

# Persistence and Degradation of 'Endosulfan'- An Organochlorine Pesticides

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### Abstract

Organochlorine pesticides are chlorinated hydrocarbons used extensively in agriculture and mosquito control. These compounds are typically very persistent in the environment, and are known for accumulating in sediments, plants and animals. Endosulfan is a characteristic organochlorine pesticide having functional groups chlorine and sulphur, controls a large spectrum of pests. In general, Endosulfan is less persistent in environment than other cyclodienes, but can persist in soil and water environment for 3 to 6 months and more.  $\alpha$ -Endosulfan showed a bi-exponential form of degradation for all water content-temperature experiments. The persistence varies depending upon the climatic conditions and physico-chemical characteristics like pH, organic matter content and particle size of the soil in the area.

Degradation of the two isomers,  $\alpha$ - and  $\beta$ -Endosulfan, does occur in temperate/tropical soil and aquatic systems, both by abiotic and biotic processes. In general, degradation of this pesticide is found to be relatively higher in the clay soil than in the sandy soil. The proposed pathway of degradation of Endosulfan by different bacterial and fungal cultures, shows its metabolic products as: endosulphate followed by endodiol, endohydroxy ether and endolactone. A soil bacterium is able to degrade 50% Endosulfan by converting it to Endosulfan sulphate within 3 days of its incubation. The major isomers of Endosulfan (Endosulfan- $\alpha$ ), are degraded by a mixed population of anaerobic microorganisms to endodiol under anaerobic and methanogenic conditions. Endosulfan is only moderately persistent in soil under aerobic conditions. *Achromobacter xylosoxidans*, an aerobic bacterium, can degrade 94.12%  $\alpha$ -Endosulfan, 84.52%  $\beta$ -Endosulfan and 80.10% Endosulfan sulphate using it as sulfur source. Isolated strains of *Aspergillus niger*, a fungus, can also degrade Endosulfan to endodiol.

Different factors, such as: Soil redox potential, Soil organic matter, Soil acidity, soil temperature, soil salinity and chemicals also affect Endosulfan degradation. Further studies on the reduction of the half-life of organochlorine pesticides in the environment can prevent their bio-magnification into the natural habitat. Also advance research on the molecular mechanism of microbial degradation can be focused for the development of genetically modified microbes for the degradation of the dead end products of these organochlorine pesticides.

**Keywords:** Organochlorine Pesticides, Endosulfan, Persistence, Pathway of Degradation, Metabolic Products, Endodiol, Endohydroxy Ether And Endolactone, *Achromobacter Xylosoxidans*, *Aspergillus Niger*, Soil Redox Potential, Soil Organic Matter, Soil Acidity, Soil Temperature, Soil Salinity, Bio-Magnification, Genetically Modified Microbes.

### Introduction

A pesticide is a chemical used to prevent, destroy, or repel pests. Pests can be insects, mice and other animals, weeds, fungi, or microorganisms such as bacteria and viruses. Pesticides also are used to kill organisms that can cause diseases. There are different types of pesticides. Among them organochlorine pesticides are most widely used.

Organochlorine pesticides are chlorinated hydrocarbons used extensively from the 1940s through the 1960s in agriculture and mosquito control. Organochlorine pesticides have a long history of widespread use in the United States and around the world. These are insecticides composed primarily of carbon, hydrogen, and chlorine. These compounds are typically very persistent in the environment, and are known for accumulating in sediments, plants and animals. Organochlorine have a wide range of both

acute and chronic health effects, including cancer, neurological damage, and birth defects. Many organochlorines are also suspected endocrine disruptors. Though many organochlorine are banned, some remain in use include lindane, endosulfan, dicofol, methoxychlor and pentachlorophenol.

Because of their chemical structure, organochlorines break down slowly, build up in fatty tissues, and remain in our bodies for a long time. Many organochlorine compounds have been isolated from natural sources ranging from bacteria to humans.

