DICOFOL TOXICITY IN RATS

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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KERALA, INDIA

2001

DECLARATION

I hereby declare that the thesis entitled **"DICOFOL TOXICITY IN RATS"** 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 University or Society.

Mannuthy
29.12.2001

Padmaraj P.K.

CERTIFICATE

Certified that the thesis entitled **"DICOFOL TOXICITY IN RATS"** is a record of research work done independently by **Shri. Padmaraj P. 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 him.

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To My
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PADMARAJ P.K

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Introduction

1. INTRODUCTION

Dicofol is an organochlorine miticide which has been used in agriculture for thirty years. It is a registered product as a broad spectrum contact, non-systemic miticide for control of plant mites. It is insoluble in water, but soluble in most aliphatic and aromatic solvents. Chemically, it is 4-chloro-α- (4-Chlorophenyl)-α- (trichloromethyl) benzene methanol. WHO has classified dicofol as moderately hazardous (WHO, 1992).

Dicofol is extensively absorbed from the gastrointestinal tract on ingestion. The *p, p'*-dicofol isomer, the main component of dicofol, was more persistent in the body than the *o, p'* –isomer. Dicofol and DDT have structural similarity and same pattern of distribution and elimination.

Metabolism of dicofol involves dechlorination and oxidation of the ethanol moiety and hydroxylation of the aromatic rings. Chlorinated hydrocarbon insecticides are non-specific stimulants of the central nervous system. As with other members of the DDT class, the mode of action is stimulation of axonal transmission of nerve signals. The signs of toxicity like

nervousness, hyperactivity, headache, nausea, vomiting, unusual sensations and fatigue were observed in dicofol poisoning (Lorgue *et al.*, 1996). Clinical signs of toxicity like behavioural aberrations, nervous disturbances, autonomic manifestations and locomotor disturbances are also noticed (Hatch, 1982).

Chlorinated hydrocarbon compounds accumulate in the body fat of animals. The amount of storage in the fat of animals appears to be proportional to the concentration of the pesticide in the diet (Booth, 1982). Because of their persistence in the environment and ability to accumulate in biologic food chain, organochlorine pesticide residues may appear naturally in animal tissues (Hatch, 1982).

Demonstration of organochlorine pesticide residues in man, birds, atmosphere, soil and water by different research workers has given credence to the popular belief that these compounds are among the most important worldwide environmental pollutants (Kahunyo *et al.*, 1986).

It has been suggested that the residues of DDT found in the eggs and tissues of raptorial birds are responsible for the

decrease in population reported in these species (Chang and Stokstad, 1975).

In the year 1999, mite infestation (Mandari) on coconuts in Kerala assumed great attention. Ninety per cent of the trees were affected in some of the districts.

One of the measures adopted to control this mite infestation is to spray dicofol. Toxicity in animals was reported due to consumption of dicofol contaminated coconut tree leaves, plantain leaves, etc.

In the light of above observations, the study of dicofol toxicity in rats was undertaken. The objectives of the study are:

1. To study the toxic effect of dicofol in rats.
2. To assess the effect of dicofol on growth, body weight, percentage of mortality and detection of residue of dicofol in tissues like liver, kidney and heart by thin layer chromatography.

The information gathered by the study will help in suggesting suitable safe dose for use of dicofol.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Toxicity of dicofol

2.1.1 Hepatotoxicity

O'Brien (1974) reported that dicofol was a liver enzyme inducer and the primary effect observed after short or long term dietary exposure of dicofol in rats was hepatomegaly.

Studies conducted by Smith and Bababunmi (1980) revealed that dicofol causes hepatocellular carcinoma and liver hypertrophy in mice.

Ohyama *et al.* (1982) studied the effects of DDT and its analogues on rat liver mitochondria and reported that dicofol inhibits mitochondrial respiration.

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

Ramalingam (1987) studied the histopathological effects of DDT on the liver, intestine and kidneys of poultry. He reported that liver showed degenerative changes and the absorptive layers of the intestine were disrupted.

Experiments conducted in rat liver by Flodstrom *et al.* (1990) showed that DDT and structurally related organohalogen pesticides were potent inducers of the phenobarbital – inducible cytochrome P450 b isoenzyme and also caused hepatomegaly.

Kostka *et al.*, (1996) revealed the hepatic changes like vacuolated cytoplasm, focal necrosis and hepatomegaly in rats administered DDT and bromopropylate.

2.1.2 Reproductive toxicity

Guillette *et al.* (1994) hypothesised that normal steroidogenesis was inhibited by DDT and dicofol in alligators.

Morozova *et al.* (1997) reported that DDT administered to female mice caused an increase in uterine weight and pseudoestrus.

Jadarmkunti and Kaliwal (1999) studied the effects of dicofol formulations on estrus cycle and follicular dynamics in albino rats and reported that there was a decrease in the number of healthy follicles with concomitant increase in atretic follicles at higher doses of dicofol treatment.

Twenty pesticides were tested by Vinggaard *et al.* (1999) for their ability to activate the estrogen receptor *in vitro* and found that dicofol had estrogenic activity. The study also revealed that dicofol feeding reduced body weight gain, food consumption and histological changes in the ovaries.

Vinggaard *et al.* (2000) screened dicofol for inhibition of cytochrome P 19 aromatase activity *in vitro* and reported that a an inhibition of aromatase activity at a concentration of 50 μm in human placental microsomes.

