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AEROBIC BIODEGRADATION OF HEXACHLOROCYCLOHEXANE (HCH) ISOMERS —ITS BIOCHEMISTRY AND GENETICS

A. A. M. Kunhi
Department of Microbiology, Central Food Technological Research Institute, Mysore-570 013, India

Abstract

Accumulation of high levels of organochlorine pesticide residues such as HCH and DDT in human adipose tissue has been reported in India and other developing countries. These toxic chemicals, which have been implicated to cause a number of health problems including cancer, birth defects, nervous disorders, pulmonary oedema, infertility, etc., reach human body mainly through the food chain in bioconcentrated forms. Although this problem has created enough alarm among scientists and policy makers, very little work has been done on developing technologies to eliminate them from the environment. In this short review, the seriousness of the problem with respect to the levels of HCH residues found in human tissues, foods and in breast milk and the work done on microbial degradation of different isomers of HCH with particular emphasis on the biochemistry and genetics of biodegradation are highlighted.

Keywords : Aerobic biodegradation, Hexachlorocyclohexane

Introduction

Hexachlorocyclohexane (HCH) commonly known as BHC has been one of the most extensively used organochlorine pesticides. Though the use of technical grade HCH has been banned in several advanced countries way back in 1970s, India and many other developing countries continue to use this pesticide for crop protection. It is only very recently the agricultural use of HCH has been banned in our country. Nevertheless, our environment has, already, been heavily polluted due to its extensive use since 1950s (Goel, 1988 ; Gupta, 1986 ; Kaushik, *et al.,* 1987 ; Matsumura and Krishnamurti, 1982 ; Ramesh *et al,* 1989 ; Ray *et al.,* 1985). All the isomers of HCH that are usually present in the technical grade HCH have been detected in soil water and air (Deo *et al.,* 1994). Almost all foodstuffs including processed foods in India have been shown to contain high levels of HCH and other insecticide residues (Agarwal, 1986 ; Appaiah, 1988 ; Attri, 1981 ; Chatterjee, 1979 ; Chawla *et al.,* 1978 ; Dikshit *et al.,* 1989 ; Kalra and Chawla ,1981 ; Kannan *et al.,* 1992; Kaphalia *et al.,* 1985 ; Lakshminarayana and Menon, 1975 ; Lal *et al.,* 1989 ; Noronha *et al.,* 1980). Table 1 gives the mean values and the range of total HCH and lindane

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Table 1. Concentration of HCH (μg/kg wet wt.) in foodstuffs from different parts of India. (Compiled from Kannan *et al.*, 1992)

Food item	Σ HCH	γ HCH
Cereals	35 (27–39)	4.8 (3.9–5.8)
Pulses	420 (5.4–1600)	34 (1.1–130)
Spices	210 (83–410)	35 (13–71)
Oil	220 (6.9–480)	35 (1.2–100)
Milk	180 (82–490)	4.2 (0.6–18)
Butter	2800 (2100–3800)	160 (87–300)
Fish & Prawn	28 (0.48–380)	2.9 (0.15–23)
Meat & Animal fat	480 (3.3–5500)	3.2 (0.14–110)

(the gamma-isomer of HCH) residues detected in foodstuffs from different parts of India. Many of these values are much higher than the permissible level of 100–300 μg/kg set by FAO and WHO. The rating of foodstuffs on the basis of the concentration of HCH detected was in the order : dairy products > meat > pulses > oils > spices > fishes (Kannan *et al.*, 1992). High levels of HCH residues have been detected in human adipose tissue, blood plasma, liver, brain and placenta (Dale *et al.*, 1985 ; Siddiqui and Saxena, 1985 ; Siddiqui *et al.*, 1981 ; Saxena *et al.*, 1983). Some representative values of HCH residue concentrations detected in various human tissues are given in Table 2. What is more disturbing is the fact that even breast milk contains very high concentrations of these toxic chemical residues (Attri, 1981; Tanabe *et al.*, 1990; Siddiqui and Saxena, 1985). The concentration of total HCH detected in human breast milk from South India has been shown to range from 1100 to 26000 μg/kg of fat (Tanabe *et al.*, 1990). It has been well established now that these chemical residues reach human body through food chain from the contaminated soil, water, animal feeds, poultry feeds, etc. Due to their high recalcitrance and persistence, the polychloroorganic compounds such as HCH get into the food chain and get bioconcentrated in fatty layers due to their highly lipophilic nature and thus find their way into human body. Their entry into crop plants, vegetables and fruits have been shown to be through absorption directly from soil. Potato and cauliflower samples showed HCH residues, though HCH was not sprayed on these crops. Similarly, it has also been shown that the entry of HCH residues to feed is also through absorption from contaminated soil, but not through

