has a significant impact on sorption (Wu et al. 1997). However, considerable biodegradation of HCHs has been observed, not only in liquid media and soil slurries, but also in non-saturated soil (i.e. soil treated in solid-phase), indicating that although moisture content is relevant to degradation rates, it is not the most influential parameter.

Doelman et al. (1988b) studied the effects of different aeration/stirring protocols, temperature and bioaugmentation, on HCH removal, using 60 kg soil samples incubated in a glasshouse. α -HCH was removed aerobically as long as temperatures were kept ambient (15–32 °C) and degradation was partially dependent on availability, which was controlled by sorption and influenced by moisture content and temperature. β -HCH was not degraded in these experiments, and bioaugmentation with indigenous microorganisms did not enhance degradation of either isomer. In a subsequent study, the effects of aeration, mixing and temperature, on the degradation of α - and β -HCH, were studied in box plots (140 × 110 × 40 cm) of a moist aerated soil at 10% moisture content, and in tubes (110 × 90 cm diameter) containing a soil slurry at 50% moisture content (Doelman et al. 1990). The purpose of this study was to determine if rates of HCH removal were fast enough to make soil bioremediation a feasible solution to full-scale treatment of a contaminated site. Removal of β -HCH (280 mg kg⁻¹) was not observed in any treatments. Degradation of α -HCH (367 mg kg⁻¹) was most rapid in the aerated slurry, with 83% removal after 23 weeks. In the moist soil, 71% removal was observed after 23 weeks. Regular tillage, aeration and covering the soil with PVC film did not enhance removal rates. Although the results indicated that degradation of α -HCH was possible in both solid phase soil and soil slurry, target concentrations of 10 mg kg⁻¹ were not attained and the authors suggested that more time for treatment or increased bioavailability of residual quantities of α -HCH may be required. It should also be considered, however, that if the enzymes or transport systems responsible for HCH degradation have relatively low affinity for their substrates

(high K_m values), low concentrations of HCH will persist in the environment indefinitely.

HCH concentration

A significant influence on biodegradation of HCHs could be the concentration at which they are present in contaminated soils. There is evidence that pesticide biodegradation rates in soil follow first-order kinetics and are concentration-dependent. As a consequence, HCH removal rates might be expected to slow as the concentrations are reduced (El Beit et al. 1981). HCH may also affect soil microbial populations, stimulating growth of certain microorganisms and exerting toxic effects and inhibiting growth of others.

Agricultural rates of HCH application to soils were reportedly as high as 29 kg Lindane $\text{ha}^{-1} \text{yr}^{-1}$ in the 1950s (Stewart & Chisholm 1971). A Japanese study in the 1970s reported using 10 kg Lindane ha^{-1} applied yearly to field plots (Wada et al. 1989), while an application rate of 0.15 kg Lindane ha^{-1} was reported as normal in the 1980s by Boehncke et al. (1990). The majority of remaining sources have cited typical application rates of Lindane, from the 1960s to present, that range from 1 to 6 kg ha^{-1} , or 1 to 15 mg kg^{-1} soil (Bharati et al. 1998; Bintein & Devillers 1996; Doelman et al. 1990; MacRae et al. 1967; Martinez-Toledo et al. 1993; Waliszewski 1993). Research on the effects of these typical application rates have included studies where γ -HCH was applied in a single dose, or in repeated applications such as the 1 kg ha^{-1} Lindane applied every 15 days to flooded and non-flooded soils by Bhuyan et al. (1992). In general, these application rates have not been found to significantly affect total numbers of soil microorganisms (Bollen et al. 1954; El Beit et al. 1981; Martinez-Toledo et al. 1993). Martinez-Toledo et al. (1993) found that total bacterial and fungal numbers were unaffected by one or two applications of 2.0 to 15.0 kg ha^{-1} Lindane in soil microcosms, during 30 days incubation.

In the study by Martinez-Toledo et al. (1993), nitrogenase activity and denitrifying bacteria were also unaffected by applications of 2.0 to 15.0 kg ha^{-1} Lindane, however, the numbers of nitrifying bacteria were significantly reduced. Further evidence that γ -HCH inhibits nitrification was provided by Ogunseitan & Odeyemi (1985). In

soil microcosms spiked with 100 mg kg^{-1} Lindane (20% active ingredient), they observed that bacterial and fungal populations were not significantly affected over a 30-day period, and soil respiration was enhanced, but nitrification of ammonium sulfate was suppressed in the first 30 days after addition of Lindane.

In soil and culture media containing higher HCH concentrations, that are more representative of what might be found at post-production sites and waste disposal sites, the numbers of some microbial species or their activities may be affected. For example, data collected from five different soil plots, spiked with 10 to 22 kg ha^{-1} γ -HCH, suggested a depressive effect on numbers of *Streptomyces* sp. despite an increase in total bacterial counts (Bollen et al. 1954). Feidieker et al. (1994) reported reduced microbial diversity in soil containing HCHs (up to 11,055 mg kg^{-1}) and other chlorinated aromatic compounds (up to 18,500 mg kg^{-1} CB and 132 mg kg^{-1} CP), using selective culture techniques, metabolic testing on microtitre plates and fatty acid analyses. In a 70-day study, Rodriguez & Toranzos (2003) used Biolog, 16S rDNA and denaturing gradient gel electrophoresis (DGGE) analyses to monitor the diversity of bacteria in a tropical soil with a history of HCH contamination and spiked with 100 mg kg^{-1} γ -HCH. Within two weeks, overall bacterial cell numbers were reduced by 50% relative to the non-spiked control. However, by the end of 70 days there was no difference between the test soil and the control, in bacterial numbers or diversity, as determined by the tests used.

Bioremediation of HCH-contaminated soil is usually only necessary on industrial post-production, or waste dumping sites, as opposed to crop fields where application rates were lower and the HCHs are expected to be degraded by naturally occurring microorganisms. There are limited studies on biodegradation of HCH at concentrations above 500 mg kg^{-1} . Doelman et al. (1985) utilized glass jars containing 25 g aliquots of a soil containing 5334 mg kg^{-1} α -HCH. In another study, soil containing 367 and 280 mg kg^{-1} α - and β -HCH, respectively, was used in small field plots (Doelman et al. 1990), and additional studies have been reported using soil containing 400 mg kg^{-1} α -HCH, 250 mg kg^{-1} β -HCH and much lower concentrations of δ - and γ -HCH (13 and 22 mg kg^{-1} , respectively), in bench-scale micro-

cosms (Bachmann et al. 1988a,b). A bench-scale laboratory study showed successful bioremediation of soil containing 4122 mg kg⁻¹ total HCHs, consisting of 2710, 826, 491 and 95 mg kg⁻¹ of α -, β -, δ - and γ -HCH isomers, respectively (Phillips et al. 2000c). Over 90% of total HCHs was degraded in 251 days in the most effective treatment. To our knowledge, there is no published information on the bioremediation of soils containing higher concentrations of HCHs in the field. Bench-scale and field studies with soils of higher concentrations of HCH are needed in order to evaluate the potential to bioremediate highly contaminated soils.

Metabolic pathways of HCH degradation microorganisms

Isolation of Lindane-degrading microorganisms by enrichment culture has confirmed the ability of specific species of bacteria to degrade HCHs either aerobically or anaerobically. Some of these strains are able to grow on HCH as a sole carbon source. Several bacteria capable of degrading Lindane and other HCH isomers have been described. These include *Clostridium sphenoides* UQM780 (Heritage & MacRae 1977), *Clostridium rectum* S-17 (Ohisa & Yamaguchi 1978), and several other *Clostridium* sp. (Jagnow et al. 1977), a strain of *E. coli* isolated from rat feces (Francis et al. 1975), two species of *Bacillus* (Yule et al. 1967), several *Pseudomonas* (Huntjens et al. 1988; Nawab et al. 2003; Sahu et al. 1990; Tu 1976) and *Sphingomonas* (Adhya et al. 1996; Senoo & Wada 1989) species, a *Pandora* sp. (Okeke et al. 2002), *Citrobacter freundii* (Jagnow et al. 1977), and *Rhodanobacter lindaniclasticus* (Thomas et al. 1996). A summary of the HCH-degrading microorganisms discussed below is provided in Table 3.

Anaerobic HCH degradation in bacteria

In the absence of a bacterial model for HCH degradation, rat liver microsomes (enriched in cytochrome P-450) were initially used to study the degradation and relative rates of dechlorination of α -, γ - and β -HCH (Beurskens et al. 1991). Metabolic intermediates detected from microsomal degradation of α - and γ -HCH, under anoxic conditions, were δ -3,4,5,6-tetrachlorocy-

clohexene (TCCH) and monochlorobenzene (MCB). TCCH was later identified as a metabolite using soil microcosms and pure cultures under both oxic and anoxic conditions, while MCB was identified as a metabolite of HCH biodegradation in anoxic soils.

The anaerobic dechlorination rates of the HCH isomers by rat liver microsomes were isomer-specific and could be explained by the proposed reaction mechanisms and the orientation of chlorine atoms on the molecule. The ring structure of HCH prevents rotation about the carbon-carbon bonds, making vicinal elimination of chlorines dependent on the presence of axial chlorines oriented opposite to each other (anti-parallel or *trans* diaxial configuration). β -HCH has no such chlorine pairs and was not dechlorinated, while α -HCH has one pair and γ -HCH has two. The rate of degradation of γ -HCH was 8-fold higher than that of α -HCH. In an aerobic assay with the microsomes, a hydrogen atom and a chlorine atom, also preferably anti-parallel, are removed. Again, β -HCH has no such pairings, but α - and γ -HCH each have two. In the case of the aerobic reaction, however, other unknown factors prevented the degradation rates of these two isomers from being equal. This study provided useful explanations for the relative reactivity of these three isomers that can be applied to microbial systems in soil.

HCH degradation studies in methanogenic glass columns packed with contaminated sediments revealed an anaerobic degradation pathway for the β -isomer that differed from those determined for α - and γ -HCH (Middeldorp et al. 1996). TCCH was identified by gas chromatography-mass spectroscopy (GC-MS) as an intermediate of β -HCH degradation, and the degradation pathway was proposed to proceed via anaerobic dihalo-elimination producing TCCH, to two end products, MCB and benzene, that accumulated in the medium. A schematic diagram depicting this degradation pathway is provided in Figure 2. MCB was detected from all four isomers, but TCCH was not detected as a metabolite of γ - and δ -HCH.

The first HCH-degrading microorganism to be isolated was *Clostridium sphenoides* UQM780, which anaerobically reduced the concentration of Lindane in minimal salts media (MacRae et al. 1969). Further studies with this microorganism

Table 3. Summary of microorganisms with demonstrated ability to degrade HCHs

Microorganism	HCH degradation characteristics	Reference
Bacteria	Aerobic degradation	
<i>Bacillus</i> sp.	Dechlorination ^a (γ -HCH)	Yule et al. (1967)
Cyanobacteria: <i>Anabaena</i> sp. strain PCC 7120, and <i>Nostoc ellipsoforum</i>	Dechlorination (γ -HCH)	Kuritz & Wolk (1995)
<i>Escherichia coli</i>	Dechlorination (γ -HCH)	Francis et al. (1975)
<i>Pandoraea</i> sp.	Utilized as sole carbon source (γ -HCH); Dechlorination (α -HCH)	Okeke et al. (2002)
<i>Pseudomonas</i> sp.	Dechlorination (γ -HCH)	Tu (1976)
<i>Pseudomonas</i> sp.	Mineralization ^b (α -, β - and γ -HCH); Dechlorination (δ -HCH)	Sahu et al. (1992, 1995)
<i>Pseudomonas</i> sp.	Dechlorination (γ -HCH)	Nawab et al. (2003)
<i>Pseudomonas putida</i>	Dechlorination (γ -HCH); Isomerization of γ -HCH to α -HCH	Benezet & Matsumura (1973)
<i>Pseudomonas vesicularis</i> P59	Mineralization (α -HCH)	Huntjens et al. (1988)
<i>Rhodanobacter lindaniclasticus</i>	Dechlorination (α - and γ -HCH)	Thomas et al. (1996)
<i>Sphingomonas paucimobilis</i> B90	Utilized as sole carbon source (α -, β -, δ - and γ -HCH); implies mineralization.	Adhya et al. (1996)
<i>Sphingomonas paucimobilis</i> UT26	Mineralization (γ -HCH); Dechlorination (α - and δ -HCH)	Nagasawa et al. (1993) and Senoo & Wada (1989)
	Anaerobic degradation	
<i>Citrobacter freundii</i>	Dechlorination (γ -HCH)	Jagnow et al. (1977)
<i>Clostridium rectum</i> S-17	Dechlorination (γ -HCH)	Ohisa & Yamaguchi (1978)
<i>Clostridium sphenoides</i> UQM780	Utilized as sole carbon source (α -, β -, δ - and γ -HCH)	MacRae et al. (1969)
<i>Desulfovibrio</i> sp.	Dechlorination (γ -HCH)	Boyle et al. (1999)
Fungi		
<i>Cyathus bulleri</i>	Aerobic dechlorination (γ -HCH)	Singh & Kuhad (2000)
DSPM95	Aerobic dechlorination (γ -HCH)	Tekere (2002)
<i>Phanerochaete chrysosporium</i> BKM-F-1767	Aerobic mineralization (γ -HCH)	Bumpus et al. (1985)
<i>Phanerochaete sordida</i>	Aerobic dechlorination (γ -HCH)	Singh & Kuhad (2000)
<i>Pleurotus eryngii</i>	Aerobic dechlorination (γ -HCH)	Arisoy (1998)
<i>Pleurotus florida</i>	Aerobic dechlorination (γ -HCH)	Arisoy (1998)
<i>Pleurotus sajor-caju</i>	Aerobic dechlorination (γ -HCH)	Arisoy (1998)
<i>Trametes hirsutus</i>	Aerobic dechlorination (γ -HCH)	Singh & Kuhad (1999)

^aDechlorination means degradation of HCH has been measured but mineralization was either not tested or not detected.

