CHAPTER 1

AN OVERVIEW: PESTICIDES AS ENDOCRINE-DISRUPTING CHEMICALS

1.1 INTRODUCTION

1.1.1 Pesticides

The United States Environmental Protection Agency (U.S. EPA) defined "pesticides" as any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. Pesticides may also be described as any physical, chemical or biological agent that will kill an undesirable plant or animal pest. The term "pesticide" is a generic name for a variety of agents that are usually more specifically classified on the basis of the pattern of use and organism killed. The major agricultural classes encompass insecticides, herbicides and fungicides (Ecobichon, 1992).

The widespread use and disposal of pesticides by farmers, institutions and the general public provide many possible sources of pesticides in the environment. Pesticides may possess different fates and behavior when they are released into the environment, namely they may be degraded by the action of sunlight, water or other chemicals or microorganisms (i.e. bacteria). Some pesticides may be resistant to degradation and remained unchanged in the environment for a certain period of time. (EXTOXNET, 1993).

Volatility is the capacity of a substance to evaporate, thus moving into the air, being easily inhaled and moving widely as its persistence permits (Briggs).

Table 1.1. Scale of rating for volatility

Volatility rating	Vapor pressure at 20 to 30°C
Non-volatile	Less than 1 x 10^{-7} mm Hg (0.0000001mm Hg)
Slightly volatile	10^{-7} to 10^{-4} mm Hg (0.0000001 to 0.0001mm Hg)
Volatile	10^{-4} to 10^{-2} mmHg (0.0001 to 0.01mm Hg)
Highly volatile	Greater than 0.01mm Hg

Water solubility is important in determining the course of a pesticide through the environment. A pesticide which is very water-soluble is more easily carried off with rainwater, as run-off or through the soil as groundwater contaminant and, will travel as far as its persistence in original form or will transform to less harmful breakdown products or more toxic products.

1.1.2 Organochlorine (OC) pesticides

The OC pesticides are a diverse group of agents belonging to three distinct chemical classes, namely the dichlorodiphenylethane-, the chlorinated cyclodiene-, and the chlorinated benzene- and cyclohexane-related structures (Table 1.2).

Table 1.2. Structural classification of OC pesticides

Structure	Compound
Dichlorodiphenylethane	DDT, DDD, DDE
Cyclodiene	Aldrin, dieldrin, endrin, heptachlor, endosulfan
Chlorinated benzene and cyclohexane	Lindane (γ-BHC), HCH

From the mid-1940s to the mid-1960s, OC pesticides were used extensively in all aspects of agriculture and forestry, in building and structural protection, and in human situations to control a wide variety of insect pests (Ecobichon, 1992). Their use was discontinued in many countries in subsequent years following their inclination to bio-accumulate in the lipid component of the biological species and their resistance to degradation (Pandit *et al.*, 2001). In humans, the OC pesticides affect the nervous system but are not cholinesterase inhibitors (Pinkston *et al.*). However, OC pesticides are still used in large quantities in some of the third-world, developing nations for the control of agricultural pests because of their low cost and versatility in industry, agriculture and public health. DDT has been used in Mexico for anti-malaria control programs since 1990 (Pardio *et al.*, 1998). It was also used in Thailand as malaria repellant and as an agricultural pesticide since the early 1950s and was finally banned for all application in 1994 (Kumblad *et al.*, 2001). In India, DDT and HCH were used extensively till recently both for agricultural and sanitary purposes (Pandit *et al.*, 2001). Lindane (γ-BHC) and endosulfan are still permitted for use in Malaysia (Pesticide Board 1991) for its agricultural purposes.

Studies in North and Latin America, Europe, South Africa and Asia-Pacific showed that OC pesticides were ubiquitous and widely distributed in both the abiotic (Arthur *et al.*, 1976; Ayas *et al.*, 1997; Coupe *et al.*, 2000; Dua *et al.*, 1996; Dua *et al.*, 1998; Olden *et al.*, 1996; Tan and Vijayaletchumy, 1994) and biotic environment, including man although most of them were banned many years ago (Abbott *et al.*, 1981; Beretta *et al.*, 1994; Bordet *et al.*, 1993; Choi *et al.*, 2001; Cok *et al.*, 1997; Dommarco *et al.*, 1987; Glynn *et al.*, 2000; Jensen, 1983; Kannan *et al.*, 1994; Kumblad *et al.*, 2001; Mes *et al.*, 1982; Pardio *et al.*, 1998; Rhainds *et al.*, 1999; Roots, 2001; Siyali, 1972; Van Wyk *et al.*, 2001).

1.1.3 Organophosphorus (OP) pesticides

OP compounds, which are derived from phosphoric acid, are one of the most important classes of pesticides. They are used as insecticides and to a lesser extent, as herbicides (Maroni *et al.*, 2000). They are widely used in agriculture or animal husbandry for crop protection and/or elimination of ectoparasites to substitute the persistent OC pesticides which are currently restricted. They have also showed some interesting features for environmental safety, such as limited persistence and selective toxicity to insects with respect to mammals (Vittozzi *et al.*, 2001). In addition, they are more specific than the OC pesticides (Galgani *et al.*, 1990) and they are considered to be less persistent and lipophilic (Schimmel *et al.*, 1983). OP pesticides exhibit two distinct features as a class of insecticides. Generally, they are much more toxic to vertebrates than the OC pesticides and they are chemically unstable or non-persistent and break down rapidly. These materials inhibit cholinesterase and prevent the termination of nerve impulse transmission (Pinkston *et al.*). Examples of OP pesticides are chlorpyrifos, diazinon, fenitrothion, malathion, parathion and methamidophos.

