CHAPTER 1

1. INTRODUCTION

Endosulfan is a chlorinated hydrocarbon insecticide and acaricide of the cyclodiene subgroup, which acts as a poison to a wide variety of insects and mites on contact. Although it may also be used as a wood preservative, it is used primarily on a wide variety of food crops including tea, coffee, fruits, and vegetables, as well as on rice, cereals, maize, sorghum or other grains. Formulations of Endosulfan include emulsifiable concentrate, wettable powder, ultra-low volume (ULV) liquid, and smoke tablets. It is compatible with many other pesticides and may be found in formulations with dimethoate, malathion, methomyl, monocrotophos, pirimicarb, triazophos, fenoprop, parathion, petroleum oils, and oxine-copper. However, it is not compatible with alkaline materials. Technical Endosulfan is made up of a mixture of two molecular forms (isomers) of Endosulfan, the alpha- and beta-isomers (Arrebola *et al.* 2001).

1.1 Trade and Other Names

Trade or other names for the product include Afidan, Beosit, Cyclodan, Devisulfan, Endocel, Endocide, Endosol, FMC 5462, Hexasulfan, Hildan, Hoe 2671, Insectophene, Malix, Phaser, Thiodan, Thimul, Thifor, and Thionex.

1.1.1 Chemical Name

6,7,8,9,10,10- hexachloro-1,5,5a,6,9,9a-hexahydro–6,9-methano-2,4,3-benzodioxathiepine-3-oxide.

1.1.2 Synonyms

Endosulfan is produced commercially by different trade names which are as mentioned in the table given below:

1	1,2,3,4,7,7 – Hexachlorobicyclo (2.2.1) hepten - 5,6 – bioxymethylenesulfite
2	1,4,5,6,7,7 - Hexachloro - 5 - norbornene - 2,3 - dimethanol cyclic sulfite
3	6,7,8,9,10,10 - Hexachloro - 1,5,5a,6,9,9a - hexahydro - 6,9 - methano - 2,4,3 - benzodioxathiepin - 3 – oxide
4	6,9 - Methano - 2,4,3 - benzodioxathiepin, 6,7,8,9,10,10 – hexachloro – 1, 5, 5a, 6, 9, 9a - hexahydro – 3, 3, dioxide.
5	Sulfurous acid, cyclic ester with 1,4,5,6,7,7 - hexachloro - 5 - norbornene - 2,3 – dimethanol
6	Hexachlorohexahydromethano 2,4,3 - benzodioxathiepin - 3 - oxide Hildan
7	BIO 5,462
8	Benzoepin

9	Beosit	26	Niagara 5,462
10	Chlorthiepin	27	OMS 570
11	Crisulfan	28	Rasayansulfan
12	Devisulphan	29	SD - 4314
13	ENT 23,979	30	Thifor
14	Endocel	31	Thimul
15	Endosulfan	32	Thiodan
16	Endosulfan (ACGIH:OSHA)	33	Thiodan
17	Endosulphan	34	Thiofor
18	Endotaf	35	Thiomul
19	FMC 5462	36	Thionate
20	Goldenleaf tobacco spray	37	Thionex
21	HOE 2,671	38	Thiosulfan
22	Insectophene	39	Thiosulfan tionel
23	Kop – thiodan Malix	40	Thiotox
24	NCI - C00566	41	Thiotox (insecticide)
25	NIA 5462	42	Tiovel

1.2 Regulatory Status

Endosulfan is a highly toxic pesticide in EPA toxicity class I. It is a Restricted Use Pesticide (RUP). Labels for products containing Endosulfan must bear the Signal Words DANGER - POISON, depending on formulation.

1.3 Physical Properties

Sr. No.	Physical Properties	
1	Appearance	Pure Endosulfan is a colorless crystal. Technical grade is yellow-brown in color (Kidd, 1991).
2	Chemical Name	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzadioxathiepin 3-oxide (Kidd, 1991).
3	CAS Number	115-29-7 (alpha-isomer, 959-98-8; beta-isomer, 33213-65-9)
4	Molecular Weight	406.96
5	Water Solubility	0.32 mg/L @ 22^0C (Kidd, 1991)
6	Solubility in other Solvents	Soluble in toluene and hexane (Kidd, 1991)
7	Melting Point	Technical material, 70-100^0C (Kidd, 1991)
8	Vapour Pressure	1200 mPa @ 80^0C (Kidd, 1991)
9	Partition Coefficient	Not Available
10	Adsorption Coefficient	12,400 (Wauchope *et al.*, 1992)

1.4 Chemical Class, Structure and Properties

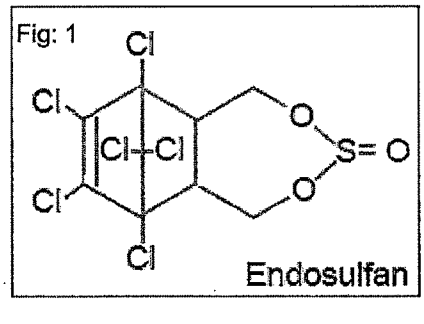
Endosulfan has been classified as a moderately hazardous chemical 5 and it has been classified as a Moderately Hazardous (class II) pesticide by World Health Organization (WHO, 1984, 2002).

The U S Environmental Protection Agency (EPA) classifies Endosulfan as Category Ib – Highly Hazardous. The European Union also rates it Highly Hazardous. However, World Health Organisation (WHO) classifies Endosulfan in Category II - Moderately Hazardous. Nevertheless classification of WHO was found to be inappropriate considering the classification followed in many countries and the available toxicological information. It has been alleged that the classification is based mainly on LD_{50} value for acute toxicity generated by the producer company (Romeo *et al.,* 2000). The Industrial Toxicological Research Centre (ITRC) in India, the nodal centre for the Regional Based Assessment of Persistent Toxic Substances (PTS) for the Indian Ocean region by the United Nations Environment Programme - Global Environment Facility (UNEP-GEF) classifies Endosulfan as Extremely Hazardous (Anon, 1989).

Chemical Formula - $C_9H_6Cl_6O_3S$

Structural Formula (figure 1)

Fig: 1

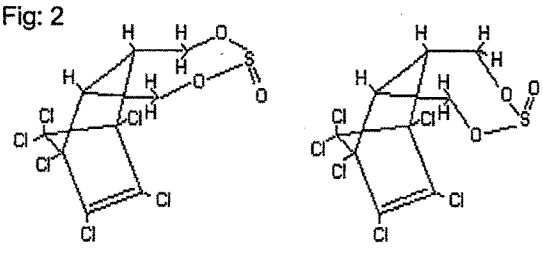


Endosulfan

In pure form Endosulfan exists as colourless crystals. But the technical product is brownish crystals with slight odour of sulphur dioxide. Technically Endosulfan is a mixture of two isomers- alpha-Endosulfan and beta-Endosulfan in the ratio of 7:3. Technical grade Endosulfan contains 94% alpha-Endosulfan and beta-Endosulfan and other related compounds like Endosulfan alcohol, Endosulfan ether and Endosulfan sulfate. Though Endosulfan is only very slightly soluble in water, it dissolves readily in xylene, chloroform, kerosene and most organic solvents and is a noncombustible solid. It is mixable with most fungicides and compatible with most pesticides (Anon, 1989).

