ASSESSMENT OF DICHLORODIPHENYLTRICHLOROETHANE (DDT) RESIDUES IN CATTLE OF ELOOR AREA

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that th s thesis entitled **ASSESSMENT OF DICHLORODIPHENYLTRICHLOROETHANE (DDT) RESIDUES IN CATTLE OF ELOOR AREA** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree diploma associateship fellowship or other similar title of any other Univers ty or Society

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CERTIFICATE

Certified that the thesis entitled **ASSESSMENT OF DICHLORODIPHENYLTRICHLOROETHANE (DDT) RESIDUES IN CATTLE OF ELOOR AREA** is a record of research work done independently by **Dr Deepa, A K** under my guidance and supervision and that it has not previously formed the basis for the award of any degree fellowship or associateship to her

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Introduction

1. INTRODUCTION

In India, livestock sector is a significant contributor to the economy and is considered as a key element in improving the socio-economic conditions of the rural poor. It is closely inter-woven with agriculture and contributes to about 25 per cent of the total gross natural product (GNP). The need for organic pesticides in Indian agriculture and public health has been well recognized in protecting the crops from pests, and human health from vector borne diseases. But their injudicious and indiscriminate use leads to contamination of environment and subsequent entry into ecosystem. Pesticide industries also contribute substantially to environmental pollution.

Insecticidal property of dichlorodiphenyltrichloroethane (DDT) was discovered in 1939 by Paul Muller of Geigy company in Switzerland. Although it is an efficient and economical pesticide, DDT is now seldom used in agriculture because of bioaccumulation and ecotoxicity, which lead to the ban of the compound worldwide. But in several tropical countries like India, DDT is still being manufactured and used in various public health programmes.

The main route of entry of DDT into the body of animals is through contaminated feed and fodder. Further the contaminated animal feed gives rise to residues in eggs, meat and milk which may be of concern to man. Once absorbed into the body, pesticide residues cannot be quickly eliminated by simple withdrawal of contaminated feed as the residues are stored in depot fat. Also, DDT residues may be concentrated in the food chain. Hence chronic

exposure to forage and fodder plants carrying residues in small quantities may pose a danger when fed to livestock in large quantities. Apart from ingestion, inhalation of contaminated air and absorption through skin are the major routes of entry of DDT into the body. In mammals DDT produces moderate toxicity characterized by abnormal susceptibility to fear, violent reaction to stimuli with definite motor unrest and appearance of spontaneous tremors. In later stages epileptiform and tonic-clonic convulsions occur followed by death (Clarke and Clarke, 1975). Chronic toxicity causes significant damage to the nervous system, liver, kidney, adrenals and immune system in a number of species.

Central nervous system is the major site of action of DDT where it produces stimulation in transmission of nerve impulses. DDT prolongs the duration of sodium channel opening at depolarization thereby decreasing the transmembrane resting potential which causes an increase in neuronal excitability.

DDT is generally classified under persistent organic pollutant (POP). Its chemical, physical and biological stability makes it available for storage and recycling in the various segments of environment like air, water, soil, plants and animals. Contamination of straw and green fodder with DDT sprayed for mosquito control was reported by Battu *et al.* (1989). DDT and its principal metabolites DDD and DDE are widespread pollutants and the detection of the residues in blood, urine, milk and dung is of extreme importance in assessing the degree of pollution (Durham *et al.*, 1963).

In Kerala 197 medium and large industries and 1721 small scale industries are present which cause varying degrees of environmental pollution (Cheeran *et al.*, 1987). Udyogamandal (Eloor) in Ernakulam district is identified as one of the critically polluted areas in the country by virtue of the several industrial units located in the area. Seven important industries form the major source of pollution in the region. Inorganic chemicals, organic wastes and hazardous chlorinated organic compounds like pesticides and their intermediates are expelled out into the surrounding water bodies and pasture lands, producing toxicities in livestock grazing the locality.

Hence the present study was undertaken to assess the environmental pollution with DDT from industrial effluents and its impact on the health status of cattle population in the study area.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Physical and chemical properties

Dichlorodiphenyltrichloroethane (DDT) is a moderately toxic organochlorine insecticide which is slowly metabolized from the body and can act both as an acute and chronic poison. It is absorbed into the body through gastrointestinal tract and by inhalation. It may also be absorbed through intact skin, especially in case of oil-based formulations (WHO, 1979). Technical DDT (1, 1, 1 – trichloro – 2, 2 – bis – (4 – chlorophenyl) ethane) contains the p,p'-isomer of DDT which contributes 63-77%, the o,p'–isomer 8-12% and the o,o'–isomer 0.1-1.0%. Pure p,p'–DDT is a white crystalline solid of melting point 108.5°C-109°C. It is practically insoluble in water, moderately soluble in polar solvents, petroleum oils and is readily soluble in most aromatic and chlorinated solvents. The compound has a vapour pressure of 0.025 mPa @ 25 degrees C and a partition coefficient of 100,000. DDT is classified in EPA toxicity as class II, moderately toxic. It is available as aerosols, dustable powders, emulsifiable concentrates, granules and wettable powders (Meister, 1992).

2.2 Toxicity of DDT in animals

2.2.1 Hepatotoxicity

Rats were maintained on a diet containing 0, 200, 400, 600 and 800 ppm of DDT for 2 years. Liver lesions consisting of hypertrophy of centrilobular

hepatocytes and focal necrosis were found at all concentrations (Fitzhugh and Nelson, 1947).

Activities of liver microsomal enzymes NADPH – cytochrome reductase, flavoprotein linked monooxygenase and o-demethylase were significantly greater in albino rats given a high protein diet along with DDT @ 100 mg/kg diet for 4 weeks (Girija *et al.*, 1985)

Brown and Casida (1987) observed that the *in vivo* metabolic dechlorination of alpha-chloro-DDT and dicofol involves a reduced porphyrin in mice liver microsomes.

Experimental studies showed that rats exposed to PCB, DDT and dieldrin had a marked reduction in liver vitamin A store, suggesting that the metabolism of vit. A may be affected by exposure to these organochlorines (Landis and Ho, 1995).

Kostka *et al.* (1996) reported that early hepatic changes induced in rats by oral administration of DDT consisted of hepatomegaly accompanied by an increase in the p-nitroanisole o-demethylase activity, hepatocyte proliferation, vacuolated cytoplasm and focal necrosis .

2.2.2 Adrenotoxicity

Weber *et al.* (1958) used Holstein-Friesian calves to study the effects of orally administered DDD @ 50 mg/kg body weight/day on adrenals. Cytological and cytochemical studies indicated that the compound exerted

cytopathogenic effect on cells of inner portions of zona fasciculata and zona reticularis, which caused massive parenchymatous degeneration, interstitial tissue replacement, fatty degeneration of cells and presence of large number of extracellular acinar birefringent crystals.

A single intravenous injection of o,p'-DDD @ 60 mg/kg body weight produced an acute effect on the adrenal cortex of dogs. Reduction in *in vitro* response of adrenal cortex to stimulation by adrenocorticotropic hormone and an inhibition of glucose-6-phosphate dehydrogenase activity was reported by Cazorla and Monoclova (1962).

Administration of DDD and DDT in guinea pigs @ 300 mg/kg and 150 mg/kg respectively, caused stimulation of cortisol metabolism followed by a decrease in cortisol production. In dogs reduction in cortisol production was demonstrated (Balazs, 1969).

Lund *et al.* (1988) reported that 3-methyl sulphonyl – DDE is highly toxic to adrenal cortex in mice. Histopathological examination of adrenals from mice treated with the compound @ 25 mg/kg body weight revealed extensive vacuolation and necrosis of the entire zona fasciculata.

2.2.3 Neurotoxicity

Wurster *et al.* (1965) observed heavy bird mortality accompanied with tremors and convulsions prior to death after spraying of DDT against Dutch Elm disease.

Evdokimov and Prigarin (1974) reported that cattle were fed with BHC and DDT at a minimum toxic dosage (200 mg/kg) daily for 22 weeks (BHC as 12% dust formulation and DDT as 10% dust). Nervous symptoms of poisoning developed after 76,111 and 150 days in the animals.

Acute toxicity of DDT was less pronounced in mammals (oral LD_{50} = 113 to 800 mg/kg in rats, 500 to 750 mg/kg in dogs, greater than 1000 mg/kg in sheep and goats). The acute toxicity was related to the presence of the compound in the central nervous system where it evokes symptoms such as hyperexcitability, tremor and convulsions leading to death. (Hirdina *et al.*, 1975)

DDT is capable of altering the transport of sodium and potassium ions across the axonal membranes, resulting in an increased negative after potential, prolonged action potentials, repetitive firing after a single stimulus and spontaneous trains of action potentials (Narahashi, 1983).

Gamez (1984) noted that 17 zebu cattle at Ibaque, Colombia developed ataxia, progressive posterior paralysis with muscular atrophy and lateral decubitus with opisthotonus. When the tissues were subjected to insecticidal analysis, high concentration of organochlorine compounds were found in the liver, nerve tissue and muscle of affected animals.

2 2 4 Immunotoxicity

Street and Sharma (1975) showed that rabbits treated with DDT carbofuran and methyl parathion had decreased counts of activated lymphocytes in the lymphnodes reduced number of germinal centers in the spleen and more pronounced atrophyof the cortex of thymus

The effect of acute and chronic doses of HCH or DDT (40 mg/kg live weight or 20 mg/kg of each compound respectively given once daily for 20 days) on the ability of white mice to react to pathogens was investigated It was found that both treatments depressed the immunobiological reactivity of the animals (Evdokimov *et al* 1980)

Influence of protein deficiency on immune responsiveness after subchronic DDT exposure in albino rats was studied by Banerjee *et al* (1995) Animals maintained on 3 percent protein diet alone showed depression in humoral and cellular immune responses to antigen

In rats a diet containing 200 ppm of DDT DDE and TDE induced differential degrees of humoral and cellular immune suppression Increase in albumin/globulin ratios suppression of IgM and IgG levels attenuation in ovalbumin induced antibody responses marked inhibition of leukocyte and macrophage migration factors and delayed type hypersensitivity reaction were observed in these animals (Banerjee *et al* 1996)

2 2 5 Reproductive toxicity

When p p DDT was administered to pregnant mice at the rate of 1 mg/kg on days 10 12 and 17 of gestation revealed that it was not teratogenic but altered the gonadal function and decreased the fertility of the young especially the females (McLachlan and Dixon 1972)

Biswas *et al* (1981) studied semen characteristics of rams during continuous feeding of DDT (3 mg and 10 mg/kg daily) for six months During the treatment period the ejaculates were characterized by reduced sperm motility decreased live sperm count and an increased incidence of tailless heads The semen characteristics attained the pre treatment levels within two months of withdrawal of the insecticide

Administration of DDT to 4 week old cockerels at 12 5 25 and 37 5 mg/kg body weight for 24 weeks produced testicular atrophy in all treated birds (Balasubramanian and Sundararaj 1993) Histopathological changes included disruption and necrosis of spermatogonial cells desquamation of spermatocytes formation of macrophages megalocytes and giant cells intertubular fibrosis and atrophy of tubules

The effects of organochlorine pesticides o p DDT p p DDT methoxychlor and lindane on ATPase activities of microsomal fraction of bovine oviductal and endometrial cells were investigated by Tiemann and Kuchenmeister (1999) After 10 minutes preincubation a significant inhibition

was found only with o p DDT at 32 μM and 64 μM in the oviductal microsomal fraction and 64 μM in the endometrial cells

2 2 6 Carcinogenicity

DDT was given to mice at the maximum tolerated dose of 46 4 mg/kg/day upto four weeks and 21 mg/kg/day for 18 months by Innes *et al* (1969) Hepatomas were observed in large number of animals lymphomas were significantly increased above the controls

Over a period of six generations BALB/c mice were maintained with an average daily intake of 0 4 – 0 7 mg/kg of DDT An increase of myeloid leukaemias and pulmonary carcinomas were reported in the population (Tarjan and Kemeny 1969)

Walker *et al* (1973) noticed a dose dependent development of liver neoplasms in CF 1 mice fed with DDT for 104 weeks The percentage of occurrence was found to be 53 per cent at a dose of 15 mg/kg/day and 37 per cent @ 7 5 mg/kg/day

2 2 7 Teratogenicity

An experimental study was conducted to understand DDT residue concentration and distribution in 22 stillborn calves from cows fed either apple pomace maize silage or both The concentrations of total residues in the depot

fat of still born calves and their dams were similar but in the former a higher proportion of DDE was present (Rumsey *et al* 1973)

Craig and Ogilvie (1974) reported impairment of learning and memory in young white mice born to or nursed by females fed a diet containing a sublethal amount of DDT (200 µg/g feed)

On the basis of studies conducted by Saxena *et al* (1981) it was found that DDT crosses the placenta and its concentration in the umbilical cord blood was in the same range as that in the blood of exposed mother

Alm *et al* (1998) established that bovine oocytes exposed to DDT and lindane *in vitro* are affected in a dose dependent manner Higher concentrations of pesticides produced higher rate of chromatin degeneration and depression of bovine oocyte maturation Further embryonic development of fertilized oocytes were significantly affected

2 2 8 Haemotoxicity

Various haematological parameters in albino rats were prominently altered after oral administration of DDT (100 mg 20 mg and 10 mg/kg body weight/day) for a total period of 48 hrs 15 days and 18 months respectively (Ali and Shakoori 1994)

2 2 9 Other effects

Incorporation of p p DDT @ 30 mg/kg body weight daily to five rum nally fistulated beef steer produced reduction in the total concentration of ruminal volatile fatty acids and protozoa DDT was converted to DDD in the rumen and had no significant effect on electrocardiograph patterns and respiratory rates (Rumsey *et al* 1970)

Laboratory mice were investigated for DDT induced chromosome mutations by Larsen and Jalal (1974) Karyotypes of bone marrow cells showed that gaps stickiness and mitotic indices were not significantly affected by DDT treatments but deletions gaps with deletions were significantly higher at 50 ppm and higher concentration

Hifazi and Chefurka (1982) used fluorescent probe 1 anilino 8 naphthalene sulfonate (ANS) to monitor the interaction of pesticides with mitochondrial membranes and submitochondrial membranes The results suggested that both DDT and dicofol were relatively ineffective inhibitors of substrate induced quenching of ANS fluroscence of submitochondrial particles

Smith and Tramontm (1995) presented a case where two 11 month old Holstein heifers were found dead due to consumption of a pesticide mixture of endosulfan dieldrin DDT and DDE The predominant postmortem findings were pulmonary oedema and muscular haemorrhage

2 3 Avian toxicity of DDT

Japanese quail were fed with diet containing 100 ppm of p p DDT or p p DDE for a period of three months Reduction in the carbonic anhydrase enzyme activity to about 16 to 19 per cent in the shell forming glands and 22 to 44 per cent in the blood of the treatment group were observed (Bitman *et al* 1970)

Effect of p p DDT in ringdoves given 10 ppm were studied by Peakall (1970) A decrease of estradiol in blood early in the breeding cycle delay in egg laying fall in egg shell weight and reduced deposition of medullary calcium were noticed Injection of p p DDE (150 mg/kg body weight) caused reduction of egg shell weight and inhibition of carbonic anhydrase in the oviduct

Subtoxic doses (5 50 500 ppm in the feed over 57 days) of technical DDT inhibited corticosterone synthesis in chicks and reduced liver glycogen concentration (Srebocan *et al* 1971)

Miller *et al* (1976) noted that DDT was responsible for increased frequency of breakage of eggs in birds as it inhibits Ca^{2+} ATPase enzyme necessary for calcification of eggs

Increased metabolism of estrogens due to induction of cytochrome P_{450} by DDT was revealed by Lundholm (1987) The resulting endocrine imbalance

affected calcium metabolism egg laying and nesting so that total reproductive success and survival of young one was reduced

Day old chicks were fed with subacute doses of DDT (10 mg of 5 per cent w p/bird) by Ramalingam (1987) Degenerative changes were seen in liver and renal tubules of kidney The absorptive layers of intestine were also disrupted