2.1.3 Neurotoxicity

Lessenger and Riley (1991) explained the neurotoxicity and behavioural changes in a 12-year-old boy exposed to dicofol. The patient demonstrated subjective and objective evidence of neurological injury.

The review on behavioural toxicology of chlorinated hydrocarbons by Evangelista and Duffard (1996) explained their developmental neurotoxicity due to alteration in motor function, cognitive abilities or changes in the ontogeny of sensorimotor reflexes.

A two-generation laboratory study conducted in captive American Kestrels by Mc Lellan *et al.* (1997) reported the behavioural alterations due to administration of *o, p'*- dicofol.

2.1.4 Osteotoxicity

Pikaliuk (1991) reported the osteotoxic effect of chlorophose and dicofol. He attributed the inhibition of bone growth due to imbalance of mineral saturation and their composition.

2.1.5 Toxicity on thyroid

Vandenberg *et al.* (1991) reported lowered plasma thyroid hormone levels through interference with hormone transport carriers.

2.1.6 Dermal toxicity

Matsumara (1976) compared the oral and dermal toxicity of various insecticidal compounds and found that dicofol penetrated rat skin with relative ease.

The Material Safety Data Sheet (Rohm and Haas Company, 1999) cautions that prolonged or repeated skin contact with dicofol causes moderate skin irritation. Skin sensitisation was also reported from susceptible individuals.

2.1.7 Toxicity to metabolic enzymes

Christensen and Riedel (1981) treated lipase preparations *in vitro* with 100 chemicals and reported that dicofol inhibits the activity of lipase.

Hijazi and Chefurka (1982) used the fluorescent probe, 1-anilino-8-naphthalene sulfonate (ANS), to monitor the interaction of pesticide chemicals with mitochondrial membranes and submitochondrial membranes. The results suggested that both DDT and dicofol were relatively ineffective inhibitors of

substrate–induced quenching of ANS fluorescence of submitochondrial particles.

Electron microscopic studies of the membranous cochlea of rats in chronic dicofol poisoning displayed mitochondrial swelling, golgi organ hyperplasia, intracellular space enlargement, perivascular edema, thickening of the capillary endothelium and basal membrane swelling (Muminov *et al.*, 1990).

2.2 Mutagenicity

Mutagenic studies conducted by Planche *et al.* (1979) revealed that dicofol caused no mutagenic effects in *Salmonella typhimurium* strains TA 100 or TA 98, either in the presence or absence of a mouse-liver microsomal fraction. But, they reported that dicofol acetate was a direct acting mutagen to strain TA 100.

Sobti *et al.* (1983) reported that dicofol and other organochlorine pesticides caused a dose dependant cytotoxicity, mitotic depression and cell cycle traverse inhibition of human lymphoid cell lines.

2.3 Avian toxicity of dicofol

DDT and its residues decrease egg shell thickness by inhibiting carbonic anhydrase in the oviduct (Bitman *et al.*, 1969). The studies conducted by Schwarzbach (1991) to determine the role of dicofol metabolites in the egg shell thinning, revealed that the dicofol metabolites are less toxic than dicofol to egg shell formation.

Mc Lellan *et al.* (1997) evaluated the reproductive and morphological effects of *o, p'*-dicofol on two generations of captive American Kestrels. High dosed birds laid thin-shelled eggs and feminization of male embryos was confirmed by the presence of primordial germ cells in the male gonad.

The field studies conducted by Blus and Henny (1997) on pesticides and birds pointed out the effects of DDT and its metabolites on egg shell thickness, reproductive success and population stability.

2.4 Detection of dicofol

Das (1981) described the technique of detection of organochlorine pesticides and detected dicofol by thin-layer chromatography.

Krynitsky *et al.* (1988) introduced a combined extraction–clean up column chromatographic procedure for high volume determination of dicofol in eggs and other avian tissues.

Mourer *et al.* (1990) stated that the recovery efficiency of dicofol and its metabolites in egg yolks can be increased by high performance liquid chromatography.

Beasley *et al.* (1998) developed a panel of immunoassays for monitoring DDT, its metabolites and analogues in food and environmental matrices. Greater specificity and sensitivity for dicofol detection were obtained by using an immunogen derived from ester hydrolysis of chlorbenzilate.

2.5 Dicofol residues

Kahunyo *et al.* (1986) conducted a survey in Kenya to estimate the organochlorine pesticide residues in chicken fat and reported that the total residue levels of DDT and its metabolites were above the respective practical residue limit values.

Surendranath and Rao (1991) monitored dicofol residues in tissues of tropical penaeid prawn, under sublethal chronic exposure. The study confirmed that dicofol is a persistent insecticide of low biodegradability and capable of accumulating in tissues as residues even at sublethal concentration under chronic exposure.

Dicofol, originally evaluated by the Joint FAO/ WHO meeting on pesticide residues in 1968 was re-evaluated for residues in 1970 and 1974, and was included in the Codex committee on Pesticide Residues periodic review programme (JMPR report, 1992).

Takatsuki *et al.* (1994) mentioned the residual levels of DDT and its metabolites in imported Australian beef.

Chen *et al.* (1995) recommended the use of pyridaben and propargite in place of dicofol for control of tea mites in china.

Clark *et al.* (1995) reported the presence of dicofol and DDT residues in lizard carcasses and avian eggs from Texas, Florida and California.

The dietary exposure of French consumers to ten pesticides was detected by Leblanc *et al.* (2000) and reported that dicofol residues were present in at least one sample at a level at or above the limits of quantification.

2.6 Haemotoxicity

DDT induced haemotoxicity was reported by Ali and Shakoori (1994) in Sprague Dawley rats.