Technical Endosulfan contains two stereoisomers, Endosulfans A and B (figure 2) in the proportion variously reported as from 4:1 to 7:3. The technical material is a 90-95 per cent pure mixture of the two isomers.

Fig: 2

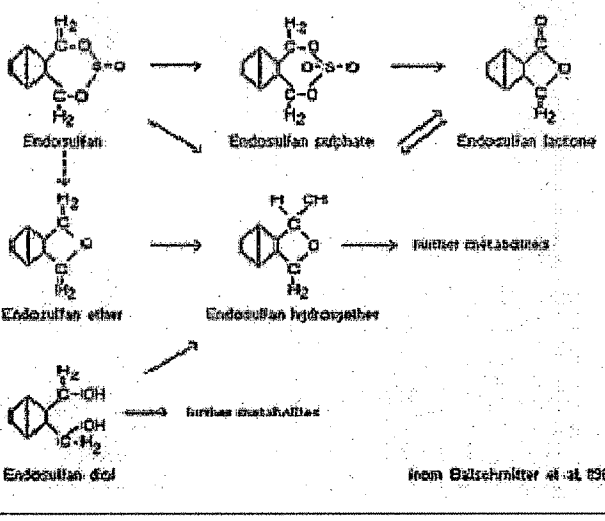


ENDOSULFAN A ENDOSULFAN B

1.4.1 Other Relative Chemical Properties Including Metabolites

Ballschmitter *et al.,* (1967) have considered all of the possible metabolites of Endosulfan which can hypothetically be formed on hydrolysis, oxidation or reduction. They have investigated Endosulfan metabolism in the mouse and rat using thin layer and gas chromatographic techniques and have identified five of the possible metabolites, including Endosulfan sulfate, diol, ether, hydroxyether and lactone as illustrated in the following figure 3:

Fig:3



1.5 Formulation

Various formulations of Endosulfan exist in the market. These formulations of Endosulfan include emulsifiable concentrate, wettable powder, ultra-low volume (ULV) liquid and smoke tablets.

1.6 Exposure Guidelines

ADI : 0.006 mg/kg/day (Lu, 2000)

MCL : Not Available

RfD : 0.00005 mg/kg/day (US EPA, 1995)

PEL : Not Available

HA : Not Available

TLV : 0.1 mg/m3 (8-hour) (American Conference of Governmental Industrial Hygienists, Inc, 1986)

1.7 Compositions and Chemical Structure of Endosulfan (selected for dissertation)

Endosulfan 35% EC	-	Insecticide
Batch Number	-	DNDO-170
Date of manufacture	-	June -2003
Date of Expiry	-	May 2005
Quantity	-	250ml
Manufactured by	-	Nothern Minerals limited
Daulatabad road, Gurgaon	-	122001 (Haryana)
Manufacturing License Number	-	3/73/PPH
Registration Number	-	V1-1375(3) (E.C.)-369

Chemical Composition

Endosulfan (active ingredient)	-	35 % (w/w)
Adjuvants	-	65 % (w/w)
		100 %

1.8 Justification for Selecting of Emulsifiable Concentrate

Most of the products of Endosulfan used for biocidal purposes are of emulsifiable concentrate. Solvents and/or emulsifiers used with Endosulfan in formulated products may influence its absorption into the system via all routes; technical Endosulfan is slowly and incompletely absorbed into the body whereas absorption is more rapid in the presence of alcohols, oils, and emulsifiers (Smith, 1991).

1.9 Production of Endosulfan

In many countries the production of Endosulfan is banned, but in India it is still produced. Though the production of Endosulfan is reducing gradually it was produced 4489, 3663 and 3657 tonnes in the year 2001-2002, 2002-2003 and 2003-2004, respectively (Ware, 1986).

Practically very little information is available of the volumes of production of Endosulfan. WHO estimated that the worldwide production of Endosulfan was 10,000 MT in the year 1984 (Anon, 2003), Current estimates of worldwide production or domestic formulations could not be located. Many countries that produced Endosulfan in the 1970's and 80's do not produce it any more. For instance, Endosulfan has not been produced in the United States since 1982, but it is still used in chemical formulations. In USA, annual average amount of 626 tonnes is used (Michael, 2003). In European Union, Germany is the only producer of Endosulfan and it is manufactured at a single site, where 5000 TPA is produced. Vast majority of this is exported to South America and South East Asia (Michael, 2003). In EU (1999), 90% of the Endosulfan was used in Mediterranean area (Michael, 2003). Especially Spain, Italy, Greece and France are the major consumers. Endosulfan is produced mainly in Israel, India, China and South Korea (Michael, 2003) and India being one of the major producers of Endosulfan. Since 1996-97 it produces an average of 8206 MTPA totaling 41033 MT during 1995-20002. India exported 12180 MT during this period and consumed on an average 3599 MTPA. The UNEP-GEF report on PTS has identified some of the producing and importing countries but there is a large data gap. No information regarding stockpiles of Endosulfan could be located.

2. USES OF ENDOSULFAN

2.1 Agricultural Uses of Endosulfan

The agricultural use of Endosulfan is very diverse and it is being used with several other insecticides or used at different intervals pre and/or post plantation. However it is difficult to draw a distinct line between the effect of Endosulfan alone or in combination since Endosulfan is often being used with other pesticides to evoke desirable end result in different crop fields against diverse groups of pest population.

2.1.1 Description

Endosulfan is a non-systemic insecticide and acaricide with contact and stomach action. It is used in the control of sucking, chewing and boring insects and mites on a very wide range of crops, including fruit (including citrus), vines, olives, vegetables, ornamentals, potatoes, cucurbits, cotton, tea, coffee, rice, cereals, maize, sorghum, oilseed crops, hops, hazels, sugar cane, tobacco, alfalfa, mushrooms, forestry, glasshouse crops, etc. It also controls tsetse flies (Tomlin, 1994).

2.1.2 High Risk Circumstance of Poisoning

Accidental poisoning of children by Endosulfan stored in the home or garage, accidental exposure among formulating plant workers and suicide attempts have a high risk

circumstance of poisoning. Additionally individuals with a history of convulsive disorders would be expected to be at increased risk from exposure (Mackison *et al.,* 1981).

2.1.3 Occupationally Exposed Populations

Factory workers involved in synthesis of Endosulfan, workers involved in formulating and dispensing Endosulfan and Public health workers involved in pest control are occupationally exposed populations.

2.2 Other Uses of Endosulfan

Endosulfan is being used as wood preservative in a variety of products with different compositions.