Reproductive toxicity of DDT in white leghorn cockerels were studied by feeding 6 25 12 5 25 37 5 and 50 mg DDT/kg body weight/day A decrease in the volume of semen gross motility live spermatozoa and sperm concentration with an increase in whole semen cholesterol was noticed (George and Sundararaj 1995a)

Blus *et al* (1997) observed significant egg shell thinning in captive American Kestrels (*Falco sparverius)* and wild brown pelicans (*Pelecanus occidentalis)* fed with a DDT contaminated diet High dietary concentration of DDE caused extreme egg shell thinning and mortality in adult mallards (*Anas platyrhynchos)* No significant effect was seen in the size mass and shape of eggs

2 4 Toxic effect on human beings

Relatively low doses of DDT induced the mixed function oxidase system (cytochrome P_{450}) of the hepatic endoplasmic reticulum This effect has been demonstrated in workers of a DDT factory (Poland *et al* 1970)

Ecobiochon (1991) observed that signs and symptoms of human poisoning from high doses of DDT include paresthesias of the tongue lips and face apprehension hyper susceptibility to stimuli irritability dizziness tremors tonic and clonic convulsions

2 5 Residues of DDT and metabolites

2 5 1 Biological sources

Milk samples from human buffalo and goat were analysed for organochlorine pesticides and p p DDT p p DDE p p DDD HCH lindane aldrin were detected Mean total DDT in human milk was 0 52 ppm where as p p DDE and p p DDD were 0 31 ppm and 0 04 ppm Average concentrations of total DDT in buffalo and goat milk were 0 049 and 0 042 ppm (Saxena and Siddiqui 1980)

Kaphalia and Seth (1984) estimated DDT HCH and aldrin residues in blood serum of goat buffalo chicken and man by gas liquid chromatography Total DDT levels in goat serum were very low (3 69 ppb) compared to buffalo (13 67 ppb) and chicken (14 89 ppb) In human serum the residue levels were very high (34 53 ppb)

Fifty samples from five brands of butter were analysed by Takroo *et al* (1985) Residues of DDT and HCH were detected in all brands of butter and the average levels of DDT were 2 01 5 94 6 15 7 14 and 10 80 ppm Levels

were higher compared to the tolerance limit of 1 25 ppm recommended by FAO/WHO

Battu *et al* (1989) observed that samples of bovine milk collected from houses where DDT and HCH were used for control of malaria, contained residues of both insecticides above the maximum residue limit of 0 05 mg/kg (whole milk basis)

Tissue samples of slaughtered pigs were found to be positive for organochlorine pesticides like DDT ∝ HCH β HCH lindane DDE DDD heptachlor heptachlor epoxide hexachlorobenzene and dieldrin (Cantoni *et al* 1990)

In an experiment to study the residue level of DDT in poultry (George and Sundararaj 1995b) they found that residues of DDT and its metabolites were highest in adipose tissue followed by liver kidney heart fat of meat blood spleen testes brain egg yolk and semen

Waliszewski *et al* (1996) analysed 345 samples of butter from Mexican supermarkets and residues of γ HCH (91%) ∝ HCH (63%) p p DDE (88%) p p DDT (42%) and o p DDT (17%) were detected Mean values of residues determined was 0 056 mg/kg fat for total DDT

DDT and BHC were detected in all human and dairy milk samples collected from in and around Banglore city and analysed by Surendranath *et al*

(2000) In human milk fat DDT ranged from 0 27 to 31 ppm but in dairy samples the levels ranged from traces to 0 47 ppm

Residues of DDT and its derivatives were detected in most foods of animal origin like meat poultry egg dairy products and fish in Thailand (1989 to 1996) But the dietary intake calculated were far below established ADI of 0 02 mg/kg body weight (Vongbuddhapitak *et al* 2002)

2 5 2 Environmental sources

A total of 244 samples of cereals pulses spices vegetables fruits milk butter deshi ghee and edible oils were analyzed by Kaphalia *et al* (1990) using gas liquid chromatography Residues of HCH and DDT were detected in 85% of total samples and very high concentrations were seen in wheat flour (4 42 and 0 12 ppm) butter (1 19 and 4 85 ppm) mustard oil (1 26 and 2 42 ppm) deshi ghee (1 10 and 3 84 ppm) vegetable oil (1 02 and 0 59 ppm) ground nut oil (0 51 and 1 49 ppm) and chilli (0 48 and 1 92 ppm)

Dogheim *et al* (1996) detected residues of o p DDT p p DDT p p DDE and p p DDD in potatoes from Egyptian local markets The mean quantities of residues detected were 0 019 ppm 0 032 ppm 0 004 ppm and 0 02 ppm respectively

Levels of DDT and HCH were monitored in five lakes of Nainital region by Dua *et al* (1998) and varying concentrations of residues were found in each

lake Mean DDT residues in all lakes ranged from 6 05 to 31 34 µg/l and exceeded the WHO recommendations of 1 µg/l for drinking water

Cows maintained at the NDRI farm consumed in their feed about 500 µg of γ BHC and 30 µg of DDT and excreted about 80 µg of γ BHC and 12 µg of DDT through milk (Surendranath *et al* 1998)

Hans *et al* (1999) estimated pesticide residues in the vegetables grown in the dry bed field of river Ganga in Kanpur Mean levels of 109 35 136 76 and 145 93 µg HCH/kg and 6 64 49 3 and 46 70 µg DDT/kg were found in the rural upstream city and down stream industrial areas respectively

Anderson and Johnson (2001) utilized gas chromatography with electron capture detection to estimate the organochlorine pesticides in water and sediments of Malheur Watershed Eastern Oregon USA Though banned 30 years ago DDT was found to be still persistent throughout the river basin as all water samples tested were positive

Samples of animal feed resources like oil seed cakes cereals cereal by products green fodder and straw were collected and analysed by HPLC for organochlorine pesticide residues by Prasad and Chhabra (2001) The order of contamination was BHC >endosulfan >heptachlor >DDT >aldrin

Materials and Methods

3 MATERIALS AND METHODS

The study was aimed to

(a) Assess the environmental pollution with DDT and its isomers in Eloor area

(b) Study the effects of DDT and its isomers on haematological and biochemical parameters of adult cattle in Eloor and to compare them with that of cattle in Mannuthy

3 1 Area of study

The study was conducted in the Eloor industrial belt of Ernakulam district in Kerala (Fig 1) A total of seven major industrial units are located within the limited area of 5 sq km contributing to its highly polluted environment The major industrial units include Fertilizers and Chemicals Travancore (FACT) Hindustan Insecticides Ltd (HIL) Travancore Chemical Manufacturing Company (TCM) Cominco Binani Indian Aluminium Company (IAC) and Indian Rare Earths (IRE) (Fig 2) Annually HIL produces 65 tons of DDT 650 tons of BHC and 10 tons of Endosulfan A number of hazardous chlorinated organic compounds are utilized in the factory and they along with the residues of manufactured pesticides form the major source of pollutants of the area (Cheeran *et al* 1987) Compared to other pesticide residues DDT is highly bioaccumulative in nature and is moderately toxic to living beings Hence the study was undertaken to detect and quantify the DDT present in environmental and biological samples using gas liquid chromatography (GLC)

Fig.1

Map of Kerala showing the proposed area of study

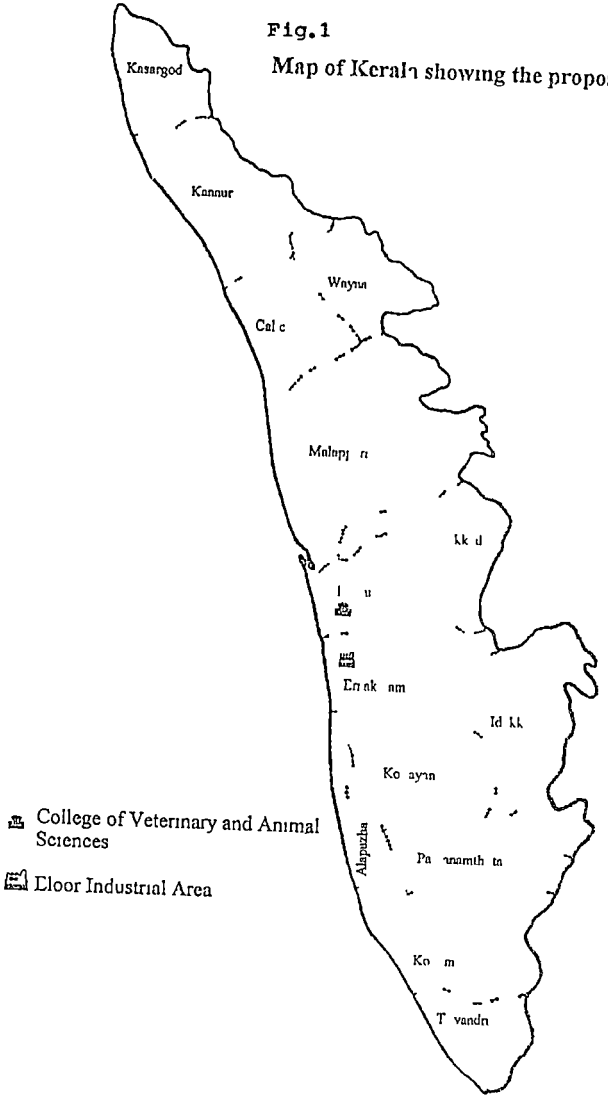
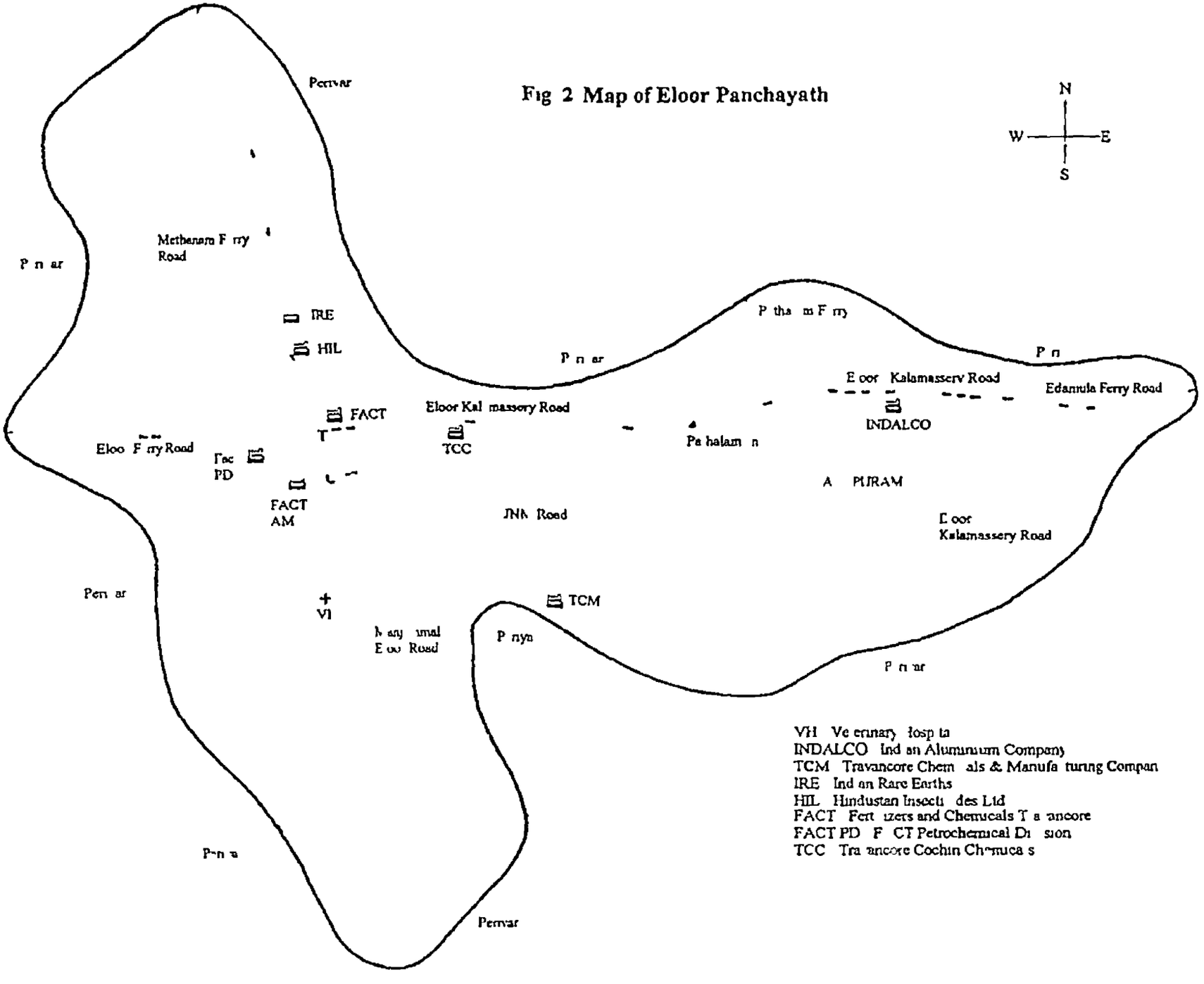


Fig 2 Map of Eloor Panchayath



VH Veterinary Hospital
INDALCO Indian Aluminium Company
TCM Travancore Chemicals & Manufacturing Company
IRE Indian Rare Earths
HIL Hindustan Insecticides Ltd
FACT Fertilizers and Chemicals Travancore
FACT PD FACT Petrochemical Division
TCC Travancore Cochin Chemicals

3 2 Collection of samples

Both environmental and biological samples were collected from the study area for detection of DDT residues Environmental samples included water sludge and fodder and the biological samples collected were blood milk dung and urine Ten cattle above three years of age which were maintained for a minimum period of three years in Eloor area were selected for collection of the biological samples

Biological samples were collected from six adult cattle of University Livestock farm Mannuthy and environmental samples were also collected from in and around the livestock farm Mannuthy for comparison

3 2 1 Environmental samples

The environmental samples were collected as per the sampling techniques suggested by Lorgue *et al* (1996) Collected samples were transported in stoppered glass vials and stored under deep freezing (20°C)

3 2 1 1 Fodder

Samples of fodder were collected from open grazing lands local vegetation which were used as fodder and dried hay routinely fed to the animals These materials were collected dried and stored in containers

3 2 1 2 Sludge

Surface sludge was collected from various locations within the study area Samples were stored in suitable containers after proper drying in the shade

3 2 1 3 Water

Water samples were collected from wells paddy fields canals ponds and marshy areas of Eloor Collected samples were stored in glass bottles under deep freezing (20°C) conditions

3 2 2 Biological samples

3 2 2 1 Blood

Whole blood was collected in heparinised vials for haematological studies Serum samples for pesticide residue detection and determination of biochemical parameters were harvested from blood collected in clean and dry centrifuge tubes

3 2 2 2 Dung

Dung samples were collected dried and stored in suitable containers

3 2 2 3 Milk

About 100 ml milk was collected in glass bottles and stored under deep freezing (20°C)

3 2 2 4 Urine

Collected urine samples were stored under deep freezing and analysed as soon as possible

3 3 Pesticide residue analysis

Specific clean up procedures were utilized for complete removal of interfering impurities and extraction of pesticide residues from the collected samples before introduction into the gas liquid chromatograph

3 3 1 Environmental samples

Collected samples were analysed as per the methods specified by FDA (1977) and Sherma (1979)

3 3 1 1 Residue extraction from water

From the collected water sample 500 ml was transferred into a one litre separating funnel Ten g of anhydrous sodium sulphate was added to the funnel and extracted twice with 50 ml dichloromethane The mixture was shaken well and allowed the solvent layer to separate Then the aqueous layer was discarded through the nozzle The dichlpromethane layer was concentrated to four millilitre by vacuum flash evaporation To this extract about 20 ml n hexane was added and vacuum flash evaporated The resulting dry matter was extracted in one millilitre hexane This was transferred to a small glass column of size 30 mm x 450 mm filled with one gram deactivated silica gel (deactivated with 20% water) The column was then eluted first with 10 ml hexane then by 15 ml 60% benzene in hexane then with 15 ml 25% acetone in dichloromethane Later all the fractions were pooled together and concentrated by vacuum flash evaporation Final extraction was done in one millilitre petroleum ether (60 80) and directly injected into the GLC