2.7 Ecotoxicology

The studies by Stark *et al.* (1997) indicated that reproductive potential is important in terms of pesticide impact on populations and should be considered when estimating the impact of pollutants on species.

Stark and Banken (1999) opined that exposure of differently structured pesticides may result in different outcomes in terms of population growth rates. This finding is significant in terms of estimating risk of toxicants to populations.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Pesticide

Technical grade dicofol* was procured for conducting the study. Dicofol was prepared as a one per cent suspension for oral administration. One gram of dicofol was mixed with five ml of Tween-80** and made upto 100ml by adding distilled water. Dicofol for external application was prepared as a 0.1 per cent emulsion in water.

3.1.2 Animals

The study was conducted in ninety adult albino rats weighing 150-200 g of either sex. The rats were maintained under standard feeding and management practices in the laboratory for one week before starting the experiment.

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* Dicofol Technical: Manufactured by Rohm and Haas Company, Philadelphia.

** Tween-80 (Polysorbate 80): Manufactured by Cita Diagnostics, Kochi, Kerala.

The rats were divided into five groups as given below:

a) The control group (Group I), consisting ten rats of either sex.

b) The experimental group (Group II, Group III, Group IV and Group V) consisting of 20 rats of either sex, in each group.

The dose of dicofol for rats in Group II, Group III and Group IV were calculated based on body weight and administered orally using the stomach tube. The dose schedule for administration of dicofol was as follows:

Group	Dose of dicofol
Group I	Control
Group II	50 mg/kg, orally, once daily for three months
Group III	75 mg/ kg, orally, once daily for three months
Group IV	100 mg/ kg, orally, once daily for three months
Group V	0.1 per cent, external application (spraying), once daily for three months.

3.2 Observations

3.2.1 Clinical symptoms

The signs of toxicity of dicofol were observed in the experimental groups of rats and compared with the control group on daily basis.

3.2.2 Dermal toxicity

The extent of dermal toxicity of dicofol in Group V was studied and compared with the control group.

3.2.3 Body weight

The body weights of rats of both the treated and control groups were taken and recorded before the commencement of the experiment, at weekly intervals during the experiment and at the end of the experiment and data was analysed by Completely Randomised Design (CRD) method (Snedecor and Cochran, 1980).

3.2.4 Necropsy findings

The gross and histopathological lesions in liver, kidneys and heart were studied.

3.2.4.1 Gross lesions

The gross lesions in liver, kidney and heart of the treated groups of rats were compared with the control group.

3.2.4.2 Histopathological study

The histopathological examination of liver, kidneys and heart was done to assess the effect of dicofol on vital organs.

Three mm thick pieces of tissue were selected randomly from liver, kidney and heart of the control and experimental groups of rats and fixed in 10 per cent formaline and processed through routine paraffin – embedding process and stained with haematoxylin and eosin and studied the histopathology.

3.2.5 Detection of dicofol

The presence of dicofol in liver, kidneys and heart was detected by Thin Layer Chromatography (TLC).

3.2.5.1 Thin Layer Chromatography Technique

a) Extraction

Liver, kidneys and heart tissues were randomly selected from the rats of control and treated groups. Ten gram of tissue was thoroughly macerated and mixed with equal amount of anhydrous sodium sulphate and 100 ml of acetone in a conical flask and then refluxed on hot water bath for one hour. After cooling, the solution was filtered. The residue was extracted twice with further 50 ml portions of acetone. The acetone fractions were combined and concentrated by evaporation to 50 ml.

b) Clean-up procedure

Fifty ml of concentrated acetone extract was taken into a separating funnel, diluted with 150 ml of water and 20 ml of saturated solution of sodium sulphate was added to it. The contents were extracted thrice with 25ml portions of chloroform

with gentle shaking. The chloroform extracts were mixed and washed with 50 ml of water. The washed chloroform layer was passed through anhydrous sodium sulphate and then evaporated to dryness.

c) Procedure of TLC

The TLC plates were thoroughly cleaned and dried, and arranged on a template board. The slurry was prepared by mixing silica gel-G and distilled water in the ratio of 1:2 and applied as a thin layer on TLC plate using a spreader. The plates were dried at room temperature and activated in a hot air oven at 110^{0}C for one hour.

The cleaned-up extract was dissolved in 0.5 ml of acetone. An aliquot of this acetone solution was spotted on the TLC plate. The plate was held vertically in a chromatographic chamber containing the solvent petroleum ether and liquid paraffin in the ratio of 9:1 and continued to run until the solvent front reaches two cm from the top of the plate. The plates were removed and after air-drying, sprayed the plate evenly and thoroughly with one per cent silver nitrate spray solution. Air

dried the plate for 10 min and exposed to ultraviolet light for 10 min (Das, 1981).

The black coloured spots developed were compared with the control and the standard.

3.2.6 Haematological study

Blood samples were collected from the retroorbital plexus of both the treated and control groups of rats at two weeks interval for the estimation of haematological parameters viz., total erythrocyte count, total leucocyte count, differential leucocyte count and haemoglobin concentration by methods described by Schalm *et al.* (1975).

3.2.6.1 Total Erythrocyte (TEC) Count

Total red blood cells were counted by using heamocytometer (Benjamin, 1978).

3.2.6.2 Total Leucocyte (TLC) Count

Total leucocytes were counted by standard dilution technique using Thomas fluid and haemocytometer (Benjamin, 1978).