^bMineralization means complete degradation to CO₂ as detected using radiolabelled tracers.

identified the degradation intermediates from α - and γ -HCH as δ - and γ -3,4,5,6-TCCH, respectively (Heritage & MacRae 1977). The primary intermediate of anaerobic Lindane degradation by *C. sphenoides* was postulated to be the reductive dechlorination product pentachlorocyclohexane (PCCH), which was too unstable to be detected.

The authors proposed that the reaction mechanism for the formation of this metabolite was similar to the mechanism for dechlorination of the pesticide DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane) to DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane) (Heritage & MacRae 1977; Sethunathan et al. 1969).

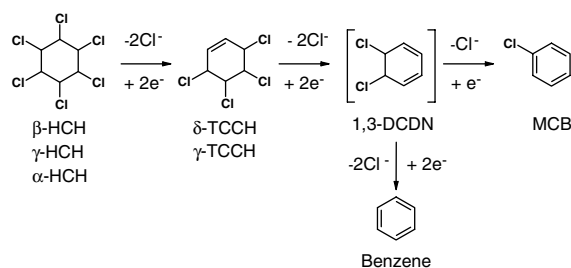


Figure 2. Proposed anaerobic degradation pathway of β -HCH, and conversion of α -, δ -, and γ -isomers to chlorobenzene. Pathway for α - and β -HCH was adapted from Middeldorp et al. (1996) and Van Eekert et al. (1998). Formation of TCCH from α - and γ -isomers was reported for *Clostridium* sp. (Heritage & MacRae 1977; Maule et al. 1987). Conversion of γ -HCH to CB was reported for cell extracts of *Clostridium rectum* (Ohisa et al. 1980), and conversion of γ -HCH to TCCH and benzene, in anaerobic sewage sludge (Beland et al. 1976). HCH: hexachlorocyclohexane, TCCH: tetrachlorocyclohexene, 1,3-DCDN: 5,6-dichloro-1,3-cyclohexadiene, MCB: monochlorobenzene.

TCCH was an intermediary metabolite of anaerobic γ -HCH degradation by several different microorganisms including other strains of *Clostridium* sp. and some *Bacillus* sp., based on GC retention times (Jagnow et al. 1977). The same authors confirmed the identity of this metabolite by GC-MS, as an intermediate of anaerobic γ -HCH degradation by *Citrobacter freundii*. The same metabolite of anaerobic γ -HCH degradation was also detected in a different mixed culture containing a large number of *Clostridium* sp. (Maule et al. 1987) and TCCH and benzene were intermediates in degradation of γ -HCH by another anaerobic mixed culture (Beland et al. 1976). Formation of small amounts of tri- and tetrachlorinated benzenes from anaerobic biodegradation of γ -HCH has also been reported (Deo et al. 1994; Jagnow et al. 1977).

Clostridium rectum S-17, isolated from a rice paddy soil, was also found to degrade γ -HCH anaerobically to γ -3,4,5,6-TCCH (Ohisa & Yamaguchi 1978). Further studies with this strain showed that expression of the enzymes required for HCH degradation appeared to be constitutive, in that there was no lag phase in the degradation of γ -HCH re-introduced to cultures that had been previously acclimated to HCHs, then subsequently grown in the absence of HCHs.

Aerobic HCH degradation in bacteria

The most well studied pathway for aerobic bacterial degradation of HCHs appears to be that found in *Sphingomonas paucimobilis* UT26 and summarized in Figure 3. In this pathway, Lindane is first subjected to dehydrochlorination to yield pentachlorocyclohexene (PCCH). Aerobic Lindane degradation by a *Pseudomonas* sp. isolated from a Canadian soil produced pentachlorobenzene, γ -PCCH and α -, β -, or γ -3,4,5,6-TCCH (Tu 1976). This organism could also degrade 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB) and 1,2,4,5-TeCB. The *Pseudomonas* sp. was not studied further, but studies with other aerobic Lindane degraders (fungi and several other bacterial species) revealed similar pathways leading to formation of dichlorophenols (DCP) and trichlorobenzenes (TCB), starting with formation of γ -PCCH (Francis et al. 1975; Nagasawa et al. 1993; Yule et al. 1967). PCCH was also detected as a metabolite of α -HCH degradation in aerobic soil slurries (Bachmann et al. 1988b) and as the product of γ -HCH degradation in moist, aerated soils (Yule et al. 1967). The *Pseudomonas putida* strain found to isomerize γ -HCH to α -HCH also produced γ -PCCH from γ -HCH (Benezet & Matsumura 1973).

An HCH-degrading *Pseudomonas vesicularis* P59 was isolated in the Netherlands (Huntjens et al. 1988) by enrichment culture from contaminated soil slurries. This strain mineralized ^{14}C - α -HCH as the sole carbon source in pure culture of mineral salts medium. The enzymes involved in HCH degradation appeared to be constitutively produced in that repeated cultures in rich media did not cause a lag phase prior to degradation when the culture was returned to minimal media containing HCHs.

A *Pseudomonas* sp. capable of aerobic growth on minimal salts media containing α - and γ -HCH as sole carbon sources was isolated from sugarcane rhizosphere soil (Sahu et al. 1990, 1992). The organism was able to degrade δ - and β -isomers in minimal salts media, but did not proliferate, nor did it degrade these isomers in inoculated soil (Sahu et al. 1993). PCCH was identified as a transient metabolite of δ - and γ -isomers by comparing GC retention times. Two metabolites of β -HCH degradation were tentatively identified as isomers of pentachlorocyclo-

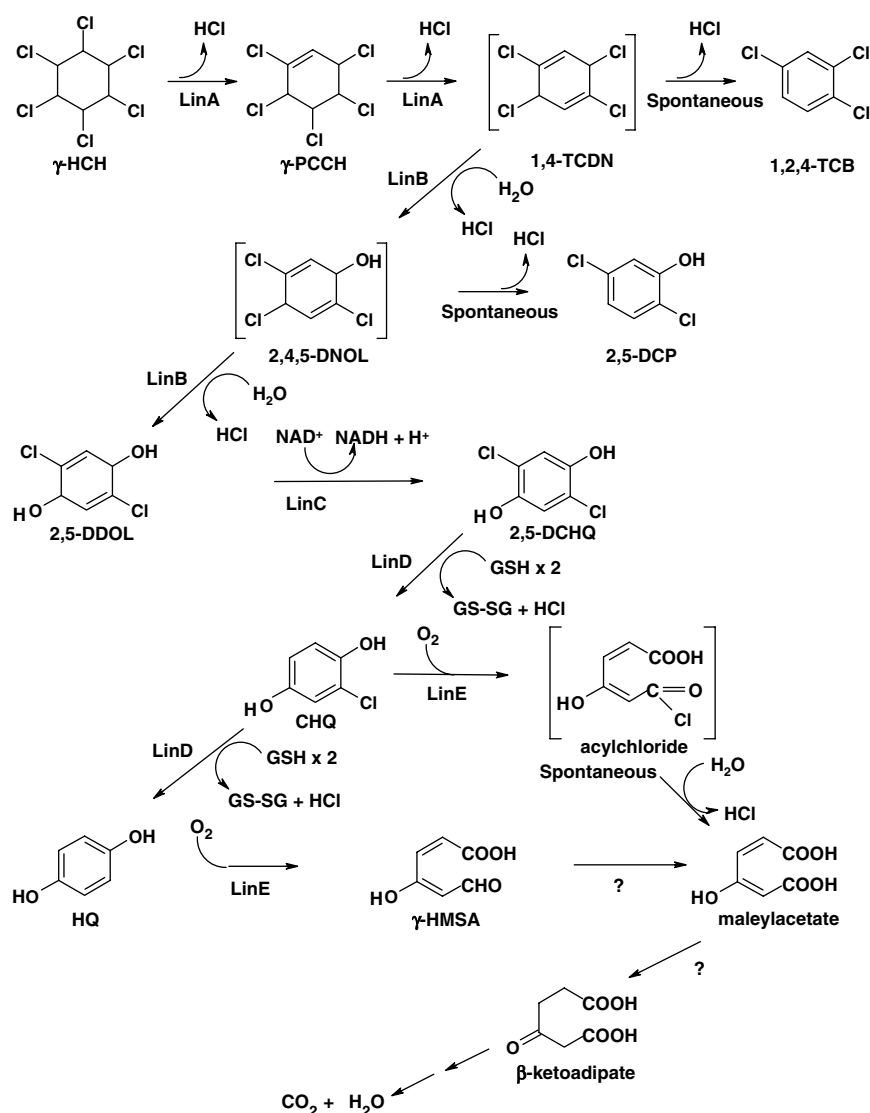


Figure 3. Proposed aerobic degradation pathway of γ -HCH by *Sphingomonas paucimobilis* UT26. Adapted from Nagata et al. (1999). HCH: hexachlorocyclohexane, PCCH: pentachlorocyclohexene, 1,4-TCDN: 1,3,4,6-tetrachloro-1,4-cyclohexadiene, 1,2,4-TCB: 1,2,4-trichlorobenzene, 2,4,5-DNOL: 2,4,5-trichloro-2,5-cyclohexadiene-1-ol, 2,5-DCP: 2,5-dichlorophenol, 2,5-DDOL: 2,5-dichloro-2,5-cyclohexadiene-1,4-diol, 2,5-DCHQ: 2,5-dichlorohydroquinone, CHQ: chlorohydroquinone, HQ: hydroquinone, HMSA: hydroxymuconic semialdehyde, GSH: reduced glutathione, LinA: γ -HCH dehydrochlorinase, LinB: 1,4-TCDN halohydrolase, LinC: 2,5-DDOL dehydrogenase, LinD: 2,5-DCHQ reductive dehalogenase, LinE: CHQ dioxygenase.

hexanol and tetrachlorocyclohexanediol by GC-MS (Sahu et al. 1995). Two additional *Pseudomonas* sp. with the ability to utilize γ -HCH as a carbon source were isolated from agricultural soils by Nawab et al. (2003). Preliminary studies indicated that the biodegradation products produced in minimal salts media by these strains were similar to those reported by Bhuyan et al. (1993).

Kuritz & Wolk (1995) studied degradation of γ -HCH using two species of cyanobacteria. Degradation intermediates included γ -PCCH and 1,2,4- and 1,2,3-TCBs. The TCBs appeared to be produced from PCCH, and did not accumulate, suggesting their subsequent degradation or volatilization. Nitrate stimulated the rate of γ -HCH degradation in *Anabaena* sp. from 1.5 to 25 ng μg^{-1} chlorophyll per hour. The degradation

rate was also enhanced when the *Anabaena* sp. was transformed with the *linA* gene from *Sphingomonas paucimobilis* (see below). Transformed cells degraded γ -HCH at rates of 10.1 and 40 ng μg^{-1} chlorophyll per hour, in the absence and presence of nitrate, respectively. TCCH was also identified by GC-MS as a metabolite of Lindane degradation by aerobic microorganisms in soil slurry (Bachmann et al. 1988a) and aerobic degradation by *Rhodanobacter lindaniclasticus* (Thomas et al. 1996). PCCH resulting from dehydrodechlorination was not detected.