3 3 1 2 Residue extraction from fodder and sludge

Sample collected was dried and powdered (in case of fodder) and two grams of the sample was taken in an extraction thimble The thimble was introduced into a soxhlet extraction un t and extracted with 200 ml petroleum ether (60 80) for six hours This extract was concentrated in vacuum flash evaporator to 10 ml and was quantitatively transferred to a 100 ml separating funnel Fifteen millilitre acetonitrile saturated with petroleum ether was added and shaken well and the layers were allowed to separate The bottom layer containing the pesticide was transferred to a one litre separating funnel having 600 ml water 100 ml petroleum ether and 40 ml saturated sodium chloride solution Extraction with acetonitrile was repeated for two more times and the bottom layer was collected in the same one litre separating funnel shaken well and allowed to separate The bottom aqueous layer was transferred to another one litre separating funnel containing 100 ml petroleum ether It was also shaken well and allowed to separate The aqueous layers were discarded and the petroleum ether layers from the two were pooled washed with 100 ml of distilled water three times and dried with anhydrous sodium sulphate then vacuum flash evaporated

Five gram anhydrous sodium sulphate was placed at the bottom of a glass column of size 30 mm x 450 mm and 25g of activated florisil was added to the top of sodium sulphate Another 10g of sodium sulphate was added above the florisil After wetting the column with petroleum ether transferred the acetomtrile clean up sample using small quantities of petroleum ether

Eluted the column first with 200 ml of 6% diethyl ether in petroleum ether followed by 200 ml of 15% diethyl ether in petroleum ether The elutes were pooled together and evaporated to dryness in vacuum flash evaporator The dry matter obtained was taken in five millilitre petroleum ether for injection into GLC

3 3 2 Biological samples

3 3 2 1 Residue extraction from blood

Two millilitre serum separated from the sample was taken in a 20 ml separating funnel and diluted with three millilitre deionised water To this added five millilitre dichloromethane and shaken well for five minutes Repeated the procedure and the extract was centrifuged to remove the emulsion obtained The organic layer was then separated and dried by passing through a column packed with three centimetre layer of anhydrous sodium sulphate The extract was evaporated to dryness under a gentle air stream and the residue was dissolved in one millilitre n hexane for injection into the GLC (Pitarch *et al* 2001)

3 3 2 2 Residue extraction from urine

Five millilitre urine sample was shaken with same quantity of d chloromethane in a 20 ml separating funnel for one minute Repeated the procedure twice and the separated organic layer was dried by passing through a column packed with three centimetre layer of anhydrous sodium sulphate The extract was then evaporated to dryness under a gentle stream of air and the

residue was dissolved in one millilitre n hexane for injection into the GLC (Pitarch *et al* 2001)

3 3 2 3 Residue extraction from milk

About 100 ml milk sample was taken in a centrifuge bottle and added 100 ml methylalcohol one gram sodium oxalate and mixed well To this mixture added 50 ml of diethyl ether and shaken for one minute Again 50 ml petroleum ether was added to the mixture and shaken for one minute The emulsion thus formed was centrifuged at 1500 rpm for five minutes The solvent layer from the top of the sample was pipetted into a one litre separating funnel with 600 ml distilled water and 30 ml saturated sodium chloride solution The remaining emulsion was extracted twice with 50ml of 50% diethyl ether – petroleum ether mixture and added to the separating funnel Contents of the separating funnel were mixed cautiously and water layer was discarded off Rewashed the solvent layer twice with 100 ml distilled water discarding water each time The solvent layer was then allowed to pass through anhydrous sodium sulphate taken in a column of 25 mm x 50 mm length The elute was collected and complete evaporation was done at steam bath temperature under air current to obtain fat Weighed about three grams of fat into 125 ml separator and added petroleum ether so that total volume in the separator becomes 15 ml Added 30 ml acetonitrile saturated with petroleum ether and shaken vigorously for one minute After the different layers separated out acetomtrile layer was drained into one litre separator containing 600 ml water 40 ml saturated sodium chloride solution and 100 ml petroleum ether

Extraction of the petroleum ether solution in 125 ml separator was repeated with three additional 30 ml portions of acetonitrile saturated with petroleum ether and collected all extracts in the one litre separator Then separator was held in horizontal position and mixed thoroughly for 30 40 seconds After the layers separated aqueous layer was transferred into the second one litre separator with 100 ml petroleum ether shaken vigorously for 15 seconds and allowed the layers to separate Discarded the aqueous layer and pooled the two petroleum ether fract ons in the original separator and washed with two 100 ml portions of distilled water The washings were discarded and petroleum ether layer was drained through anhydrous sodium sulphate taken in a column of 25mm x 50mm length The separator and column were rinsed thrice with small portions of petroleum ether The pooled extracts were concentrated to 10ml under gentle air stream

Prepared a column that contains four inches of activated florisil topped with about half an inch anhydrous sodium sulphate Prewetting of the column was done with 50 ml petroleum ether Transferred the petroleum ether extract to the column and allowed it to pass through at the rate of five millilitre/minute Rinsed the container twice with small quantities of petroleum ether and passed through the column The column was then eluted with 6% diethyl ether with petroleum ether at the rate of five millilitre/minute The elute was concentrated to dryness under gentle air stream and the residue was extracted in two millilitre n hexane for injection into the GLC

3 3 2 4 Residue extraction from dung

The dung samples were sun dried and extracted adopting the same procedure for extraction and cleanup of fodder and sludge as per FDA (1977)

3 4 Analysis on gas liquid chromatography

Quantification of DDT residues in the collected environmental and biological samples were done using gas liquid chromatography as per the method specified by Sherma (1979) and FDA (1977) GLC analysis was performed on a Hewlett Packard Agilent 6890 series GC with electron capture detector (ECD) having ^{63}Ni as the radioactive source and equipped with HP enhanced integrator algorithm (Fig 3)

3 4 1 Operating conditions of GLC

System condition	DDT analysis
Column	HP I 30m x 0 32mm x 0 25µm
Injection port	250°C split mode 1 30 split ratio
Oven temperature	210°C
Detector	ECD 300°C
Carrier	Nitrogen 1 5 ml/min constant flow
Sampler	Agilent analytical syringe 1µl injection
Analysis time	40 minutes

Fig 3 Hewlett Packard Agilent 6890 GC with Electron Capture Detector

3 4 2 Calibration of instrument

Calibration standards of o p DDT p p DDT p p DDD o p DDE and p p DDE were acquired from Merck and stored under refrigeration conditions Stock solutions of 1 ppm were prepared for each sample using hexane as solvent The ECD response for all the five somers were analysed indiv dually at a range of concentrations from 0 001 ppm to 1 ppm Excellent linearity was observed for all the five isomers upto a concentration of 0 100 ppm Then a multilevel calibration using three different concentrat ons 0 01, 0 05 and 0 1 ppm was given The calibration curve showed a linearity through the origin for all the five isomers with an average correlation of 0 99

3 4 3 Detection and Estimation

The chromatograph of samples and pestic de standard were obtained under identical operation condit on of GLC The residues were detected by the comparison of their retention time with the standard and the amount by comparing the area with the standard using the HP enhanced integrator algorithm Sum total of DDT and its isomers in the samples were quantified by the formula

$$\text{DDT residue in ppm} = \frac{X \quad V \quad I}{V \quad M \quad 10^3}$$

X – integrator reading in picogram

V – µl of sample injected

V – Total volume of cleaned up sample in ml

M – Weight (g) or Volume (ml) of sample taken for extraction

3 5 Haematological parameters

Haematological parameters were studied using heparinised blood samples

3 5 1 Total leukocyte count

The leukocytes were counted by standard dilution technique using Thomas fluid as diluent Counting of leucocytes was done in the zone for leucocytes in the haemocytometer placed under low power of the m croscope (Benjamin 1985)

3 5 2 Differential leukocyte count (DLC)

Blood smears were prepared from freshly drawn blood (without anticoagulant) by using slide method After staining with Wright s stain counting was done under oil immersion (Benjamin 1985)

3 5 3 Haemoglobin

The haemoglobin concentration was estimated by acid haematin method described by (Benjamin 1985)

3 5 4 Packed cell volume (PCV)

Packed cell volume was estimated by filling Wintrobe haematocrit tube with a uniform column of blood devoid of air bubbles The tubes were centrifuged at 6000 rpm for 15 minutes (Benjamin 1985)

3 5 5 Haemoglobin indices

Mean corpuscular volume (MCV) Mean corpuscular haemoglobin (MCH) mean corpuscular haemoglobin concentration (MCHC) were calculated using the following formulas

$$\text{MCV in } \mu m^3 = \frac{\text{PCV} \times 10}{\text{No of erythrocytes per ml of blood} \times 10^{-6}}$$

$$\text{MCH in } \mu g \text{ or } pg = \frac{\text{Hb in (g/dl)} \times 10}{\text{No of erythrocytes} \times 10^{-6} \text{ per ml of blood}}$$

$$\text{MCHC in g\%} = \frac{\text{Hb in (g/dl)} \times 100}{\text{PCV in (ml/dl)}}$$

3 6 Serum Biochemistry

3 6 1 Total serum protein (albumin globulin ratio)

The estimation of total serum protein (albumin globulin ratio) was carried out in semi automatic blood analyzer (Microlab 200) using Ecoline® kits manufactured by E Merck (India) Limited

3 6 2 Serum enzymes

Estimation of serum enzymes like Alanine am no transferase (ALT) and Aspartate aminotransferase (AST) were done using Ecoline® kits manufactured by E Merck (India) Limited in semi automatic blood analyzer (Microlab 200)

3 7 Comparison of data

Comparison of data obtained by the analysis of environmental and biological samples from cattle of Eloor area and the data from environmental samples of cattle at University Livestock Farm Mannuthy was done

3 8 Statistical analysis

The data was analysed statistically by students t test (Rangaswamy 1995)

Results

4 RESULTS

4 1 Analytical method for estimation of DDT and isomers

Gas liquid chromatography (GLC) was used for the estimation of DDT and its isomers from the collected samples The chromatogram of DDT mixed standard depicts each isomer of DDT namely o p DDE p p DDE p p DDD o p DDT and p p DDT as a separate and well defined peak (Fig 4) Under the operating conditions for GC stated in Section 3 the retention time (Rt) for each component was 11 289 13 090 15 752 16 512 and 19 642 minutes respectively Linearity of isomers were tested at different concentrations of the standard (10 50 and 100 ppm) and shown in Fig 5 The limit of detection of the instrument was found to be 1 ppb

4 2 Recovery study

The procedures for extraction and clean up of field and biological samples were tested for recovery percentage by fortifying the samples with a known quantity of DDT Recovery studies revealed the percentage of recovery to be about 75 to 80 per cent

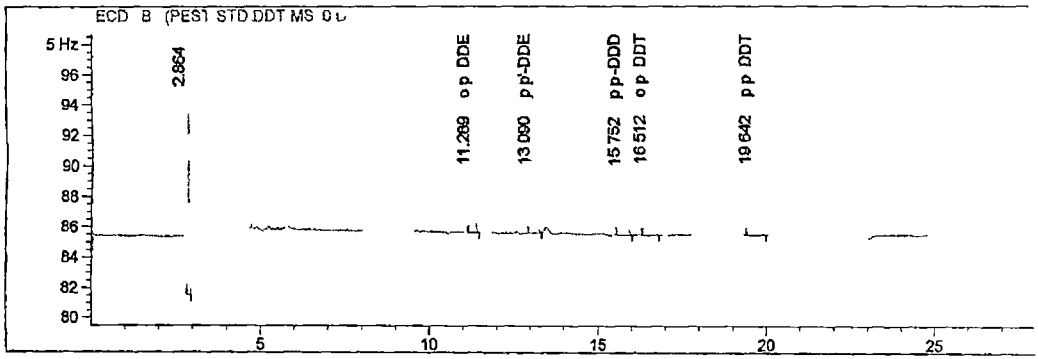
4 3 DDT residues in field samples

4 3 1 Fodder

The mean level of o p DDE p p DDE p p DDD o p DDT and p p DDT and total DDT (ppm) in fodder samples from Eloor were 0 266 ± 0 124 0 347 ± 0 133 0 185 ± 0 087 0 132 ± 0 070 0 587 ± 0 349 and 1 463 ± 0 555

Data File Name C \HPCHEM\1\DATA\PEST STD\DDT MS10 D
Method Name C \HPCHEM\1\METHODS\BMG M
Operator Name bmg
Sample Name DDT MS100ppb
Injection Date 11/7/01 10 33 23 AM

Fig. 4



Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2.864	30.572	7.771	0.000	
2	11.289	32.338	8.220	98.607	o p DDE
3	13.090	100.606	25.572	97.876	p p DDE
4	15.752	65.248	16.585	97.100	p p DDD
5	16.512	88.032	22.376	97.758	o p DDT
6	19.642	76.627	19.477	97.234	p p DDT

Instrument Name HPGC 6890

Operator's Signature

Calibration Table

Calib Data Modified 10/16/02 11 28 46 AM

Calculate Area Percent

Fig.5

Rel Reference W ndow 3 000 %
Abs Reference Window 0 000 min
Rel Non ref Window 3 000 %
Abs Non ref W ndow 0 000 m n
Uncal brated Peaks not reported
Part al Calibrat on Yes identified peaks are recalibrated
Correct All Ret Times No only for identified peaks

Curve Type Linear
Origin Included
Weight Equal

Recalibrat on Settings
Average Response Average all calibrat ons
Average Retent on Time Float ng Average New 75%

Calibration Report Options
Printout of recal brat ons with n a sequence
Calibrat on Table after Recal brat on
Normal Report after Recal bration
If the sequence is done w th bracket ng
Results of f rst cycle (ending prev ous bracket)

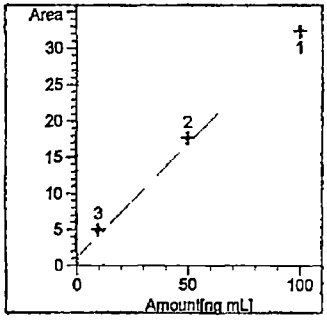
Signal 1 ECD2 B

RetTime [m n]	Sig	Lvl	Amount [ng/mL]	Area	Amt/Area	Ref	Grp	Name
11 261	1	3	10 00000	4 99494	2 00203		o p	DDE
	2		50 00000	17 64754	2 83326			
	1		100 00000	32 33765	3 09237			
13 063	1	3	10 00000	17 05291	5 86410e-1		p p	DDE
	2		50 00000	57 13910	8 75057e-1			
	1		100 00000	100 60629	9 93974e-1			
15 713	1	3	10 00000	11 09729	9 01121e-1		p p	DDD
	2		50 00000	38 50749	1 29845			
	1		100 00000	65 24811	1 53261			
16 472	1	3	10 00000	14 48480	6 90379e-1		o p	DDT
	2		50 00000	50 25632	9 94900e-1			
	1		100 00000	88 03216	1 13595			
19 596	1	3	10 00000	12 13991	8 23729e-1		p p	DDT
	2		50 00000	44 85704	1 11465			
	1		100 00000	76 62736	1 30502			

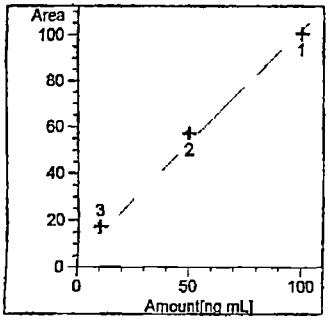
Peak Sum Table

No Entries in table

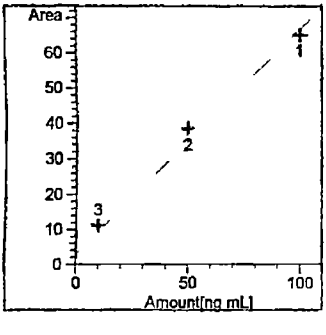
Calibration Curves



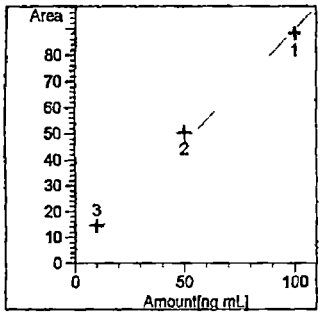
o p DDE at exp RT 11 261
ECD2 B
Correlation 0 99806
Res dual Std Dev 1 10241
Formula y – mx + b
m 3 17240e-1
b 1 05544
x Amount[ng/mL]
y Area