3. EXPOSURE OF ENDOSULFAN

3.1 Exposure Routes of Endosulfan

3.1.1 Oral

Ingestion occurs through accidental or deliberate ingestion or accidental ingestion of contaminated foodstuffs.

3.1.2 Inhalation

Endosulfan vapor is absorbed by inhalation.

3.1.3 Dermal

Endosulfan is readily absorbed after dermal contact, at a degree depending on the type of solvent used.

3.1.4 Eye

Eyes are exposed to vapors, dust and aerosols.

3.1.5 Parenteral

No data available on parenteral exposure.

4. KINETICS

4.1 Absorption by Route of Exposure

The percentage of Endosulfan absorbed after oral dosing would appear to have been moderate to high. Single oral doses of 0.3 mg Endosulfan and its two isomers administered to male Balb-c mice were not completely absorbed from the gastrointestinal tract but were excreted with the metabolites Endosulfan sulfate and diol in the faeces (IPCS, 1998a).

4.2 Distribution by Route of Exposure

The autopsy and toxicological findings in a fatal case caused by ingestion of Endosulfan dispersed in a colorless liquid containing about 55% of xylene (w/v) is reported by Bernardelli and Gennari (1987). The following concentrations of Endosulfan were found: blood 30 mg/L, gastric contents 0.5g in the total 50 mL, liver 20 mg/kg, kidney 2.0 mg/kg , brain 0.3 mg/kg, xylene (solvent) was detected only in stomach contents (0.4 g in the total 50 mL). When Endosulfan was fed to Balb-c mice in the diet at a concentration of 10 ppm for up to 49 days, the sulfate metabolite was detected in the liver and visceral fat of all animals. Both isomers and the sulfate and diol metabolites of Endosulfan were detected in the faeces, while the only Endosulfan product detected in the urine of these animals was the diol metabolite. After a single dose of 0.3 mg of ^{14}C-labelled Endosulfan to Balb-c mice the highest concentrations followed, in rank order, by visceral fat > urine > small intestine > kidney > brain> expired carbon dioxide > blood (Deema *et al.,* 1966).

At the end of a 24 - month study in which NMRI mice were given diets containing 0, 2, 6, or 18 ppm technical - grade Endosulfan, the concentrations of Endosulfan and its main metabolites Endosulfan hydroxyether, sulfate, lactone, and diol were measured in the liver and kidneys. No Endosulfan was detected in either the liver or the kidney. In mice that were given 18 ppm Endosulfan, the concentrations of the hydroxyether, lactone, and diol metabolites were at or below the level of detection (0.02 ppm), while the Endosulfan sulfate concentrations were 0.1 to 0.2 ppm in kidney and 0.7 to 1.1 ppm in liver. The tissue concentrations of Endosulfan sulfate in mice at 2, 6 and 18 ppm, respectively, were: kidney, 0.2 to 0.4 ppm, 0.04 ppm and 0.1 to 0.2 ppm; and liver, 0.06 to 0.07 ppm, 0.12 to 0.45 ppm, and 0.7 to 1.1 ppm (Leist, 1989).

Following acute over-exposure, high Endosulfan concentrations can temporarily be found in the liver; the concentration in the plasma decreases rapidly (IPCS, 1984).

4.3 Biological half-life by route of exposure

The half lives for urinary and faecal elimination for male and female rats were biphasic, with an earlier half life of 6 to 14 hour and a later half life of 33 to 67.5 hour (IPCS, 1998a).

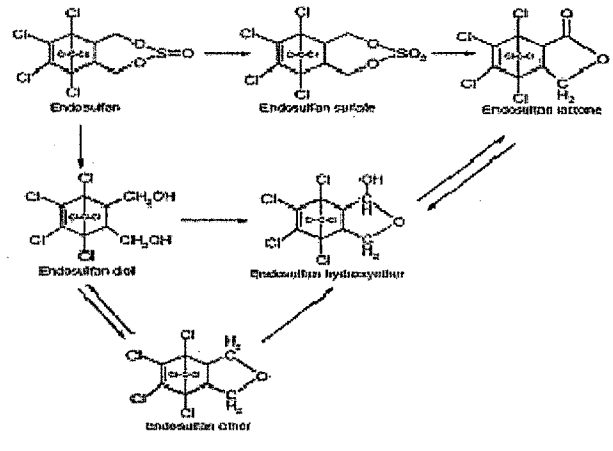
4.4 Metabolism

Proposed Metabolic Pathway for Endosulfan

No information is available on the metabolism of Endosulfan in adult humans or children. Endosulfan is readily metabolized in animals following exposure (Deema *et al.,* 1966; Dorough *et al.,* 1978; Gorbach *et al.,* 1968). It exists in two stable stereoisomeric forms,

which can be converted to Endosulfan sulfate and Endosulfan diol (WHO 1984). These can be further metabolized to Endosulfan lactone, hydroxyether, and ether. Figure 4 shows the pathway for the degradation of Endosulfan. Dorough *et al.,* (1978) indicated that the major portion of residues in the excreta and/or tissues consisted of unidentified polar metabolites that could not be extracted from the substrate, whereas the nonpolar metabolites, including sulfate, diol, α-hydroxyether, lactone, and ether derivatives of Endosulfan, represented only minor amounts. Excretion data from an acute dermal study in rats showed that, after 24 hours, a dose-related decrease in excretion occurred at higher doses, suggesting saturation of the metabolic pathway of Endosulfan (Hoechst, 1986).

Fig:4



High concentrations of Endosulfan sulfate were found primarily in the liver, intestine, and visceral fat, 24 hours after mice were exposed to a single dose of 14^{C}-Endosulfan (Deema *et al.,* 1966).

Xenobiotic Metabolism in animals is by oxidation and hydrolysis. When given to rats by various routes, Endosulfan is metabolized to the sulfate, diol, hydroxyether, lactone, ether, hydroxy Endosulfan carboxylic acid. Most Endosulfan metabolites are polar and yet to be identified (IPCS, 1998a).

4.5 Elimination and Excretion

After oral and intravenous administration of ^{14}C - Endosulfan to male and female Wistar rats at a dose of 2 or 0.5 mg/kg body weight respectively, >80% (intravenous) or 90% (oral)

of the dose was eliminated in the urine and faeces within seven days; elimination however was essentially complete within 1 - 2 days (IPCS, 1998a).

^{14}C-Endosulfan (alpha or beta isomer) was rapidly excreted by female rats after a single oral dose of 2 mg/kg body weight or administration in the diet at a concentration of 5ppm. After a single dose, > 85% was excreted within 120 hours (> 70% after 48 hours), mainly in the faeces and to a lesser extent in the urine. After dietary administration for 14 days, followed by a 14 day recovery period, >72% of the administered dose was recovered. Biliary excretion of radiolabel in male rats given 1.2 mg/kg body weight as a single dose approached 50% for the alpha isomer and 30 % for the beta isomer over 48 hours. There appeared to be little enterohepatic circulation. The largest proportion of the radiolabel administered was metabolized to highly polar products, most of which could not be extracted from faeces (28%) or tissues (71%). Of the extractable fraction, unidentifiable polar metabolites constituted 6.2 % in faeces and 13 % in urine. The apolar metabolites of Endosulfan identified in faeces and urine were the diol, the lactone, the alpha-hydroxyether, and the sulphate (Dorough *et al.*, 1978).