3.2.6.3 Differential Leucocyte Count (DLC)

Blood smears were prepared from freshly drawn blood (without anticoagulant) by using slide method. After staining with Wright's stain, differential leucocyte count was done by counting and classifying 200 leucocytes under oil immersion (Benjamin, 1978).

3.2.6.4 Haemoglobin concentration (Hb)

Haemoglobin concentration was estimated by acid haematin method (Benjamin, 1978).

The haematological parameters were analysed by Completely Randomised Design (CRD) method (Snedecor and Cochran, 1980).

Results

4. RESULTS

The data of the study conducted are presented in Tables 1 to 5.

The plates of hisotpathological sections and thin layer chromatography are shown in pages 27, 29 and 31.

4.1 Incidence of Mortality

Mortality was recorded in experimental groups during the study. In the Group II one rat died in the second week and one rat died in the Group III on the third week. Two rats died in the Group IV, one each at the second and eighth week of experiment. One mortality was recorded in Group V at the third week of external application of dicofol.

Rats from both the treated and control groups exhibited gasping and convulsion towards death. Post-mortem was conducted but no lesions could be observed in the organs like liver, heart and kidneys.

4.2 Signs of toxicity

The rats from Group II, Group III and Group IV (treated groups) showed clinical symptoms of toxicity. After administration of dicofol, the rats, huddled together in the cages. No change in body temperature was noticed.

In the first week of study, the rats showed decreased appetite and towards the end of experiment, total loss of appetite and stopped feeding. It was also found that the rats were more thirsty. As consequence to decreased feed intake, a decrease in body weight was observed throughout the experiment. Some of the rats of Group IV showed sluggish movements after eight weeks of administration of dicofol.

4.3 Dermal toxicity

Dermal toxicity studied in Group V showed moderate skin irritation initially, later resulting in loss of hair. Conjunctivitis was also noticed in some rats due to eye contact of dicofol during spraying.

4.4 Assessment of body weight

The mean body weight of rats of both the control and treated groups at weekly intervals were recorded and statistically analysed and presented in Table 1.

The mean body weight of rats belonging to Group II, Group III and Group IV (experimental groups) showed a progressive decline when compared to the control group.

The mean body weight of rats of Group III (169.89 ± 4.88) was significantly ($p<0.05$) lower than that of control (1888.00 ± 5.86) at sixth week of experiment.

The mean body weight of rats of Group II, Group III and Group IV were significantly ($p<0.01$) lower than that of control from the seventh week of experiment to the end of experiment.

The mean body weight of rats of Group V showed significant decrease ($p<0.05$) when compared to that of control from 10^{th} week of experiment.

Table 1 Effect of dicofol on body weights (g) in rats

Sl. No.	Interval (week)	Group I (control)	Group II (50mg/kg)	Group III (75 mg/kg)	Group IV (100 ml/kg)	Group V (external application)
1.	Before the experiment	182.66 ± 5.99	187.16 ± 3.55	184.70 ± 3.49	183.47 ± 3.97	185.47 ± 2.34
2.	1st	180.00 ± 6.27	186.00 ± 3.86	182.42 ± 3.74	180.74 ± 4.22	180.63 ± 2.38
3.	2nd	181.11 ± 6.23	183.67 ± 4.18	180.00 ± 3.81	179.78 ± 4.08	177.67 ± 2.43
4.	3rd	184.20 ± 5.66	182.00 ± 4.03	177.47 ± 3.88	176.42 ± 3.96	178.63 ± 2.21
5.	4th	185.78 ± 5.72	179.44 ± 4.04	175.44 ± 4.25	173.78 ± 4.67	179.44 ± 2.22
6.	7th	186.89 ± 5.62	178.33 ± 3.96	171.67 ± 4.55	171.00 ± 4.74	181.44 ± 2.30
7.	6th	188.00 ± 5.86^{ab}	176.22 ± 3.99	169.89 ± 4.81^{a}	168.22 ± 4.88^{b}	182.33 ± 2.34
8.	7th	191.78± 5.84^{ABC}	173.56 ± 3.99^{A}	167.11 ± 4.94^{B}	165.00 ± 4.97^{c}	183.44 ± 2.46
9.	8th	193.11 ± 5.26^{ABC}	171.11 ± 3.90^{A}	163.89 ± 5.19^{B}	161.65 ± 5.26^{c}	185.11 ± 2.59
10.	9th	196.44 ± 4.73^{ABC}	169.56 ± 3.93^{A}	161.00 ± 4.98^{B}	158.12 ± 5.39^{C}	186.78 ± 2.56
11.	10th	200.00 ± 4.98^{ABCd}	165.33 ± 3.83^{A}	158.67 ± 4.94^{B}	154.70 ± 5.55^{C}	189.11± 2.63^{d}
12.	11th	203.11 ± 4.20^{ABCd}	162.00 ± 3.99^{A}	154.44 ± 5.12^{B}	150.94 ± 5.48^{C}	191.00 ± 2.60^{d}
13.	12th	204.44 ± 4.12^{ABCd}	158.00 ± 4.16^{A}	148.97 ± 5.25^{B}	142.47± 5.69^{C}	193.33 ± 2.85^{d}
14.	13th	207.33 ± 4.28^{ABCd}	154.33 ± 4.15^{A}	138.78 ± 5.60^{B}	134.82 ± 5.94^{C}	195.22 ± 2.33^{d}

Mean bearing different superscripts in a row differ significantly

ABD - at $p<0.01$

abd - at $p<0.05$

4.5 Detection of dicofol

Dicofol was detected in liver, heart and kidney by thin layer chromatography (TLC).