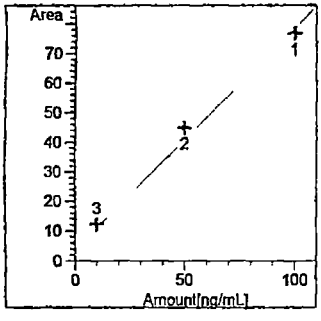
p p DDE at exp RT 13 063
ECD2 B
Correlat on 0 99630
Residual Std Dev 4 72187
Formula y = mx + b
m 9 83255e-1
b 4 36938
x Amount[ng/mL]
y Area



p p DDD at exp RT 15 713
ECD2 B
Correlation 0 99468
Residual Std Dev 3 68789
Formula y = mx + b
m 6 39846e-1
b 3 11940
x Amount[ng/mL]
y Area



o p DDT at exp RT 16 472
ECD2 B
Correlation 0 99636
Residual Std Dev 4 11130
Formula y = mx + b
m 8 62895e-1
b 3 67752
x Amount[ng/mL]
y Area



p p DDT at exp RT 19 596
ECD2 B
Correlation 0 99554
Residual Std Dev 3 98324
Formula y = mx + b
m 7 55164e-1
b 3 19953
x Amount[ng/mL]
y Area

respectively and that of samples from Univers ty Livestock Farm (ULF) Mannuthy were 0 054 ± 0 019 0 002 ± 0 002 0 0005 ± 0 0005 0 0 005 ± 0 002 and 0 062 ± 0 021 o p DDT was absent in all the samples collected from University Livestock Farm (ULF) Mannuthy Stat stical analysis showed that there was significant (P<0 05) increase n case of p p DDE and total DDT The results are represented in Table 1 and Fig 6 The chromatogram of a fodder sample collected from the Eloor industrial area and University Livestock Farm Mannuthy area each is shown in Fig 7 and Fig 8 respectively

4 3 2 Sludge

The DDT levels in sludge samples are presented in Table 2 and F g 9 Mean values of o p DDE p p DDE p p DDD o p DDT and p p DDT and total DDT of sludge samples from Eloor area 2 479 ± 1 229 4 693 ± 2 530 16 199 ± 10 25 1 651 ± 0 908 10 135 ± 5 569 and 35 157 ± 19 783 (ppm) respectively These values were higher than the corresponding values of samples from University Livestock Farm Mannuthy (0 0 002 ± 0 001 0 004 ± 0 003 0 0 005 ± 0 002 and 0 011 ± 0 006 ppm) o p DDE and o p DDT were not present in any of the University Livestock Farm samples Statistical analysis showed no significant difference between the two areas Fig 10 and Fig 11 presents the chromatogram of a sludge sample each from Eloor industrial and University Livestock Farm Mannuthy

Table 1 DDT level in fodder from Eloor area and University Livestock Farm Mannuthy

	Sl No	o p DDE (ppm)	p p DDE* (ppm)	p p DDD (ppm)	o p DDT (ppm)	p p DDT (ppm)	Total * (ppm)
Samples from Eloor	1	0 097	0 062	0 029	0 007	0 040	0 235
	2	0 160	0 118	0 044	0	0 038	0 360
	3	0 087	0 60	0 106	0 106	0 420	0 779
	4	1 137	0 474	0 180	0 133	0 404	2 328
	5	0	0 140	0	0	0	0 140
	6	0	0 490	0 910	0 590	3 570	5 560
	7	0 144	0 175	0 319	0 485	1 139	2 262
	8	0 826	1 386	0 232	0	0 168	2 612
	9	0	0 031	0 029	0	0 080	0 140
	10	0 204	0	0	0	0 009	0 213
	Mean ± SE	0 266 ± 0 124	0 347 ± 0 133	0 185 ± 0 087	0 132 ± 0 070	0 587 ± 0 349	1 463 ± 0 555
Samples from ULF	1	0 105	0 013	0 003	0	0 008	0 129
	2	0 093	0	0	0	0 002	0 095
	3	0 053	0	0	0	0 007	0 060
	4	0 075	0	0	0	0 011	0 086
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	Mean ± SE	0 054 ± 0 019	0 002 ± 0 002	0 0005 ± 0 0005	0	0 005 ± 0 002	0 062 ± 0 021

* bearing column differ significantly (P<0 05)

Fig 6 DDT levels in fodder from Eloor & Mannuthy

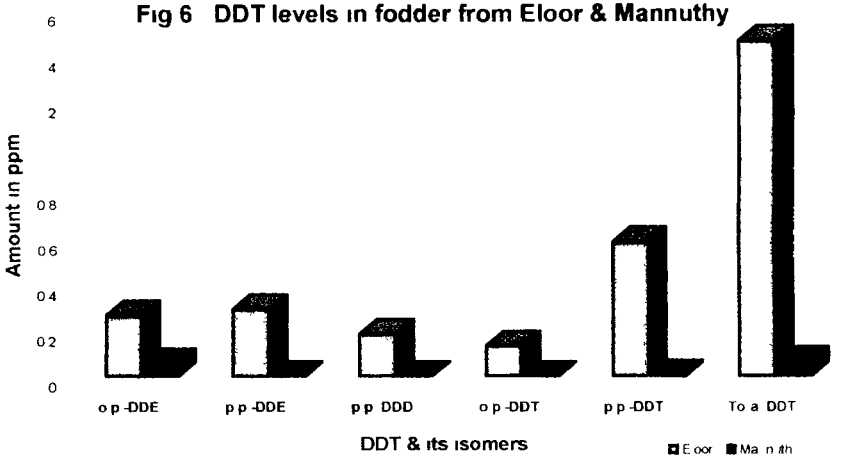
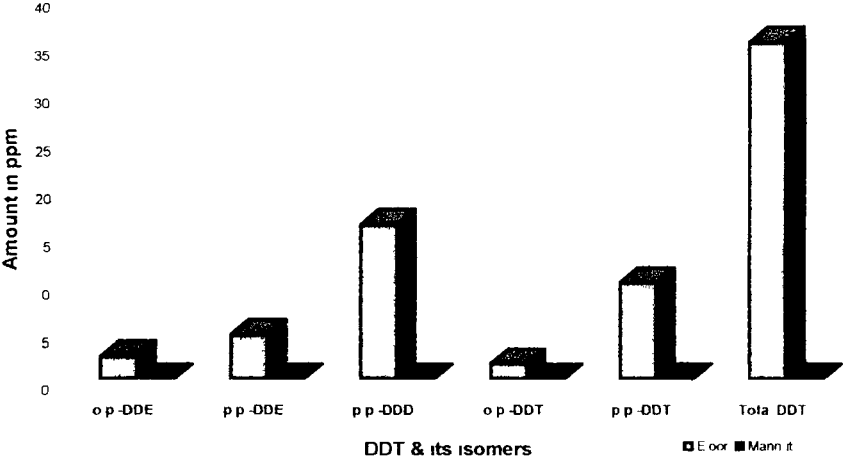


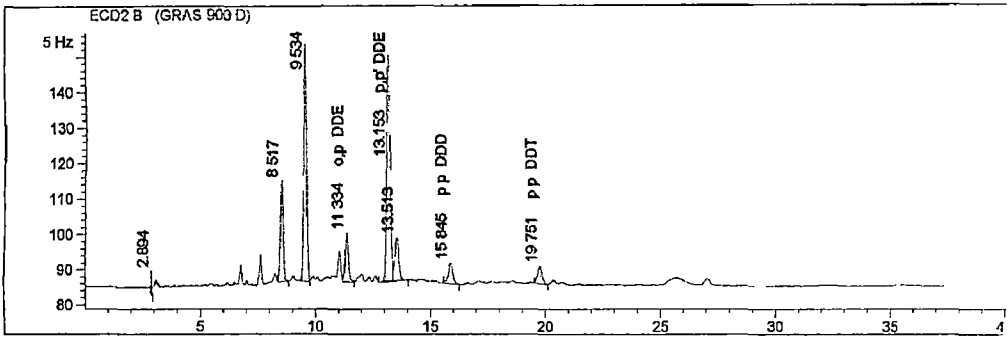
Fig 9 DDT levels in sludge from Eloor & Mannuthy



COLLEGE OF VETERINARY AND ANIMAL SCIENCES

Data File Name	C \HPCHEM\1\DATA\GRAS 900 D
Method Name	C \HPCHEM\1\METHODS\BMG M
Operator Name	bmg
Sample Name	GRASS-90R
Injection Date	11/17/01 12 25 50 PM

Fig 7



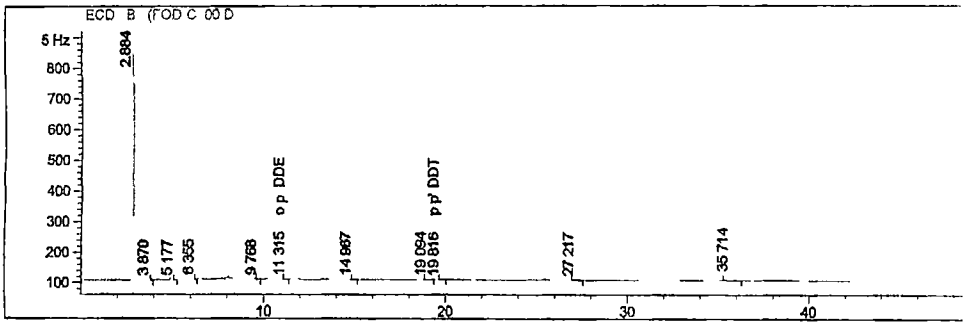
Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2 894	13 593	0 722	0 000	
2	8 517	218 998	11 630	0 000	
3	9 534	548 170	29 110	0 000	
4	11 334	132 068	7 013	412 976	o p DDE
5	13 153	686 991	36 482	694 247	p p DDE
6	13 513	139 337	7 399	0 000	
7	15 845	76 971	4 087	115 422	p p DDD
8	0 000	0 000	0 000	0 000	o p DDT
9	19 751	66 963	3 556	84 437	p p DDT

Instrument Name HPGC-6890

Operator's Signature

Data File Name C \HPCHEM\1\DATA\FOD-C300 D
Method Name C \HPCHEM\1\METHODS\BMG M
Operator Name bmg
Sample Name Fod-C3
Injection Date 9/7/02 1 52 38 PM

Fig.8



Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2 884	1 733e3	77 715	0 000	
2	3 870	5 551	0 249	0 000	
3	5 177	14 497	0 650	0 000	
4	6 355	27 785	1 246	0 000	
5	9 768	4 027	0 181	0 000	
6	11 315	15 901	0 713	46 796	o p DDE
7	0 000	0 000	0 000	0 000	p p DDE
8	14 967	6 969	0 312	0 000	
9	0 000	0 000	0 000	0 000	p p DDD
10	0 000	0 000	0 000	0 000	o p DDT
11	19 094	15 336	0 688	0 000	
12	19 816	5 898	0 264	3 573	p p DDT
13	27 217	18 595	0 834	0 000	
14	35 714	382 492	17 149	0 000	

Instrument Name HPGC-6890

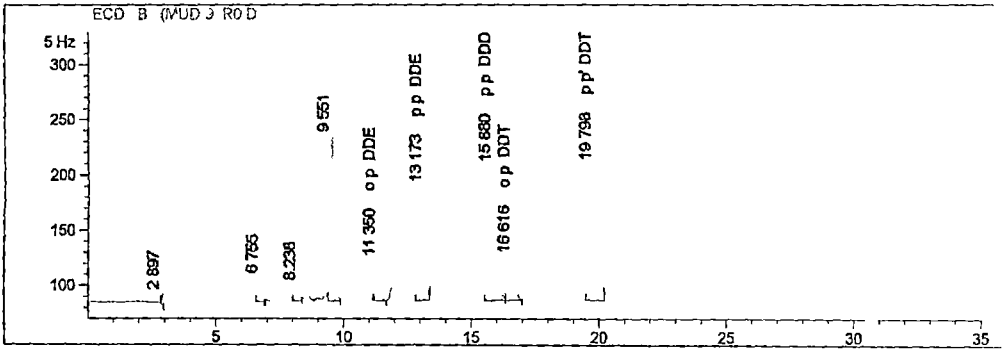
Operator's Signature

Table 2 DDT level in sludge from Eloor area and University Livestock Farm Mannuthy

	Sl No	o p DDE (ppm)	p p DDE (ppm)	p p DDD (ppm)	o p DDT (ppm)	p p DDT (ppm)	Total (ppm)
Samples from Eloor	1	2 838	4 252	1 474	0 129	0 673	9 366
	2	0 078	0	0	0	0 019	0 097
	3	0 019	0	0	0	0 007	0 026
	4	0 023	0 027	0 107	0	0 066	0 223
	5	11 76	11 880	43 280	3 870	18 670	89 460
	6	4 600	24 600	99 700	8 700	53 00	190 600
	7	0	0 016	0 101	0	0 035	0 152
	8	0	0 520	0 820	0 580	3 340	5 260
	9	5 460	5 615	16 240	3 220	25 470	56 005
	10	0 016	0 018	0 268	0 011	0 067	0 380
	Mean ± SE	2 479 ± 1 229	4 693 ± 2 530	16 199 ± 10 250	1 651 ± 0 908	10 135 ± 5 569	35 157 ± 19 783
Samples from ULF	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0 008	0 008
	4	0	0 005	0 011	0	0 010	0 026
	5	0	0 006	0 014	0	0 012	0 032
	6	0	0	0	0	0	0
	Mean ± SE	0	0 002 ± 0 001	0 004 ± 0 003	0	0 005 ± 0 002	0 011 ± 0 006

Data File Name C \HPCHEM\1\DATA\MUD 92R0 D
Method Name C \HPCHEM\1\METHODS\BMG M
Operator Name bmg
Sample Name MUD 92R
Injection Date 11/17/01 11 07 10 AM

19 10



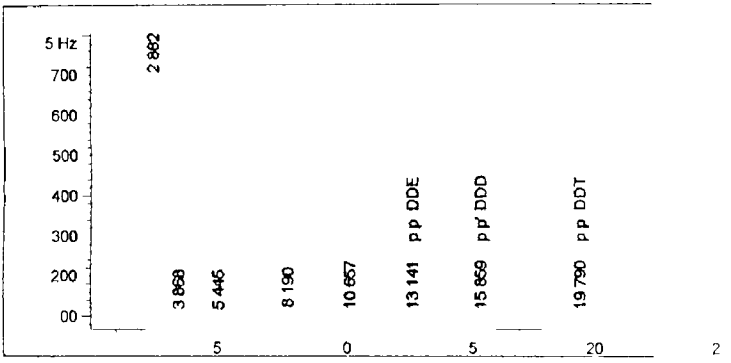
Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2 897	24 157	0 255	0 000	
2	6 755	147 567	1 558	0 000	
3	8 238	105 242	1 111	0 000	
4	9 551	1 248e3	13 181	0 000	
5	11 350	347 079	3 664	1090 732	o p DDE
6	13 173	1 110e3	11 722	1124 726	p p DDE
7	15 880	2 080e3	21 959	3245 644	p p DDD
8	16 616	558 851	5 900	643 385	o p DDT
9	19 798	3 850e3	40 649	5094 049	p p DDT

Instrument Name HPGC-6890

Operator's Signature

Data F le Name C \HPCHEM\1\DATA\MUD C500 D
Method Name C \HPCHEM\1\METHODS\BMG M
Operator Name bmg
Sample Name Mud C5
Inject on Date 9/9/02 12 16 51 PM