5. TOXICITY

5.1 Human Data

5.1.1 Adults

In general, the doses of Endosulfan, involved in cases of poisoning have been poorly characterized. In a summary of case reports (Lehr, 1996), the lowest reported dose that resulted in death was 35 mg/kg body weight; deaths have also been reported after ingestion of 295 and 467 mg/kg body weight, within 1hour of ingestion in some cases. Intensive medical treatment within 1 hour was reported to be successful after ingestion of doses of 100 and 1000 mg/kg body weight. The clinical signs in these patients were consistent with those seen in laboratory animals, dominated by tonic clonic spasms. In a case where a dose of 1000 mg/kg body weight was ingested, neurological symptoms requiring anti-epileptic theraphy was still required one year after exposure (IPCS, 1998a).

5.1.2 Children

No data available.

5.1.3 Relevant Animal Data

Acute oral LD_{50} for rats	:	80 mg/kg (IPCS, 1998b)
Acute oral LD_{50} for mice	:	14 - 35 mg/kg
Acute dermal LD_{50} for rabbits	:	290 mg/kg

Inhalation LC_{50} (1 hour) for rats	:	> 21 mg/L air
(4 hours) male rats	:	0.0345 mg/L
(4 hours) female rats	:	0.0126 mg/L (Tomlin, 1994)
NOEL	:	30 mg/kg (2 year feeding trials in rats)
NIOSH REL	:	Ca TWA 0.1 mg/m^3 (skin)
Acceptable daily intake (ADI)	:	0 - 0.006 mg/kg body weight.

5.2 Toxicity Types

5.2.1 Acute Toxicity

Endosulfan is highly toxic via the oral route, with reported oral LD_{50} values ranging from 18 to 160 mg/kg in rats, 7.36 mg/kg in mice, and 77 mg/kg in dogs (Kidd, 1991; Smith, 1991). It is also highly toxic via the dermal route, with reported dermal LD_{50} values in rats ranging from 78 to 359 mg/kg (Kidd, 1991; Smith, 1991). Endosulfan may be only slightly toxic via inhalation, with a reported inhalation LC_{50} of 21 mg/L for 1 hour, and 8.0 mg/L for 4 hours (Smith, 1991). It is reported not to cause skin or eye irritation in animals (Smith, 1991). The alpha-isomer is considered to be more toxic than the beta-isomer (Smith, 1991). Animal data indicates that toxicity may also be influenced by species and by level of protein in the diet; rats which have been deprived of protein are nearly twice as susceptible to the toxic effects of Endosulfan (Smith, 1991). Solvents and/or emulsifiers used with Endosulfan in formulated products may influence its absorption into the system via all routes; technical Endosulfan is slowly and incompletely absorbed into the body whereas absorption is more rapid in the presence of alcohols, oils, and emulsifiers (Smith, 1991). Stimulation of the central nervous system is the major characteristic of Endosulfan poisoning (U.S. Agency for Toxic Substances and Disease Registry, 1990). Symptoms noted in acutely exposed humans include those common to the other cyclodienes, e.g., incoordination, imbalance, difficulty in breathing, gagging, vomiting, diarrhea, agitation, convulsions, and loss of consciousness (Smith, 1991). Reversible blindness has been documented for cows that grazed in a field sprayed with the compound. The animals completely recovered after a month following the exposure (Smith, 1991). In an accidental exposure, sheep and pigs grazing on a sprayed field suffered a lack of muscle coordination and blindness (Smith, 1991).

5.2.2 Chronic Toxicity

In rats, oral doses of 10 mg/kg/day caused high rates of mortality within 15 days, but doses of 5 mg/kg/day caused liver enlargement and some other effects over the same period (Smith, 1991). This dose level also caused seizures commencing 25 to 30 minutes following dose administration that persisted for approximately 60 minutes (Smith, 1991). There is

evidence that administration of this dose over 2 years in rats also caused reduced growth and survival, changes in kidney structure, and changes in blood chemistry (U.S. Agency for Toxic Substances and Disease Registry, 1990; Smith, 1991).

5.2.3 Reproductive Toxicity

Rats fed doses of Endosulfan of 5 mg/kg/day for three generations showed no observable reproductive effects (Smith, 1991), but 5.0 mg/kg/day caused increased dam mortality and resorption (U.S. Agency for Toxic Substances and Disease Registry, 1990; Smith, 1991). Female mice fed the compound for 78 weeks at 0.1 mg/kg/day had damage to their reproductive organs (National Cancer Institute, 1978). Oral dosage for 15 days at 10 mg/kg/day in male rats caused damage to the semeniferous tubules and lowered testes weights (Hurt, 1991; Smith, 1991). It is unlikely that Endosulfan will cause reproductive effects in humans at expected exposure levels.

5.2.4 Teratogenic Effects

An oral dose of 2.5 mg/kg/day resulted in normal reproduction in rats in a three-generational study, but 5 and 10 mg/kg/day resulted in abnormalities in bone development in the offspring (U.S. Agency for Toxic Substances and Disease Registry, 1990; Smith, 1991). Teratogenic effects in humans are unlikely at expected exposure levels.

5.2.5 Carcinogenic Effects

In a long-term study done with both mice and rats, the males of both groups experienced such a high mortality rate that no conclusions could be drawn (National Cancer Institute, 1978). However, the females of both species failed to develop any carcinogenic conditions 78 weeks after being fed diets containing up to about 23 mg/kg/day. The highest tolerated dose of Endosulfan did not cause increased incidence of tumors in mice over 18 months, and a later study also showed no evidence of carcinogenic activity in mice or rats (National Cancer Institute, 1978; Smith, 1991). Therefore, it infers that Endosulfan is not carcinogenic (IPCS, 1998a).

5.2.6 Organ Toxicity

Data from animal studies reveal that the organs most likely to be affected include kidneys, liver, blood, and the parathyroid gland (U.S. Agency for Toxic Substances and Disease Registry, 1990).

5.3 Genotoxicity of Endosulfan

5.3.1 Mutagenic Effects

Endosulfan is mutagenic to bacterial and yeast cells (U.S. Agency for Toxic Substances and Disease Registry, 1990). The metabolites of Endosulfan have also shown the ability to

cause cellular changes (U.S. Agency for Toxic Substances and Disease Registry, 1990; Smith, 1991). This compound has also caused mutagenic effects in two different mammalian species (U.S. Agency for Toxic Substances and Disease Registry, 1990). Thus, evidence suggests that exposure to Endosulfan may cause mutagenic effects in humans if exposure is great enough.