Sphingomonas paucimobilis UT26 is a naldixic acid-resistant mutant of *Pseudomonas* (reclassified *Sphingomonas*) *paucimobilis* SS86, which was initially isolated from an upland soil in Japan (Senoo & Wada 1989). UT26 is capable of aerobically degrading α -, γ - and δ -HCH isomers and using γ -HCH as a sole carbon source (Nagasawa et al. 1993). The degradation pathway for *S. paucimobilis* shown in Figure 3 involves several novel enzymes encoded by *linA*, *linB*, *linC*, *linD*, *linE*, *linR* and *linX*, leading to eventual mineralization of γ -HCH via maleylacetate and β -ketoadipate, common metabolites in the microbial degradation pathways of aromatic compounds. The genes, gene products and their reactions have all been studied in detail (Imai et al. 1989, 1991; Miyauchi et al. 1998, 1999, 2002; Nagasawa et al. 1993; Nagata et al. 1993, 1994, 1997) and summarized by Nagata et al. (1999).

In *S. paucimobilis* UT26, the 465-bp *linA* gene encodes a 155 amino acid protein with a predicted MW of 17.2 kDa (Imai et al. 1991), and catalyzes the dehydrochlorination of HCH to form PCCH. PCCH is converted by LinA to the unstable metabolite 1,3,4,6-tetrachloro-1,4-cyclohexadiene (1,4-TCDN) which may spontaneously dechlorinate to 1,2,4-TCB (Nagasawa et al. 1993). The pathway proceeds if 1,4-TCDN is converted by the *linB* gene product 1,4-TCDN halohydrolase to 2,4,5-trichlorocyclohexenol (2,4,5-DNOL), which is further oxidized by the same enzyme to 2,5-dichloro-2,5-cyclohexadiene-1,4-diol (2,5-DDOL) (Nagata et al. 1993). 2,4,5-DNOL may also spontaneously dechlorinate to form 2,5-DCP. 1,2,4-TCB and 2,5-DCP are considered dead end products and the stable result of non-enzymatic conversion of the unstable diene intermediates (Nagata et al. 1993).

The *linA* gene appeared to be expressed constitutively from genomic DNA. However, the ability of the organism to degrade γ -HCH was often lost after repeated subculturing in LB medium (Imai et al. 1991). The LinA enzyme, designated γ -HCH dehydrochlorinase, has narrow substrate specificity and has not been found to be homologous to any other known enzymes despite early suggestions that it was a member of the glutathione-S-transferase (GST) superfamily (Imai et al. 1991; Nagata et al. 1997). This enzyme removes HCl molecules from its substrate, much like the reaction catalyzed by DDT dehydrochlorinase, an enzyme that belongs to the GST superfamily. However, LinA does not have GST or DDT dehydrochlorinase activity in the presence of glutathione, nor does it require any other cofactors. As a result, LinA has been tentatively classified as a novel dehydrochlorinase and not a GST-type enzyme (Imai et al. 1991; Nagata et al. 1999).

The *linB* gene, like *linA*, appears to be encoded on chromosomal DNA and constitutively expressed in *S. paucimobilis* UT26. The 885-bp *linB* encodes a 32 kDa polypeptide which has 29.3% amino acid sequence identity and similar properties (conserved residues in the putative active site) to the haloalkane dehalogenase, DhIA, of *Xanthobacter autotrophicus* GJ10 (Nagata et al. 1993). However, the LinB enzyme, designated 1,4-TCDN halohydrolase, prefers long chain haloalkanes, while DhIA prefers short chain haloalkanes (Nagata et al. 1997). Also, DhIA is not active toward HCH (Beurskens et al. 1991). We have attempted dechlorination of γ -HCH in a colorimetric plate assay using cell lysate from an *Escherichia coli* strain expressing the GJ10 *dhIA* gene without success (Phillips et al. 2001). LinB is likely of a separate substrate specificity class altogether.

Unlike the enzymes encoded by *linA* and *linB* (above), and *linD* and *linE* (below), the *linC* product (and related gene *linX*) catalyzes only one reaction. LinC (2,5-DDOL dehydrogenase) activity is also constitutively expressed in *S. paucimobilis* UT26. LinC and LinX appear to catalyze the same reaction, conversion of 2,5-DDOL to 2,5-dichlorohydroquinone (2,5-DCHQ) via a NAD⁺-dependent dehydrogenase reaction (Nagata et al. 1994), although LinX activity is much lower and the amino acid sequence identity between the two proteins is only 33.1%. These enzymes have been classified as members of the short-chain alcohol

dehydrogenase superfamily based on sequence comparison (Nagata et al. 1994).

The *linD* gene encodes the enzyme which carries out a two-step conversion of 2,5-DCHQ to hydroquinone (HQ) via chlorohydroquinone (CHQ) (Miyauchi et al. 1998). The 1038-bp gene encodes an enzyme of 346 amino acids with a predicted MW of 38.7 kDa. LinD is a reductive dehalogenase whose expression is induced by 2,5-DCHQ. LinD activity expressed in *E. coli* was increased 3.7-fold in the presence of glutathione. The amino acid sequence of LinD has some similarity to that of tetrachlorohydroquinone reductive dehalogenase (PcpC) from *Sphingobium chlorophenolicum* ATCC 39723 and *Sphingomonas* sp. UG30 (Habash et al. 2002, 2004; Nagata et al. 1999). Although LinD can convert CHQ to HQ, the reaction is very slow and an alternate pathway for CHQ degradation exists in *S. paucimobilis* UT26 (Miyauchi et al. 1999).

The fifth gene in the initial HCH degradation pathway of *S. paucimobilis* UT26 is *linE*, encoding a dioxygenase that cleaves the aromatic ring of both CHQ and HQ. LinE has lower activity against HQ than CHQ (Miyauchi et al. 1999). CHQ cleavage by LinE occurs between C-1 and C-2, which have hydroxyl and chlorine substituents, respectively, producing an intermediate acylchloride product that reacts spontaneously with water with loss of HCl to form maleylacetate, which is subsequently converted to β -ketoadipate. Maleylacetate and β -ketoadipate were identified in extracts of cell suspensions incubated with CHQ by GC-MS. γ -Hydroxymuconic semialdehyde (γ -HMSA) was identified as a metabolite by GC-MS in extracts of cell suspensions incubated with HQ alone. Mineralization of the metabolites of CHQ and HQ is indicated in Figure 3 because *S. paucimobilis* UT26 is known to completely mineralize γ -HCH.

LinE is classified as a member of the *meta*-cleaving dioxygenases, based on amino acid sequence similarity and the conservation of putative active site residues. The amino acid sequence of LinE was most closely matched to PcpA from *S. chlorophenolicum* ATCC 39723. PcpA has ring cleavage activity on 2,6-dichlorohydroquinone (2,6-DCHQ), an intermediate in the pentachlorophenol (PCP) degradation pathway also with two hydroxyl groups *para* to each other, and is also capable of cleaving CHQ and HQ (Ohtsubo et al.

1999). Like *linD*, *linE* expression is inducible by its substrates. The two genes appear to be organized within an operon with an upstream transcriptional regulator named LinR (Miyauchi et al. 2002).

A second HCH-degrading *S. paucimobilis* strain, designated B90, was isolated from flooded rice-field soil in India that had been repeatedly treated with a commercial Lindane pesticide formulation (Bhuyan et al. 1993). This strain was the first organism reported to aerobically degrade all four commonly found isomers of HCH (Johri et al. 1998; Kumari et al. 2002). GC-MS was used to identify PCCH as a metabolite of all four isomers (Johri et al. 1998). Rates of degradation were slightly higher than for *S. paucimobilis* UT26, which did not degrade the β -isomer.

When exposed to γ - or α -HCH, *S. paucimobilis* B90 produced seven polypeptides which were required for the degradation of the two HCH isomers (Adhya et al. 1996). The degradation kinetics of the α - and γ -HCH isomers were similar suggesting the two isomers followed the same degradation pathway. Induction of the polypeptides did not affect degradation of β -HCH, nor did the presence of β -HCH induce the polypeptides required for degradation of α - or γ -HCH, thus it appeared α - or γ -HCH were degraded by a different pathway than β -HCH. Although the enzyme responsible for γ -HCH dehydrochlorination was inducible, none of the seven induced polypeptides were of similar size to the LinA protein (17 kDa) expressed by *S. paucimobilis* UT26. This prompted studies to compare HCH-catabolic genes between UT26 and B90 strains (Kumari et al. 2002). Homologous genes for *linA*, *linB* and *linC* were found in both strains. The UT26 *linA* gene and the preceding *linX* sequence were similar to two genes in *S. paucimobilis* B90 designated *linA1* and *linA2*. Although expression of the two *S. paucimobilis* B90 *linA* genes in *E. coli* BL21 conferred the ability to convert α -, δ -, and γ -HCH to α -, δ -, and γ -PCCH, respectively (Kumari et al. 2002), degradation of β -HCH was not observed. This provided further evidence that β -HCH degradation in *S. paucimobilis* B90 is initiated by enzymes other than those encoded by *linA*.

Another species of α - and γ -HCH-degrading bacterium, named *Rhodanobacter lindaniclasticus* (Nalin et al. 1999), was also found to express two genes with homology to *linA* and *linB* of *S. paucimobilis* UT26 (Thomas et al. 1996). *R.*

lindaniclasticus was isolated from soil in France and was able to grow in minimal media with γ -HCH as the sole carbon source. Degradation of γ -HCH produced a tetrachlorinated metabolite in addition to two metabolites tentatively identified by GC-MS as 1,2,4-TCB and 2,5-DCP. Filter-sterilized media from actively dechlorinating cultures possessed the ability to dechlorinate γ -HCH at the same rate as the cell cultures, suggesting that enzymes involved in dechlorination were extracellular. The production of extracellular enzymes for HCH degradation has relevance in its potential to enhance bioavailability and degradation rates of these hydrophobic compounds in a soil environment by eliminating the need for transport across the cell membrane.

A recent comparison of the organization of the *lin* genes in *R. lindaniclasticus*, *S. paucimobilis* UT26 and *S. paucimobilis* B90A (a parent strain to B90 that contains *linD* and *linE* genes homologous to those of *S. paucimobilis* UT26) has provided evidence that horizontal gene transfer between the strains may have occurred via the insertion sequence IS6100 (Dogra et al. 2004). Each of the three strains contains multiple copies of IS6100. Instability of *lin* genes and generation of mutants lacking either *linA* or *linD*, *linE* and *linR* is coupled with a change in IS6100 hybridization patterns and copy numbers. In each of the *Sphingomonas* strains, the GC content of the *linA* genes is considerably lower than the remainder of the genome, supporting the theory that *linA* was obtained from a different microorganism with a lower GC content (Kumari et al. 2002; Nagata et al. 1999). Constitutive expression of *linA* and *linB* versus a requirement for induction of *linC*, *linD* and *linE*, different GC contents within the *linA* genes, and different locations within the genome, all point to a patchwork assembly of enzymes with alternate functions, leading to the evolution of HCH-degrading microorganisms similar to that proposed for PCP-degrader *Sphingobium chlorophenolicus* (Copley 2000). The sequence similarities between the related genes of each strain suggest that each originated from a single source fairly recently and have not yet acquired strain-specific mutations (Dogra et al. 2004). Recent evolution of genes for the degradation of HCHs is to be expected considering the comparatively recent introduction of this compound into the environment. The results presented by Dogra

(2004) suggest that soil microorganisms around the globe have potential to adapt to HCH contamination and that genetic transfer of genes for the degradation of HCHs is also possible.

In some species of bacteria, non-enzymatic molecules or cofactors might be involved in HCH degradation (Boyle et al. 1999; Jagnow et al. 1977; Marks et al. 1989). γ -HCH degradation in autoclaved cultures of some marine bacteria has been observed, suggesting that a cofactor is responsible (Boyle et al. 1999). Lindane dehalogenation by a number of hemeproteins, porphyrins and corrins has been documented (Marks et al. 1989). Finding a means of utilizing these macromolecules in bioremediation treatment cells and permeable reactive barriers is the focus of some laboratories (Assaf-Anid et al. 1992, 1994, Marks et al. 1989). It does not appear, however, that β -HCH is susceptible to dechlorination by metal-containing cofactors (Van Eekert et al. 1998).