the spray treatment and this is the main source of HCH contamination of milk and milk products (Deo *et al.*, 1994).

Table 2. Concentration of HCH detected in human adipose and other tissues in different geographical areas (compiled from Appaiah, 1988, and Tanabe *et al* 1990)

Tissue	Geographical area/ sex/age group	Average concentration of total HCHm g/kg)	Year of reporting
Adipose Tissue	India (Ahmedabad)	3,870	1984
	India (Bangalore)	5.050	1984
	India (Calcutta)	1.600	1984
	India (Delhi)	10.110	1984
	India (Lucknow)	2.334	1983
	India (Lucknow) males	2.536	1981
	The United Kingdom	0.430	1972
	The U.S.A.	0.480	1986
Blood Plasma	India – Children	0.038	1973
	India – Females	0.034	1973
	India–Males	0.075	1973
Liver	India	2.000	1985
Brain	India	3,500	—
Breast Milk	India	0.195	1981
	India (Tamil Nadu)	1.1–26.0 (on fat basis)	1990

All the major four isomers of HCH, *viz.*, alpha-, beta-, gamma- and delta- are detected in the environment. But, the levels of gamma-isomer detected in the environment do not, usually, correspond with the levels applied on the field. Earlier, it was thought that this phenomenon might be due to their faster degradation in the soil. However, now it has become evident that it is not only because of that, but also due to its conversion to alpha-, beta-, and delta-isomers through microbial action. This fact is very important from the environmental pollution point of view, because these alpha-, beta- and delta-isomers are more persistent and are more toxic to plants, animals and human beings.

Microbial Degradation of HCH

There are not too many reports on the microbial degradation of HCH and a majority of the available reports deal with anaerobic degradation of HCH in soil under flooded conditions (MacRae, 1989). Raghu and MacRae (1966) have shown degradation of alpha-, beta-, gamma- and delta-isomers of HCH in tropical soils under flooded conditions. Yoshida

and Castro (1970) have demonstrated the advantage of flooding the soil as an effective means of accelerating the degradation of HCH-isomers. Many other workers also have reported anaerobic degradation of HCH-isomers in soil and they have implicated the role of *Clostridium sphenoides, Cl. butyricum, Cl. rectum, Cl. pasteurianum, Citrobacter freundii* and species of *Bacillus* in this process (MacRae, *et al.,* 1969; Beland *et al.,* 1976; Jagnow *et al.,* 1977; Heritage and MacRae, 1977; Hill and McCarty, 1967; Ohisa and Yamaguchi, 1978a, b). It is also generally believed that the rates of bioconversion of HCH-isomers decrease in the following order: gamma–HCH>alpha–HCH> delta–HCH >beta–HCH (Ohisa *et al.,* 1980).

Contrary to the common belief that HCH-isomers are degraded only under anaerobic conditions and that they are recalcitrant to biodegradation aerobically, recent reports have shown degradation of most of the HCH-isomers under aerobic conditions. Bachmann *et al.* (1988a and 1988b) have shown efficient degradation of alpha-HCH in heavily contaminated soil slurries under aerobic conditions. These workers, however, observed that beta-HCH was recalcitrant to biodegradation under aerated conditions. Japanese workers have reported the isolation and characterization of an aerobic bacterium *Pseudomonas paucimobilis* (presently placed under *Sphingomonas paucimobilis)* capable of utilizing gamma-HCH as the sole carbon source from a gamma-HCH plot of a long-term experimental upland field (Senoo and Wada, 1989).