Endosulfan was not mutagenic in *E. coli* or *S. typhimurium* (Fahrig, 1976; Moriya *et al.,* 1983). It did not induce mitotic conversion in *Saccharomyces cerevisae* (Fahrig, 1976). However, in one study technical grade Endosulfan was reported to induce reverse mutations, cross overs, and mitotic gene conversions in *Saccharomyces cerevisiae* (Yadav *et al.,* 1982).

Endosulfan did not induce chromosomal aberrations in bone marrow cells or spermatogonia of male rats treated with 5 daily oral doses of 11 to 55 mg/kg body weight (Dikshith and Datta, 1978).

An increased number of micronuclei induced in the bone marrow erythrocytes of mice treated with Endosulfan in the drinking water (43.3 mg/litre) for 2 consecutive days was not statistically significant (Usha Rani *et al.,* 1980). Negative results were observed in a dominant lethal test in mice (Canada, National Research Council, 1975).

5.3.2 Genotoxicity Issues

The Spanish conclusions about genotoxicity of Endosulfan were presented at the Working Group on the Classification and Labelling of Dangerous Substances: Meeting on Pesticides-Health Effects (25-27 April 2001) as documents ECBI/11/01 and ECBI/11/01 Add. 1. This evaluation had taken into account all studies that were included both in the monograph and in the first addendum (July 2001). It was concluded that although Endosulfan was non-mutagenic *in vitro* and *in vivo* for somatic cells, it could not be precluded its mutagenicity for germ cells. In this sense, the notifier was requested by the ECCO 102-Peer Review Meeting to address the significance of published studies showing genotoxicity to germ cells.

5.4 Neurotoxicity

5.4.1 Mode of Action to the Nerve Cell

Chlorinated hydrocarbon insecticides act by altering the electrophysiological and associated enzymatic properties of nerve cell membranes, causing a change in the kinetics of Na^+ and K^+ ion flow through the membrane. Disturbances of calcium transport of Ca^{2+}-ATPase activity may also be involved, as well as phosphokinase activities (Hayes and Laws, 1991).

The cyclodiene compounds antagonize the action of the neurotransmitter gamma-aminobutyric acid (GABA), which induces the uptake of chloride ions by neurons. The blockage of this activity by cyclodiene insecticides results in only partial repolarization of the neuron and a state of uncontrolled excitation (Klassen and Watkins, 1999).

5.5 Interactions

The report of Arnold *et al.,* (1996) indicated that even estrogen mimics low potency, such as Endosulfan, could have important effects because of interactions with other chemicals. The estrogenic properties of combinations of chemicals were screened in a system in which the human estrogen receptor sequence is incorporated into the yeast genome. Combinations of two weak environmental estrogens, such as Dieldrin, Endosulfan, and Toxaphene, were 1000 times more potent in human estrogen receptor – mediated transactivation than any chemical alone. This result was not produced in another laboratory in which the same assay was used or in a uterotropic assay in which sexually immature rats were treated with Endosulfan or Dieldrin alone or in a combination of three successive days and the uterine mass weighed on the following day. Both chemicals were inactive in either assay, and there was no evidence of synergism (Ashby *et al.,* 1997). In a further study with the human estrogen receptor assay, however, 0.1 mmol/L Endosulfan increased the activity of beta-glycosidase (Ramamurthy *et al.,* 1997).

More doubt was cast upon the thesis of synergism by an independent study in which Endosulfan and dieldrin showed no additive effect in displacing ^{3}H-17-estradiol from rat uterine estrogen receptors or in inducing the proliferation of MCF-7 breast cancer cells. The weak proliferative potential described by Soto *et al.,* (1994, 1995) was, however, confirmed in this assay *in vitro*. Endosulfan or dieldrin alone at 3 mg/kg body weight per day or in combination, injected intraperitoneally daily for three days, did not stimulate uterotrophic activity and had no effect on pituitary prolactin or other endocrine related end-points in immature female rats, indicating that these weakly estrogenic compounds did not interact in a synergistic fashion in binding to estrogen receptors or in activating estrogen receptors-dependent responses in mammalian tissues or cells (Wade *et al.,* 1997). The paper in which synergism was originally proposed was later withdrawn, since the results could not be reproduced, even in the same laboratory (McLachalan, 1997). Overall, these suggest that concomitant exposure to weakly estrogenic compounds probably does not result in reproductive toxicity related to estrogen action.

5.6 Fate in Humans and Animals

Endosulfan is rapidly degraded into mainly water-soluble compounds and eliminated in

mammals with very little absorption in the gastrointestinal tract (Smith, 1991). In rabbits, the beta-isomer is cleared from blood plasma more quickly than the alpha-isomer, with reported blood half-lives of approximately 6 hours and 10 days, respectively (Smith, 1991), which may account in part for the observed differences in toxicity. The metabolites are dependent on the mixture of isomers and the route of exposure (Smith, 1991). Most of the Endosulfan seems to leave the body within a few days to a few weeks.

6. ECOLOGICAL EFFECTS

6.1 Effects on Birds

Endosulfan is highly to moderately toxic to bird species, with reported oral LD_{50} values in mallards ranging from 31 to 243 mg/kg (Hudson, 1984; Kidd, 1991) and in pheasants ranging from 80 to greater than 320 mg/kg (Hudson, 1984). The reported 5-day dietary LD_{50} is 2906 ppm in Japanese quail (Hill, 1986). Male mallards from 3 to 4 months old exhibited wings crossed high over their back, tremors, falling, and other symptoms as soon as 10 minutes after an acute, oral dose. The symptoms persisted for up to a month in a few animals (Hudson, 1984).

6.2 Effects on Aquatic Organisms

The effect of Endosulfan on non-target species can be swift and devastating. Farmers in Benin have observed birds and frogs dying following consumption of insects sprayed with Endosulfan (Ton *et al.*, 2000). According to one such farmer, "*fields smell awful two or three days after spraying because virtually every living thing has been killed and starts to rot*" (Myers, 2000).

Endosulfan is also extremely toxic to aquatic life. Research has shown that exposure to Endosulfan, even at sublethal doses (50% of LC_{50}), induces behavioural and biochemical changes in fish (Abu Zeid *et al.*, 2000).

Endosulfan is very highly toxic to four fish species and both of the aquatic invertebrates studied; in fish species, the reported 96-hour LC_{50} values were (in µg/L): rainbow trout, 1.5; fathead minnow, 1.4; channel catfish, 1.5; and bluegill sunfish, 1.2. In two aquatic invertebrates, scuds (*G. lacustris*) and stoneflies *(Pteronarcys*), the reported 96-hour LC_{50} values were, respectively, 5.8µg/L and 3.3µg/L (Johnson, 1980). The bioaccumulation for the compound may be significant; in the mussel (*Mytillus edulis*) the compound accumulated 600 times to the ambient water concentration (U.S. National Library of Medicine, Hazardous Substances Data Bank, 1995).