1.2 Pesticides in Malaysia

1.2.1 Pesticide usage

Agriculture is an important sector in the Malaysian economy. In 1997, it accounted for 13.2% of Gross National Product (GNP), 14.8% of total export earnings and employed 21.3% of total work force. Hence, pesticide industry is an essential contribution to agriculture. In 1996, the pesticide market was valued at RM301.0 million (end-user level). Most pesticides used are herbicides (75%) followed by insecticides (16%) and fungicides (5%) (Table 1.3) (MACA, 1997) and they are mainly applied in the plantation industry,

vegetable growing, rice cultivation and public health control (Department of Statistics, 1994).

The first few OC insecticides to appear in the market were DDT, lindane and the cyclodienes (aldrin, dieldrin, endrin). They were well received by farmers because they were cheap, effective, persistent and had a wide spectrum of activity that could give total kill.

Table 1.3. Pesticides market in Malaysia (The Malaysian Agricultural Chemicals Association [MACA], 1997)

	RM million						
Pesticide	1990	1991	1992	1993	1994	1995	1996
Herbicides	261.3	230.0	210.0	200.0	201.0	220.0	227.0
Insecticides	42.8	40.0	41.0	39.0	41.0	43.0	47.0
Fungicides	14.5	13.0	13.0	13.0	14.0	15.0	16.0
Rodenticides	10.5	10.0	12.0	10.0	11.0	11.0	11.0
Total	329.1	293.0	276.0	262.0	267.0	289.0	301.0

In 1967, the implemented Malaria Eradication Program (MEP) in Malaysia was principally based on the attempted eradication of the female mosquito, the carrier of malaria. DDT was the most widely used insecticide for this purpose in Malaysia as well as in other countries such as India and Thailand (Sahabat Alam Malaysia, 1981).

Most of the manufacturers of pesticides in Malaysia import the active ingredients and formulate them locally (Table 1.4) (Department of Statistics, 1994). The local production of pesticides is illustrated in Table 1.5 (Department of Statistics, 1994).

Table 1.4. Imports of pesticides in Malaysia, 1992-1993 (Department of Statistics, 1994)

Pesticide	RM '000	
	1992	1993
Herbicides	23, 479	23, 290
Insecticides	48, 540	52, 291
Fungicides	19, 832	23, 463

Table 1.5. Local production of pesticides in Malaysia, 1992-1993 (Department of Statistics, 1994)

Pesticide	RM '000	
	1992	1993
Herbicides	203, 526	216, 173
Insecticides	69, 347	57, 698
Fungicides	4, 202	3, 623

1.2.2 Pesticides in the Malaysian environment

Due predominantly to its persistent characteristic, OC pesticides continue to be detected in the Malaysian environment despite the banned and restricted status of many of these chemicals. In addition, the use of OP pesticides, though less persistent also contaminate the environment due to its current and sometimes indiscriminate application. Hence, OC and OP residues have been detected in water, sediment and biota including foodstuff such as agricultural products and seafood. Tables 1.6a - 1.8 show the extent of contamination of OC and OP pesticides in both biotic and abiotic components in the Malaysian environment.

Table 1.6a. OC pesticide and phthalates residues in the Malaysian aquatic environment (adapted from Abdullah *et al.*, 2000)

Location	Survey Year	Chemical	Concentration (ng ml^{-1} / ng g^{-1})			References
			Water	Sediment	Fish	
Krian River basin	1981	Dieldrin	nd-0.5	nd-4.7	6.6-24.9	Meier *et al.*,1983
		Chlordane	nd-0.6	1.8-6.6	5.0-27.8	
		Lindane	nd-0.6	0.4-0.8	1.4-3.5	
		p,p'-DDT	nd-1.6	1.0-4.0	2.2-6.0	
Tanjong Karang	1982	Lindane	0.1	nil	10-100	Soon and Hock, 1987
		α- Endosulfan	nil	nil	5130	
		β- Endosulfan	nil	nil	1700	
Penang	1984-1987	Dieldrin	nil	nil	0.2	Jothyl *et al.*,1987
		p,p'-DDT	nil	nil	0.8	
		α- Endosulfan	nil	nil	3.4	
		β- Endosulfan	nil	nil	2.0	
Sabah	1988	Lindane	nil	nd-1.1	nil	Heng *et al.*,1989
		Heptachlor	nil	nd-0.5	nil	
		total DDT	nil	nd-34.7	nil	
Major rivers in Peninsular Malaysia	1989-1990	Dieldrin	nd-0.0003	nil	nil	Tan and Vijayaletchumy, 1994
		p,p'-DDT	nd-0.067	nil	nil	
		Heptachlor	nd-0.0034	nil	nil	
		α- Endosulfan	nd-0.044	nil	nil	
		β- Endosulfan	nd-0.010	nil	nil	
	1990-1991	*t-PCB	nd	nil	2.1	Kanapathippillai, 1992