Repeated addition of commercial HCH to rice fields resulted in accelerated aerobic degradation of gamma-HCH under flooded and non-flooded conditions (Bhuyan *et al.,* 1992). Sahu *et al.* (1990a) have reported rapid degradation of alpha-, beta- and gamma-isomers of HCH by a suspension of soil from the rhizosphere of sugarcane under aerobic condition. A *Pseudomonas* sp. isolated from the sugarcane rhizosphere soil degraded alpha-, gamma- and the thermodynamically more stable beta-isomer of HCH (Sahu *et al.,* 1990b). Very recently, Bhuyan *et al.* (1993) have reported the isolation of a *Sphingomonas paucimobilis* strain from commercial HCH-acclimatized flooded soil, which degraded alpha-, beta-, gamma- as well as delta-isomers of HCH under aerobic conditions.

Microbial consortia capable of degrading HCH-isomers were developed in the author's laboratory by long term enrichment of HCH-contaminated soil and sewage in a semi-continuous column reactor followed by enrichment in shake flask, using different isomers of HCH as sole source of carbon and energy (Chandrashekaraiah, 1993 ; Chandrashekaraiah *et al.,* 1993 ; Reddy, 1994 ; Reddy and Kunhi, 1994). Alpha-HCH-enriched consortium, AHR degraded upto 50 ppm of alpha-HCH. The beta-, gamma- and delta-HCH-enriched consortia, BHR, GHR and DHR, degraded their respective substrates beta-HCH (10 ppm), gamma-HCH (25 ppm) and delta-HCH (10 ppm), respectively both under shaken and stationary conditions, with the release of stoichiometric amounts of chloride. The effect of co-substrates such as glucose, cellulose, saw dust, acetone, ethanol

and benzoate and the physical conditions such as temperature and pH on the degradation of alpha-HCH by the consortium AHR was studied. Cellulose, saw dust and low levels of glucose significantly improved the rate of degradation of alpha-HCH. The optimal temperature and pH were 30° C and 7.5, respectively.

Biochemistry of HCH Degradation

The biochemistry of degradation of lindane (gamma-HCH) and other isomers of HCH has been reviewed by Deo *et al.* (1994), Engst *et al.* (1979) and Macholz and Kujawa (1985). Most of the available data pertain to their catabolism in animal and plant systems. Information on the microbial catabolism of HCH isomers is rather patchy. Available data indicate that their degradation produces two series of metabolites : isomeric chlorobenzenes and chlorophenols. These intermediates are then mineralized to chlorine-free end products. Formation of pentachlorocyclohexene (PCCH) and tetrachlorocyclohexene (TeCCH) during the biodegradation of HCH by bacteria, fungi and algae has been reported (Bachmann *et al.,* 1988b; Benezet and Matsumura, 1973; Engst *et al.,* 1979; Francis *et al.*, 1975; Heritage and MacRae, 1979; Macholz and Kujawa, 1985; Yule *et al.,* 1967). Mathur and Saha (1975) have reported detection of gamma-2, 3, 4, 5,