Fig 11



Peak #	Ret Tme (m n)	Area	Area %	Amount	Compound Name
1	2 882	1 831e3	85 645	0 000	
2	3 868	6 489	0 303	0 000	
3	5 445	6 972	0 326	0 000	
4	8 190	37 050	1 733	0 000	
5	10 657	6 350	0 297	0 000	
6	0 000	0 000	0 000	0 000	o p DDE
7	13 141	5 686	0 266	1 339	p p DDE
8	15 859	6 793	0 318	5 742	p p DDD
9	0 000	0 000	0 000	0 000	o p DDT
10	19 790	10 939	0 512	10 249	p p DDT
11	35 626	226 698	10 601	0 000	

Instrument Name HPGC 6890

Operator's S gnature

4 3 3 Water

Mean content of o p DDE p p DDT p p DDD p DD1 p p DI and total DDT n ater sa np es f om Eloo a 041 0 0004 0 0004 0 00062 ± 0 000 0 00 8 0 00 ɔɔ 0 00 16 ppn p c e a pa l 0 000014 0 00007 0 0 0 00006 0 00006 0 00008 0 0 pp L e s t L vestock Fa m Mannuth Sta l lo d ha was no s g f ca t d ffere ce be e n le Sa p n L L es ock Farm Mann thv d d o ha e p p DDD a d p DD Chromatogram of vater sample collec ed fro l d a a d L L estock Farm Mannu h are presented n F g a d F g 4 Ile sho vn n Table ɔ and F g 12

4 4 DDT residues in biological samples

4 4 1 Serum

DD1 level (pp) n the serum of cattle l l o a d Ln e L estock Farm Mannu l are sl o n n Table 4 d l 15 C h a serum samples collected fro n the s d area and L t l ck F n Mannuthv are sho vn n F g 16 and F g 7 Ihe m a p DDL p p DI l p p DDD o p DD1 p p DD1 and o al DD1 d l n tl e ca le f om study area were 0 140 ± 0 078 0 087 ± 0 060 0 00? 0 00 0 0 01 0 0 1 ± 0 011 a d 0 268 0 160 esp l l l e a u co para el h gher tl a he samp es colle ed ro n L n l o

I 1le 3 DD I level in water from Eloor are and Un rs I ve t ck far M

	Sl No	o p DDE (ppm)	p p DDE (ppm)	p p DDD (pp n)	o p DD I (ppm)	p p DD I pp)	pl
Sa pl fr 11 or	1	0 00039	0		0		
	?	0 00034	0 0001ɔ	0 00064	(00014	0 00	
	3	0 000046	0	0	0 000012	0 0001	7
	4	0 00009	0	0	0	0	9
	5	0 00010	0		0	0	
	6	0 00005	0 00008	0 000016	0	0 000	
	7	0 00037	0 00009	0 00031	0 00009	0 000 6	
	8	0 00007	0	0	0	0 00017	
	9	0 00265	0 00380	0 00530	0 00195	0 008 0	
	10	0	0 00010	0 00046	0 0000)	0 00019	٨4
	Mean ± SE	0 00041 ± 0 0003	0 00047 ± 0 0004	0 0006 ± 0 0005	0 00 0 000	0 0008) 0 0008	
S p fo 1 11	1	0	0 00002	0	0	0	
	2	0 00005	0 00003	0	0	0	8
	3	0 00004	0 0000ɔ	0	0	0 000	
	4	0	0		0	0	
	ɔ	0	0		0	0	
	6	0	0	0	0	0	
	Mean ± SE	0 00002 ± 0 00009	0 000014 ± 0 00007	0	0	0 00006 0 00006	٨

Fig 12 DDT levels in water from Eloor & Mannuthy

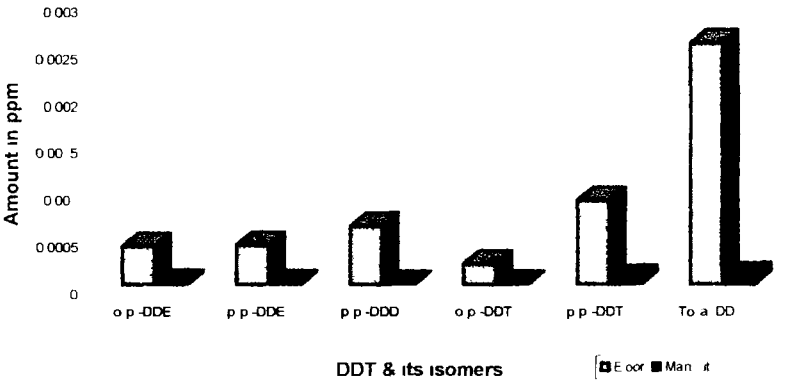
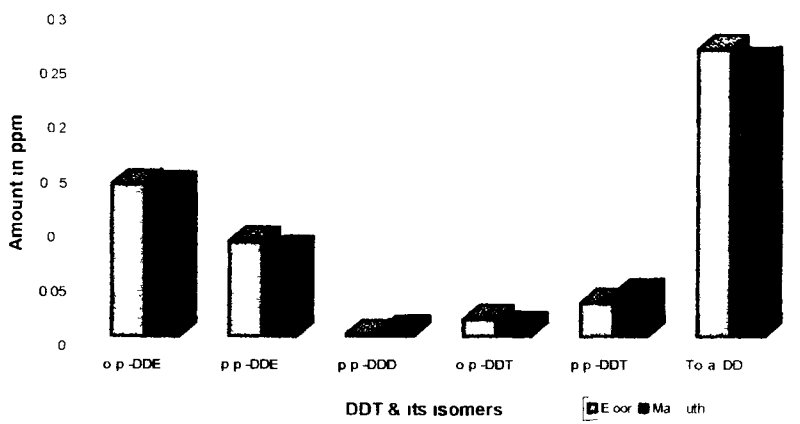
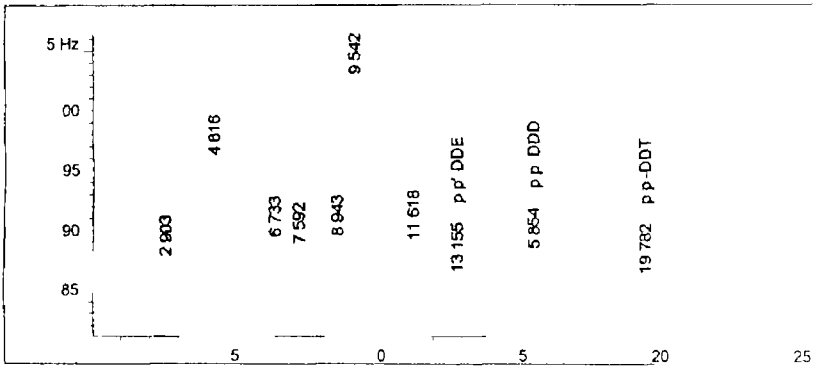


Fig 15 DDT levels in serum from cattle of Eloor & Mannuthy



COLLEGE OF VETERINARY AND ANIMAL SCIENCES

Data F le Name	C \HPCHEM\1\DATA\WAT93R00 D
Method Name	C \HPCHEM 1\METHODS BMG M
Operator Name	bmg
Sample Name	WATER 93R
Inject on Date	11 20/01 12 32 01 PM



Peak #	Ret Tme (m n)	Area	Area %	Amount	Compound Name
1	2 903	9 107	1 965	0 000	
2	4 616	51 537	11 120	0 000	
3	6 733	25 066	5 409	0 000	
4	7 592	33 623	7 255	0 000	
5	8 943	32 961	7 112	0 000	
6	9 542	150 925	32 565	0 000	
7	0 000	0 000	0 000	0 000	o p DDE
8	11 618	37 786	8 153	0 000	
9	13 155	14 116	3 046	9 913	p p DDE
10	15 854	32 367	6 984	45 711	p p DDD
11	0 000	0 000	0 000	0 000	o p DDT
12	19 782	17 773	3 835	19 298	p p DDT
13	27 088	58 196	12 557	0 000	

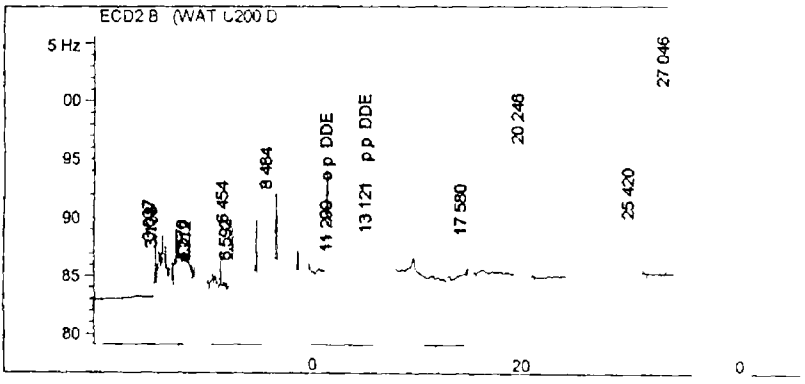
Instrument Name HPGC 6890

Operator's S gnature

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

Data F le Name C \HPCHEM\1\DATA\WAT C200 D
Method Name C \HPCHEM\1\METHODS BMG M
Operator Name bmg
Sample Name WATER C2
Inject on Date 12/5/01 9 11 16 AM

Fig 14



Peak #	Ret T me (m n)	Area	Area %	Amount	Compound Name
1	3 037	25 296	3 710	0 000	
2	3 138	15 577	2 284	0 000	
3	4 570	13 121	1 924	0 000	
4	4 712	12 492	1 832	0 000	
5	6 454	20 729	3 040	0 000	
6	6 592	7 286	1 069	0 000	
7	8 484	40 750	5 976	0 000	
8	11 299	7 722	1 133	21 015	o p DDE
9	13 121	20 783	3 048	16 693	p p DDE
10	0 000	0 000	0 000	0 000	p p DDD
11	0 000	0 000	0 000	0 000	o p DDT
12	17 580	28 112	4 123	0 000	
13	0 000	0 000	0 000	0 000	p p DDT
14	20 246	123 274	18 079	0 000	
15	25 420	58 220	8 538	0 000	
16	27 046	308 498	45 244	0 000	

Instrument Name HPGC 6890

Operator's S gnature

Table 4 DDT level in serum from cattle of Eloor area and University Livestock Farm Mannuthy

	Sl No	o p DDE (ppm)	p p DDE (ppm)	p p DDD (ppm)	o p DDT (ppm)	p p DDT (ppm)	Total (ppm)
Samples from Eloor	1	0 085	0 017	0	0	0	0 102
	2	0	0	0	0	0 010	0 010
	3	0	0 009	0	0	0	0 009
	4	0	0 003	0	0	0	0 003
	5	0 025	0 013	0	0	0	0 037
	6	0 067	0	0	0	0	0 067
	7	1 133	0 853	0 013	0 160	0 145	2 303
	8	0	0	0	0	0 013	0 013
	9	0 012	0	0	0	0 054	0 066
	10	0 512	0 398	0 011	0 076	0 075	1 072
	11	0 115	0 005	0	0	0 061	0 088
	12	0	0	0	0	0 005	0 005
	13	0 057	0	0	0	0 083	0 139
	14	0 092	0	0	0	0 018	0 110
	15	0	0	0	0	0 003	0 003
	Mean ± SE	0 140 ± 0 078	0 087 ± 0 060	0 002 ± 0 001	0 016 ± 0 011	0 031 ± 0 011	0 268 ± 0 160
Samples from ULF	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0 282	0	0 032	0 001	0 302	0 616
	4	0	0	0	0	0	0
	5	0 473	0 334	0 011	0 036	0 040	0 892
	6	0	0 003	0	0	0	0 003
	7	0	0	0	0	0	0
	8	0 628	0 450	0 015	0 057	0 057	1 005
	9	0	0	0	0	0	0
	10	0	0	0	0	0	0
	Mean ± SE	0 138 ± 0 075	0 079 ± 0 053	0 006 ± 0 003	0 009 ± 0 006	0 040 ± 0 030	0 252 ± 0 131

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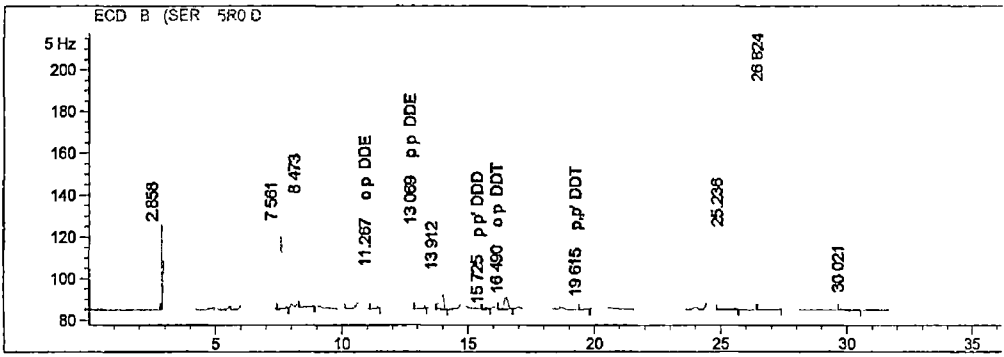
Data File Name C \HPCHEM\1\DATA\SER135R0 D
Method Name C \HPCHEM\1\METHODS\BMG M
Operator Name bmg
Sample Name Ser 135R
Injection Date 11/7/01 2 14 40 PM

Fig 16



Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2 858	80 862	1 724	0 000	
2	7 561	279 396	5 958	0 000	
3	8 473	463 644	9 886	0 000	
4	11 267	144 713	3 086	452 837	o p DDE
5	13 069	340 438	7 259	341 792	p p DDE
6	13 912	151 864	3 238	0 000	
7	15 725	6 256	0 133	4 903	p p DDD
8	16 490	59 146	1 261	64 282	o p DDT
9	19 615	46 653	0 995	57 542	p p DDT
10	25 236	643 743	13 727	0 000	
11	26 824	2 359e3	50 293	0 000	
12	30 021	114 422	2 440	0 000	

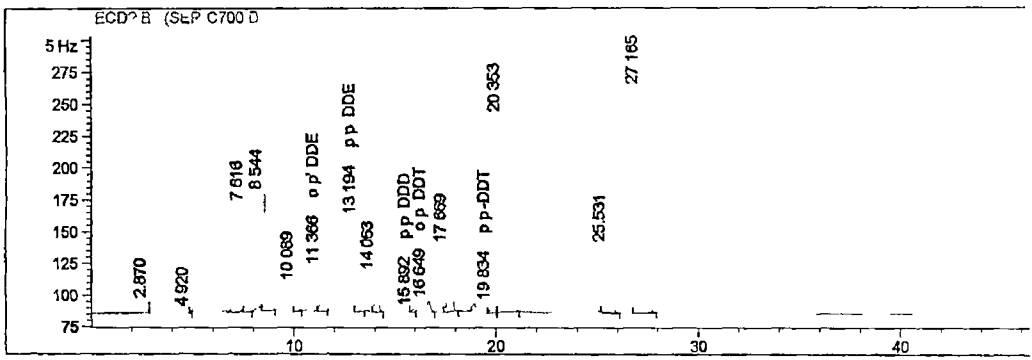
Instrument Name HPGC 6890

Operator's Signature

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

Data File Name C \HPCHEM\1\DATA\SER C700 D
Method Name C \HPCHEM\1\METHODS\BMG M
Operator Name bmg
Sample Name SERUM C862
Injection Date 11/28/01 12 23 04 PM

Fig.17



Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2 870	16 051	0 133	0 000	
2	4 920	7 697	0 064	0 000	
3	7 616	647 491	5 346	0 000	
4	8 544	986 843	8 147	0 000	
5	10 089	169 270	1 397	0 000	
6	11 366	325 607	2 688	1023 047	o p DDE
7	13 194	745 772	6 157	754 029	p p DDE
8	14 063	315 689	2 606	0 000	
9	15 892	20 011	0 165	26 399	p p DDD
10	16 649	95 553	0 789	106 474	o p DDT
11	17 669	709 002	5 853	0 000	
12	19 834	84 818	0 700	108 080	p p DDT
13	20 353	2 740e3	22 620	0 000	
14	25 531	982 232	8 109	0 000	
15	27 165	4 267e3	35 225	0 000	