Lindane degradation by fungi

Pure cultures of the lignin-degrading fungi *Phanerochaete chrysosporium* and *Trametes hirsutus* have been shown to degrade Lindane (Bumpus et al. 1985; Kennedy et al. 1990; Mougin et al. 1997; Singh & Kuhad 1999). Mineralization of nanomolar quantities of ^{14}C -Lindane by a *P. chrysosporium* inoculum was reported in both liquid media and in soil amended with corn cobs at a level of 4 g/g soil (Kennedy et al. 1990). When the soil-corn cob mixture was spiked with 4.7 nmol (0.254 μCi) ^{14}C -Lindane, 22.8% of the radiolabel was recovered as $^{14}\text{CO}_2$ during a 60-day incubation period. In a 10 ml aqueous culture of nitrogen-deficient, ligninolytic *P. chrysosporium* spiked with 1.25 nmol ^{14}C -Lindane (Bumpus et al. 1985), 190.8 pmol were converted to $^{14}\text{CO}_2$ in 30 days. The conditions required for degradation of highly substituted aromatic or aliphatic compounds were similar to those that promoted lignin degradation by this fungus: nitrogen-deficiency and the presence of a co-substrate, in this case, glucose. Suppression of lignin degradation was observed in nitrogen-rich environments, as observed for degradation of DDT. Therefore the proposed mechanism of degradation of organochlorine pesticides by the fungus was similar to that of lignin degradation by lignin peroxidases, i.e., multiple non-specific oxidative reactions

resulting from generation of carbon-centered free-radicals (Bumpus et al. 1985).

Use of a lignin degrader such as *P. chrysosporium* to treat Lindane-contaminated soil might result in a synergistic relationship with other soil microorganisms in which HCH uptake or degradation is slowed by its poor solubility and low bioavailability (Bumpus et al. 1985; Mougin et al. 1997). The fungi initiate Lindane degradation by extra-cellular lignin-degrading peroxidases, which are relatively non-specific and have broad substrate specificity (Bumpus et al. 1985). Evidence of such synergism has been reported by Mougin et al. (1997) who observed mineralization of ^{14}C -Lindane in a non-sterile soil containing 0.8 mg kg^{-1} Lindane, and reported that the cumulative percent recovery of $^{14}\text{CO}_2$ more than doubled in soil inoculated with *P. chrysosporium*. These data suggest that a fungal inoculum might be used during soil bioremediation to enhance initial HCH dechlorination rates and the more hydrophilic dechlorination products may become more readily available to indigenous microorganisms with the ability to complete the mineralization process.

The ability of several white rot fungi to degrade Lindane was tested by Arisoy (1998) who reported that *Phanerochaete chrysosporium*, *Pleurotus sajorajua*, *Pleurotus florida* and *Pleurotus eryngii* were all able to degrade significant ($> 10\%$) amounts of Lindane during 20 days incubation in culture media under oxic conditions. A sub-tropical white rot fungi designated DSPM95 from Zimbabwe also degraded Lindane in bench-scale bioreactors (Tekere et al. 2002).

Rates of Lindane degradation, and metabolic intermediates, were compared for *T. hirsutus* and *P. chrysosporium* (Singh & Kuhad 1999) and for *Cyathus bulleri* and *Phanerochaete sordida* (Singh & Kuhad 2000). The initial mechanisms of degradation in the four fungi appeared to be similar, due to the appearance of identical metabolites in the culture media. TCCH and TCCOL were identified by GC-MS and appeared in the culture media of *T. hirsutus*, *P. chrysosporium*, and *P. sordida*, while only TCCOL was detected in flasks of *C. bulleri*. Evidence that HCH dechlorination was extracellular was provided in both studies. These studies provide convincing evidence that the ability of white rot fungi to degrade complex organopollutants might be profitably applied to the treatment of highly contaminated

soils containing organochlorine pesticides such as Lindane.

Bioremediation of HCH-contaminated soil

Bioremediation as a technology for reclaiming chemically contaminated land has been steadily growing in acceptance since the 1980's, but detailed strategies for optimizing treatments on sites containing HCHs remain to be discovered. Bioremediation strategies typically involve enhanced natural attenuation, or optimization of the environmental conditions discussed previously, to stimulate growth and biodegradation of HCHs by indigenous microorganisms. Supplemental nutrients and organic amendments may be added to enrich the habitat for degrading organisms. Bioaugmentation (inoculation with previously acclimated HCH-degrading microorganisms that are not necessarily indigenous to the site) is a less popular approach, albeit a potentially effective one. Bioaugmentation and enhanced natural attenuation have been successful approaches for enhancing remediation of soils contaminated with a variety of xenobiotic compounds (Cunningham & Philp 2000; Juhasz et al. 2000; Manzano et al. 2003; Newcombe & Crowley 1999; Runes et al. 2001; Top et al. 1999). Each of these approaches can enhance bioremediation, either by increasing the population of microorganisms in soil capable of degrading the target contaminant, or by rendering the contaminant more bioavailable. Combinations of these strategies might be used to further enhance the effectiveness of a treatment protocol. The soil environmental conditions that best support biodegradation of HCHs were discussed above. The following section addresses what is known to date of nutrient amendment and bioaugmentation strategies, as they have been applied to soil microcosms, pilot-scale or full-scale projects. Many projects have focused only on establishing that HCHs applied to soil would eventually disappear without added amendment i.e. natural attenuation. Those studies have addressed the fate of HCHs in terms of volatilization, movement within the soil, biodegradation and determination of intermediary metabolites, as discussed above. Studies that have focused on improving HCH removal by amendment or inoculation are rare, and results for laboratory

microcosm studies are not easily extrapolated to the field. Doelman et al. (1988a) demonstrated how a field-scale treatment protocol might go awry, should unforeseen complications alter the environmental conditions in the soil from what was intended. Technical problems hindered their efforts to aerate soil slurry consisting of 90 kg soil, which contained an active HCH-degrading population that required aerobic conditions. The complications resulted in failure to remove HCHs from the contaminated soil on a larger scale, although laboratory studies using 25 g soil samples had indicated that complete removal of α -HCH was possible.

Inorganic and organic amendments

Amendments for bioremediation of HCH-contaminated soil can be divided into two general classes. The inorganic amendments include nutrients in the form of fertilizers, salts or metals, and might also include emulsifiers/surfactants to improve bio-availability of the contaminant, and lime or other amendments for adjusting pH and improving soil drainage properties. The organic amendments are carbon-based nutrient sources. Often, for economic reasons, organic amendments used in field-scale bioremediation protocols are derived from plant or other natural sources although commercially manufactured organic fertilizers also fall under this category. Before amendments are utilized *in situ* at field-scale they are typically tested in bench-scale microcosms of soil or water.

Inorganic macronutrient sources of nitrogen (N), phosphorus (P), magnesium (Mg) or potassium (K) might be added in pure form or as constituents of organic fertilizers in a scaled-up bioremediation study. Doelman et al. (1990) utilized soil that was enriched with a fertilizer (N : P : K ratio = 12:10:18), however, they did not compare the rates of HCH degradation in this soil with unamended controls. MacRae et al. (1984) found that urea, a source of nitrogen, stimulated the removal of all four HCH isomers when applied at a level of 0.25 mg ml⁻¹ to either oxic or anoxic soil suspensions, with the exception of aerobic removal of α -HCH and anaerobic removal of β -HCH, which were inhibited, and aerobic removal of δ -HCH, which was unaffected.

Nitrate, sulfate and ferric iron are commonly used as alternative electron acceptors, in the ab-

sence of oxygen (i.e. under anoxic conditions) and might be used to enhance anaerobic degradation processes. Potassium nitrate (0.2%) was tested in buffered cell suspensions of *Clostridium* sp. but was found to inhibit γ -HCH degradation (Sethunathan et al. 1969). In contrast, potassium chloride and potassium sulfate enhanced γ -HCH removal. Pure cultures of sulfate-reducing bacteria from marine sources were reported to have the ability to dehalogenate γ -HCH (Boyle et al. 1999). Bachmann et al. (1988b) reported inhibition of α -HCH degradation under nitrate- and sulfate-reducing conditions in soil slurries and proposed an inhibitory effect of nitrate and sulfate resulting from the competition for electrons required by both of these processes and reductive dechlorination. In a different study, they found that ammonium nitrate did not inhibit degradation of α -HCH under aerobic conditions (Bachmann et al. 1988a).

In several cases, enriched pure cultures and consortia have been unable to degrade HCHs without another source of carbon in the media. An example of this were washed cells of *Clostridium rectum*, which anaerobically degraded γ -HCH in a buffered suspension containing 0.2% glucose, pyruvate or some amino acids but not in buffer alone (Ohisa et al. 1980). Peptone or yeast extract (2 g l⁻¹) were also required for anaerobic degradation of γ -HCH by a mixed consortium of microorganisms containing *Clostridium* sp. (Maule et al. 1987). Degradation by this mixed culture was enhanced by additions of formate (1% w/v), proline + leucine (0.2% ea.), leucine, proline, mannitol, acetate, pyruvate, citrate or lactic acid (1% w/v), in order of greatest to least effect. Certain *Clostridium* sp. catalyzes the Stickland reaction, an oxidation/reduction reaction between pairs of amino acids, of which leucine and proline are a typical couple. It was suggested that a Stickland-type reaction might be linked to the dechlorination of γ -HCH in some microorganisms (MacRae et al. 1984; Maule et al. 1987; Ohisa et al. 1980, 1982). Yeast extract applied at 0.5 mg ml⁻¹ also enhanced degradation of β -, δ - and γ -HCH in anaerobic soil suspensions, and α - and γ -HCH in aerobic soil suspensions, while a mixture of amino acids enhanced aerobic degradation of α -, β - and γ -HCH (MacRae et al. 1984). The yeast extract presumably enhanced cell growth, resulting in higher cell numbers, thus increased degradation, whereas the contribution of the amino acids was

proposed to be via the Stickland-type reaction mechanism. HCH, TCCH, PCCH and HCCH appear to act as electron acceptors in the Stickland-type reaction and contribute to the formation of ATP in *C. rectum* (Ohisa et al. 1982).

Other auxiliary carbon sources, added to enrichment cultures or soils where microorganisms are capable of utilizing HCHs for growth, might either enhance or retard HCH degradation. A summary of the reported effects of different organic amendments on HCH degradation is provided in Table 4. For example, while glucose was required for anaerobic γ -HCH removal by *C. rectum*, and enhanced anaerobic γ -HCH degradation when applied (at 18 mg ml⁻¹) to a buffered suspension of *Citrobacter freundii* cells (Jagnow et al. 1977), application of 10 mg ml⁻¹ glucose to the a mixed culture inhibited anaerobic γ -HCH degradation (Maule et al. 1987). Glucose applications ranging from 500 to 4000 mg kg⁻¹ had nearly no effect on α -HCH degradation in aerobic and anaerobic soil slurries, but a slight initial negative effect under anaerobic conditions, that later changed to slight enhancement of degradation (Doelman et al. 1985). Inhibition of α -HCH degradation was observed in a different study when glucose was applied to aerobic soil slurries at levels ranging from 2.2 to 3.4 mg ml⁻¹ (Bachmann et al. 1988a). α -HCH degradation, by an aerobic mixed culture, was inhibited by high concentrations of glucose (1.6 mg ml⁻¹), in cultures that had not been progressively acclimated to higher HCH concentrations, but not in those that had been acclimated (Manonmani et al. 2000). Cell growth was enhanced in both acclimated and unacclimated cultures, and low concentrations of glucose (0.1 mg l⁻¹) stimulated dechlorination. Enhanced degradation of α -, β -, δ - and γ -HCH was observed in soil with low organic carbon content, when amended with 5000 mg kg⁻¹ glucose under anaerobic conditions (Castro & Yoshida 1974). In another soil with higher organic carbon, the same application level of glucose inhibited HCH removal.