*t-PCB: Arochlor 1254, 1260, 1242

Table 1.6b. OC pesticide and phthalates residues in the Malaysian aquatic environment (adapted from Abdullah *et al.*, 2000)

Location	Survey Year	Chemical	Concentration (ng ml^{-1} / ng g^{-1})			References
			Water	Sediment	Fish	
Klang River	1990-1991	Pentachlorophenol (PCP)	nd	nil	nil	Tan and Chong, 1993
Klang River	1992-1993	Di-butyl phthalate (DBP)	0.8-3.2	67-637	nil	Tan, 1995
		Di-2-ethylhexyl phthalate (DEHP)	3.1-64.3	493-15015	nil	
Jeram	1991	Lindane	1.8	nil	nil	Iwata *et al.*, 1994
		total DDT	0.001	nil	nil	
		Chlordane	0.002	nil	nil	
		PCBs	0.0005	nil	nil	
Selangor River	1992-1993	α, β, γ-HCH	nil	4.0	nil	Tan and Vijayalechumy, 1994
		Heptachlor	nil	0.9	nil	
		α, β-Endosulfan	nil	5.4	nil	
Estuarine waters in Peninsular Malaysia	1992	TBT	nd-0.3	nd-217	nd-0.02	Tan, 1995
Major rivers in Peninsular Malaysia	2001-2002	DEP	nd-0.009	nil	nil	Mustafa *et al.*, 2002
		DBP	nd-0.006	nil	nil	
		DOA	nd	nil	nil	
		DEHP	nd-0.001	nil	nil	

Table 1.7a. OC pesticide residues in marine biota from Malaysian waters (adapted from Abdullah *et al.*, 2000)

Location	Survey Year	Chemical	Concentration (ng/g)	References
Coastal waters off, the Straits of Malacca	1977			Jothyl *et al.*, 1983
Cockles (*Anadara granosa*)		DDT	50	
Fish		Lindane	1.0-12.0	
		Dieldrin	<1-4	
		DDT	0.4-0.8	
Jeram, West Coast, off Peninsular Malaysia	1985			Everaarts *et al.*, 1991
Shrimp		γ-HCH	3	
Crab		γ-HCH	4	
Polychaete worm		γ-HCH	8	
Bivalve molluscs		γ-HCH	17	
Shrimp		Dieldrin	94	
Crab		Dieldrin	232	
Polychaete worm		Dieldrin	57	
Bivalve molluscs		Dieldrin	52	

Table 1.7b. OC pesticide residues in marine biota from Malaysian waters (adapted from Abdullah *et al.*, 2000)

Location	Survey Year	Chemical	Concentration (ng/g)	References
Penang	1990			Ismail *et al.*, 1992
Mussels (*Perna veridis*)		Lindane	180.9	
Cockles (*Anadara granosa*)		Lindane	0.222-3.01	
		DDT	1.23	
Muar, Johor	1990			
Oysters (*Cronsostrea belcherei*)		Lindane	27.50-66.46	
		DDT	1.46-7.41	
Batu Lintang, Kedah	1990			
Mussels		α-Endosulfan	0.05	

Table 1.8. Concentration of pesticide residues in human blood and milk (adapted from Abdullah *et al.*, 2000)

Location/ Year of Survey	Matrix (No. of sample)	Chemical	Concentration (ng/ml; ng/g)	References
River Basin, 1993	Blood (372)*	Malathion	0.966	Yusof *et al.*, 1995
	Milk (31)*		0.789	
Various locations in	Blood (577)*	Lindane	nd-5.7	Zulkifli *et al.*, 2000
Peninsular Malaysia,		Heptachlor	nd-3.1	
1999		Endosulfan#	nd-0.6	
		Aldrin	nd-47.6	
		o,p'-DDE	nd-1.4	
		p,p'-DDT	nd-3.4	
		Chlorpyrifos	nd-10.3	
		Diazinon	nd-103	

*Number of sample

#α, β and sulfate

1.3 Pesticides as endocrine-disrupting chemicals

1.3.1 Definition of endocrine-disruptors

An environmental or hormone disruptors may be defined as an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes (Kavlock, *et al.*, 1996). They include a diverse group of chemicals, synthetic or natural products that can affect hormonal activity in living organisms. In 2000 at the international symposium on EDCs sponsored by the United Nations University, an endocrine disrupting chemical (EDC) is defined as an exogenous substance that alters functions of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or subpopulations (Kavlock, 2000).

Environmental pollutants which have endocrine-disrupting properties and are still in commercial use, currently number more than 50. These include industrial chemicals, pesticides and byproducts of manufacturing processes as well as products of incineration of industrial and household wastes. Known EDCs include PCBs, organochlorine pesticides and other types of pesticides, dioxins, alkylphenols. polyethoxylates, pentachlorophenols, bisphenol A, styrenes and phthalate esters (Colborn *et al.*, 1996; Gascón *et al.*, 1997; Castillo and Barcelo, 1997) (Table 1.9). Because of the widespread use of many of these chemicals, exposure of biota, including man, to EDCs is generally considered significant (Jimènez, 1997). The ability of a chemical to disrupt hormones is unlikely to be ever predictable from its structure alone (McLachlan, 1993; Katzenellenbogen, 1997).