Instrument Name HPGC 6890

Operator's Signature

Farm Mannuthy (0 138 ± 0 075 0 079 ± 0 053 0 006 ± 0 003 0 009 ± 0 006 0 040 ± 0 030 and 0 252 ± 0 131 ppm) Statistical analysis showed that there was no significant difference in DDT levels between the samples from Eloor and Mannuthy

4 4 2 Urine

Mean values of o p DDE p p DDE p p DDD o p DDT p p DDT and total DDT in ppm detected in the urine samples of cattle from Eloor area were 0 029 ± 0 006 0 004 ± 0 001 0 003 ± 0 0002 0 0 001 ± 0 0008 and 0 034 ± 0 006 respectively o p DDT was not detected in any of the samples from Eloor Compared to Mannuthy samples (0 015 ± 0 012 0 012 ± 0 008 0 001 ± 0 001 0 001 ± 0 001 0 002 ± 0 001 and 0 030 ± 0 022 ppm) the level of DDT and its isomers in samples from study area were h gh Statistically no significant difference were found between the samples of two areas Results are presented in Table 5 and Fig 18 The chromatogram of a urine sample each collected from the study area and University Livestock Farm Mannuthy are shown in Fig 19 and Fig 20

4 4 3 Dung

DDT content (ppm) in dung samples are shown in Table 6 and Fig 21 The mean o p DDE p p DDE p p DDD o p DDT p p DDT and total DDT in dung samples from study area were 0 116 ± 0 052 0 108 ± 0 056 0 241 ± 0 197 0 038 ± 0 035 0 407 ± 0 353 and 0 910 ± 0 689 respectively as compared to 0 017 ± 0 017 0 003 ± 0 003 0 003 ± 0 003 0 0 004 ± 0 003 and

DDT l ur ca l l l k l
M l

Sl No	o DDF ppm)	p p DDE (ppm	p p DDD pp n	p DD pl n	p p DDT p
1	0 018	0			0
	41	0			
	4				
4	0 069	0 009			0
5	0	0 004	0		0 0
6	0 070	0 004	0 001	0	0 0 1
7	0 018	0 003	0 002		0 0 9
8	04	0	0		
9	0 009	0 01			
0	0 04	0 004			
M an	0 0 9	0 004 ±	0 00		
SE	0 00	0 001	0 0		
1	0	0			
	0 1 6	0 083	0 004	07	0 8
3	0 01 4	0 011	0 000	0 00	0 01
4	0	0	0		0
5	0	0	0		
6	0 01	0 0 0	04		
7		0			
8	0	0			
9		0 0 4			
1	9	0		0	
M n	0 01 ±	0 01 ±	0 0 1	0 0 1	
SE	0 01	0 008	00	0	

Fig 18 DDT levels in urine of cattle from Eloor & Mannuthy

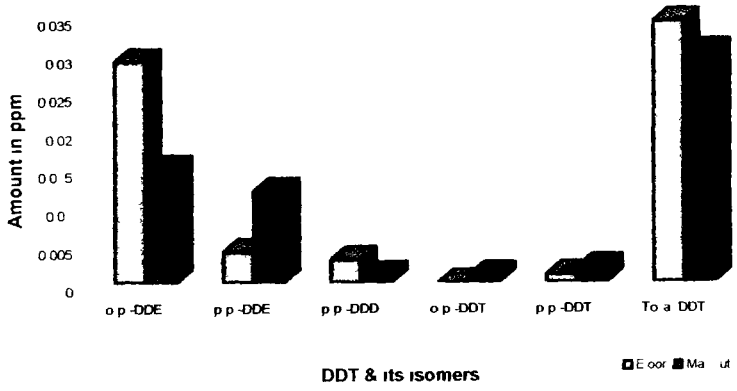
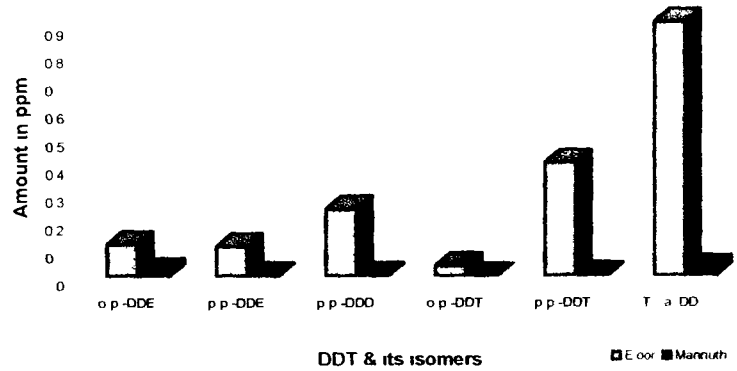
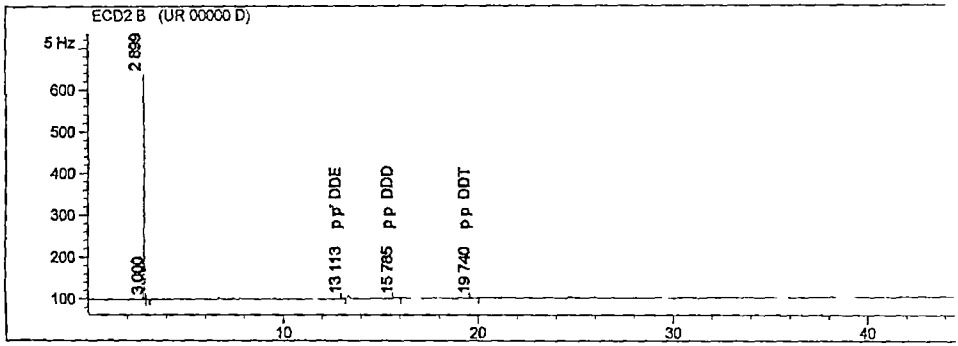


Fig 21 DDT levels in dung from cattle of Eloor & Mannuthy



Data File Name C \HPCHEM\1\DATA\URI00000 D
Method Name C \HPCHEM\1\METHODS\BMG M
Operator Name bmg
Sample Name Urine-005
Injection Date 7/27/02 10 22 07 AM

Fig.19



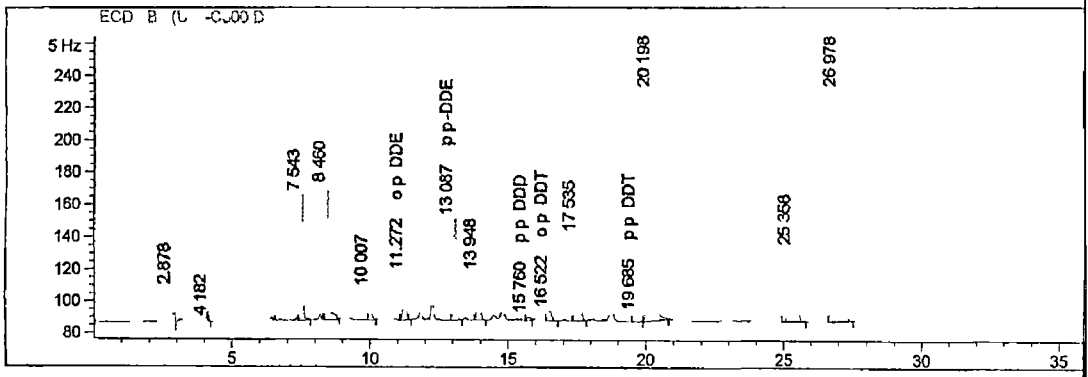
Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2 899	1 442e3	96 303	0 000	
2	3 000	10 205	0 681	0 000	
3	0 000	0 000	0 000	0 000	o p DDE
4	13 113	25 053	1 673	21 036	p p DDE
5	15 785	8 457	0 565	8 342	p p DDD
6	0 000	0 000	0 000	0 000	o p DDT
7	19 740	11 651	0 778	11 191	p p DDT

Instrument Name HPGC 6890

Operator's Signature

Data File Name	C \HPCHEM\1\DATA\URI C300 D
Method Name	C \HPCHEM\1\METHODS\BMG M
Operator Name	bmg
Sample Name	URINE C1143
Inject on Date	12/3/01 9 09 20 AM

Fig.20



Peak #	Ret Time (mn)	Area	Area %	Amount	Compound Name
1	2 878	78 677	0 936	0 000	
2	4 182	1 621	0 019	0 000	
3	7 543	357 938	4 258	0 000	
4	8 460	498 558	5 931	0 000	
5	10 007	110 215	1 311	0 000	
6	11 272	200 522	2 386	628 757	o p DDE
7	13 087	452 814	5 387	456 082	p p DDE
8	13 948	229 663	2 732	0 000	
9	15 760	17 134	0 204	21 903	p p DDD
10	16 522	51 132	0 608	54 994	o p DDT
11	17 535	564 796	6 719	0 000	
12	19 685	49 724	0 592	61 608	p p DDT
13	20 198	2 197e3	26 143	0 000	
14	25 358	695 098	8 270	0 000	
15	26 978	2 900e3	34 503	0 000	

Instrument Name HPGC 6890

Operator's Signature

0 026 ± 0 017 in the University Livestock Farm Mannuthy samples o p DDT was not detected in any of the samples from University Livestock Farm Mannuthy The samples from study area had comparatively higher values but no statistical significance was observed Chromatogram of a dung sample from Eloor area and Mannuthy area each are shown in Fig 22 and Fig 23

4 4 4 Milk

The mean o p DDE p p DDE p p DDD o p DDT p p DDT and total DDT in milk samples of cattle from Eloor area were 0 0072 ± 0 0072 0 037 ± 0 011 0 008 ± 0 005 0 0 010 ± 0 004 and 0 058 ± 0 019 (ppm) respectively o p DDT was absent in all the milk samples None of the samples from University Livestock Farm Mannuthy contained DDT or its isomers Hence significant difference between the two groups could not be determined Chromatogram of a milk sample from study area and Mannuthy area each are shown in Fig 25 and Fig 26 Results are presented in the Table 7 and Fig 24

4 5 Haemogram

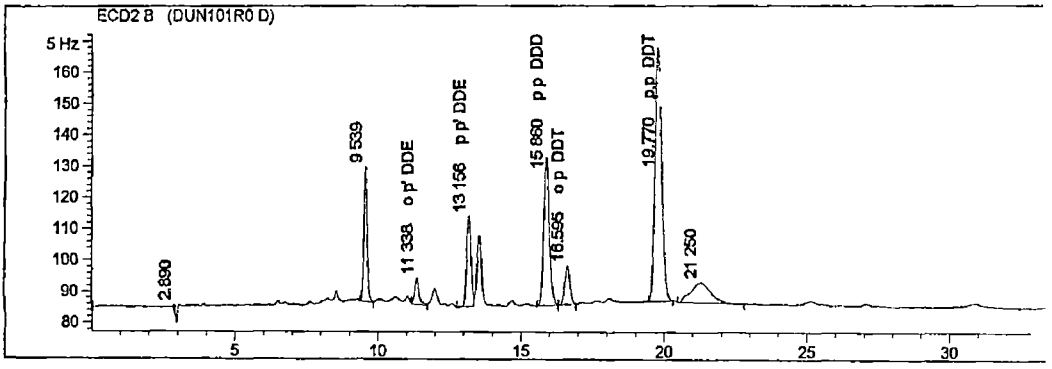
Total leucocyte count total erythrocyte count haemoglobin packed cell volume mean corpuscular volume mean corpuscular haemoglobin mean corpuscular haemoglobin concentration and differential leucocyte count were estimated and the observations are presented in Table 8

Table 6 DDT level in dung from cattle of Eloor area and University Livestock Farm Mannuthy

	Sl No	o p DDE (ppm)	p p DDE (ppm)	p p DDD (ppm)	o p DDT (ppm)	p p DDT (ppm)	Total (ppm)
Samples from Eloor	1	0 050	0 035	0 009	0	0 012	0 106
	2	0 109	0 145	0 016	0	0 009	0 279
	3	0 215	0 111	0 223	0 034	0 378	0 961
	4	0 025	0 023	0 007	0	0 009	0 064
	5	0 042	0 055	0 021	0	0 011	0 129
	6	0 026	0 047	0 017	0	0 009	0 099
	7	0 544	0 600	1 998	0 348	3 570	7 060
	8	0 032	0 049	0 120	0	0 061	0 262
	9	0	0 012	0	0	0 009	0 021
	10	0 114	0	0	0	0	0 114
	Mean ± SE	0 116 ± 0 052	0 108 ± 0 056	0 241 ± 0 197	0 038 ± 0 035	0 407 ± 0 353	0 910 ± 0 689
Samples from ULF	1	0 100	0	0	0	0	0 100
	2	0	0	0	0	0	0
	3	0	0 016	0 016	0	0 015	0 047
	4	0	0	0	0	0 007	0 007
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	Mean ± SE	0 017 ± 0 017	0 003 ± 0 003	0 003 ± 0 003	0	0 004 ± 0 003	0 026 ± 0 017

Data File Name C \HPCHEM\1\DATA\DUN101R0 D
Method Name C \HPCHEM\1\METHODS\BMG M
Operator Name bmg
Sample Name DUNG-101R
Injection Date 11/17/01 11 48 44 AM

Fig.22



Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2.890	6.180	0.194	0.000	
2	9.539	339.076	10.638	0.000	
3	11.338	87.285	2.738	271.811	o p DDE
4	13.156	299.618	9.400	300.276	p p DDE
5	15.860	641.582	20.129	997.839	p p DDD
6	16.595	154.143	4.836	174.373	o p DDT
7	19.770	1.351e3	42.388	1784.852	p p DDT
8	21.250	308.399	9.676	0.000	

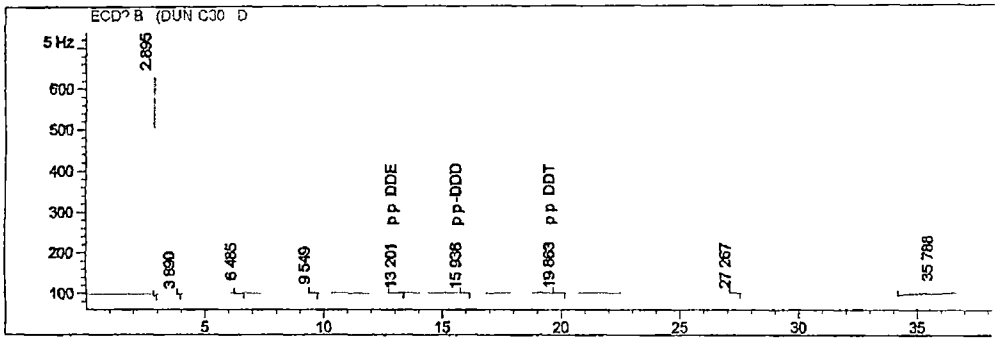
Instrument Name HPGC-6890

Operator's Signature

COLLEGE OF VETERINARY AND ANIMAL SCIENCES

Data File Name	C \HPCHEM\1\DATA\DUN C300 D
Method Name	C \HPCHEM\1\METHODS\BMG M
Operator Name	bmg
Sample Name	Dung C3 1205
Injection Date	9/7/02 10 46 22 AM

Fig.23



Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2 895	1 480e3	61 295	0 000	
2	3 890	4 400	0 182	0 000	
3	6 485	105 764	4 382	0 000	
4	9 549	66 167	2 741	0 000	
5	0 000	0 000	0 000	0 000	o p DDE
6	13 201	16 461	0 682	12 297	p p DDE
7	15 936	7 610	0 315	7 018	p p DDD
8	0 000	0 000	0 000	0 000	o p DDT
9	19 863	12 914	0 535	12 863	p p DDT
10	27 267	10 229	0 424	0 000	
11	35 788	710 710	29 444	0 000	