A number of studies have compared multiple sources of carbon for their effect on HCH removal. Ethanol (1 ml l⁻¹) enhanced bacterial growth in an unacclimated aerobic mixed culture, while adversely affecting the rate of chloride release from α -HCH (Manonmani et al. 2000). Acetone (2 ml l⁻¹) also inhibited chloride release

and growth of the unacclimated culture. Sodium benzoate (1.9 mg ml⁻¹) caused a longer lag period before growth or chloride release and reduced the total chloride ion accumulation (Manonmani et al. 2000). In contrast, ethanol (0.2 mmol ml⁻¹) had no effect on anaerobic γ -HCH removal in washed cell suspensions of *Citrobacter freundii* (Jagnow et al. 1977). In this study, glucose enhanced degradation to the greatest extent (see above), followed by pyruvate, formate or succinate (0.2 mmol ml⁻¹ each). Decreased removal of α -HCH was observed in aerobic soil slurries amended with acetate, glucose or both (2.2 to 3.4 mg ml⁻¹), with the greatest effect being observed for acetate (Bachmann et al. 1988a). Sodium acetate also inhibited γ -HCH removal in cultures of *Pseudomonas* sp. that used γ -HCH as a carbon source to promote aerobic growth in minimal salts media (Sahu et al. 1993). The same microorganism was able to degrade β -HCH in minimal media, without growth, resulting in slight (5%) mineralization (Sahu et al. 1995).

Addition of 2 g kg⁻¹ glutamic acid or peptone had minimal effect on α -HCH degradation in microcosms of aerobic and anaerobic soil slurries containing soil from a waste site with 5334 mg kg⁻¹ α -HCH (Doelman et al. 1985). Under aerobic conditions, a slight enhancement of degradation was observed as a result of treatment with glutamic acid. In an aerobic sediment-water system spiked with ¹⁴C- γ -HCH, application of nettle leaf extract to provide additional dissolved organic carbon did not significantly affect mineralization (Kalsch et al. 1998).

With the exception of fertilizers and other compounds made for agricultural use (e.g., urea), nutrient amendments consisting of manufactured chemicals are not cost-effective enough for full-scale bioremediation projects. Plant-derived organic amendments are often used in bioremediation because of the availability of large quantities of raw material at low cost. The amendments are selected on the basis of desirable C:N:P ratios and not only serve as a nutrient source, but improve aeration or water retention in the soil where required, reduce toxicity and create habitats for indigenous microorganisms. Also, because of the high carbon content in plant matter, organic amendments tend to create a respiratory burst, thereby contributing to the generation of anoxic conditions in the soil, especially at high moisture

Table 4. Summary of organic amendment effects on HCH degradation

Reference	Organic amendment	Medium	HCH isomer	Effect on degradation
MacRae et al. (1984)	(1) Urea (0.25 mg ml ⁻¹)	Oxic and anoxic soil suspensions	α , β , δ , γ	(1) \uparrow all except: no effect δ \downarrow β anaerobic \downarrow α aerobic
MacRae et al. (1984)	(2) Yeast extract (0.5 mg ml ⁻¹)			(2) \uparrow β , δ , γ anaerobic, \uparrow α , γ aerobic
	Amino acids: glutamine and serine (0.5 mg ml ⁻¹ ea.), proline and leucine (0.25 mg ml ⁻¹ ea.)	Oxic and anoxic soil suspensions	α , β , δ , γ	\uparrow α , β , γ aerobic
Ohisa et al. (1980)	Glucose, sodium pyruvate, alanine, leucine or proline (2 mg ml ⁻¹) or leucine + proline (2 mg ml ⁻¹ ea.)	<i>Clostridium rectum</i> washed cell suspension, anoxic	γ	Nutrients required, suspension alone did not degrade
Maule et al. (1987)	Peptone + yeast extract (2 mg ml ⁻¹)	Mixed culture, anoxic	γ	Nutrients required, suspension alone did not degrade
Maule et al. (1987)	(1) Glucose (10 mg ml ⁻¹)	Mixed culture containing	γ	(1) \downarrow
	(2) Formate, proline, leucine,			(2) \uparrow , formate >
Jagnow et al. (1977)	Lactic acid, mannitol, pyruvate, citrate or acetate (10 mg ml ⁻¹) or proline + leucine (2 mg ml ⁻¹ ea.)	Peptone + yeast extract, anoxic		Proline + leucine > leucine > proline > mannitol > acetate > pyruvate > citrate > lactic acid
	(1) Ethanol (0.2 mmol ml ⁻¹)	<i>Citrobacter freundii</i> washed cell suspension, anoxic	γ	(1) No effect
	(2) Glucose (0.1 mmol ml ⁻¹)			(2) \uparrow greatest effect
Bachmann et al. (1988a)	(3) Pyruvate, formate or succinate (0.2 mmol ml ⁻¹)			(3) \uparrow , pyruvate > formate > succinate
	Acetate, glucose or both (2.2–3.4 mg ml ⁻¹)	Aerated (oxic) soil slurry	α	\downarrow , acetate > glucose
Doelman et al. (1985)	Glucose (0.5, 2, 4 g kg ⁻¹), glutamic acid or peptone (2 g kg ⁻¹)	Oxic and anoxic soil slurries	α	Nearly no effect; glutamic acid slight \uparrow aerobic; glucose \downarrow initially, then \uparrow anaerobic
Manonmani et al. (2000)	(1) Glucose (1.6 mg ml ⁻¹)	Unacclimated and acclimated mixed culture, oxic	α	(1) unacclimated: \downarrow , \uparrow growth acclimated: no effect, \uparrow growth
Manonmani et al. (2000)	(2) Glucose (0.1 mg l ⁻¹)			(2) \uparrow
	(1) Ethanol (1 ml l ⁻¹)	unacclimated mixed culture, oxic	α	(1) \downarrow , \uparrow growth..
	(2) Acetone (2 ml l ⁻¹)			(2) \downarrow , \downarrow growth
	(3) Sodium benzoate (1.9 mg ml ⁻¹)			(3) \downarrow , \uparrow lag period for growth and degradation

Table 4. (Continued.)

Reference	Organic amendment	Medium	HCH isomer	Effect on degradation
Sahu et al. (1993)	Sodium acetate (1 mg ml ⁻¹)	<i>Pseudomonas</i> sp. in minimal media, oxic	(1) γ (2) β	(1) \downarrow (2) \uparrow
Kalsch et al. (1998)	Nettle-leaf extract (to a COD of 18 mg l ⁻¹)	Oxic sediment/water system	γ	No effect
Siddaramappa & Sethunathan (1975)	(1) Rice straw (5 mg g ⁻¹ dry soil) (2) Ethanol (5 μ l g ⁻¹ dry soil)	Flooded soil	γ	(1) \uparrow (2) \uparrow
Castro & Yoshida (1974)	Glucose, cellulose or rice straw (5 mg g ⁻¹)	Anoxic soils (high and low organic carbon)	α , β , δ , γ	low organic carbon soil: \uparrow , rice straw > glucose or cellulose high organic carbon soil: glucose \downarrow
Manonmani et al. (2000)	Cellulose or teakwood sawdust (1 mg ml ⁻¹)	Unacclimated and acclimated mixed culture, oxic	α	\uparrow , \uparrow growth
Phillips et al. (2000c)	Daramend [®] organic amendment (1) D6386 (3 mg g ⁻¹) (2) D6390 (2 mg g ⁻¹)	(1) Oxic soil (2) Soil to which an-oxic/ oxic cycled technology was applied	α , β , δ , γ	(1) \uparrow (2) \uparrow

contents. The general observation that auxiliary carbon sources enhance anaerobic HCH degradation but inhibit aerobic degradation can be explained by this phenomenon.

Siddaramappa & Sethunathan (1975) reported that application of rice straw to tubes containing flooded soil (5 g kg⁻¹ dry soil), and spiked with γ -HCH in aqueous solution, significantly enhanced γ -HCH removal. Soil redox potentials were considerably more negative in the amended soil than in unamended soil. Rice straw presumably enhanced HCH degradation by creating a burst of bacterial growth in the tubes, thus resulting in reducing conditions as the supply of oxygen was depleted. Carbon sources such as rice straw may be useful when lower soil redox potentials are desired, particularly for bioremediation of a soil containing significant amounts of β -HCH. Degradation of this isomer under oxic conditions is generally reported as minimal (Sahu et al. 1995) while removal under anoxic conditions appears to

be possible when sufficiently low redox potentials are achieved (Van Eekert et al. 1998).

Although addition of rice straw can speed reduction and lower the redox potential in flooded soil to more negative values than previously measured, Bhuyan et al. (1993) observed that soil treated in this manner was still effective at promoting aerobic HCH degradation when used to inoculate minimal salts medium containing Lindane. The authors proposed that, despite lowered redox potentials further down the soil column, surface diffusion of oxygen from the atmosphere to the standing water in their soil slurries was sufficient to allow continued proliferation of an aerobic microbial population. Addition of rice straw to flooded and non-flooded soils (5 kg) in pots, had no effect on the development of the observed 'enhancement factor' (i.e., suspected biological organisms) that mediated aerobic HCH degradation once the soils were used to inoculate minimal salts medium.

Castro & Yoshida (1974) compared glucose, cellulose and rice straw as organic amendments to two different soils under submerged/anoxic conditions. One soil contained approximately one half as much organic carbon (OC) (2.3%) as the other (4.9%), and the authors reported that all three amendments, at 5 g kg⁻¹, enhanced removal of all four isomers from the soil with the lower OC. The effect of rice straw was most pronounced. In the soil with the higher organic carbon content, glucose retarded degradation of all four isomers. It was also found that addition of high amounts (100 mg g⁻¹) of organic matter interfered with extraction recovery and biodegradation, likely due to sorption.

Manonmani et al. (2000) found that aerobic degradation of α -HCH by a mixed microbial consortium in minimal media was enhanced by addition of 1 mg ml⁻¹ cellulose or sawdust. A more marked effect was observed in cultures that had not been acclimated to higher HCH concentrations, than in those that had. Glucose was detected in the cultures in which cellulose powder or sawdust was used, at concentrations up to 210 μ g ml⁻¹, suggesting that its release from the more complex carbohydrate sources served as an easily available co-substrate, without competing with HCH as a carbon source.

The Daramend[®] technology for bioremediation of HCH-impacted soils (Phillips et al. 2000c), involves application of plant-derived organic amendments which provide nutrients and a non-toxic habitat for indigenous microorganisms. It is also postulated that organic amendments in the form of solid plant matter will create a concentration gradient that facilitates diffusion of organic contaminants from pockets of higher, possibly toxic concentrations, to lower, less toxic concentrations on the amendment surface, where they are more bioavailable. Addition of certain plant-derived organic amendments (Phillips et al. 2000a; Seech et al. 1991), including the Daramend[®] products (Bucens et al. 1996; Seech et al. 1994), have been demonstrated to improve the rate of bioremediation of organochlorine pesticides, such as toxaphene, pentachlorophenol (PCP) and DDT, and other chemical contaminants, such as petroleum products, polycyclic aromatic hydrocarbons (PAHs), phthalates and nitroaromatic compounds, in soil. Daramend[®] amendments were demonstrated to be effective at enhancing the re-

moval of HCH isomers in microcosms of soil containing 4122 mg kg⁻¹ total HCHs (Phillips et al. 2000c).

There are no published field-scale studies of bioremediation of HCH-contaminated soil by organic amendment addition. A field-scale project was proposed for a site in the Netherlands to treat an HCH-contaminated plume of groundwater using an electron donor to first create anaerobic conditions for dechlorination of HCHs, followed by introduction of oxygen, or oxygen and nitrate, to stimulate aerobic mineralization of the resulting benzene or chlorobenzenes (Langenhoff et al. 2002). This proposal was based on the cumulative findings that HCH degradation proceeds both anaerobically and aerobically. The strategy of this project was to optimize the existing conditions for biodegradation of β -HCH in the plume in order to treat it before it exits the ground into a neighboring water body.

Osterreicher-Cunha et al. (2003) investigated the effects of lime (calcium oxide) application on volatilization, leaching and microbial degradation of HCHs, in soil from a former HCH manufacturing facility in Brazil. α -, β -, and δ -HCHs had been discarded outside the factory and, after 25 years, lime was applied as an attempt at inexpensive remediation. Fifteen years following the application of lime, its effects on soil microorganisms and HCH biodegradation were assessed using soil microcosms. As expected, the application of lime increased soil drainage by 50%. While 4% w/w HCH addition to non-contaminated soil stimulated respiratory activity in the soil, application of 4% lime raised the soil pH from 5 to 9 and nearly halted respiration for a period of 1 week. Overall leached quantities of HCH were similar, however, the concentration of HCH per ml of leachate was reduced in soil containing lime. This was likely due, not only to enhanced partitioning toward soluble organic matter and reduced mobility of the organic matter in the soil resulting from liming, but to the reduced amount of soluble organic matter made available by soil microorganisms that had been stressed by the application of lime. Since most of the HCH remained in the soil, the results of this study strongly suggested that liming was an unsuccessful approach to remediation of the contaminated site.