The TLC plates of Group I, Group II, Group III, Group IV and Group V are given on pages 27 and 29 respectively.

The presence of dicofol in tissues was compared with that of standard 1 (0.1 per cent dicofol), standard 2 (0.05 per cent dicofol) and control (blank).

Group I (control group) showed absence of dicofol in tissues when compared to standards (Plate 1).

In Group II, liver tissue showed presence of dicofol comparable to that of standards (Plate 2).

TLC plates of Group III clearly showed the presence of dicofol in tissues of liver and kidney and slight presence in heart when compared to that of standards (Plate 3).

Plate 3 TLC Plate – Group III, presence of dicofol in tissues of liver and kidney and slight presence in heart

Plate 2 TLC Plate – Group II, presence of dicofol in liver tissue

Plate 1 TLC Plate – Group I (control), absence of dicofol in tissues

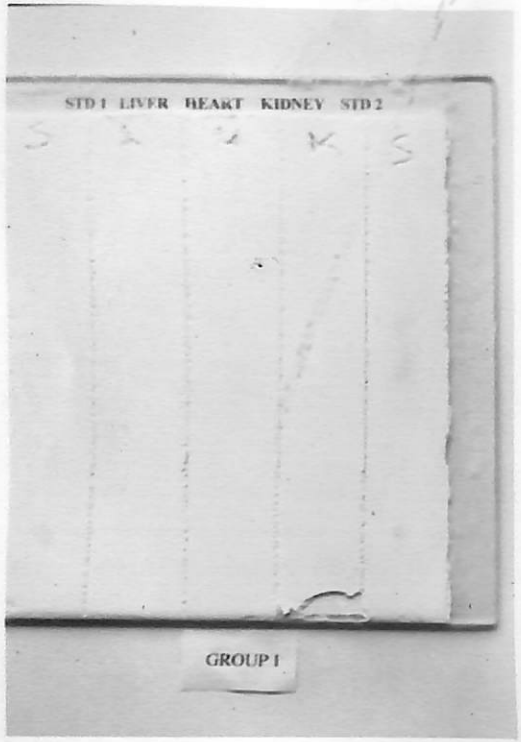

Plate 1

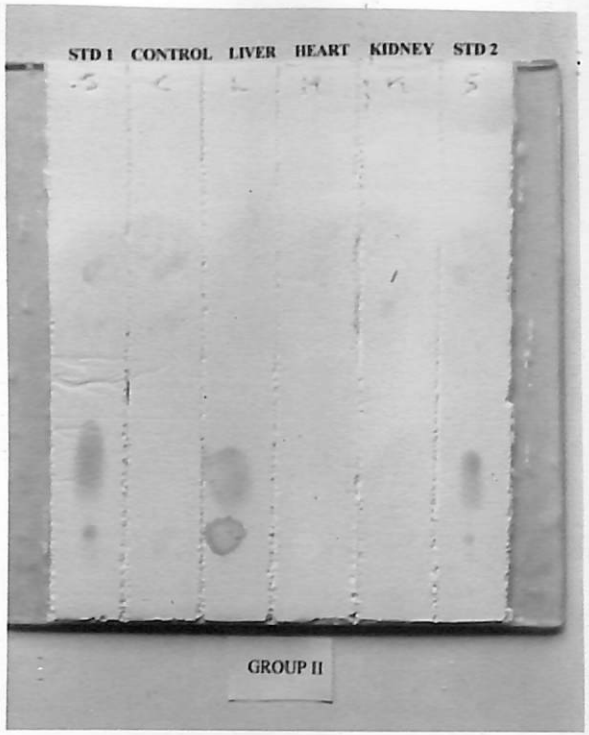

Plate 2

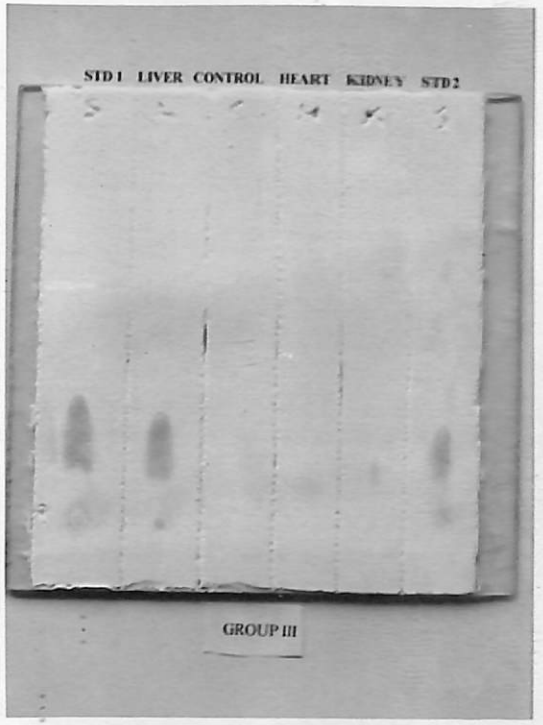

Plate 3

The tissues of liver, kidney and heart clearly showed the presence of dicofol in the TLC plate of Group IV when compared to that of standards (Plate 4).

In Group V, a small spot of dicofol could be observed in the area of liver on TLC plate when compared to standards (Plate 5).

4.6 Post-mortem findings

4.6.1 Gross lesions

Post-mortem conducted at the end of the experiment in both the control and treated groups of rats revealed gross lesions in the liver, kidney and heart. All the experimental groups of rats showed lesions in these organs but the intensity, of the lesions varied with the dose schedule.