Table 1.9. Examples of organic pollutants with reported endocrine-disrupting effects (Gascón *et al.*, 1997; Castillo and Barcelo, 1997)

Source	Chemical
Pesticide	DDT and metabolites
	Alochlor
	Atrazine
	Endosulfan
	Kepone
	Lindane
	Vinclozolin
Industry	
Surfactants	Pentachlorophenol
	Penta-nonylphenols
Polymer	Phthalates
	Styrenes
	Bisphenol A
Others	PCBs (Polychlorinated biphenyls)
	Polybrominated biphenyls
	Dioxin
	Butyl hydroxyanisole
	Organotin compounds
	Phytoestrogens

1.3.2 Mode of action of EDCs

Chemicals that have very different structures can interfere with the way in which steroid hormones and thyroid hormones normally work. EDCs can mimic by binding to hormone receptors in cells, and thereby triggering the same biological effect as the hormone (McLachlan, 1993). In addition, EDCs may enhance (an agonist) or inhibit (an antagonist) the action of hormones. Under some circumstances, they may act as hypertrophy (stimulatory) agents and tumor promoters (U.S. EPA, 1997). They may block the effects

of a hormone in parts of the body normally sensitive to it by preventing the hormone from binding to the receptors and exerting their normal biological effects (McLachlan, 1993). They may also alter or block the signaling pathways of the cell to disrupt the cell function, for instance, their synthesis and natural breakdown and elimination from the body. These chemicals do not interfere directly with hormones or their receptors. Hence, they are sometimes termed imposters.

1.3.3 Transport and distribution of EDCs in the environment

It has been estimated that there are approximately 70 000 synthetic chemicals in everyday use, with between 500-1000 new chemicals being added and introduced to the list annually (Gascón *et al.*, 1997). Many of these chemicals are discharged into the environment through industrial wastewater, municipal wastes, agricultural runoffs and atmospheric emissions (Abdullah *et al.*, 2000).

Chemicals can become airborne either by their direct emission to air, such as from factory or incinerator stacks, or by evaporation from the ground, water, or the leaves of sprayed crops. Once airborne, they may be easily transported for thousands of kilometres in the atmosphere before condensing and falling once more to the earth's surface (Allosopp *et al.*, 1997). Generally, OC pesticides are distributed into the environment as a result of spray drift from aerial spraying and runoff from agricultural areas after rainfall.

Research has shown that many persistent OC pesticides appear to be carried on air currents from warmer regions of the globe towards polar regions where they are subsequently deposited are now found in Arctic and Antarctic regions, where they have never even been used (Allosopp *et al.*, 1997; Iwata *et al.*, 1993; Wania and Mackay,

1996). This gives rise to the possibility of long-term pollution and dissipation throughout the global ecosystem.

Most endocrine-disrupting chemicals which are persistent, such as the dioxins, PCBs, organochlorine pesticides and alkylphenols, are also fat-soluble. Due to its lipophilic characteristic, and because of the way that animals and humans deal with these toxic substances once they enter the body, they can become stored in fatty tissues. The levels of chemicals build up or "bioaccumulate", in the animal's fat as more of the chemical is taken in. For many of these chemicals, the levels in fat increase as one animal eats another, so that the highest levels are found in predator animals at the top of food webs, for example, humans, seals, dolphins and fish-eating birds (Allosopp *et al.*, 1997).

Environmental pollutants which have endocrine-disrupting properties currently number more than 50 and include industrial chemicals, pesticides and byproducts of manufacturing processes as well as products of incineration of industrial and household wastes. Known EDCs include PCBs, organochlorine pesticides and other types of pesticides, dioxins, alkylphenols. polyethoxylates, pentachlorophenols, bisphenol A, styrenes and phthalate esters (Colborn *et al.*, 1996; Castilló and Barceló, 1997). Table 1.10 provides an indication of the extent of pollution of EDCs in the environment.

1.3.4 Effects on wildlife populations

Wildlife studies have revealed male reproductive disorders in several species that have been associated with exposure to EDCs. EDCs are able to act by means of various mechanisms, some of which may only operate during specific developmental periods. Research activities on the effects of EDCs have been confined to reproductive toxicology,

carcinogenic effects and immunotoxicology. Hormonal actions that have been studied include estrogenic, antiestrogenic and antiandrogenic effects, growth factor modulation, cytokine modulation as well as modulation of hormone metabolism. Table 1.11 (Toppari *et al.*, 1996; Jimènez, 1997) summarises the EDCs effects that have been observed in the wildlife populations.

Documented effects of EDCs on wildlife include decreased hatching success of eggs in fish, birds and turtles, feminisation of male fish, embryo death as well as reproductive defects in reptiles, birds and mammals, in some cases leading to population decline. In addition, some of the EDCs are also able to disrupt the development of the immune and nervous systems in wildlife (Jimènez, 1997; Raloff, 1998).