Chlorinated organic compounds are found in nearly every class of biomolecules including alkaloids, terpenes, aminoacids, flavonoids, steroids and fatty acids (Gribble 1998; Engvild 1986). Organochlorides, including dioxins, are produced in the high temperature environment of forest fires, and dioxins have been found in the preserved ashes of lightning-ignited fires that predate synthetic dioxins. In addition, a variety of simple chlorinated hydrocarbons including dichloromethane, chloroform, and carbon tetrachloride have been isolated from marine algae. A majority of the chloromethane in the environment is produced naturally by biological decomposition, forest fires, and volcanoes. The natural organochloride epibatidine, an alkaloid isolated from tree frogs, has potent analgesic effects and has stimulated research into new pain medication.

Table-1

Five Groups of Organochlorine Pesticides

Major groups	Examples
Dichlorodiphenylethanes	DDT, DDD, Dicofol, Methoxychlor (DDE).
Cyclohexanes	Hexachlorocyclohexane (HCH)
Chlorinated cyclodienes	Aldrin, dieldrin, endrin, endosulfan
Toxaphene	Chlorinated camphene
Mirex and chlordecone	

#### Persistence of Endosulfan

Endosulfan is a characteristic pesticide having functional groups chlorine and sulphur, controls a large spectrum of pests. Endosulfan, a mixture of 2 isomers  $\alpha$  and  $\beta$  at around 2:1 ratio has been widely used for broad spectrum cyclodiene insecticide on a wide range of crops including upland cotton and wetland rice crops. It persists in soil and water environment for 3 to 6 months and more (Rao and Murty 1980; Kathpal et al. 1997; Awasthi et al. 2000).

#### Persistence of Endosulfan in soil

In an experiment by Sunitha et al. (2011), reported that,  $\alpha$ -Endosulfan and  $\beta$ -Endosulfan were found to be persistent in soil along with their degradation product Endosulfan sulphate after spraying of Endosulfan on food crop. The persistence of Endosulfan in cashew planted soil of Kerala was found to be 1.5 to 2 years (Harikumar et al. 2014). An experiment by Nasreen et al. (2012) reported an increased enzymatic activities of cellulase and amylase in presence of 5Kg/ hectare of Endosulfan in 20 days of incubation.

#### Persistence of Endosulfan in flooded soil

Rao and Murty (1980) studied on the persistence (at >0.05ppm) of total Endosulfan residues under natural conditions of wet and dry cultivation.

When the initial dose of application was the same, the residue persisted for 120 days in wet soils and 100 days in dry soils. In an experiment by Singh et al. (1999) resulted the persistence of the isomers of Endosulfan:  $\alpha$ - Endosulfan,  $\beta$ - Endosulfan and Endosulfan sulphate was more in non-flooded soil as compared to flooded soil. The degradation rates of both Endosulfan isomers were greatly affected by changes in soil water content and temperature.

#### Persistence of Endosulfan in Environment

In general, Endosulfan is less persistent in environment than other cyclodienes (Sethunathan et al. 2002). When sprayed on food crops,  $\alpha$ -Endosulfan and  $\beta$ -Endosulfan were found to be persistent in air and water along with their degradation product Endosulfan sulphate (Sunitha et al. 2011). An experiment conducted by Harikumar et al. (2014) on water and sediment samples of Kerala was resulted that the concentration of Endosulfan was below the detection limit. Methane-5, 6-sulfite, a chlorinated pesticide of the cyclodiene group contains two major stereoisomers i.e.  $\alpha$ - and  $\beta$ -Endosulfan in an approximate ratio of 70:30, was used extensively for the protection of cotton, tea and sugarcane crops (Bode 1968; Goebel 1983). It is extremely toxic to fish and aquatic invertebrates (Verschueren 1983) and is a priority pollutant for international environmental agencies (Kieth et al. 1979). A study conducted by Puneeta Pandey et al. (2011) in the surface sediments of river Yamuna, Delhi reported the concentration of Endosulfan 1+ $\alpha$ -Chlordane 0.11-23.54ng/g, Endosulfan 2 11.34-60.51ng/g and Endosulfan sulphate+DDT 20.59-86.19ng/g. Because of its abundant usage and potential transport, Endosulfan contamination is frequently found in the environment at considerable distances from the point of its original applications (singh and Wilson 1995; Miles and Pfeuffer 1997).