A great deal remains unknown about the use of inorganic and organic amendments for bioreme-

diation of HCH-contaminated soils. Since the texture, nutrient requirements and microbial populations of each soil are different, the usefulness of amendments may need to be assessed on a case-by-case basis. Trends from the above-mentioned studies suggest that some generalizations can be made about the use of amendments to optimize full-scale bioremediation processes. For example, a balanced C:N:P ratio is conducive to faster biodegradation rates, since it promotes optimal microbial growth rates. Certain inorganic amendments such as nitrate appear to be inhibitory to HCH removal, for reasons yet unknown. Auxiliary carbon sources might enhance HCH degradation in a soil where carbon is lacking, but will inhibit HCH removal if added in excess. The benefits of using plant-derived organic amendments make them a more feasible choice than commercial products such as yeast extract or peptone, which are more costly. Considerable success has been reported using rice straw as an amendment at bench-scale, particularly for anaerobic treatments, in soil slurry or pure cultures of bacteria. However, more research is required to assess the efficacy of organic amendments for bioremediation of HCH-contaminated soils *in situ* at field-scale.

Bioaugmentation

In a soil with no previous history of HCH exposure, a period of acclimation might be required before mineralization can commence (Bachmann et al. 1988b; Manonmani et al. 2000; Marwood et al. 1998; Phillips et al. 2000b). Theoretically, a number of changes to the soil ecosystem might be responsible for the period of adaptation following initial exposure, or exposure to higher concentrations of HCHs. These include the selective proliferation of microorganisms capable of degrading the contaminant, transfer of genetic material, and induction of enzymes in the appropriate degradation pathways in the existing population. Degradation by indigenous microorganisms might be expected to begin slowly, whereas degradation in a soil where the microorganisms have already acclimated would likely proceed more quickly (Bachmann et al. 1988a). Wada et al. (1989) observed an increase in the rate and extent of Lindane degradation after repeated annual applications to an agricultural soil over 3–4 years. Singh (2003) re-

ported enhanced degradation of α -, β -, and γ -HCH in rhizosphere soils of *Kochia* sp. that had been pretreated with HCHs prior to testing. Pre-exposure of other field sites to HCHs, through multiple applications, was also found to accelerate degradation of the pesticide (Bharati et al. 1998; Bhuyan et al. 1993). In pure culture, pre-exposure of *S. paucimobilis* UT26 to HCHs resulted in an increased rate of degradation of all four major isomers (α , β , δ , γ) (Johri et al. 2000). Enhanced acclimation of a mixed consortium capable of degrading α -HCH was also achieved by enrichment in progressively higher concentrations of HCH in minimal media (Manonmani et al. 2000).

Bioaugmentation refers to the inoculation of a contaminated soil with a previously contaminated soil or with pure microbial cultures, in order to introduce already acclimated competent HCH-degrading microorganisms and minimize the lag period before biodegradation begins. Inoculation of soil with a history of HCH contamination, that already contains acclimated microorganisms, might result in enhanced biodegradation as cell numbers are increased. Pure culture inoculants might consist either of acclimated microorganisms indigenous to that soil, acclimated microorganisms from another source, or genetically engineered microorganisms (GEMs). However, processes involving the release of foreign microorganisms and GEMs are strictly regulated and methods for monitoring the survival and spread of the released microorganisms are still being developed. In either case, survival and activity of the inoculum are not always guaranteed, and present another issue to be addressed in bioremediation studies. *S. paucimobilis* SS86 was isolated from an acclimated soil (Wada et al. 1989) and was able to survive and degrade γ -HCH when used to inoculate other soils. The ability of this microorganism to survive in soil with and without γ -HCH has been studied. The microorganism flourishes when γ -HCH is added but cell numbers decrease to a lower plateau once the γ -HCH has been depleted (Senoo & Wada 1990).

Future perspectives

We have uncovered a number of gaps in our existing knowledge of the biodegradation of HCHs in the environment. There is very little

information on actual field-scale treatment of contaminated soil containing HCHs, whether by bioaugmentation or the optimization of environmental conditions to enhance degradation by indigenous microorganisms. There is a sound base of knowledge describing individual environmental parameters and how they affect HCH degradation in soil microcosms. However, this information is sometimes not applicable to field-scale studies where other parameters are not controlled or are less predictable. In addition, what is known of the biodegradation pathways of microorganisms at the molecular level is based on studies with a limited number of species. More work is needed to allow us to fully understand the interactions of the soil environment with HCH-degrading microorganisms and how these interactions regulate HCH removal. We recommend further study of the effects of multiple soil parameters on HCH degradation, the use of soil amendments to modify those parameters and the applicability of this new information to full-scale bioremediation protocols for highly contaminated sites where HCHs have accumulated or persisted for many years.

Acknowledgements

HL and JTT gratefully acknowledge the Natural Sciences and Engineering Research Council (NSERC) of Canada for supporting their general research programs through individual Discovery grants.

References

- Adhya TK, Apte SK, Raghu K, Sethunathan N & Murthy NBK (1996) Novel polypeptides induced by the insecticide lindane (gamma-hexachlorocyclohexane) are required for its biodegradation by a *Sphingomonas paucimobilis* strain. *Biochem. Biophys. Res. Commun.* 221: 755–761
- Arisoy M (1998) Biodegradation of chlorinated organic compounds by white-rot fungi. *Bull. Environ. Contam. Toxicol.* 60: 872–876
- Assaf-Anid N, Nies L & Vogel TM (1992) Reductive dechlorination of a polychlorinated biphenyl congener and hexachlorobenzene by vitamin B12. *Appl. Environ. Microbiol.* 58: 1057–1060
- Assaf-Anid N, Hayes KF & Vogel TM (1994) Reduction dechlorination of carbon tetrachloride by cobalamin(II) in the presence of dithiothreitol: mechanistic study, effect of redox potential and pH. *Environ. Sci. Technol.* 28: 246–252
- ATSDR (1989) Toxicological Profile for Alpha-, Beta-, Gamma- and Delta-Hexachlorocyclohexane. Agency for Toxic Substances and Disease Registry, US Public Health Service, Atlanta, GA
- Bachmann A, DeBruin W, Jumelet JC, Rijnaarts HHN & Zehnder AJB (1988a) Aerobic biomineralization of alpha-hexachlorocyclohexane in contaminated soil. *Appl. Environ. Microbiol.* 54: 548–554
- Bachmann A, Wijnen P, DeBruin W, Huntjens JLM, Roelofsen W & Zehnder AJB (1988b) Biodegradation of alpha- and beta-hexachlorocyclohexane in a soil slurry under different redox conditions. *Appl. Environ. Microbiol.* 54: 143–149
- Beland FA, Farwell SO, Robocker AE & Geer RD (1976) Electrochemical reduction and anaerobic degradation of Lindane. *J. Agr. Food Chem.* 24: 753–756
- Benezet HJ & Matsumura F (1973) Isomerization of γ -BHC to α -BHC in the environment. *Nature* 243: 480–481
- Beurskens JEM, Stams AJM, Zehnder AJB & Bachmann A (1991) Relative biochemical reactivity of three hexachlorocyclohexane isomers. *Ecotoxicol. Environ. Safety* 21: 128–136
- Bharati K, Padhy S & Adhya TK (1998) Accelerated biodegradation of γ -hexachlorocyclohexane (γ -HCH) in a flooded alluvial soil retreated with γ -HCH or its metabolite 1,2,4-trichlorobenzene (TCB). *Bull. Environ. Contam. Toxicol.* 60: 858–863
- Bhuyan S, Sahu SK, Adhya TK & Sethunathan N (1992) Accelerated aerobic degradation of gamma-hexachlorocyclohexane in suspensions of flooded and non-flooded soils pretreated with hexachlorocyclohexane. *Biol. Fertil. Soils* 12: 279–284
- Bhuyan S, Sreedharan B, Adhya TK & Sethunathan N (1993) Enhanced biodegradation of γ -hexachlorocyclohexane (γ -HCH) in HCH (commercial) acclimatized flooded soil: factors affecting its development and persistence. *Pestic. Sci.* 38: 49–55
- Bintein S & Devillers J (1996) Evaluating the environmental fate of Lindane in France. *Chemosphere* 32: 2427–2440
- Boehncke A, Siebers J & Nolting HG (1990) Investigations of the evaporation of selected pesticides from natural and model surfaces in field and laboratory. *Chemosphere* 21: 1109–1124
- Bollen WB, Morrison HE & Crowell HH (1954) Effect of field treatments of insecticides on numbers of bacteria, *Streptomyces*, and molds in the soil. *J. Econ. Ent.* 47: 302–307
- Boyle AW, Haggblom MM & Young LY (1999) Dehalogenation of lindane (γ -hexachlorocyclohexane) by anaerobic bacteria from marine sediments and by sulfate-reducing bacteria. *FEMS Microbiol. Ecol.* 29: 379–387
- Bucens P, Seech AG & Marvan I (1996) Pilot-scale demonstration of DARAMENDTM enhanced bioremediation of sediment contaminated with polycyclic aromatic hydrocarbons in Hamilton Harbour. *Water Qual. Res. J. Canada* 31: 433–451
- Bumpus JA, Tien M, Wright D & Aust SD (1985) Oxidation of persistent environmental pollutants by a white rot fungus. *Science* 228: 1434–1436
- Buser H, Rudolf & Muller MD. (1995) Isomer and enantioselective degradation of hexachlorocyclohexane isomers in sewage sludge under anaerobic conditions. *Environ. Sci. Technol.* 29: 664–672
- Castro TF & Yoshida T (1974) Effect of organic matter on the biodegradation of some organochlorine insecticides in submerged soils. *Soil Sci. Plant Nutr.* 20: 363–370