4.6.1.1 Liver

Necrotic changes were observed in the liver of experimental groups of rats. Patchy areas of necrosis was observed on parietal and visceral surfaces. In Group V, echymosis was also seen.

Plate 5 TLC Plate – Group V, small spot of dicofol in area of liver tissue

Plate 4 TLC Plate – Group IV, clear presence of dicofol in tissues of liver, kidney and heart

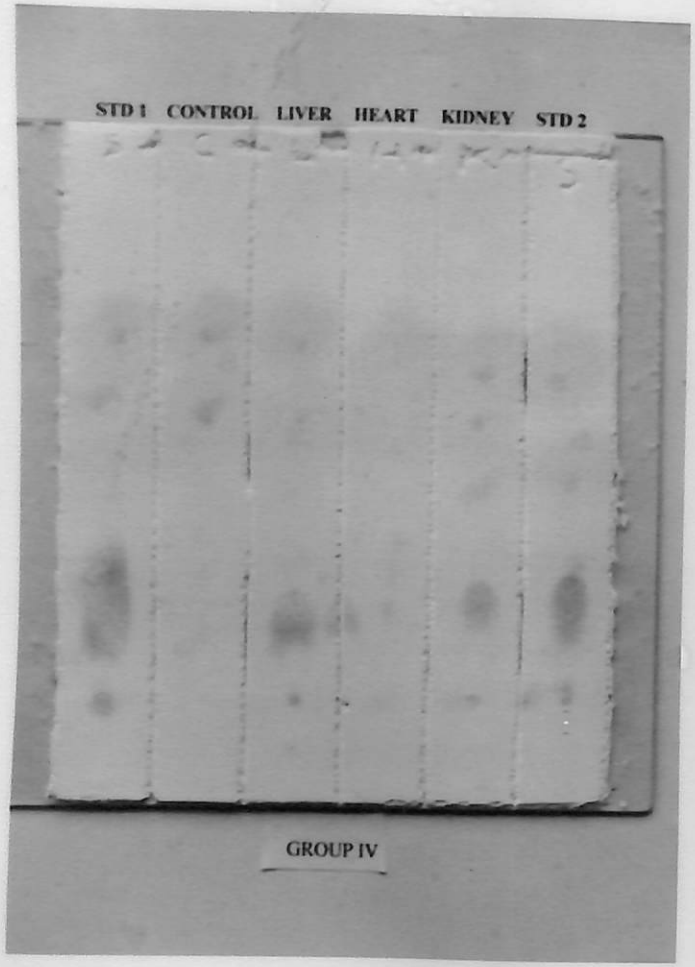

Plate 4

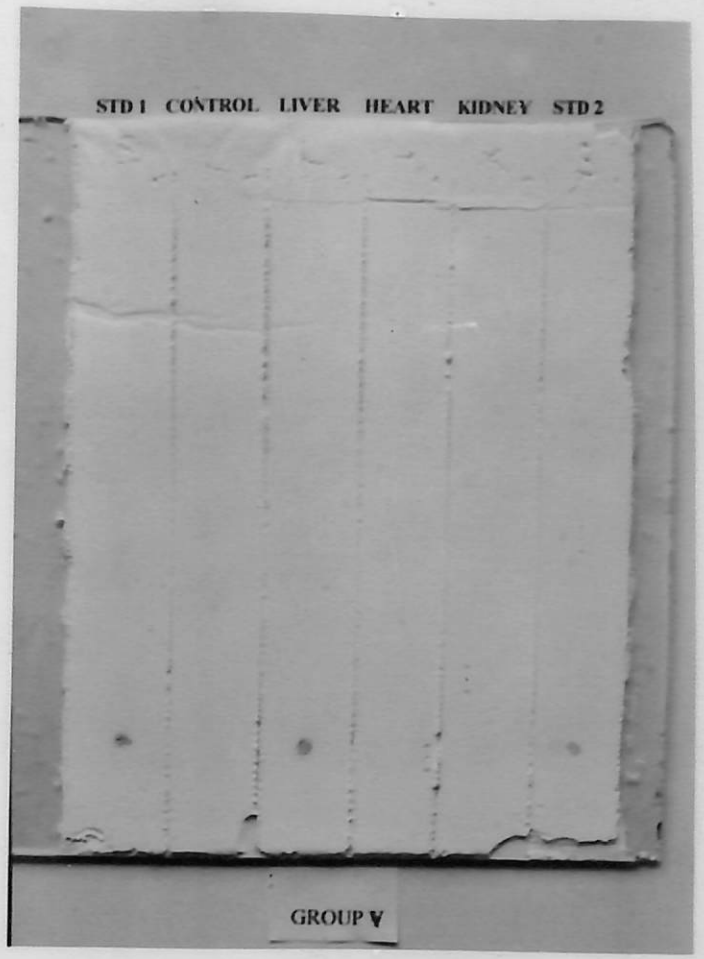

Plate 5

4.6.1.2 Kidney

The kidneys in the treated groups were found to be congested.

4.6.1.3 Heart

In all the treated groups, the coronary blood vessels were congested. Petechae were seen in the heart of experiment groups IV and V.

4.6.2 Histopathology

The plates of microscopic lesions observed in tissues of liver, kidney and heart are given on page 31.

The histopathological examination of liver, kidney and heart of experimental groups of rats showed microscopic lesions in these organs. The intensity of lesions were higher in Groups IV and Group V.