1.3.4.1 Reptiles

A significant decline in the population of juvenile alligators from Lake Apopka in Florida was reported in 1980 due to the contamination by a spill of dicofol and DDT whereas alligator populations elsewhere were increasing or stable at the same time. Further contamination of the lake by agricultural sewage dumping has made this lake as one of Florida's most polluted wetlands. Young male alligators have altered sex hormone levels and suffer from reproductive abnormalities. The females have also been affected. They also have altered levels of sex hormones and abnormalities in the structure of their reproductive systems. These abnormalities are most likely caused by permanent

Table 1.10. Distribution of EDCs in the general environment (Abdullah *et al.*, 2000)

Location	Year of Survey	Chemical	Concentration μg/L; ng/g
Malaysia			
Rice field fish	1981	Dieldrin	6.6-24.9
		ΣDDT[#]	0.3-1.1
Oyster	1992	Lindane	27.5-66.5
		ΣDDT[#]	1.46-7.41
Mussels	1992	α-endosulfan	0.05
	1990	Dibutylphthalate ester	613.6
Thailand			
Soil	1990	ΣDDT[#]	0.61-98
Vietnam			
Soil	1990	ΣDDT[#]	19-330
River sediments	1996	PCBs	223.2
The Philippines			
Water	1990	Endosulfan	0.307
Bangladesh			
Rice field fish	1993	p,p'-DDT	7.86-142.7
Kenya			
Crocodile eggs	1990	ΣDDT[#]	550
		Dieldrin	30
USA			
Fish	1996	PCBs	270-800
		Chlordane	240-560
Bald eagle eggs	1993-1997	p,p'-DDT	<10-30
		PCBs	20-54
Fish	1996	PCBs	4.9-8.1
Frog			9.1-229.3
Mexico			
Human milk	1996-1997	ΣDDT[#]	7820
Australia			
Fish	1986-1987	t-endosulfan*	1.4-251.4
Hokkaido, Japan			
Black-tailed gull eggs	2001	PCBs	2.4-7.4

[#]ΣDDT: p,p'-DDD, p,p'-DDE, o,p'-DDT and p,p'-DDT

*t-endosulfan: α-endosulfan, β-endosulfan and endosulfan sulfate

modification during development in the egg as a result of exposure to endocrine-disrupting chemicals (Guillette *et al.*, 1994). The data from Lake Apopka suggested that the gonads of alligators have been permanently modified, altering steroidogenesis and inhibiting normal sexual maturation (Jimènez, 1997).

Table 1.11. Reported endocrine-disrupting effects in wildlife populations (Toppari *et al.*, 1996; Jimènez, 1997)

Effect	Biota	Compound
Reproductive effects		
Reproductive failure	Bird	DDT
Abnormal sexual development	Fish, reptile, bird, mammals	Alkylphenols, DDT
Uterine occlusion	Marine mammals	PCBs
Reduction in testosterone levels	Marine mammals	PCBs
Impaired gonad development and reduced fecundity		
Feminization	Fish	DDT, DDE
Embryonic deformities	Bird	DDT, DDE
Reproductive failure	Mammals	TCDD, PCBs, atrazine
Pseudohermaphroditism	Marine gastropods	Alkylphenols
Carcinogenic effects		
Liver tumors	Fish	PAHs, PCBs, DDT
Immunological effects		
Autoimmune syndromes	Bird	DES
Immune suppression	Mammals	DES, TCDD, PCBs
Population decline		
Associated with reproductive disorders	Reptile	DDT

1.3.4.2 Fish

Fish living in the Laurentian Great Lakes of North America have experienced a number of developmental and reproductive abnormalities due to the exposure of OC pesticides. Male coho salmon (*Onchorhynchus kisutch*) living in Lake Erie exhibited a number of abnormalities including decreased fertility, lower plasma concentrations of gonadotrophins and steroids, poor expression of secondary sex characteristics, and highly precocious sexual maturations (Jimènez, 1997).

1.3.4.3 Birds

Pollutants which have been found in bird's eggs, including OC pesticides and heavy metals, have been reported to cause reduced hatchability of eggs, death and deformities in the embryo, and reduced survival of chicks hatched from eggs. Some of the effects may be due to the endocrine-disrupting properties of these chemicals. The most dramatic effect on the reproductive performance of wild birds was eggshell thinning caused by DDE, the breakdown product of DDT. Feminization of gulls and terns in several locations along the Pacific coast of the United States has also been associated with DDT and DDE pollution (Jimènez, 1997). From the 1950s to 1970s, this resulted in the population decline of many predatory and fish-eating wild bird species (Fry, 1995). In addition, severely reduced breeding success in cormorants has been reported in the area of the Rhine and Meuse delta in the south-western part of The Netherlands (Allosopp *et al.*, 1997).

1.3.4.4 Mammals

It was reported that Florida panthers exhibited a number of developmental abnormalities and reproductive defects and these include low ejaculate volume, low sperm concentrations, poor sperm motility and a very high proportion of sperm with morphological abnormalities due to high body burdens of various contaminants such as p,p'-DDE, PCBs oxychlordane and trans-nonachlor, methoxychlor and other OC compounds (Toppari *et al.*, 1996). In addition, seals inhabiting the Wadden Sea, The Netherlands and Beluga whales from St. Lawrence estuary in Quebec, Canada also suffered from reproductive problems due to the exposure of OC pesticides (Allosopp *et al.*, 1997).