- Chessells MJ, Hawker DW, Connell DW & Papajcsik IA (1988) Factors influencing the distribution of lindane and isomers in soil of an agricultural environment. *Chemosphere* 17: 1741–1749
- Cohen Y & Ryan PA (1989) Chemical transport in the top soil zone – the role of moisture and temperature gradients. *J. Haz. Mat.* 22: 283–304
- Copley SD (2000) Evolution of a metabolic pathway for degradation of a toxic xenobiotic: the patchwork approach. *Trends Biochem. Sci.* 25: 261–265
- Cunningham CJ & Philp JC (2000) Comparison of bioaugmentation and biostimulation in ex situ treatment of diesel contaminated soil. *Land Contam. Reclam.* 8: 261–269
- Deeb R, A. & Alvarez-Cohen L (2000) Aerobic biotransformation of gasoline aromatics in multicomponent mixtures. *Bioremed. J.* 4: 1–9
- Deo PG, Hasan SB & Majumder SK (1980) Isomerization of β -HCH in aqueous solution. *J. Environ. Sci. Health, Part B* 15: 147–164
- Deo PG, Karanth NG & Karanth NGK (1994) Biodegradation of hexachlorocyclohexane isomers in soil and food environment. *CRC Crit. Rev. Microbiol.* 20: 57–78
- Doelman P, Haanstra L, deRuiter E & Slangen J (1985) Rate of microbial degradation of high concentrations of α -hexachlorocyclohexane in soil under aerobic and anaerobic conditions. *Chemosphere* 14: 565–570
- Doelman P, Haanstra L & Vos A (1988a) Microbial degradation by the autochthonous soil population of alpha and beta HCH under anaerobic field conditions in temperate regions. *Chemosphere* 17: 481–487
- Doelman P, Haanstra L & Vos A (1988b) Microbial sanitation of soil with alpha and beta HCH under aerobic glasshouse conditions. *Chemosphere* 17: 489–492
- Doelman P, Haanstra L, Loonen H & Vos A (1990) Decomposition of alpha and beta-hexachlorocyclohexane in soil under field conditions in a temperate climate. *Soil Biol. Biochem.* 22: 629–634
- Dogra C, Raina V, Pal R, Suar M, Lal S, Gartemann KH, Holliger C, van der Meer JR & Lal R (2004) Organization of *lin* genes and IS6100 among different strains of hexachlorocyclohexane-degrading *Sphingomonas paucimobilis*: evidence for horizontal gene transfer. *J. Bacteriol.* 186: 2225–2235
- Edwards EA & Grbic-Galic D (1992) Complete mineralization of benzene by aquifer microorganisms under strictly anaerobic conditions. *Appl. Environ. Microbiol.* 58: 2663–2666
- El Beit IOD, Wheelock JV & Cotton DE (1981) Factors affecting soil residues of dieldrin, endosulfan, γ -HCH, dimethoate, and pyrethrin. *ecotoxicol. Environ. Safety* 5: 135–160
- Fairlee JR, Burbach BL & Perry JJ (1997) Biodegradation of groundwater pollutants by a combined culture of *Mycobacterium vaccae* and a *Rhodococcus* sp. *Can. J. Microbiol.* 43: 841–846
- Feidieker D, Kampfer P & Dott W (1994) Microbiological and chemical evaluation of a site contaminated with chlorinated aromatic compounds and hexachlorocyclohexanes. *FEMS Microbiol. Ecol.* 15: 265–278
- Francis AJ, Spanggaard RJ & Ouchi GI (1975) Degradation of lindane by *Escherichia coli*. *Appl. Microbiol.* 29: 567–568
- Galiulin RV, Bashkin V & Galiulina RA (2002) Behavior of persistent organic pollutants in the air-plant-soil system. *Water Air Soil Pollut.* 137: 179–191
- Glottfelty DE & Schomburg CJ (1989) Volatilization of pesticides from soil. In: Sawhney BL & Brown K (Eds) *Reactions and Movement of Organic Chemicals in Soils* (pp 181–207). Soil Science Society of America and American Society of Agronomy, Madison, WI
- Habash MB, Beaudette LA, Cassidy MB, Leung KT, Hoang TA, Vogel HJ, Trevors JT & Lee H (2002) Characterization of tetrachlorohydroquinone reductive dehalogenase from *Sphingomonas* sp. UG30. *Biochem. Biophys. Res. Commun.* 299: 634–640
- Habash MB, Trevors JT & Lee H (2004) Bacterial reductive dehalogenases. In: Singh A & Ward OP (Eds) *Biodegradation and Bioremediation* (pp 197–233). Springer-Verlag, Heidelberg, Germany
- Helm PA, Diamond ML, Semkin R & Bidleman TF (2000) Degradation as a loss mechanism in the fate of α -hexachlorocyclohexane in Arctic watersheds. *Environ. Sci. Technol.* 34: 812–818
- Heritage AD & MacRae IC (1977) Identification of intermediates formed during the degradation of hexachlorocyclohexanes by *Clostridium sphenoides*. *Appl. Environ. Microbiol.* 33: 1295–1297
- Hühnerfuss H, Faller J, König WA & Ludwig P (1992) Gas chromatographic separation of the enantiomers of marine pollutants. 4. Fate of Hexachlorocyclohexane isomers in the Baltic and North Sea. *Environ. Sci. Technol.* 26: 2127–2133
- Huntjens JLM, Brouwer W, Grobbsen K, Jansma O, Scheffer F & Zehnder AJB (1988) Biodegradation of alpha-hexachlorocyclohexane by a bacterium isolated from polluted soil. Contaminated Soil '88. In: Wolf K, van der Brink WJ & Colon FJ (Eds) (pp 733–737). Kluwer Academic Publishers, Dordrecht, The Netherlands
- Imai R, Nagata Y, Senoo K, Wada H, Fukuda M, Takagi M & Yano K (1989) Dehydrochlorination of gamma-hexachlorocyclohexane (gamma-BHC) by gamma-BHC-assimilating *Pseudomonas paucimobilis*. *Agric. Biol. Chem.* 53: 2015–2017
- Imai R, Nagata Y, Fukuda M, Takagi M & Yano K (1991) Molecular cloning of a *Pseudomonas paucimobilis* gene encoding a 17-kilodalton polypeptide that eliminates HCl molecules from gamma-hexachlorocyclohexane. *J. Bacteriol.* 173: 6811–6819
- Jagnow G, Haider K & Ellwardt P (1977) Anaerobic dechlorination and degradation of hexachlorocyclohexane isomers by anaerobic and facultative anaerobic bacteria. *Arch. Microbiol.* 115: 285–292
- Johri AK, Dua M, Tuteja D, Saxena R, Saxena DM & Lal R (1996) Genetic manipulations of microorganisms for the degradation of hexachlorocyclohexane. *FEMS Microbiol. Rev.* 19: 69–84
- Johri AK, Dua M, Tuteja D, Saxena R, Saxena DM & Lal R (1998) Degradation of alpha, beta, gamma and delta-hexachlorocyclohexanes by *Sphingomonas paucimobilis*. *Biotechnol. Lett.* 20: 885–887
- Johri AK, Dua M, Saxena DM & Sethunathan N (2000) Enhanced degradation of hexachlorocyclohexane isomers by *Sphingomonas paucimobilis*. *Curr. Microbiol.* 41: 309–311
- Juhász AL, Stanley GA & Britz M, L. (2000) Degradation of high molecular weight PAHs in contaminated soil by a bacterial consortium: effects on Microtox and mutagenicity bioassays. *Bioremed. J.* 4: 271–283

- Jury WA, Spencer WF & Farmer WJ (1983) Behavior assessment model for trace organics in soil: I. Model description. *J. Environ. Qual.* 12: 558–564
- Kalsch W, Knacker T, Robertz M, Studinger G & Franke C (1998) Partitioning and mineralization of [¹⁴C]Lindane in a laboratory sediment-water system. *Environ. Toxicol. Chem.* 17: 662–669
- Kennedy DW, Aust SD & Bumpus JA (1990) Comparative biodegradation of alkyl halide insecticides by the white rot fungus, *Phanerochaete chrysosporium* (BKM-F-1767). *Appl. Environ. Microbiol.* 56: 2347–2353
- Kohnen R, Haider K & Jagnow G (1975) Investigations on the microbial degradation of Lindane in submerged and aerated moist soil. In: Coulston F & Korte F (Eds) *Environmental Quality and Safety: Pesticides* (pp 222–225). Georg Thieme Publishers, Stuttgart, Germany
- Kumari R, Subudhi S, Suar M, Dhingra G, Raina V, Dogra C, Lal S, van der Meer JR, Holliger C & Lal R (2002) Cloning and characterization of *lin* genes responsible for the degradation of hexachlorocyclohexane isomers by *Sphingomonas paucimobilis* strain B90. *Appl. Environ. Microbiol.* 68: 6021–6028
- Kuritz T & Wolk CP (1995) Use of filamentous cyanobacteria for biodegradation of organic pollutants. *Appl. Environ. Microbiol.* 61: 234–238
- Langenhoff AAM, Staps JJM, Pijls C, Alphenaar A, Zwiap G & Rijnaarts HHM (2002) Intrinsic and stimulated *in situ* biodegradation of hexachlorocyclohexane (HCH). *Water Air Soil Pollut.* 2: 171–181
- Law SA, Bidleman TF, Martin MJ & Ruby MV (2004) Evidence of enantioselective degradation of α -hexachlorocyclohexane in groundwater. *Environ. Sci. Technol.* 38: 1633–1638
- Lide DR (Ed) (2003) *CRC Handbook of Chemistry and Physics*. CRC Press, Boca Raton
- Ma J, Daggupaty S, Harner T & Li Y-F (2003) Impacts of Lindane usage in the Canadian Prairies on the Great Lakes ecosystem. 1. Coupled atmospheric transport model and modeled concentrations in air and soil. *Environ. Sci. Technol.* 37: 3774–3781
- MacRae IC, Raghu K & Castro TF (1967) Persistence and biodegradation of four common isomers of benzene hexachloride in submerged soils. *J. Agr. Food Chem.* 15: 911–914
- MacRae IC, Raghu K & Bautista EM (1969) Anaerobic degradation of the insecticide Lindane by *Clostridium* sp. *Nature* 221: 859–860
- MacRae IC, Yamaya Y & Yoshida T (1984) Persistence of hexachlorocyclohexane isomers in soil suspensions. *Soil Biol. Biochem.* 16: 285–286
- Malaiyandi M, Shah SM & Lee P (1982) Fate of alpha- and gamma-hexachlorocyclohexane isomers under simulated environmental conditions. *J. Environ. Sci. Health, Part A* A17: 283–297
- Manonmani HK, Chandrashekaraiiah DH, Reddy NS, Elcey CD & Kunhi AA (2000) Isolation and acclimation of a microbial consortium for improved aerobic degradation of α -hexachlorocyclohexane. *J. Agr. Food Chem.* 48: 4341–4351
- Manzano MA, Perales JA, Sales D & Quiroga JM (2003) Enhancement of aerobic microbial degradation of polychlorinated biphenyl in soil microcosms. *Environ. Toxicol. Chem.* 22: 699–705
- Marks TS, Allpress JD & Maule A (1989) Dehalogenation of Lindane by a variety of porphyrins and corrins. *Appl. Environ. Microbiol.* 55: 1258–1261
- Mars AE, Kasberg T, Kaschabek SR, vanAgteren MH, Janssen DB & Reineke W (1997) Microbial degradation of chloroaromatics: Use of the *meta*-cleavage pathway for mineralization of chlorobenzene. *J. Bacteriol.* 179: 4530–4537
- Martinez-Toledo MV, Salmeron V, Rodelas B, Pozo C & Gonzalez-Lopez J (1993) Studies on the effects of a chlorinated hydrocarbon insecticide, lindane, on soil microorganisms. *Chemosphere* 27: 2261–2270
- Marwood TM, Knoke K, Yau K, Suchorski-Tremblay A, Flemming CA, Hodge V, Liu D, Seech AG, Lee H & Trevors JT (1998) Comparison of toxicity detected by five bioassays during bioremediation of diesel-spiked soils. *Environ. Toxicol. and Water Qual.* 13: 117–126
- Maule A, Plyte S & Quirk AV (1987) Dehalogenation of organochlorine insecticides by mixed anaerobic microbial populations. *Pest. Biochem. Physiol.* 27: 229–236
- Middeldorp PJM, Jaspers M, Zehnder A & Schraa G (1996) Biotransformation of alpha-, beta-, gamma-, and delta-hexachlorocyclohexane under methanogenic conditions. *Environ. Sci. Technol.* 30: 2345–2349
- Miyauchi K, Suh S-K, Nagata Y & Takagi M (1998) Cloning and sequencing of a 2,5-dichlorohydroquinone reductive dehalogenase gene whose product is involved in degradation of gamma-hexachlorocyclohexane by *Sphingomonas paucimobilis*. *J. Bacteriol.* 180: 1354–1359
- Miyauchi K, Adachi Y, Nagata Y & Takagi M (1999) Cloning and sequencing of a novel *meta*-cleavage dioxygenase gene whose product is involved in degradation of γ -hexachlorocyclohexane in *Sphingomonas paucimobilis*. *J. Bacteriol.* 181: 6712–6719
- Miyauchi K, Lee H-S, Fukuda M, Takagi M & Nagata Y (2002) Cloning and characterization of *linR*, involved in regulation of the downstream pathway for γ -hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26. *Appl. Environ. Microbiol.* 68: 1803–1807
- Mougin C, Pericaud C, Dubroca J & Asther M (1997) Enhanced mineralization of Lindane in soils supplemented with the white rot basidiomycete *Phanerochaete chrysosporium*. *Soil Biol. Biochem.* 29: 1321–1324
- Nagasawa S, Kikuchi R, Nagata Y, Takagi M & Matsuo M (1993) Aerobic mineralization of gamma-HCH by *Pseudomonas paucimobilis* UT26. *Chemosphere* 26: 1719–1728
- Nagata Y, Nariya T, Ohtomo R, Fukuda M, Yano K & Takagi M (1993) Cloning and sequencing of a dehalogenase gene encoding an enzyme with hydrolase activity involved in the degradation of gamma-hexachlorocyclohexane in *Pseudomonas paucimobilis*. *J. Bacteriol.* 175: 6403–6410
- Nagata Y, Ohtomo R, Miyauchi K, Fukuda M, Yano K & Takagi M (1994) Cloning and sequencing of a 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase gene whose product is involved in degradation of gamma-hexachlorocyclohexane by *Sphingomonas paucimobilis*. *J. Bacteriol.* 176: 3117–3125
- Nagata Y, Miyauchi K, Damborsky J, Manova K, Ansorgova A & Takagi M (1997) Purification and characterization of a haloalkane dehalogenase of a new substrate class from a gamma-hexachlorocyclohexane-degrading bacterium, *Sphingomonas paucimobilis* UT26. *Appl. Environ. Microbiol.* 63: 3707–3710