Endosulfan runoff from cotton fields killed over 240,000 fish in Alabama (USA) in 1995, despite the pesticide reportedly having been applied according to label instructions (PAN-UPS, 1996). Similarly, mass fish deaths have been reported in India (PAN-UPS, 1996), Benin (Ton *et al.,* 2000), Sudan (Dinham, 1993), Germany (WHO/UNEP/ILO,1984) and Australia (www.mp.wa.gov.au/giz-watson/speeches/fitzroy.html).

Dr Michael Berrill of Ontario's Trent University conducted seminal research into the effects of Endosulfan on amphibians (Raloff, 1998). Frogs and toads hatched from eggs exposed to low Endosulfan concentrations exhibit a depressed "avoidance behaviour", increasing their likelihood of predation. Tadpoles exposed after hatching experienced elevated mortality, with death being considerably more likely for two-week old tadpoles than those just hatched. Symptoms of sub-lethal poisoning were also observed and included: hyperactivity, whip-like convulsions, temporary paralysis and slow growth rates. Berrill concluded that the hazard posed by Endosulfan is "*sufficiently great to warrant its replacement by less toxic alternatives wherever possible.*" (Raloff, 1998) In a separate experiment with red-spotted newts, low-concentration exposure to Endosulfan impaired the pheromonal system, thereby disrupting mate choice and reducing mating success (Park, *et al.,* 2001).

6.3 Effects on Other Organisms

It is moderately toxic to bees and is relatively nontoxic to beneficial insects such as parasitic wasps, lady bird beetles, and some mites (Kidd, 1991; U.S. National Library of Medicine Hazardous Substances Data Bank, 1995). Toxicity for bees is low to moderate (IPCS, 1984)

7. ENVIRONMENTAL FATE

7.1 Degradation of Endosulfan

Microbial degradation of Endosulfan may play an important role in detoxifying the Endosulfan- contaminated sites in the environment. There are a few reports on degradation of Endosulfan by different groups of microorganisms. However, recent reports indicated that microbial conversion of Endosulfan to Endosulfan diol by hydrolytic pathway is a detoxification process whereas Endosulfan sulfate was found to be a terminal degradation product. In this study, they reported the degradation of Endosulfan by a soil fungus *M. thermohyalospora* MTCC 1384 in culture medium under laboratory conditions (Shetty, *et al.,* 2000).

7.2 Breakdown in Soil and Groundwater

Endosulfan is moderately persistent in the soil environment with a reported average field half-life of 50 days (Wauchope, 1992). The two isomers have different degradation times in soil. The half-life for the alpha-isomer is 35 days, and is 150 days for the beta-isomer under

neutral conditions. These two isomers will persist longer under more acidic conditions. The compound is broken down in soil by fungi and bacteria (Kidd, 1991). Endosulfan does not easily dissolve in water, and has a very low solubility (Kidd, 1991; Wauchope, 1992). It has a moderate capacity to adhere or adsorb to soils (Wauchope, 1992). Transport of this pesticide is most likely to occur if Endosulfan is adsorbed to soil particles in surface runoff. It is not likely to be very mobile or to pose a threat to groundwater. It has, however, been detected in California well water (Howard, 1991).

7.3 Breakdown in Water

In raw river water at room temperature and exposed to light, both isomers disappeared in 4 weeks (Howard, 1991). A breakdown product first appeared within the first week. The breakdown in water is faster i.e. within 5 weeks, under neutral conditions than at more acidic conditions or basic conditions i.e. within 5 months (Howard, 1991). Under strongly alkaline conditions the half-life of the compound is 1 day. Large amounts of Endosulfan can be found in surface water near areas of application (U.S. Agency for Toxic Substances and Disease Registry, 1990). It has also been found in surface water throughout the country at very low concentrations (Howard, 1991).

7.4 Breakdown in Vegetation

In plants, Endosulfan is rapidly broken down to the corresponding sulfate (Kidd, 1991). On most fruits and vegetables, 50% of the parent residue is lost within 3 to 7 days (Kidd, 1991). Endosulfan and its breakdown products have been detected in vegetables (0.0005-0.013 ppm), in tobacco, in various seafoods (0.2 ppt-1.7 ppb), and in milk (Howard, 1991).

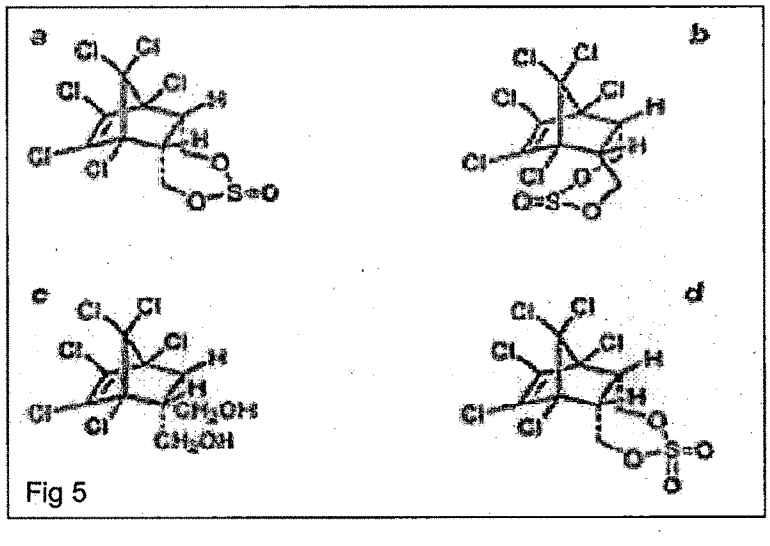


Figure 5: Degradation (Endosulfan isomers and its metabolites); ***a***, *α*-Endosulfan; ***b***, *β*-Endosulfan; ***c***, endodiol and ***d***, endosulfate.

8. ISSUES WITH ENDOSULFAN

8.1 Risks of Endosulfan

Pesticide safety is classified by the World Health Organization (WHO) according to the results of LD_{50} tests, which document the amount of a chemical required to kill 50 % of a population of laboratory rats. Under this system, Endosulfan is currently classified as Class II – Moderately Hazardous to human health. However, the United States' Environmental Protection Agency (EPA) rates Endosulfan as Category Ib – Highly Hazardous. LD_{50} data for Endosulfan are equivocal, with some published results indicating that the chemical should be in the WHO's Class Ib, according to the organization's own criteria. Evidence of the threats to human health posed by Endosulfan is abundant, and the chemical has been banned outright or severely restricted in a number of countries as a result (see box). Independent of LD_{50} results, these threats warrant the immediate upgrading of Endosulfan to WHO Class Ib.

Figure 6: A boy showing cerebral palsy. The boy cannot walk or talk. **Figure 7:** A lady showing acute epilepsy and severe neural disfunction. The village was exposed to aerial spraying of Endosulfan for over 15 years.