#### Persistence of Endosulfan Isomers

Under a high water content-high temperature regime the concentration of  $\alpha$ -Endosulfan in the soil fell rapidly during the first 4 weeks of application, followed by a prolonged period of slower rate of degradation.  $\alpha$ -Endosulfan showed a bi-exponential form of degradation for all water content-temperature experiments except for extremes in both these two factors. Degradation of beta-Endosulfan was significantly slower than for the alpha-isomer under all conditions studied. A half-life of more than a year was recorded for the beta-isomer when both water content and temperature were low (Ghadiri and Rose 2001). Castro et al. (2002) conducted laboratory and field experiments throughout the growing season and the residual levels of these chemicals after harvest of tomato in the Spanish region of Badajoz. An increase in the pesticide degradation rate with temperature was observed while correlation of the degradation rate with the soil moisture content was only observed for  $\alpha$ -Endosulfan. Endosulfan sulphate was found to be the most persistent metabolite of Endosulfan in soil, but undergoes rapid degradation in cotton foliage (Kennedy et al. 1998).  $\alpha$ - Endosulfan decreased more rapidly in non-flooded soil than flooded soil in both unamended and straw amended soil during 120 days incubation. Thus Endosulfan decreased to <45% of the original level on non-flooded conditions compared

to a decrease of 15-18% in flooded conditions (Sethunathan et al. 2002).

#### **Factors Affecting Persistence of Endosulfan Isomers**

The persistence depending upon the climatic conditions and physico-chemical characteristics like pH, organic matter content and particle size of the soil in the area (Harikumar et al. 2014).

#### **Moisture**

Castro et al. (2002) reported that the half-life of  $\alpha$  and  $\beta$ - Endosulfan decreased to 27 and 89 days respectively in presence of 8% soil moisture content. Ghadiri and Rose (2001) resulted from their study that under high water content of soil,  $\alpha$ - Endosulfan degraded rapidly within 4 weeks of application followed by a prolonged period of slower rate of degradation in comparison to lower water content.  $\beta$ - isomer degradation was comparatively slow than  $\alpha$ - isomer.

#### **pH and Eh**

Sea water having pH 5.76 medium and also having a high electrical conductivity, which adversely affects the physical properties and bio efficacy of Endosulfan. So, when the physical properties of Endosulfan changed than it will lose its effectiveness against control of insect pests (Rathod and Butani 2008).

#### **Temperature**

Castro et al. (2002) reported that the half-life of  $\alpha$  and  $\beta$  Endosulfan decreased to 27 and 89 days respectively in 35°C.

#### **Organic Matter-Native or Added**

Addition of straw retarded the degradation of Endosulfan under both flooded and non-flooded conditions. Half-life values of Endosulfan were 137 days under non flooded, unamended conditions and 125 days under non flooded straw-amended conditions, 430 days under flooded, unamended conditions and 501 days under flooded straw-amended conditions (Sethunathan et al. 2002).

#### **Degradation of Endosulfan**

Degradation of the two isomers,  $\alpha$ - and  $\beta$ - Endosulfan, does occur in temperate/tropical soil and aquatic systems, both by abiotic and biotic processes.

#### **Chemical degradation of Endosulfan Isomers**

In environment, Endosulfan can undergo either oxidation or hydrolysis reaction to form Endosulfan sulphate and Endosulfan-diol respectively. Endosulfan sulphate is as toxic and as persistent as its parent isomers (Singh et al. 2011).