1.3.5 Human exposure to EDCs

Exposure to both synthetic and natural chemicals is presently unavoidable due to its ubiquitous characteristic in the environment. EDCs have been found and detected in contaminated foodstuff, in polluted drinking water and in some plastics. Humans are therefore exposed to EDCs through oral ingestion, skin penetration or inhalation through the lungs (Allosopp *et al.*, 1997; EXTONET, 1993; Rivas *et al.*, 1997). Some individuals, i.e., farmers may also be exposed to EDCs as a result of handling of chemicals at work. In this regard, those responsible for occupational health and safety should ensure that such workers fully understand the toxic properties of the chemicals they handle and follow proper procedures before application. Since many of these chemicals are lipophilic, they tend to accumulate in the fatty tissues of animal meats, fat-rich milk and dairy products. Hence, residues of OC pesticides have been detected in human fat (Kannan *et al.*, 1994; Mes *et al.*, 1982), breast milk (Beretta and Dick, 1994; Bordet *et al.*, 1993; Cok *et al.*, 1997; Dommarco *et al.*, 1987; Fürst *et al.*, 1994; Pardio *et al.*, 1998; Stacey *et al.*, 1985), urine (Anderson *et al.*, 1998; To-Figueras *et al.*, 1997) and blood (Abbott *et al.*, 1981; Anderson *et al.*, 1998; Asplund *et al.*, 1994; Glynn *et al.*, 2000; Radomski *et al.*, 1971; Rhainds *et al.*, 1999; Sala *et al.*, 1999; Soliman and Ismail, 1997; To-Figueras *et al.*, 1997).

Other dietary exposure may also come from pesticide residues which remain on sprayed crops and contaminate drinking water. Spraying pesticides on crops means that a proportion of them may remain on the crops by the time they are harvested. Pesticides can leach from sprayed land into water courses which are used for drinking water supplies. Monitoring in the Selangor River basin in Malaysia has shown that the most commonly

detected pesticides in surface water include several EDCs namely the OC pesticides lindane, DDT, aldrin and heptachlor (Mustafa *et al.*, 2000).

Some endocrine-disrupting chemicals are used in the production of food packaging materials. Several studies have shown that these chemicals can leach out of packaging into food they are in contact with. For example, Bisphenol-A (BPA) is a monomer used in the manufacture of a multitude of chemical products, including epoxy resins and polycarbonate for packaging of food and drinks. In addition, BPA, an estrogenic chemical, is also present in lacquer coatings which are used to line the inside of some food cans. Tests on tins of peas, artichokes, green beans, mixed vegetables, corn and mushrooms found the liquid surrounding vegetables in food cans had estrogenic properties due to BPA (Brotons *et al.* 1995).

A research by Harris *et al.* (1997) has demonstrated that the isomeric phthalate DINP (diisononyl phthalate), commonly used at high concentrations in consumer products such as PVC toys, can also show weak estrogenic activity *in vitro* with human breast cancer cell lines. DEHP and DBP which are used widely in the manufacture of plastics cause apoptosis and loss of spermatogenic cells, resulting in the testicular atrophy (Park *et al.*, 2002; Gray *et al.*, 1982).

1.3.6 Health effects of EDCs on humans

It has been hypothesized that EDCs may be causing adverse effects to wildlife and humans alike. In some cases it is almost certain that detrimental effects in wildlife populations are caused through endocrine disruption. There is also growing evidence that EDCs could be

implicated in human health effects, particularly the reproductive system, at levels currently found in the environment.

Over the last 50 years or so there has been a dramatic increase in the incidence of several reproductive disorders in men. Studies show that, testicular cancer, a disease most common in men aged 20 - 45, has increased world-wide, rising by as much as 4-fold in some areas. It is now the most common form of cancer in men in some countries (Toppari *et al.*, 1996). The incidence of testicular maldescent (undecended testicles) and the incidence of boys' born with urethral abnormalities also appear to have increased in several countries (Toppari *et al.*, 1996). Other reproductive disorders in male include prostate cancer, reduced sperm count or quality, reproductive abnormalities and reduced proportion of male babies.

Presently, there is some evidence that exposure to EDCs (e.g. DES, dioxin and DDT) in the environment, both during development and during adult life, may affect the female reproductive system. Many of the girls suffered from vaginal cancer in their teens, had reduced fertility and a high incidence of structural abnormalities of the reproductive system (Allosopp *et al.*, 1997). EDCs has also been associated with female reproductive disorders such as early puberty, increases in the incidence of breast cancer as well as a shorter duration of lactation in women (Toppari *et al.*, 1996; Rivas *et al.*, 1997). An example is a synthetic hormone, diethylstilbesterol (DES), which was at one time prescribed to expectant mothers to prevent miscarriages and was shown to cause cancer in the offspring. Declines in the duration of lactation have also been reported throughout the world. This represents a serious public health concern because of the associated implications for increased infant illness and death, especially in developing countries.