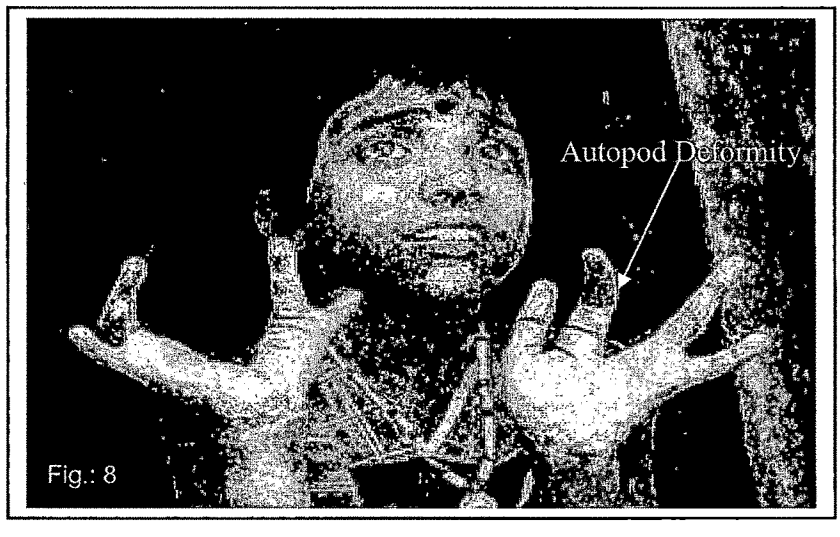


Figure 8: A girl exposed to Endosulfan shows autopod deformity

8.2 Other cases

Endosulfan has been linked to dozens of accidental deaths in the USA, Colombia, Benin, India, Malaysia, Sudan, and the Philippines (PANAP, 1996).

In the USA, Endosulfan exposure was linked to the death of one farmer and permanent neurological impairment of another (Brandt *et al.,* 2001).

In Benin's Borgou province, Endosulfan poisoning caused many deaths during the 1999/2000 cotton season. Official records state that at least 37 people died and a further 36 became seriously ill, although an independent report estimated that nearly 70 people actually lost their lives (Ton *et al.,* 2000). In 1999, a boy in Benin died after eating corn sprayed with Endosulfan (Myers, 2000).

In southern Sulawesi, Indonesia, Endosulfan was the leading cause of pesticide poisoning between 1990 and 1993. Of 153 reported poisoning cases, 32 were due to Endosulfan (PANAP, 1996).

In Sudan, in 1988, Endosulfan barrels washed in irrigation canals caused fish mortalities and three people died after drinking water from the canal (Dinham, 1993). In 1991, also in Sudan, 31 people died after eating food containing seed sprayed with Endosulfan (PAN-UK, 1995).

Colombia's Departmental Committee of Coffee Growers recorded 155 cases of poisoning due to pesticide exposure in 1994, most of which were due to Endosulfan (PAN-UK, 1995).

Pesticides Action Network North American reported that in 1993, 60 poisonings and one death occurred in Colombia due to Endosulfan use on coffee (PANUPS, 16th June 1994).

Chronic, sub-lethal effects of Endosulfan exposure manifested in experimental rats include liver enlargement, seizures and retarded growth (EXTOXNET. 1996).

The EPA states that "*available scientific literature suggests that Endosulfan may act as a potential endocrine disruptor.*" This means that the chemical has the potential to interfere with normal hormone production and activity. Implications of endocrine disruption may include disruption of development, and promotion of certain types of cancer. A major concern, especially in developing countries, is that low protein diets may increase people's sensitivity to the effects of this pesticide (PANAP, 1996).

A further concern stems from the evidence that Endosulfan may cause mutagenic effects in humans if exposure is great enough; Endosulfan has been shown to be genotoxic to human cells under experimental conditions (Lu *et al.,* 2000).

In Kerala, India, Endosulfan has been linked to hundreds of deaths and disorders among cashew nut plantation workers and villagers (THANAL, 2001). In Kasaragod province, where aerial spraying of Endosulfan occurred for at least 15 years, alarmingly high levels of Endosulfan residues have been detected in the blood and breast milk of villagers and cancers and disorders of the reproductive and central nervous systems are very common. A survey of only 123 houses found 49 cancer cases, 43 psychiatric cases, 23 epileptics, 9 with congenital abnormalities and 23 with mental retardation (Joshi, 2001).

A case-controlled study comparing 170 children exposed to Endosulfan with 92 unexposed children found, among the former, significantly poorer academic performance, elevated prevalence of congenital abnormalities and learning difficulties, delayed puberty in boys, and very high levels of menstrual disorders in girls (Yadav and Jeevan, 2002).

Romeo Quijano (2000), Professor of Pharmacology and Toxicology (University of Philippines), recently led an investigation of health defects in Kasaragod District and stated

that, "no other reasonable cause can explain the illnesses experienced by the people, except Endosulfan (THANAL, 2002).

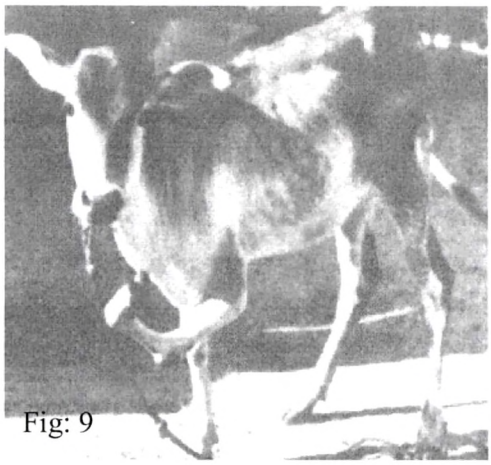


Fig: 9

Fig: 10

Figure 9: Shows deformed cow from area of heavy Endosulfan use in Kerala, southern India. Endosulfan residues measured in cow milk and flesh in Kasaragod province were over 100 times the permissible level (Vankar *et al.,* 2001). **Figure 10**: shows that Endosulfan has caused mass mortalities of fish.

8.3 Incidence of Symptoms Linked to Endosulfan Exposure

In a study conducted by Yadav and Jeevan (2002) the people exposed to Endosulfan showed increased incidences of learning disability, congenital abnormalities, menstrual disorders, compared to people free from exposure.

8.4 A Persistent Problem

Like the widely banned pesticides DDT and dieldrin, Endosulfan is an organochlorine and, as such, is persistent in the environment. Endosulfan degrades relatively quickly in water (half life: 2-22 days) (PANAP, 1996) but persists longer in soil (half life: 60-800 days) (PANAP, 1996), and its major degradation product, Endosulfan sulphate, is not only more persistent but is equally toxic (Park *et al.,* 2001). The pesticide Endosulfan, bioaccumulates in humans and other animals (particularly in their liver, kidneys and fatty tissue). Experiments have shown Endosulfan to accumulate 600 times more to the ambient water concentration in mussels (*Mytillus edulis*) (PANAP, 1996).