#### **Microbial Degradation of Endosulfan Isomers**

Bioremediation of pesticides in contaminated soil and water environments has become one of the most sensational issues due to their deleterious effect on public health and environment. In natural habitat, certain microbes are capable of metabolizing those persistent compounds or detoxify them which could be employed for bioremediation. Hence the direct use of such microorganisms capable of degrading xenobiotics is also becoming a popular approach to safeguard the environment (Vivekanandhan and Duraisamy 2012). Microbial degradation plays a major role in degradation of Endosulfan contaminated sites in the environment (Shetty et al. 2000). Ismail et al. (2005) carried out a study of the degradation of Endosulfan (6, 7, 8, 9, 10, 10-hexachloro – 1, 5, 5a, 6, 9, 9a – hexahydro – 6, 9- methano – 2, 4, 3 –

benzodioxanthiepin 3 – oxide) in Malaysian sandy loam and clay soils was carried out using a radio isotopic technique under laboratory conditions. This study indicates that microorganisms are involved in the degradation of Endosulfan. In general, degradation of the pesticide was relatively higher in the clay soil than in the sandy soil. Apart from the parent compounds,  $\alpha$ - and  $\beta$ -isomers, the degradation products include Endosulfan sulphate and three minor unidentified products. Martens (1976) studied the degradation of Endosulfan by different bacterial and fungal cultures, and found that endodiol and endosulphate, respectively were the major metabolites accumulated. Besides, small amounts of endohydroxy ether and endolactone were also formed. Miles and Moy (1979) have proposed a pathway, wherein, the Endosulfan is converted to endosulphate followed by endodiol, endohydroxy ether and endolactone. Formation of these metabolites has also been confirmed by other investigators (Kshemkalyani et al. 1987, Katayama and Matsumura 1993, Kullman and Matsumura 1996). Siddique et al. (2003) has reported that fungus degraded more of beta Endosulfan than alpha Endosulfan and bacteria degraded more of alpha Endosulfan than beta Endosulfan. In a study by Sivaramaiah and Kennedy (2006), a soil bacterium was able to degrade 50% Endosulfan by converting it to Endosulfan sulphate within 3 days of incubation. The enriched mixed culture aerobic and anaerobic bacteria from agricultural soils were used to study the degradation of Endosulfan in aqueous and soil slurry environments (Tiwari 2013).

#### **Anaerobic microbial degradation**

Guerin (1999) had reported the degradation of major isomer of Endosulfan ( $\alpha$ -Endosulfan) by mixed population of anaerobic microorganisms to Endodiol under anaerobic and methanogenic conditions. Endosulfan detoxification in anaerobic condition occurs through two different pathways:

(i) Endosulfan hydrolyzed to Endosulfan diol by enzymatic/chemical hydrolysis; (ii) formation of Endosulfan mono-aldehyde by bacterial metabolism (Kumar and Philip, 2006). The extent of biodegradation was ~80% in aqueous and ~60% in soil slurry during 60 days incubation in anaerobic condition (Tiwari, 2013).

#### **Aerobic Microbial Degradation**

Endosulfan is only moderately persistent in soil under aerobic conditions. Under several studies with radio-labelled Endosulfan, it was shown that degradation of  $\alpha$ - isomer proceeds faster than that of  $\beta$ - isomer. The half-life of  $\alpha$  Endosulfan in various soils is 15-20 days,  $\beta$  Endosulfan is 50-80 days and the technical mixture (70% alpha and 30% beta) is about 30 days. Singh et.al (2011) in an experiment, isolated Endosulfan degrading bacteria *Achromobacter xylosoxidans* strain C8B from soil through selective enrichment technique using sulphur-free medium with Endosulfan as sole Sulphur source. *Achromobacter xylosoxidans* was found to degrade 94.12%  $\alpha$ -Endosulfan, 84.52%  $\beta$ -Endosulfan and 80.10% Endosulfan sulphate using it as sulphur source. Martens (1976), in his study found that Endosulfan can be degraded by different bacterial and fungal cultures to endodiol and Endosulfan sulphate