Studies on the general population suggest that exposure in the womb to PCBs and /or dioxins can reduce intellectual capacity and alter immune systems. Studies indicate that the levels of PCBs and dioxins present in body tissues in some women of the general population are sufficient to cause subtle effects on the nervous and immune systems of their children. Effects on the nervous system include slightly reduced IQ, attention deficits, poorer memory and slight adverse effects on psychomotor and neurological function. Whether such effects on the nervous and immune systems are caused by endocrine-disrupting mechanisms is uncertain (Allosopp *et al.*, 1997).

1.4 A short review: Endosulfan

1.4.1 Introduction

Endosulfan was developed and introduced by Farbwerke Hoechst A. G. in 1954 under the registered trade mark "Thiodan". It belongs to the cyclodiene group. The other alternative names of endosulfan are Cyclodan, Thimol, Thiofar and Malix. It is chemically known as 6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepine-3-oxide or a, b, 1, 2, 3, 4, 7, 7-hexachloro-bicyclo-(2, 2, 1)-heptene-(2)-bis-hydroxymethylene (5-6) sulfide. The insecticide endosulfan is obtained by the action of thionylchloride on the addition product from hexachlorocyclopentadiene and *cis*-butene-diol-1, 4 (Martin and Worthing, 1977).

Endosulfan is a mixture of two stereoisomers, the alpha of m. p. 108-110°C, the beta of m. p. 208-210°C, having a molecular weight of 407. The structural formulae of endosulfan and its main metabolites are given in Figure 2.9, 2.10, 2.11 and 3.2. It is stable to sunlight but subject to slow hydrolysis by alcohol and sulphur dioxide. It is compatible with most non-alkaline pesticides but incompatible with strongly alkaline materials.

Usually, the technical grade consists of α- and β-isomers in the ratio 70:30. The pure mixture (90-95%) of isomers is a brownish crystalline with a terpene like odour (m. p. 70-100°C, v. p. 1 x 10^{-5} torr at 25°C). Endosulfan is practically insoluble in water but moderately soluble in most organic solvents. Under normal conditions, it is stable on storage, non-inflammable and be hydrolysed slowly by aqueous alkali and acids with the formation of the diol and sulphur dioxide (Gupta and Gupta, 1979; Briggs).

Endosulfan is a non-systemic contact and stomach insecticide. It is used to control the sucking, chewing and boring insects and mites on a very wide range of crops, such as fruit (including citrus), vines, olives, vegetables, ornamentals, potatoes, cucurbits, cotton, tea, coffee, rice, cereals, maize, sorghum, oilseed crops, hops, hazels, sugar cane, tobacco, lucerne, mushrooms, forestry, glasshouse crops and tsetse flies (Briggs).

1.4.2 Effects of endosulfan on mammals (rats)

The LD_{50} of endosulfan (isomeric mixture) for rats varies markedly depending upon the route of administration, species, dosing vehicle and the sex of the animal. The LD_{50} ranges from 47 – 89 mg/kg for male rats and 8-49mg/kg for female rats Gupta, 1976; Gupta and Gupta, 1977). The inhalation lethal dose (LC_{50}) in male rats was 350 mg/m^3 when exposed for 4 hours. The dermal LD_{50} varied from 74 – 681 mg/kg depending on the vehicle and sex used. When rats were fed on diets containing 0%, 3.5%, 9%, 26% or 81% protein as casein, the LD_{50} was 5.1, 24.0, 57.0, 102.0 and 98.0 mg/kg, respectively, but the prominent signs and symptoms of intoxication remained unchanged (Gupta and Gupta, 1977). The prominent signs of intoxication include hypersensitivity, respiratory distress, diarrhea, tremors, hunching and convulsions followed by death. Rats fed two years on a diet containing 30 ppm suffered no ill effect (Martin and Worthing, 1977).

The isomers of endosulfan show acute oral toxicity profiles similar to that of technical grade endosulfan. The acute toxicity of formulations containing endosulfan was dependent upon the concentration of the active ingredient in the end-used products, and was similar to those seen following administration of the active ingredient. The toxicity of the metabolites varies depending upon vehicle and species used. Generally, the toxicities of the metabolites were similar to the parent compound, except for endosulfan diol which has low acute oral toxicity in the mouse (NRA, 1998).

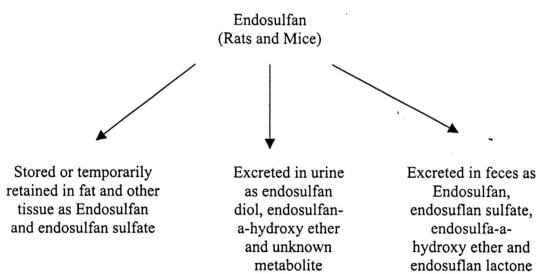

Figure 1.1. Metabolism of endosulfan in rats and mice (adapted from Gupta and Gupta, 1979)

Metabolism studies in rats, after an intraperitoneal injection of 20 mg/kg of technical endosulfan in an oil solution revealed the presence of endosulfan diol and an unknown compound in the urine as a water soluble conjugation metabolite. It was also reported that endosulfan was not excreted in rat urine as endosulfan diol, but as endosulfan-α-hydroxy ether. In addition, transient amounts of endosulfan and endosulfan sulfate was also detected in the body fat and liver of mice after the administration of [^{14}C] endosulfan where endosulfan metabolites were detected in feces (Gupta and Gupta, 1979). There is no accumulation in milk, fat or muscle and it is excreted as conjugates of the diol and other highly polar compounds depending on the species (Martin and Worthing, 1977).