Instrument Name HPGC 6890

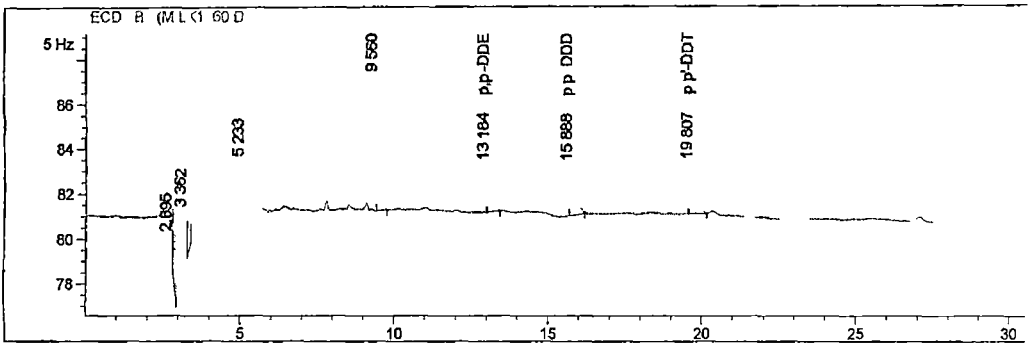
Operator's Signature

Table 7 DDT level in milk from cattle of Eloor area and University Livestock Farm Mannuthy

	Sl No	o p DDE (ppm)	p p DDE (ppm)	p p DDD (ppm)	o p DDT (ppm)	p p DDT (ppm)	Total (ppm)
Samples from Eloor	1	0	0 002	0	0	0	0 002
	2	0	0	0	0	0	0
	3	0 143	0 060	0	0	0 017	0 219
	4	0	0 036	0	0	0 029	0 065
	5	0	0 212	0 042	0	0 074	0 328
	6	0	0 033	0 003	0	0 008	0 045
	7	0	0 103	0 105	0	0 006	0 124
	8	0	0 038	0	0	0	0 038
	9	0	0 025	0	0	0 008	0 033
	10	0	0	0	0	0	0
	11	0	0 012	0	0	0 002	0 013
	12	0	0 029	0	0	0 006	0 035
	13	0	0 0004	0	0	0	0 0004
	14	0	0 016	0	0	0 009	0 024
	15	0	0 0004	0	0	0	0 0004
	16	0	0 070	0 001	0	0 018	0 089
	17	0	0 087	0	0	0 011	0 098
	18	0	0 005	0	0	0	0 005
	19	0	0 018	0	0	0 003	0 021
	20	0	0	0	0	0 003	0 003
	Mean ± SE	0 0072 ± 0 0072	0 037 ± 0 011	0 008 ± 0 005	0	0 010 ± 0 004	0 058 ± 0 019
Samples from ULF	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	Mean ± SE	0	0	0	0	0	0

Data File Name C \HPCHEM\1\DATA\MILK1160 D
Method Name C \HPCHEM\1\METHODS\BMG M
Operator Name bmg
Sample Name MILK 116
Injection Date 11/19/01 10 58 05 AM

Fig.25



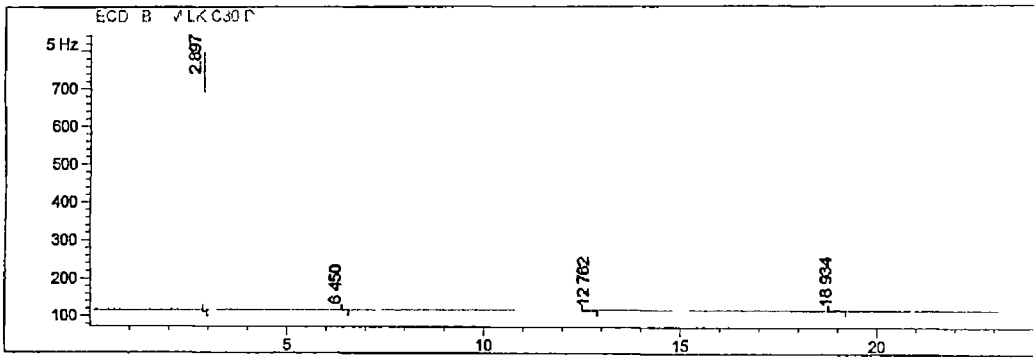
Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2 895	9 362	5 121	0 000	
2	3 362	11 489	6 284	0 000	
3	5 233	9 984	5 461	0 000	
4	9 560	49 193	26 906	0 000	
5	0 000	0 000	0 000	0 000	o p DDE
6	13 184	37 220	20 357	33 410	p p DDE
7	15 888	26 960	14 746	37 261	p p DDD
8	0 000	0 000	0 000	0 000	o p DDT
9	19 807	38 622	21 125	46 908	p p DDT

Instrument Name HPGC 6890

Operator's S gnature

Data File Name	C \HPCHEM\1\DATA\MILK C30 D
Method Name	C \HPCHEM\1\METHODS\BMG M
Operator Name	bmg
Sample Name	MILK C3
Injection Date	7/19/02 10 30 08 AM

Fig.26



Peak #	Ret Time (min)	Area	Area %	Amount	Compound Name
1	2 897	1 554e3	98 333	0 000	
2	6 450	5 237	0 331	0 000	
3	0 000	0 000	0 000	0 000	o p DDE
4	12 762	10 588	0 670	0 000	
5	0 000	0 000	0 000	0 000	p p DDE
6	0 000	0 000	0 000	0 000	p p DDD
7	0 000	0 000	0 000	0 000	o p DDT
8	18 934	10 520	0 666	0 000	
9	0 000	0 000	0 000	0 000	p p DDT

Instrument Name HPGC-6890

Operator's Signature

Table 8 Haematological parameters of cattle in Eloor area and University Livestock Farm Mannuthy (Mean ± SE)

Sl No	Parameters	Eloor	Mannuthy
1	RBC (millions/mm^3)	6 095 ± 0 481	5 95 ± 0 705
2	WBC (numbers/mm^3)	7592 ± 806 77	8810 ± 1341 43
3	PCV (%)	35 92 ± 2 014	33 9 ± 1 027
4	Hb (g%)	10 27 ± 0 475	9 25 ± 0 762
5	Differential leucocyte count (%)		
	Lymphocytes *	63 15 ± 0 798	66 7 ± 1 850
	Neutrophils *	22 5 ± 1 019	29 5 ± 1 327
	Eosinophils *	10 75 ± 0 817	1 9 ± 0 379
	Monocytes	3 55 ± 0 500	2 2 ± 0 554
	Basophils	0 00	0 00
6	MCV (μm^3)	35 92 ± 2 014	33 9 ± 8 451
7	MCH (pg)	19 549 ± 2 342	17 209 ± 1 928
8	MCH (g%)	28 753 ± 1 190	27 39 ± 2 181

* bearing row differ significantly (P<0 05)

Table 9 Serum biochemistry of cattle in Eloor area and University Livestock Farm Mannuthy (Mean ± SE)

Sl No	Parameters	Eloor	Mannuthy
1	Total protein (g/dl)	7 94 ± 0 294	7 97 ± 1 132
2	Albumin (g/dl)	3 36 ± 0 194	3 79 ± 0 37
3	Globulin (g/dl)	4 58 ± 0 397	4 18 ± 0 974
4	ALT* (u/l)	23 3 ± 1 021	17 6 ± 1 056
5	AST* (u/l)	49 3 ± 4 97	74 5 ± 3 34

* bearing column differ significantly (P<0 05)

4 5 1 Red blood cell (RBC) count

Mean RBC count (millions/mm^3) of the samples from University Livestock Farm Mannuthy and samples from Eloor were 5 950 ± 0 705 and 6 095 ± 0 481 respectively There was no significant difference observed between samples from both areas

4 5 2 White blood cell (WBC) count

The mean WBC count (thousands/mm^3) of the cattle from study area was 7592 ± 806 77 and that of the University Livestock Farm Mannuthy was 8810 ± 1341 43 Statistical analysis showed that there was no significant difference between the two groups

4 5 3 Volume of packed red cells (VPRC)

Packed cell volume (%) of blood samples from Eloor and Mannuthy area were 35 92 ± 2 014 and 33 9 ± 1 027 respectively No significant statistical difference was observed between the two groups

4 5 4 Haemoglobin (Hb) concentration

Average haemoglobin concentration (g%) of samples from University Livestock Farm Mannuthy and study areas were 9 25 ± 0 762 and 10 27 ± 0 475 respectively Though the mean haemoglobin value of samples from study area was comparatively higher than that from University Livestock Farm samples no significant difference was observed

4 5 5 Differential leucocyte count (DLC)

The mean neutrophil percentage in blood samples from Eloor area (22 5 ± 1 019 per cent) was significantly (P<0 05) lower than samples of University Livestock Farm Mannuthy (29 5 ± 1 327 per cent) The result is shown in Table 8 and Fig 27

The mean lymphocyte percentage in blood samples from Eloor area (63 10 ± 0 798 per cent) was significantly (P<0 05) lower than the samples of University Livestock Farm Mannuthy (66 7 ± 1 850 per cent) Figure 27 shows that samples from study area is comparatively lower than that of the other group

Mean eosinophil percentage of blood samples from study area and Mannuthy area were 10 75 ± 0 817 per cent and 1 9 ± 0 379 per cent respectively There was significant (P<0 05) difference (increase) between the two groups as shown in Table 8 and Fig 27

The mean monocyte count in blood samples of University Livestock Farm Mannuthy and study samples were 2 2 ± 0 554 per cent and 3 55 ± 0 500 per cent respectively No significant difference exists between the two groups

4 5 6 Erythrocyte indices

Mean corpuscular volume (MCV) of cattle from Eloor and Mannuthy areas were 35 92 ± 2 014 and 33 9 ± 8 45 (um^3) respectively There was no significant difference between the two groups (Table 8)

Fig 24 DDT levels in milk from cattle of Eloor & Mannuthy

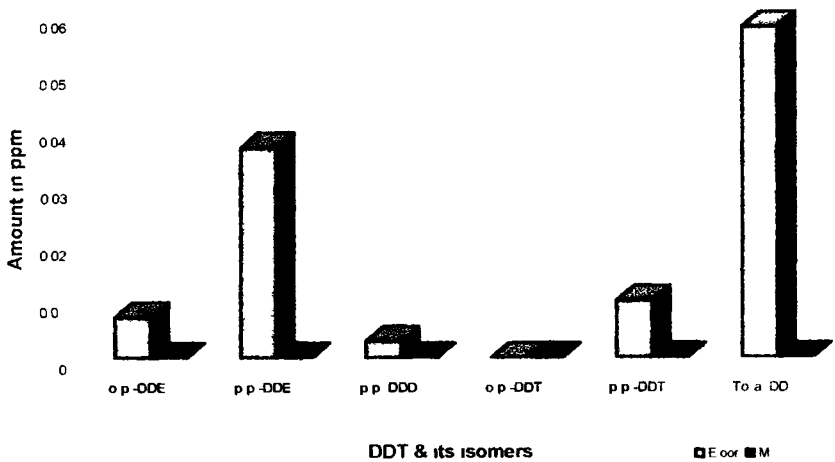
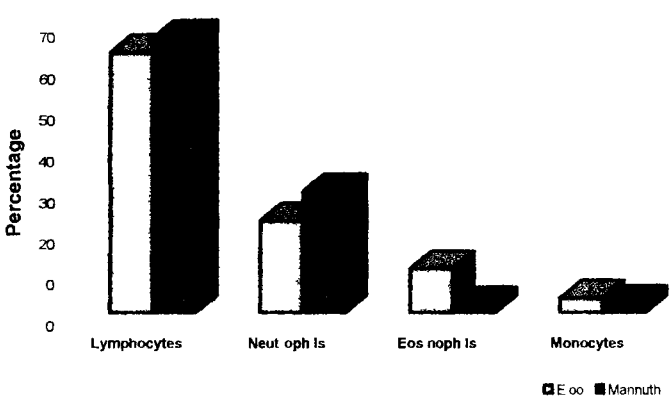


Fig 27 Differential leucocyte count of cattle from Eloor & Mannuthy



The mean corpuscular haemoglobin (MCH) values of cattle from Eloor and Mannuthy samples were 19 549 ± 2 342 and 17 209 ± 1 928 (pg) respectively No significant difference was observed between the values (Table 8)

Mean corpuscular haemoglobin concentration (MCHC) values of cattle from Eloor and Mannuthy were 28 75 ± 1 19 and 27 39 ± 2 18 (g%) respectively Statistical analysis showed that there was no significant difference observed between the two groups (Table 8)

4 6 Serum biochemistry

Total protein albumin globulin alanine aminotransferase and aspartate aminotransferase were estimated and the observations are given in Table 9

4 6 1 Total serum protein

Mean total serum protein (g/dl) in cattle of Eloor and University Livestock Farm Mannuthy were 7 94 ± 0 294 and 7 97 ± 1 132 respectively No significant difference was observed between the two groups

4 6 2 Serum albumin

Mean serum albumin (g/dl) concentration in cattle of Eloor and Mannuthy were 3 36 ± 0 194 and 3 79 ± 0 37 respectively There was no significant difference between the two group of samples

4 6 3 Serum globulin

The mean serum globulin(g/dl) concentration was calculated using the total serum protein and serum albumin values Mean value for study area was 4 58 ± 0 397 as compared to the values of Mannuthy area (4 18 ± 0 974) Statistical analysis showed that there was no significant difference between the two groups

4 6 4 Serum alanine aminotransferase (ALT)

Mean serum alanine aminotransferase level in cattle of study area and Mannuthy area were 23 3 ± 1 021 and 17 6 ± 1 056 (u/l) respectively The samples from study area were significantly (P<0 05) higher than other group

4 6 5 Serum aspartate ammo transferase (AST)

Mean serum aspartate aminotransferase (u/l) level in cattle of Eloor area (49 3 ± 4 97) was significantly (P<0 05) lower than that of University Livestock Farm Mannuthy (74 5 ± 3 34)

Discussion

5 DISCUSSION

In the present study environmental and biological samples from Eloor and Mannuthy were compared with reference to the presence of DDT residues As DDT is highly lipid soluble and non biodegradable organisms at the top of natural food chain like livestock man etc can be adversely affected by their accumulation in the body

DDT levels in environmental and biological samples

DDT levels in environmental samples like fodder sludge water and biological samples like serum urine dung milk collected from the Eloor area were very high compared to the samples from University Livestock Farm Mannuthy This indicates that the DDT eliminated from the industrial units accumulate in the environment which in turn leads to residues in animals of the study area

Environmental samples

Fodder samples

The mean level of total DDT in fodder samples from Eloor was 1 463 ± 0 555 ppm which is significantly higher than 0 062 ± 0 021 ppm obtained from University Livestock Farm Mannuthy DDT contamination of fodder plants from Nainital area with levels varying from 0 16 mg/kg to 0 63 mg/kg with p p DDT as the major residue due to recent contamination was noted by Kaphalia and Seth (1982) Findings from Eloor area indicate that the mean

p p DDT values (0 587 ± 0 349 ppm) is the highest of all isomers of DDT present Pesticide residues may be found in fodder plants from accidental or incidental contamination or spillage or volatilisation of the residues from contaminated soil through wind blown dust and by direct absorption via roots and leaves (Kaphalia and Seth 1982) When DDT was sprayed in orchards residues were detected in significant amounts in soil grass milk of grazing cows and eggs of feeding chicken (Lykov 1975) The high concentration of DDT in fodder samples of study area may be due to contamination of the pastures by effluents from the pesticide manufacturing factories like HIL

As DDT belongs to the persistent organic pollutant (POP) group excretion of residues through milk and accumulation of residues in the adipose tissue may pose public health hazard Accumulation of DDT residues was seen in rats fed on minced beef from bullock carcasses contaminated with DDT (Martin *et al* 1976) According to Willet *et al* (1993) DDT could be detected in milk fat and adipose tissue of cattle through ingestion of contaminated fodder In the present study also ingestion of highly contaminated fodder plants may be the primary source of DDT to the cattle population of Eloor In most of the collected samples from Eloor the detected levels exceeded the maximum permissible limit of 0 5 ppm in fodder plants (WHO 1979) Acute toxicity with central nervous system symptoms were not seen in the cattle reared in such pastures as the oral LD_{50} in cattle is greater than 1000 mg/kg body weight (Hirdina *et al* 1975) which is much higher than the detected levels

Sludge samples

In the present study the highest concentrat on of DDT residues was obtained in sludge samples from Eloor north The mean value of total DDT obtained was 35 157±19 783 ppm which is very h gh compared to the samples from Mannuthy but is not significantly different Wan *et al* (1989) reported that the soil samples from horticultural area of New South Wales yielded 0 24 to 0 66 ppm of DDT Such low levels were seen as residues of pesticides applied on plants were only detected unlike the soil contamination through industrial pollution in Eloor Higher concentration of DDT in Eloor may be due to strong adsorption of DDT onto soil particles (Dogheim *et al* 1996)