Plate 6	Group IV – Liver – diffuse vacuolar changes in the cytoplasm of hapatocytes and infiltration of mononuclear cells in the periportal areas and dilatation of portal vein
Plate 7	Group IV – Kidney – congestion of the renal blood vessels, degenerative changes in renal tubular epithelial cells
Plate 8	Group V – Liver – Fatty changes in the hepatocytes and dilatation of hepatic sinusoids
Plate 9	Group V – Heart – congestion of coronary blood vessels and extravasation of erythrocytes
Plate 10	Group V – Kidney – Hyperaemia of renal blood vessels and extravasation of erythrocytes in renal interstitium

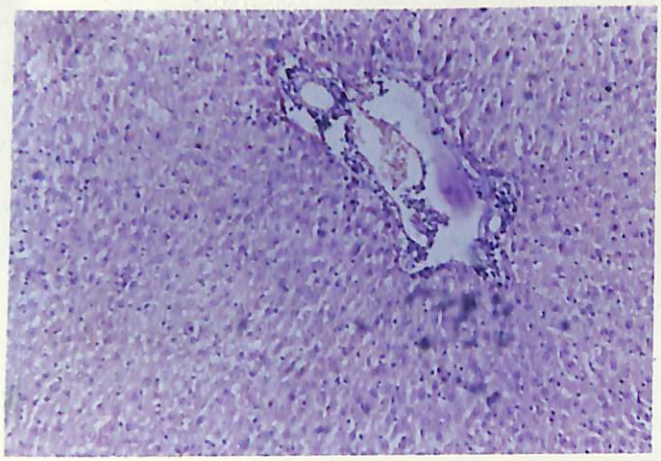

Plate 6

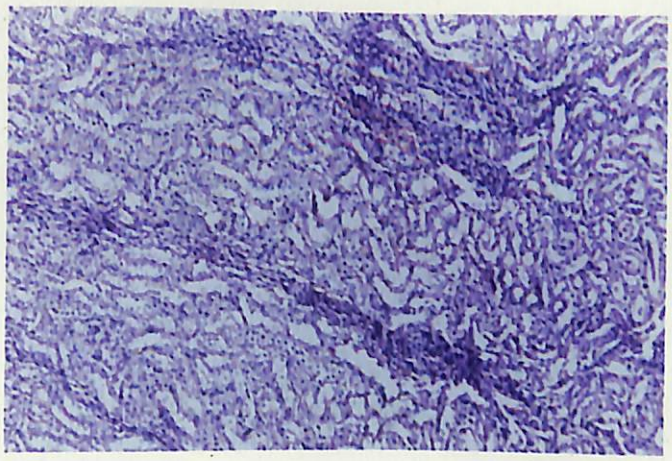

Plate 7

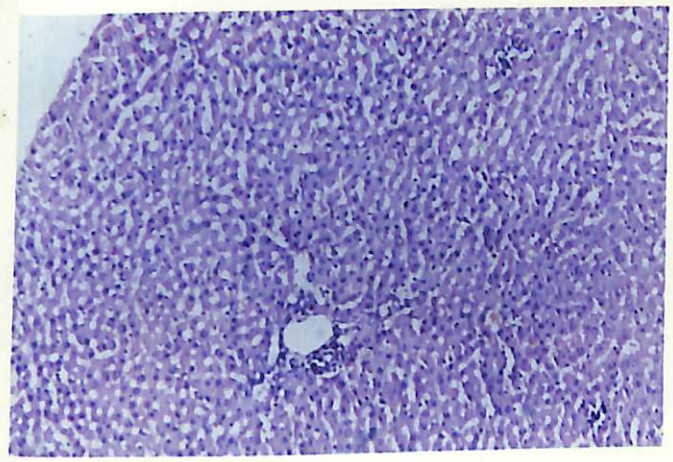

Plate 8

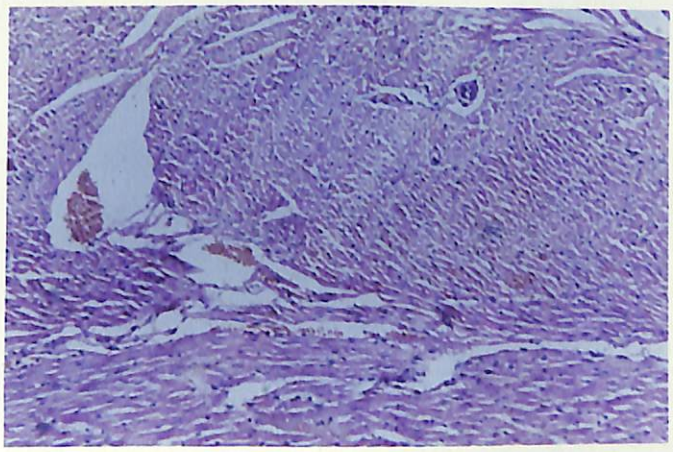

Plate 9

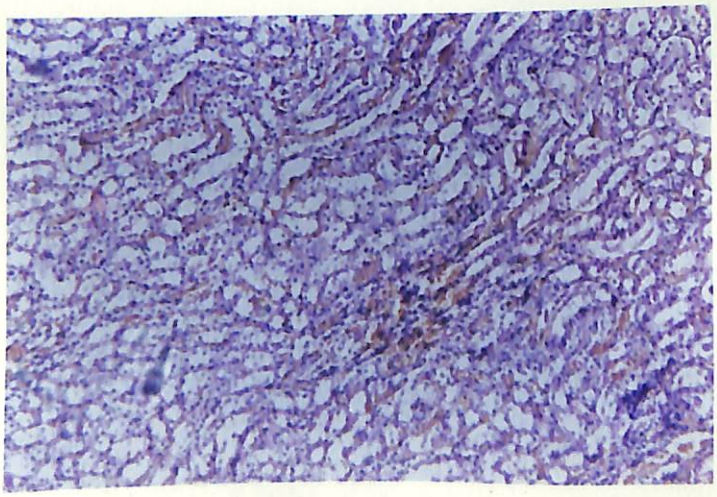

Plate 10

4.6.2.1 Liver

Swelling of the hepatocytes along with venous stasis was noticed. The hepatocytes showed diffuse vacuolar changes in the cytoplasm in focal areas. Occasional foci of necrosis could be observed. Infiltration of mononuclear cells were observed in the periportal areas.

4.6.2.2 Kidneys

Kidneys showed moderate hyperaemia characterised by the distended of the renal blood vessels. The tubular epithelial cells exhibited degeneration and the presence of albuminous casts in the kidney tubules.

4.6.2.3 Heart

Congestion of the coronary blood vessels and extravasation of erythrocytes were seen. Occasional hyaline degeneration of the cardiac myocyte could be observed.

4.7 Haematological parameters

The results of the haematological parameters were analysed and tabulated and presented in Tables 2 to 5.

4.7.1 Total Erythrocyte (TEC) count

The mean TEC values per mm^3 of the control and treated groups at two weeks interval is given in Table 2. The values were all in the normal range and were comparable to control group.

4.7.2 Total Leucocyte (TLC) count

The mean TLC values per mm^3 of the control and treated groups of rats at two weeks interval was in the normal range as showed in Table 3. There was no significant difference ($P>0.05$) between TLC values of the control and experimental groups.

4.7.3 Differential leucocyte count (DLC)