Table 3. Aerobic Biodegradation of HCH Isomers

Organism	HCH isomers	Intermediates detected	Reference
Soil microbes	γ-HCH	PCCH, tetrachloro-cyclohexene pentachloro-benzene, TeCB, TCB	Tu, 1976
Soil microbes	α-HCH	PCCH, 1, 4-DCB, 1, 2-DCB, 1, 2, 4-TCB, TeCB	Bachmann, et al.
Pseudomonas paucimobilis SS88	γ-HCH	2,3 and 2,4-Dichloro-phenols, 2, 3, 5- and 2, 4, 5-trichlorophenols	Senoo and Wada, 1989
Pseudomas sp.	,α-, -β-, and γ-HCH	γ-PCCH	Sahu *et al.,* 1990
P. paucimobilis UT26	γ-HCH	γ-PCCH, 1, 2, 4-TCB	Imai *et al.,* 1991
Soil microbes	γ-HCH	—	Bhuyan *et al.,* 1992
Sphingomonas paucimobilis	α-, ,β-, γ- and δ-HCH	α- and γ-PCCH	Bhuyan *et al.,* 1993
Microbial Consortium, AHR	α-HCH	—	Reddy & Kunhi, 1994
Microbial Consortium, BHR	β-HCH	—	Reddy & Kunhi, 1994
Microbial Consortium, GHR	γ-HCH	—	Reddy & Kunhi, 1994
Microbial Consortium, DHR	δ-HCH	—	Reddy & Kunhi, 1994

6-pentachlorocyclo-hex-1-ene, gamma-3, 4, 5, 6-tetrachlorocyclohexene (gamma-TeCCH) and small amounts of 1, 2, 3, 4-tetrachlorobenzene (1, 2, 3, 4-TeCB) and 1, 2, 4-trichlorobenzene (1, 2, 4-TCB) in lindane treated and flooded sandy loam soil. Formation of TeCCH, TeCB, TCB and monochlorobenzene (MCB) from gamma-HCH by microbial degradation under anaerobic conditions have, also, been reported by other workers (Haider and Jagnow, 1975; Heritage and MacRae, 1977; Jagnow *et al.*, 1977; Macholz and Kujawa, 1985; Hill and MaCarty 1967; Ohisa and Yamaguchi, 1978; Ohisa *et al.*, 1980). Several aerobic micro-organisms also metabolized lindane to these intermediate compounds (Tu 1967), Bachmann *et al.* (1988a & b) have reported the formation of PCCH, 1, 4-DCB, 1, 2-DCB, 1, 2, 4-TCB and TeCB from alpha-HCH by a mixed aerobic microbial population in soil slurry and have proposed a pathway for the initial steps of alpha-HCH degradation as given in Fig. 1.