Dogheim *et al* (1996) reported high levels of p p DDT in soil compared to other DDT isomers from Kafr El Zayat governorate which has one of the biggest pesticide factories in Egypt A similar hike in p p DDT content was seen in sludge samples from Eloor indicating recent residues of DDT in the area As the use of DDT in India is restricted to malaria control alone by Central Insecticide Board the possible source of DDT residues in Eloor may be the effluents given out from Hindustan Insecticides Limited as they are the only licensed manufacturers of DDT in India

Tissue stores of DDT present in cattle reared in Eloor area may be through ingestion of soil along with fodder laden with DDT residues This is substantiated by Fries and Marrow (1982) who concluded that concentration of DDT in ewes maintained on pesticide contaminated pasture is dependent on the

concentration of pesticide on soil surface Harrison *et al* (1970) also detected DDT residues in sheep by feeding of contaminated topsoil and lambs born to such animals contained twice the DDT levels in their body fat compared to their dams This indicates biomagnification of DDT and its ability to produce hazardous effects on the future generation of livestock in Eloor

Water samples

In the present study water samples from Eloor has higher concentration of total DDT (0 00255 ± 0 00216 ppm) than those from Mannuthy (0 000085 ± 0 00007 ppm) Eventhough a marked difference is observed between the two samples the quantity of DDT in water samples is considerably low compared to other environmental samples Organochlorine compounds are very soluble in fatty tissues but has low solubility in water This lipophilic hydrophobic character was largely responsible for their accumulation and persistence in aquatic biota than in water (Livingston 1977) This may be the reason for low concentration of DDT in water samples from Eloor In a similar study Dogheim *et al* (1996) estimated the DDT residues in water samples around a pesticide factory of Kafr El Zayat Governorate in Egypt to be varying from 0 269 to 0 013 ppb

DDT residues gain entry into the water bodies of Eloor region by direct discharge of effluents to water seepage through soil layers and runoff from the soil during rainy season Residues of several organochlorine insecticides including DDT were detected in the ground water of a rural area at levels

exceeding the WHO guideline for drinking water Seepage of pollutants to groundwater during monsoons were thought to be the source of contamination (Mohapatra *et al* 1995) DDT residues detected in water samples from Eloor exceeded the WHO permissible limit of 5 ppb for drinking water (WHO 1979) Hence consumption of the contaminated water can produce deleterious effects in both livestock and human beings

Biological samples

The results of environmental samples analysed were supported by results of analysis of biological samples like blood serum dung urine and milk from cattle of Eloor area

Blood samples

The serum DDT values from Eloor cattle ranged from 0 003 to 2 303 ppm Storage of DDT residues in blood liver kidney heart and central nervous system of rats was reported by Smith and Stohlman (1944) DDT HCH and lindane were detected in the blood serum of workers involved in the spraying of insecticides (Minelli and Ribeiro 1996) Organochlorine levels measured in blood have good correlation with their concentration in adipose tissue of humans (Mes 1993) Similarly analysis of blood could give an accurate estimate of DDT residues in carcass fat of bullocks (Ware *et al* 1975) Hence serum levels of DDT from cattle of Eloor area can be used as markers for the total body burden of DDT Indian Rare Earths (IRE) and Hindustan

Insecticides ltd (HIL) are the two major industries in Eloor north Out of the two HIL may be the main source of DDT residues in cattle of the area

Urine samples

The urine DDT concentration from Eloor north was 0 034 ± 0 006 ppm which was not significantly different from the Mannuthy samples Faecal excretion is the major route of elimination of DDT from the body and the major metabolite in urine DDA was not quantified in the present study This may be the reason for the low level of DDT in the urine of cattle from the study area This is consistent with the finding of Rothe *et al* (1957) who reported that after giving radioactive DDT to rats by stomach tube less than 0 1% of the activity was found in urine 7 4 to 37 1% was found in faeces or intestinal contents DDA is the major urinary metabolite of DDT in all mammals including man (Spicer *et al* 1947)

Dung samples

DDT content in dung samples from the study area ranged from 0 021 to 7 060 ppm There was no significant difference between the DDT levels of dung from Eloor and Mannuthy areas In rat faecal excretion of DDT exceeded urinary excretion irrespective of the route of administration (Hayes 1965) The concentration of residues in the dung was considerably higher than in the urine from cattle of Eloor area This is consistent with the finding of Reif and Sinsheimer (1975) that when metabolism of oral DDD was studied in rats an average 7 1 per cent and 87 8 per cent of the excretion was seen in urine and

faeces respectively The concentration of DDT in dung samples from Eloor is higher than that from animals maintained at Mannuthy Chronic exposure to DDT residues expelled from HIL located at Eloor north may be the reason for such high levels being excreted in faeces

Milk samples

Milk samples collected from the study area had a mean DDT concentration of 0 058 ± 0 019 ppm No DDT residues could be detected in the milk samples from Mannuthy None of the samples from Eloor had DDT levels higher than the maximum residue limit of 1 25 ppm (WHO 1979) Similar residue levels were detected from cow s milk by Saxena and Siddiqui (1980) Battu *et al* (1989) and Surendranath *et al* (2000) As DDT belongs to persistent organic pollutant (POP) group accumulation of residues to a significant level will be expected in the fatty tissues and fat rich samples like milk Cows fed substantial but nontoxic residues of DDT commonly excrete 10 per cent or slightly more of the total dose in the r milk (Hayes 1959) The DDT values from the study area is not in agreement with Vreman *et al* (1980) that long term oral administration of DDT at low levels results n higher excretion in milk than short term feeding at higher doses

Haemogram

Total erythrocyte count (TEC)

The total erythrocyte count in cattle of Eloor area was not significantly different from the Mannuthy samples The values were in accordance with the

normal erythrocyte count in cattle of 5 to 10 millions/mm^3 (Kaneko *et al* 1997) These findings were not in agreement with the observation of Ali and Shakoori (1994) that in albino rats fed with DDT significant reduction in total erythrocyte count was present However Cranmer *et al* (1972) could not observe any prominent alteration of erythrocyte count in squirrel monkeys even at fatal doses

Total leucocyte count (TLC)

In the present study the total leucocyte count in the cattle of Eloor was less than that of Mannuthy animals but the values lie within the normal range of 4000 to 12 000 numbers/mm^3 (Kaneko *et al* 1997) This was in discordance with the findings of Ali and Shakoori (1994) They reported a significant increases in total leucocytic count in rats fed with different doses (100 mg 20 mg and 10 mg/kg body weight/day) of DDT

Packed cell volume (PCV)

There was no significant difference in the packed cell volume between cattle of Eloor and Mannuthy areas The samples from Eloor exhibited values coming towards the lower limit of the normal range of 24 to 46 per cent (Benjamin 1985) This is in agreement with the observation of Ali and Shakoori (1994) that slight decrease was seen in the PCV values of rats fed with DDT for 18 months @ 10 mg/kg body weight

Haemoglobin concentration

Haemoglobin concentration of cattle from study area and Mannuthy showed no significant difference The values from Eloor cattle were found to be towards the lower limit of the normal range of 8 to 15 g per cent (Benjamin 1985) A reduction of haemoglobin concentration upto 11 per cent was observed in rats fed with DDT (Ali and Shakoori 1994) Decrease in haemoglobin with or without a decrease in concentration of red cells was seen in animals fed with large doses of DDT (Hayes 1959)

Differential leucocyte count (DLC)

In the present study there was significant neutropenia and eosinophilia in samples from Eloor in comparison with samples from Mannuthy The findings are not in agreement with that of Cranmer *et al* (1972) who could not observe any significant change in the DLC values in squirrel monkeys orally dosed with DDT

Erythrocyte indices

The values for erythrocyte indices like mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) from the cattle of Eloor were not significantly different from that of Mannuthy Similar results were obtained by Cranmer *et al* (1972) in squirrel monkeys However Ali and Shakoori (1994) observed a significant decline in MCHC values and increase in MCV and MCH in rats administered DDT orally

The haematological values obtained in the study shows variation from related works conducted earlier The probable explanation for such discrepancy may be due to the fact that a variety of effluent chemicals are released from the industries located in Eloor and hence the haematological changes observed were not comparable with the results of exper mental studies conducted under controlled conditions

Serum biochemistry

The serum total protein and albumin values from Eloor samples were not significantly different from the Mannuthy samples The values are within the normal range of 5 7 to 8 1 g/dl for total protein and 2 1 to 3 6 g/dl for albumin (Kaneko *et al* 1997) This is in agreement with the findings of Laws *et al* (1973) who observed that no significant change in the total protein and albumin content on long term exposure to DDT in human beings A decrease in serum albumin and increase in β and γ globulins in the blood of rats and rabbits exposed to DDT for 11 months was noticed by Kagen *et al* (1969) The normal values of total protein and albumin indicate that no significant toxicopathological changes are present in Eloor cattle

Serum enzymology

The values of serum enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) exhibited significant variation between Eloor and Mannuthy samples ALT AST and SAP are membrane bound enzymes of liver and any membrane damage results in their leaching to the blood serum

(Kaneko *et al* 1997) DDT and metabolites were reported to be responsible for the derangement of biological membranes and increasing the permeability of cell membrane (Antunes Madeira and Madeira, 1979) Morgan and Lin (1978) found that there was a tendency for SGOT and SGPT to increase with increasing concentration of DDT and DDE in serum In the present study the serum concentration of DDT and metabolites were very low and likewise the serum enzyme levels were within the normal range (ALT 11 to 40 u/l AST 78 to 132 u/l) observed by Kaneko *et al* (1997) Hence the effect elucidated by DDT residues may be too low to produce any clinically significant rise in serum enzymes

The observed biochemical values were within the normal range but were more towards the upper limits of normal values So the accumulated DDT in the tissues may not be able to produce any tissue damage

The conclusions that can be arrived at from the present study are

DDT was detected above maximum permissible levels in the soil fodder water and cattle of Eloor Presence of significant levels of DDT and its metabolites indicate that both recent and aged residue accumulations are present in environmental and biological samples Fodders may be the main source of DDT in adult cattle of Eloor industrial area The haematological serum biochemical values and serum enzyme levels reveal no substantial change in cattle of study area and it can be concluded that the DDT accumulated in these animals is not high enough to produce prominent adverse

effects Whole body concentration of DDT residues in cattle and environment is high due to constant exposure through effluents discarded from industrial units Hindustan Insecticides Limited (HIL) in Eloor is suspected to be major source of DDT contamination The persistent subchronic exposure to DDT in cattle can suppress the immune system and make the animal susceptible to a variety of physiologic and external stressors in the long run Residues can also pose a threat to humans residing in the area Hence precautionary measures should be taken by the concerned industries public and veterinarians to prevent further environmental contamination with DDT Appropriate measures must be adopted for the safer residue disposal and education of the public about the possible hazards from this POP

Summary

6 SUMMARY

The existence of organochlorine pesticides like DDT in the environment can produce a variety of adverse effects to livestock and humans in the region The present study was therefore undertaken to assess the environmental contamination with DDT and its impact on cattle of Eloor industrial area in Kerala State Seven major industrial units are located in Eloor namely Fertilizers and Chemicals Travancore (FACT) Indian Aluminium Company (INDAL) Travancore Cochin Chemicals (TCC) Travancore Chemicals Manufacturing Company (TCM) Hindustan Insecticides Limited (HIL) and Com ncobinani Of these industries DDT s being manufactured by HIL and t discharges pesticide laden effluents into the surrounding area Hence the study was concentrated mainly around this industry

Samples collected from Eloor industrial area included both environmental and biological samples Environmental samples collected were sludge water and fodder plants Blood urine dung and milk were the biological samples collected from adult cattle of Eloor Whole blood and serum from the respective cattle were collected for haematology and serum b ochemistry Similar samples were collected from the University Livestock Farm Mannuthy

The environmental and biological samples were subjected to a series of cleanup procedures and analysed using gas liquid chromatography (GLC) for

DDT residues Haematological parameters like total erythrocyte count total leucocyte count differential leucocyte count packed cell volume haemoglobin concentration mean corpuscular haemoglobin mean corpuscular volume and mean corpuscular haemoglobin concentration were recorded Serum samples were analysed for total prote n albumin serum enzymes like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) Data obtained by analysis of environmental and biological samples from cattle of Eloor industrial area was compared with that of cattle in University Livestock Farm (ULF) Mannuthy Statistical analysis of the data was done using students t test described by Rangaswamy (1995)

The levels of DDT in fodder water and sludge samples collected from Eloor were higher than that of Mannuthy samples The levels of DDT in both fodder and water samples from Eloor exceeds the maximum permissible level Biological samples like blood dung urine and milk also showed higher values of DDT compared to the samples from University Livestock Farm Mannuthy

Neutropenia and eosinophilia were observed in samples from study area and were significantly different from the Mannuthy samples Other haematological parameters including RBC count WBC count PCV Hb and erythrocyte indices (MCV MCHC MCH) did not show any significant changes

Serum biochemical values like total protein and albumin had no significant difference from the Mannuthy samples Significant difference in

serum alanine aminotransferase and serum aspartate aminotransferase were noticed in cattle of study area than Mannuthy samples But the values were found to be within the normal range

The present investigation has lead to the salient observations that DDT residues in environmental and biological samples from Eloor is higher than that in Mannuthy samples Effluent discharged from pesticide factories is mainly responsible for accumulation of DDT in Eloor region Significant variation in haematological and biochemical values was not observed in animals of Eloor industrial area As DDT is a highly persistent chemical with biomagnification potential it may pose serious threat to human and livestock health thereby suitable control measures must be adopted to reduce the environmental load of DDT residues in Eloor industrial area

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ASSESSMENT OF DICHLORODIPHENYLTRICHLOROETHANE (DDT) RESIDUES IN CATTLE OF ELOOR AREA

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ABSTRACT OF THE THESIS

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ABSTRACT

A study was conducted to assess the impact of environmental pollution with DDT in cattle of Eloor industrial belt Hindustan Insecticides Limited (HIL) is the major pesticide manufacturing factory of the region producing pesticides like DDT Hence Eloor was selected as the study area

Environmental samples like sludge water and fodder biological samples like blood urine dung and milk were collected from the cattle of Eloor area Whole blood and serum samples were collected for haematology and serum biochemistry respectively The collected samples were analysed for DDT residues in the gas liquid chromatograph (GLC) The values obtained were compared statistically with the samples collected from University Livestock Farm Mannuthy

Higher levels of DDT residues were obtained from the environmental and biological samples from Eloor than the corresponding samples from Mannuthy The mean levels of total DDT in environmental samples of Eloor were 1 463 ± 0 555 ppm (fodder) 0 00255 ± 0 00002 ppm (water) and 35 157 ± 0 198 ppm (sludge) Biological samples from Eloor contained mean total DDT of 0 201 ± 0 123 ppm (serum) 0 023 ± 0 006 (urine) 0 910 ± 0 689 ppm (dung) and 0 058 ± 0 019 ppm (milk) Environmental samples from Mannuthy contained mean total DDT of 0 062 ± 0 021 ppm 0 011 ± 0 006 ppm 0 000085 ± 0 00007 ppm in fodder sludge and water respectively Serum urine and

dung from cattle of Mannuthy contained mean total DDT of 0 252 ± 0 145 ppm 0 030 ± 0 003 ppm and 0 026 ± 0 017 ppm respectively No DDT residues were detected in the milk samples from Mannuthy

The haematological values from Eloor cattle remained within the normal range except differential leucocyte count which exhibited marked neutropenia and eosinophilia Values of total protein and albumin were normal in both groups of animals Serum enzymes like alanine aminotransferase and aspartate aminotransferase were significantly different from Mannuthy sample but were within the normal range It can be inferred that the DDT residues present in the field and biological samples were not high enough to cause toxicity in cattle Variation in the haematological and biochemical parameters may be due to the presence of other environmental pollutants along with DDT in the discharged industrial effluents