Such persistent organic pollutants (POPs) are of concern because of their long-term subtle effects on hormones, the immune system, and reproduction. Because of Endosulfan's toxicity to fish, Canadian regulations discourage farmers from using

Endosulfan near open water. However, aerial drifting of the pesticide can leave residues up to three meters beyond the perimeter of sprayed agricultural fields (Raloff, 1998). Ultra low volume Endosulfan products were banned in Australia, where spray drift had been resulting in residue problems for the beef industry (Cattle Council of Australia, 2001). Indeed, Endosulfan residues led to South Korea's rejection of Australian beef in the past (Myers, 1999). Similarly, in 1999, the European Union temporarily suspended imports of fish from Tanzania, Uganda and Kenya because of contamination with pesticides, including Endosulfan (European Commission, Directorate-General Health & Consumer Protection, Directorate D – Food And Veterinary Office, 1999) Given the serious health concerns associated with Endosulfan exposure, it is highly worrying that a report by the International Programme on Chemical Safety stated that Endosulfan has been shown to persist on the hands of pest control operators for up to 31 days after exposure (WHO/UNEP/ILO, 1984).

9. WORLD-WIDE RESTRICTIONS ON ENDOSULFAN USE

(www.indiatogether.org/petitions/Endosulfan/worldwide.htm, Cattle Council of Australia, 2001)

9.1 Countries Where Endosulfan is Banned

Endosulphan is banned in Singapore, Tonga, Syria, Germany, USA, the Brazilian state Rondonia, UK, Sweden, Netherlands, Colombia, and the Indian state Kerala.

Endosulfan is severely restricted in: Australia, Bangladesh, Indonesia, Cambodia, Japan, Korea, Khazakhstan, Kuwait, Philippines, Lithuania, Sri Lanka, Taiwan, Thailand, Denmark, Yugoslavia, Norway, Finland, Russia, Venezuela, Dominica, Canada.

Endosulfan has been identified as a pesticide of concern due to health and environmental problems associated with its use in Ecuador, Mauritius and Paraguay (PRC, 1994).

10. REQUIREMENT AND RELEVANCE OF ASSAY METHODOLOGIES

(*In vitro and in vivo* screening of Endosulfan)

The requirement and relevance of assay methodologies selected for determination of genotoxic and systemic effects of pesticide / combination: *in vitro and in vivo* screening of Endosulfan is based completely on regulatory requirement, environmental concerns and controversies related to its toxicity and genotoxicity.

The safety assessment of new chemical substances includes the requirement for an assessment of genotoxic potencial based on the following guidelines:

1. International Conference on Harmonisation guidelines for testing of pharmaceuticals,
2. EU Technical Guidance Document for testing of industrial chemicals,
3. German BfR overview of strategies for testing of industrial chemicals,
4. UK Committee on Mutagenicity Guideline for testing of chemicals,
5. Food and Drug administration (FDA) Redbook,
6. Updated Recommended Strategy for Testing Oxidative Hair Dye Substances for their potential Muatgenicity/Genotoxicity,
7. Recommended Mutagenicity/Genotoxicity Tests for the Safety Testing of Cosmetic Ingredients to be included in the Annexes Council Directive 76/768/EEC and
8. FDA Guidance for Industry recommended Approaches to Integration of Genetic Toxicology Study Results.

Many assay systems have been developed and introduced for safety assessment of chemicals. More than a half of them are *in vitro* assay systems, therefore we can say that the field of genotoxicity started from the alternatives of animal experiments. Although there are many kinds of assay systems but none can detect chemical genotoxicity. Assays are generally endpoint specific, so we usually use several assays in combination referred to as "battery".

In addition to the regulatory guidelines (*viz.* OECD, ICH etc.) various workshops have been organized by professional scientists on Genotoxicity Testing (IWGT), International Association of Environmental Mutagen Societies (IAES) and its workshops (International Conferences on Environmental Mutagens i.e. ICEM) have given the following recommendations which is summarized briefly:

1. Bacterial Tests
2. Mammalian Cell Gene mutation Tests
3. In Vitro chromosomal aberration Tests
4. Bone marrow micronucleus and chromosomal aberration tests
5. Unscheduled DNA synthesis tests and
6. Germ cell tests

In conjunction to the above mentioned tests following tests are performed in present research work. Two most important endpoints are "gene mutation" and "chromosomal aberration". This battery was proposed for pharmaceutical drugs in international

harmonization. This also includes gene mutation and chromosomal aberration *in vitro*, and one *in vivo* assay.

The experimental protocol of this work includes *in vitro* and *in vivo* tests to assess genotoxic and systemic effects of Endosulfan.

10.1 Mutagenic Assays

These assays are performed to assess mutagenicity (point mutations) in prokaryotic (Non – mammalian - *Salmonella typhimurium*) and eukaryotic (Mammalian – Chinese Hamster Ovary Cell lines) or Mouse lymphoma forward mutation assay.

10.1.1 Bacterial Tests (Ames assay)

There are inconclusive reports on its Genotoxic (Mutagenic) effects. As differences in the results of various investigators in different types of studies, *In vitro* and *In-vivo* studies were performed to assess Genotoxic and Systemic effects of Endosulfan (Refer Chapter-II for further details). An Ames test can be performed as Endosulfan falls in organochlorine group and there is no restriction or modification required as per OECD guideline 471.

10.1.2 Mammalian Cell Gene Mutation Tests

The Chinese hamster ovary cell/hypoxanthine-guanine phophoribosil transferase (CHO/HGPRT) assay has been widely applied to the toxicological evaluation of industrial and environmental chemicals.(refer Chapter-II for further details).

10.1.3 *In Vitro* Chromosomal Aberration Tests

This assay is performed to assess clastogenicity and anugenecity of the test compound by grossly examining the aberrations directly under the microscope (refer Chapter-II for further details).

10.1.4 Bone Marrow Micronucleus Tests

Clastogenicity and Aneugenicity Assays

For a test of clastogenicity *in vitro* chromosomal aberration test was performed in Chinese Hamster Ovary cell lines as *in vitro* assay and 28 days repetitive test for micronucleus assay was performed as *in vivo* assay (refer Chapter-II for further details).

10.2 *In Vivo* Screening of Endosulfan

In vivo screening includes an acute and sub acute exposure in mice. In case of acute test mice were treated once with the compound and observed for five days. This part of the study was referred to as dose selection study.

The second test applied included 28 days repetitive treatment with Endosulfan 35% EC. The study of 28 days repetitive treatment with Endosulfan 35% EC was evaluated for gross pathological symptoms after treatment, various biochemical parameters, hematological examinations, differential leukocyte counts, sperm morphology and 28 days micronucleus assay as a test for *in vivo* clastogenicity.