- Nagata Y, Miyauchi K & Takagi M (1999) Complete analysis of genes and enzymes for γ -hexachlorocyclohexane degradation in *Sphingomonas paucimobilis* UT26. *J. Ind. Microbiol. Biotechnol.* 23: 380–390
- Nalin R, Simonet P, Vogel TM & Normand P (1999) *Rhodanobacter lindaniclasticus* gen. nov., sp. nov., a lindane-degrading bacterium. *Int. J. Syst. Bacteriol.* 49: 19–23
- Nawab A, Aleem A & Malik A (2003) Determination of organochlorine pesticides in agricultural soil with special reference to γ -HCH degradation by *Pseudomonas* strains. *Biores. Technol.* 88: 41–46
- Newcombe DA & Crowley DE (1999) Bioremediation of atrazine-contaminated soil by repeated applications of atrazine-degrading bacteria. *Appl. Microbiol. Biotechnol.* 51: 877–882
- Nishiyama M, Senoo K, Wada H & Matsumoto S (1992) Identification of soil micro-habitats for growth, death and survival of a bacterium, gamma-1,2,3,4,5,6-hexachlorocyclohexane-assimilating *Sphingomonas paucimobilis*, by fractionation of soil. *FEMS Microbiol. Ecol.* 101: 145–150
- Ogunseitan OA & Odeyemi O (1985) Effects of lindane, captan and malathion on nitrification, sulphur oxidation, phosphate solubilisation and respiration in a tropical soil. *Environ. Poll. Series A* 37: 343–354
- Ohisa N & Yamaguchi M (1978) Gamma BHC degradation accompanied by the growth of *Clostridium rectum* isolated from paddy field soil. *Agric. Biol. Chem.* 42: 1819–1823
- Ohisa N, Yamaguchi M & Kurihara N (1980) Lindane degradation by cell-free extracts of *Clostridium rectum*. *Arch. Microbiol.* 125: 221–225
- Ohisa N, Kurihara N & Nakajima M (1982) ATP synthesis associated with the conversion of hexachlorocyclohexane related compounds. *Arch. Microbiol.* 131: 330–333
- Ohtsubo Y, Miyauchi K, Kanda K, Hatta T, Kiyohara H, Senda T, Nagata Y, Mitsui Y & Takagi M (1999) PcpA, which is involved in the degradation of pentachlorophenol in *Sphingomonas chlorophenolica* ATCC39723, is a novel type of ring-cleavage dioxygenase. *FEBS Lett.* 459: 395–398
- Okeke BC, Siddique T, Arbestain MC & Frankenberger WT (2002) Biodegradation of γ -hexachlorocyclohexane (Lindane) and α -hexachlorocyclohexane in water and a soil slurry by a *Pandora* species. *J. Agr. Food Chem.* 50: 2548–2555
- Osterreicher-Cunha P, Langenbach T, Torres JPM, Lima ALC, deCampos TMP, Vargas Jr. EdA & Wagener AR (2003) HCH distribution and microbial parameters after liming of a heavily contaminated soil in Rio de Janeiro. *Environ. Res.* 93: 316–327
- Padma TV, Dickhut RM & Ducklow H (2003) Variations in α -hexachlorocyclohexane enantiomer ratios in relation to microbial activity in a temperate estuary. *Environ. Toxicol. Chem.* 22: 1421–1427
- Paraiba LC & Spadotto CA (2002) Soil temperature effect in calculating attenuation and retardation factors. *Chemosphere* 48: 905–912
- Phillips TM, Liu D, Seech AG, Lee H & Trevors JT (2000a) Bioremediation in field box plots of a soil contaminated with wood-preservatives: a comparison of treatment conditions using toxicity testing as a monitoring technique. *Water Air Soil Pollut.* 121: 173–187
- Phillips TM, Seech AG, Liu D, Lee H & Trevors JT (2000b) Monitoring biodegradation of creosote in soils using radiolabels, toxicity tests, and chemical analysis. *Environ. Toxicol.* 15: 99–106
- Phillips TM, Seech AG, Trevors JT & Piazza M (2000c) Bioremediation of soil containing hexachlorocyclohexane. In: Wickramanayake GB, Gavaskar AR, Gibbs JT & Means JL (Eds) *Case Studies in the Remediation of Chlorinated and Recalcitrant Compounds* (pp 285–292). Battelle Press, Columbus, Ohio, USA
- Phillips TM, Seech AG, Lee H & Trevors JT (2001) Colorimetric assay for Lindane dechlorination by bacteria. *J. Microbiol. Met.* 47: 181–188
- Phillips TM, Seech AG, Lee H & Trevors JT (2004) Mineralization of hexachlorocyclohexane during solid-phase soil bioremediation. *J. Ind. Microbiol. Biotechnol.* 31: 216–222
- Prakash S, Tandon GS, Seth TD & Joshi PC (1994) The role of reactive oxygen species in the degradation of lindane and ddt. *Biochem. Biophys. Res. Commun.* 199: 1284–1288
- Rijnaarts HHM, Bachmann A, Jumelet JC & Zehnder AJB (1990) Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of α -hexachlorocyclohexane in a contaminated calcareous soil. *Environ. Sci. Technol.* 24: 1349–1354
- Rodriguez RA & Toranzos GA (2003) Stability of bacterial populations in tropical soil upon exposure to Lindane. *Int. Microbiol.* 6: 253–258
- Rooney-Varga JN, Anderson RT, Fraga JL, Ringelberg D & Lovley DR (1999) Microbial communities associated with anaerobic benzene degradation in a petroleum-contaminated aquifer. *Appl. Environ. Microbiol.* 65: 3056–3063
- Runes HB, Jenkins JJ & Bottomley PJ (2001) Atrazine degradation by bioaugmented sediment from constructed wetlands. *Appl. Microbiol. Biotechnol.* 57: 427–432
- Sahu SK, Patnaik KK, Sharmila M & Sethunathan N (1990) Degradation of α -, β -, and γ -hexachlorocyclohexane by a soil bacterium under aerobic conditions. *Appl. Environ. Microbiol.* 56: 3620–3622
- Sahu SK, Patnaik KK & Sethunathan N (1992) Dehydrochlorination of delta-isomer of hexachlorocyclohexane by a soil bacterium, *Pseudomonas* sp. *Bull. Environ. Contam. Toxicol.* 48: 265–268
- Sahu SK, Patnaik KK, Bhuyan S & Sethunathan N (1993) Degradation of soil-applied isomers of hexachlorocyclohexane by a *Pseudomonas* sp. *Soil Biol. Biochem.* 25: 387–391
- Sahu SK, Patnaik KK, Bhuyan S, Sreedharan B, Kurihara N, Adhya TK & Sethunathan N (1995) Mineralization of α -, γ -, and β -isomers of hexachlorocyclohexane by a soil bacterium under aerobic conditions. *J. Agr. Food Chem.* 43: 833–837
- Seech AG, Trevors JT & Bulman TL (1991) Biodegradation of pentachlorophenol in soil: the response to physical, chemical, and biological treatments. *Can. J. Microbiol.* 37: 440–444
- Seech AG, Marvan IJ & Trevors JT (1994) On-site/ex situ bioremediation of industrial soils containing chlorinated phenols and polycyclic aromatic hydrocarbons. In: Ong SK (Ed) *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbons* (pp 451–455). CRC Press Inc., Boca Raton, Florida, USA
- Senoo K & Wada H (1989) Isolation and identification of an aerobic gamma-HCH-decomposing bacterium from soil. *Soil Sci. Plant Nutr.* 35: 79–87

- Senoo K & Wada H (1990) Fate of a bacterium, *Pseudomonas paucimobilis* SS86, in upland field. *Soil Sci. Plant Nutr.* 36: 593–598
- Sethunathan N, Bautista EM & Yoshida T (1969) Degradation of benzene hexachloride by a soil bacterium. *Can. J. Microbiol.* 15: 1349–1354
- Sethunathan N, Rao VR, Adhya TK & Raghu K (1983) Microbiology of rice soils. *CRC Crit. Rev. Microbiol.* 10: 125–172
- Siddaramappa R & Sethunathan N (1975) Persistence of gamma-BHC and beta-BHC in Indian rice soils under flooded conditions. *Pestic. Sci.* 6: 395–403
- Siddique T, Okeke BC, Arshad M & Frankenberger WT (2002) Temperature and pH effects on biodegradation of hexachlorocyclohexane isomers in water and a soil slurry. *J. Agr. Food Chem.* 50: 5070–5076
- Singh BK & Kuhad RC (1999) Biodegradation of lindane (γ -hexachlorocyclohexane) by the white-rot fungus *Trametes hirsutus*. *Lett. Appl. Microbiol.* 28: 238–241
- Singh BK & Kuhad RC (2000) Degradation of insecticide lindane (γ -HCH) by white-rot fungi *Cyathus bulleri* and *Phanerochaete sordida*. *Pest Manag. Sci.* 56: 142–146
- Singh N (2003) Enhanced degradation of hexachlorocyclohexane isomers in rhizosphere soil of *Kochia* sp. *Bull. Environ. Contam. Toxicol.* 70: 775–782
- Spencer WF & Cliath MM (1973) Pesticide volatilization as related to water loss from soil. *J. Environ. Qual.* 2: 284–289
- Stewart DKR & Chisholm D (1971) Long-term persistence of BHC, DDT and Chlordane in a sandy loam soil. *Can. J. Soil Sci.* 51: 379–383
- Tekere M, Ncube I, Read JS & Zvauya R (2002) Biodegradation of the organochlorine pesticide, Lindane by a sub-tropical white rot fungus in batch and packed bed bioreactor systems. *Environ. Technol.* 23: 199–206
- Thomas J-C, Berger F, Jacquier M, Bernillon D, Baud-Grasset F, Truffaut N, Normand P, Vogel TM & Simonet P (1996) Isolation and characterization of a novel γ -hexachlorocyclohexane-degrading bacterium. *J. Bacteriol.* 178: 6049–6055
- Top EM, Maila MP, Clerinx M, Goris J, DeVos P & Verstraete W (1999) Methane oxidation as a method to evaluate the removal of 2,4-dichlorophenoxyacetic acid (2,4-D) from soil by plasmid-mediated bioaugmentation. *FEMS Microbiol. Ecol.* 28: 203–213
- Tu CM (1976) Utilization and degradation of lindane by soil microorganisms. *Arch. Microbiol.* 108: 259–263
- Van Eekert MH, Van Ras NJP, Mentink GH, Rijnaarts HHM, Stams AJM, Field JA & Schraa G (1998) Anaerobic transformation of β -hexachlorocyclohexane by methanogenic granular sludge and soil microflora. *Environ. Sci. Technol.* 32: 3299–3304
- Vonk JW & Quirijns JK (1979) Anaerobic formation of α -hexachlorocyclohexane from γ -hexachlorocyclohexane in soil and by *Escherichia coli*. *Pest. Biochem. Physiol.* 12: 68–74
- Wada H, Senoo K & Takai Y (1989) Rapid degradation of γ -HCH in upland soil after multiple applications. *Soil Sci. Plant Nutr.* 35: 71–77
- Waliszewski SM (1993) Residues of lindane, HCH isomers and HCB in the soil after lindane application. *Environ. Poll.* 82: 289–293
- Walker K, Vallero DA & Lewis RG (1999) Factors influencing the distribution of Lindane and other hexachlorocyclohexanes in the environment. *Environ. Sci. Technol.* 33: 4373–4378
- Wania F, Mackay D, Li Y-F, Bidleman TF & Strand A (1999) Global chemical fate of α -hexachlorocyclohexane. 1. Evaluation of a global distribution model. *Environ. Toxicol. Chem.* 18: 1390–1399
- Willett KL, Ulrich EM & Hites RA (1998) Differential toxicity and environmental fates of hexachlorocyclohexane isomers. *Environ. Sci. Technol.* 32: 2197–2207
- Wilson LP & Bouwer EJ (1997) Biodegradation of aromatic compounds under mixed oxygen/denitrifying conditions: a review. *J. Ind. Microbiol. Biotechnol.* 18: 116–130
- Wu WZ, Xu Y, Schramm KW & Ketttrup A (1997) Study of sorption, biodegradation and isomerization of HCH in stimulated sediment/ water system. *Chemosphere* 35: 1887–1894
- Yule WN, Chiba M & Morely HV (1967) Fate of insecticide residues. Decomposition of lindane in soil. *J. Agr. Food Chem.* 15: 1000–1004