The distribution pattern of endosulfan was estimated in the plasma and brain of the rats when they were fed daily doses of endosulfan (5 or 10 mg/kg) for 15 days. The animals were sacrificed 24 hours later. The concentration of α-isomer was in the order of cerebrum (3.76 μg/g) > remaining parts of the brain (2.66 μg/g) > cerebellum (2.04 μg/g). The concentration of the β-isomer was 0.06 μg/g in the cerebrum and 0.02 μg/g in the cerebellum, where as no β-isomer was detected in the "remaining part" of the brain. The plasma concentration of α- and β-isomers was 2.26 and 0.46 μg/g respectively and endosulfan sulfate was the only metabolite detected in the plasma (Gupta, 1978).

1.4.3 Effects on humans

In general, characterization of the dose of endosulfan in poisoning cases has been poor. The lowest reported dose that resulted in death in humans was 35 mg/kg body weight, and deaths have also been reported after ingestions of approximately 295 and 467 mg/kg, with death occurring within 1 hour of administration in some cases. Intensive medical

treatment within 1 hour of endosulfan administration was reportedly successful at doses of 100 and 1000 mg/kg with clinical signs in these patients consistent with those seen in laboratory animals, dominated by tonoclonic spasms. In a case where the dose was 1000 mg/kg, neurological symptoms requiring anti-epileptic therapy, which resulted from anoxia during treatment, were still required one year after endosulfan exposure (NRA, 1998).

1.4.4 Environmental fate of endosulfan

Endosulfan has low water solubility. It is strongly absorbed onto soil particles and hence immobile in the soil column. Transport of this insecticide is most likely in the form of surface runoff. Large amounts of endosulfan can be found in surface water near areas of application (Farms Chemical Handbook, 1992).

The breakdown of endosulfan in water is more rapid under neutral conditions with the half-life of five weeks that at more acidic condition with the half-life of five months. Under strongly alkaline conditions, the half-life of the insecticide is one day. The two isomers have different degradation times in soil. The half-life for the α-isomer is 35 days and 150 days for the β-isomer under neutral conditions.

The half-live on plants is three to seven days for most fruits and vegetables (Martin and Worthing, 1977). Endosulfan residues have been found in numerous food products at very low concentrations as documented in Table 1.10.

1.4.5 Summary of the characteristics of endosulfan

1.4.5.1 Chemical identity

Endosulfan is an OC insecticide. Technical endosulfan consists of a mixture of two stereoisomers, α-endosulfan stereochemistry 3α, 5aβ, 6α, 9α, 9aβ-; β-endosulfan stereochemistry 3α, 5aα, 6β, 9β, 9aα-.

Common name	Endosulfan
IUPAC name	1, 4, 5, 6, 7, 7-hexachloro-8, 9, 10-trinorborn-5-en-2, 3-ylenebismethylene) sulfite
CAS name	6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide
Empirical formula	$C_9H_6Cl_6O_3S$
Molecular weight	406.9

1.4.5.2 Physical and chemical properties

Physical and chemical properties of pure active constituent

Color	Colorless crystalline solid
Odour	Odourless
Physical state	Pure α-isomer-crystalline solid; pure β-isomer-crystalline solid
Melting point	α-: 109.2°C; β-: 213.3°C
Vapor pressure	α-: 1.9; β-: 0.09 mPa at 25°C. α-: 0.96; β-: 0.04 mPa at 20°C
Specificity gravity	1.745 at 20°C
Solubility in water	α-: 0.33; β-: 0.32 mg/L at 22°C
Solubility in organic solvents (100g solvent at 20°C)	Ethyl acetate, Dichloromethane, Toluene (200 g/L), Ethanol (65 g/L), Hexane (24 g/L)

Physical and chemical properties of technical grade endosulfan

Color	Brown
Odour	Terpene odour
Physical state	Crystalline flakes
Melting point	70 - 100°C
Vapor pressure	1 x 10^{-5} mm Hg at 25°C; 1.7 mPa
Specificity gravity	1.745 at 20°C
Solubility in water	60 – 150 µg/L
Solubility in organic solvents (100g solvent at 20°C	Chloroform (50 g); Xylene (45 g); Benzene (37 g); Acetone (33 g); Carbon tetrachloride (29 g); Kerosene (20 g); Methanol (11 g); Ethanol (5 g)

1.4.5.3 Exposure guidelines

ADI	0.006 mg/kg/day (WHO)
LEL	0.75 mg/kg/day (rat)
NOEL	0.15 mg/kg/day
RfD	0.00005 mg/kg/day (EPA)
TLV-STEL	0.3 mg/m^3
TLV-TWA	0.1 mg/m^3