respectively. These major metabolites were accumulated along with small amounts of endohydroxy ether and endolactone. A pathway was proposed, wherein, the Endosulfan was converted to endosulfate followed by endodiol, endohydroxy ether and endolactone (Miles and Moy (1979); Kshemkalyani et al. 1987; Katayama and Matsumura 1993; Kullman and Matsumura 1996). In an experiment carried out by (Jo et al. 2010) on Endosulfan degrading ability of *Klebsiella oxytoca* KE8 by immobilizing it with activated carbon resulted that the immobilized *K. oxytoca* KE-8 with the cell density of 4 mg (dry weight) degraded 22.18 µg Endosulfan within 5 days at pH 7.0, in batch shake flask cultures and in a laboratory scale pack bed column with support beads were able to degrade Endosulfan completely in defined minimal salt medium at a maximum rate of 129.6 µg per day.

*Pseudomonas aeruginosa* was able to achieve 94% degradation of Endosulfan in contaminated soil (Weir et. al., 2006). The extent of biodegradation was ~95% in aqueous and ~65% in soil slurry during 15 days incubation in aerobic condition (Tiwari, 2013). Microbial degradation of both the isomers of the cyclodienes insecticide Endosulfan by Pseudomonadaceae in water has been investigated by Perscheid et al (2014). Verma et al (2005) reported the degradation of Endosulfan to free chlorides by *Rhodococcus sp.* at 45°C.

#### Fungal Degradation

In an experiment, Zorghani & Omer (1974) observed that isolated strains of *Aspergillus niger* can degrade Endosulfan to endodiol. In an experiment by Katayama and Matsumura (2009) observed the degradation of Endosulfan by *Trichoderma harzianum* to *Endosulfan sulphate* and *Endosulfan diol*.

Table-2

List of Endosulfan Degrading Bacteria and Fungi

Bacteria	References
Corynebacterium sp., Nocardia sp., Mycobacterium sp., Pseudomonas fluorescens, Penicillium sp.	Jayashree et. al., 2007.
Klebsiella oxytoca, Bacillus spp., Pandoraea spp., and Micrococcus spp.	Bhalerao et. al., 2007.
Pseudomonas spinosa, P. aeruginosa, and Burkholderia cepacia.	Hussain, et al., 2007.
Staphylococcus, Micrococcus, Bacillus, Pseudomonas.	Mohanasrinivasan V. et al 2013.
Penicillium sp.	Mariana Romero Aguilar et al 2014.
Fungi	
Aspergillus sp., Phanerochaete chrysosporium.	Jayashree et. al., 2007.
Aspergillus niger, Aspergillus terreus, Cladosporium oxysporum, Mucor thermohyalospora, Fusarium ventricosum, Phanerochaete chrysosporium, Trichoderma harzianum.	Bhalerao et. al., 2007.
Phanerochaete chrysosporium, Aspergillus terreus, and Verticillium sp.	Xu, 2007; Nair and Pradeep, 2007.
Phanerochaete chrysosporium.	Kullman, and Matsumura, 1995.
Botryosphaeria laricina, Aspergillus tamaritii.	Silambarasan and Abraham, 2013.
Mucor, Penicillium, Aspergillus fumigates, Candida, Yeast.	Mohanasrinivasan V. et al., 2013.
Trichoderma harzianum	Katayama and Matsumura, 2009.

#### Factors Affecting Endosulfan Degradation

##### Soil Redox Potential

Addition of organic matter reduces the Soil redox potential, however, the native organic matter (Gardiner and James 2012). Growth of microorganisms on Endosulfan reduces the pH of the medium (Silambarasan and Abraham 2013).

##### Soil Organic Matter

Elsaid et al. (2009) have studied the degradation of α- and β -Endosulfan by incubation of 2 groups of microbes (organic nitrogen bacteria and inorganic nitrogen bacteria and *Actinomycetes*) in presence or absence of fertilizers (urea, triple super phosphate, urea + triple super phosphate and cow manure) for 45 days with sample drawn every 15 days, which resulted an increase in microbial growth.