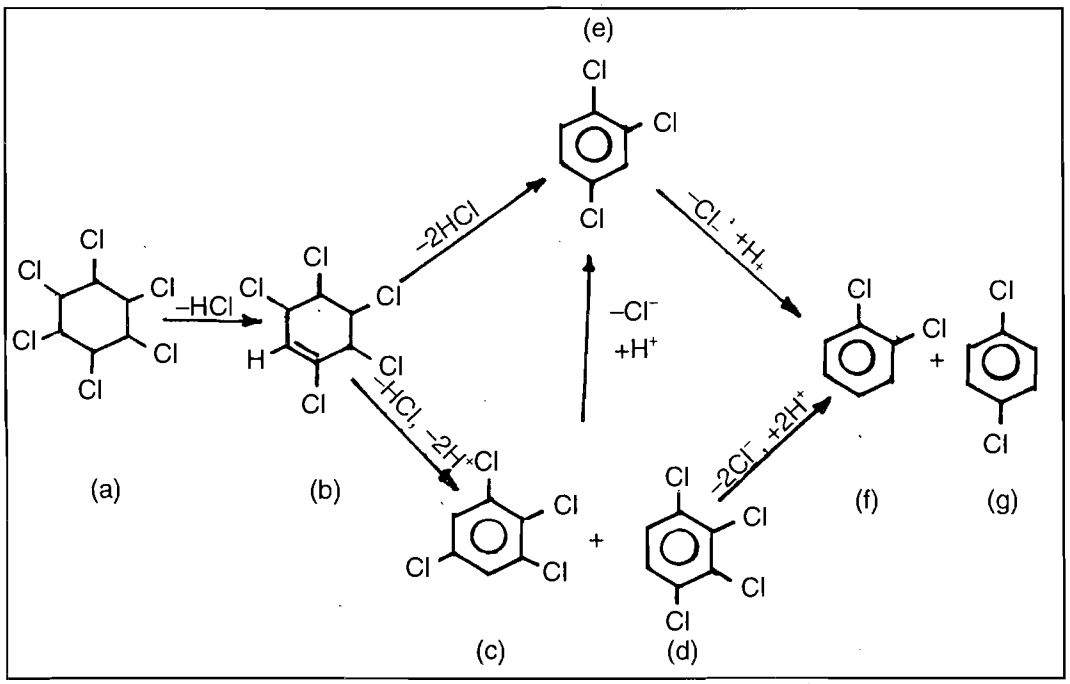

Fig. 1. Proposed initial steps of alpha-HCH degradation under aerobic conditions in a slurry of contaminated soil. (a) alpha- HCH ; (b) PCCH ; (c) 1, 2, 3, 5-TeCB ; (d) 1, 2, 3, 4-TeCB ; (e) 1, 2, 4-TCB ; (f) 1, 2-DCB ; (g) 1, 4-DCB. (Bachmann *et al.*, 1988 b).

On the other hand, Senoo and Wada (1989) have reported the formation of chlorophenols as intermediates of gamma-HCH degradation by a *P. pauciomobilis* strain. They have confirmed by TLC and GC analysis the presence of 2, 5-dichlorophenol

(2, 5-DCP) and 2, 4, 5-trichlorophenol (2, 4, 5-TCP) in the culture filtrate of *P. paucimobilis* SS86 grown on gamma-HCH. However, a mutant named UT26 isolated from the strain SS86, which also utilized gamma-HCH as the sole source of carbon and energy formed 1, 2, 4-TCB *via* gamma-PCCH from gamma-HCH (Imai *et al.,* 1991; Nagata *et al.,* 1993).

Genetics of HCH Degradation

A number of workers have shown that several biodegradation pathways are encoded by native plasmids called catabolic plasmids present in the degrading organism. Several such plasmids encoding degradative pathways of aliphatic, aromatic including chloroaromatics and other compounds have been thoroughly studied (Frantz and Chakraborty, 1986 ; Kunhi, 1991 ; Pemberton, 1983 ; Pemberton and Wynne, 1984). It has also been shown that when these plasmids or parts thereof are transferred to other bacterial systems, they get expressed in the new systems (Franklin *et al.,* 1981, Kunhi, 1991; Nakazawa and Inouye, 1986 ; Pemberton and Wynne, 1984 ;). However, it has been generally observed that many genes particularly catabolic genes from soil bacteria, *viz., Pseudomonas, Alcaligenes, Flavobacterium*, etc., encoding degradation of xenobiotics (Franklin *et al.,* 1981 ; Frantz and Chakraborty, 1986) as well as some of the biosynthetic genes (Clarke and Laverack, 1983; Kunhi, 1985) are either poorly or not at all expressed in *E. coli.* Now, however, very efficient host-vector systems of *Pseudomonas* are available, which can be effectively used for cloning and analysis of the genes from *Pseudomonas* and other Gram-negative soil bacteria (Kunhi, 1991 ; Mermod *et al.,* 1986 ; Pemberton and Wynne, 1984).

Work on genetics of HCH-degradation, however, is in its initial stages. There are but a few reports from the Japanese workers on this aspect. Senoo and Wada (1990) have shown by curing experiments that some of the genes involved in the degradation of gamma-HCH by *P. paucimobilis* SS86 might be borne by a plasmid. Imai *et al.* (1991) have reported the molecular cloning of a gene from a mutant UT26 of the strain SS86 encoding a 16.5 KDa protein that eliminates HCl molecules from gamma-HCH. The enzyme responsible for the conversion of gamma-HCH to 1, 2, 4-TCB *via* gamma-PCCH and named as gamma-HCH dehydrochlorinase is coded by a 465 bp DNA fragment. The nucleotide sequence of this gene (lin A) and the deduced amino acid profile showed no similarity to any known sequences. Over-production of this enzyme in the *E. coli* host and its purification and characterization also have been reported (Nagata *et al.,* 1993). Besides these, there is no report available on the genetics of any of the HCH-isomer biodegradation.

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