##### Soil Acidity

Cong et al. (2014) reported the accelerated degradation of Endosulfan in acidic conditions of soil. In the absence of acid, higher degradation of Endosulfan was observed using MgO/Pd(+4) doses of 10/0.5 (Agnihotri et al. 2011).

##### Soil Temperature

*Pseudomonas aeruginosa* degrades 85% α and β isomers of Endosulfan at incubation temperature 30°C (Arshad et al. 2007). Tariq et al. (2005) had reported that increase in moisture and temperature decreases the half-life of pesticides. Dominant microbial populations at higher temperatures have the ability to metabolize substrates that are not used by members of the microbial community at lower temperatures (Zogg 1997). In water Endosulfan (α-and β-) decays at a faster rate with increase in temperature (Kaur 1998).

##### Soil Salinity

Kwon et al. (2005) had reported the increase in biomass of *Klebsiella oxytoca* in a mineral salt medium containing Endosulfan (150mg/L) and Endosulfan sulphate (173mg/L) as a sole source of carbon and energy. Salinity may induce hydrolyzing enzymes to produce Endosulfan diol from Endosulfan (Park 2012).

**Chemicals**

Cong et al. (2014) observed the presence of zero valent Zinc (0) was more effective in degradation of Endosulfan than Fe (0) with half-life 110min and 330 min. The half-life of Endosulfan was decreased from 130.75 min to 41.75 min with the increment of Zn (0) from 0.1 g to 1 g in soil. An ozone dosage of 57 mg/min was found to be optimal for the degradation of Endosulfan (89%) (Begum and Gautam 2012). The degradation of alpha and beta Endosulfan by *Pseudomonas aeruginosa* with Tween 80 and different moisture regimes (flooded and non-flooded conditions) was studied. The rate of degradation was maximum (92 %) in non-flooded and Tween 80 added soil. The addition of synthetic surfactant Tween 80 enhanced the solubility and degradation of Endosulfan (Jayashree and Vasudevan 2009).

**Accelerated Biodegradation of Endosulfan**

Sunitha et al. (2012) used the enriched strains of *Pseudomonas putida* differ in 16Sr RNA sequence, isolated from an Endosulfan contaminated soil of coffee cultivated area for the study of biodegradation of Endosulfan and its metabolite. The strains were grown on Endosulfan and Endosulfan sulphate by using as sole source of sulphur and the degradation was increased to more than 70% and 90% respectively to Endosulfan diol, Endosulfan lactone and Endosulfan sulphate. *Phanerochaete chrysosporium* degraded Endosulfan >90% and >95% in absence of nitrogen source and carbon source respectively in 50 hours of incubation (Kullman and Matsumura 1996). Elsaid and Abdelbagi (2008) reported that the mutant strains of bacteria *Bacillus sp.* and fungi *Aspergillus fumigates* could tolerate 1000mg/L of Endosulfan and in absence of carbon source they converted it to Endosulfan sulphate. Kwon et al. (2005) isolated one bacterial strain, *Klebsiella oxytoca* by 16S rDNA sequence analysis, can be used as a biocatalyst for Endosulfan and Endosulfan sulphate.

**Conclusion**

The use of pesticides has become an integral part of modern agricultural system. However, government regulations for the safe usage and disposal of pesticides have become stringent in many countries. But still the pesticides are persistent in soil, sediment and aquatic systems. These studies imply the impact and biomagnifications of organochlorine pesticides in living habitats. As microbial biodegradation play a major role in eradicating these organochlorine pesticides and many bacteria, fungi degrade and detoxify HCH and Endosulfan, further studies can be made in specifying their role in degradation of many recalcitrant metabolites of these organochlorine pesticides in agricultural prospective. Further studies can be made to reduce the half-life of organochlorine pesticides in the environment to prevent their bio-magnification into the natural habitat. Also further studies on the molecular mechanism of microbial degradation can also be focused for the development of genetically modified microbes for the degradation of the dead end products of these organochlorine pesticides.

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