The mean neutrophil(%), lymphocyte(%), eosinophil(%) and monocyte(%) counts of the blood samples of the control and treated groups at two weeks interval showed in Table 4 were all in the normal range and were comparable to control group. There was no significant difference ($P>0.05$) between the differential leucocyte count of the control and experimental groups.

4.7.4 Haemoglobin concentration

The mean haemoglobin concentration (g %) of the control and experimental groups at two weeks interval was given in Table 5. The values were all in the normal range

Table 2. Effect of dicofol on Total Erythrocyte Count per mm^3 in rats.

Sl. No.	Interval (week)	Group I (control)	Group II (50mg/kg)	Group III (75 mg/kg)	Group IV (100 ml/kg)	Group V (external application)
1	2^{nd}	8.56 ± 0.26	8.67 ± 0.37	9.20 ± 0.29	9.10 ± 0.13	8.83 ± 0.34
2	4^{th}	8.41 ± 0.41	8.99 ± 0.39	9.10 ± 0.32	9.10 ± 0.20	8.68 ± 0.20
3	6^{th}	8.65 ± 0.24	8.64 ± 0.43	8.70 ± 0.54	9.36 ± 0.32	9.09 ± 0.29
4	8^{th}	9.17 ± 0.33	9.47 ± 0.20	8.95 ± 0.43	8.44 ± 0.48	8.27 ± 0.45
5	10^{th}	8.43 ± 0.40	8.49 ± 0.55	8.92 ± 0.57	8.86 ± 0.51	8.55 ± 0.44
6	12^{th}	8.72 ± 0.30	8.23 ± 0.59	8.93 ± 0.45	8.79 ± 0.30	8.79 ± 0.42
7	End of Experiment	8.29 ± 0.30	8.91 ± 0.48	8.37 ± 0.64	8.53 ± 0.12	8.67 ± 0.20

Table 3. Effect of dicofol on Total Leucocyte Count per mm^3 in rats.

Sl. No.	Interval (week)	Group I (control)	Group II (50mg/kg)	Group III (75 mg/kg)	Group IV (100 ml/kg)	Group V (external application)
1	2nd	8936 ± 102.01	7328 ± 431.98	8312 ± 618.44	9430 ± 557.99	8596 ± 591.17
2	4th	8726 ± 501.15	8474 ± 404.34	8278 ± 418.73	8110 ± 532.61	9682 ± 294.42
3	6th	8938 ± 548.04	9068 ± 442.05	8848 ± 592.30	8444 ± 732.17	9532 ± 379.20
4	8th	8956 ± 448.97	8068 ± 813.13	9284 ± 460.15	8832 ± 741.25	8964 ± 499.58
5	10th	8762 ± 437.20	8860 ± 490.27	8310 ± 487.59	8816 ± 115.65	8502 ± 625.50
6	12th	8426 ± 404.66	8772 ± 434.54	8394 ± 336.77	9372 ± 346.72	8896 ± 498.45
7	End of Experiment	8244 ± 473.27	8992 ± 474.69	9594 ± 241.19	8416 ± 471.02	8516 ± 349.38

Table 4. Effect of dicofol on Differential Leucocyte Count (%) in rats.

Sl. No	Interval (week)	Group I (control)				Group II (50mg/kg)				Group III (75mg/kg)				Group IV (100ml/kg)				Group V (external application)			
		N	L	E	M	N	L	E	M	N	L	E	M	N	L	E	M	N	L	E	M
1	2nd	21 ± 0.99	76 ± 0.94	3 ± 0.25	0	24 ± 0.18	72 ± 0.82	3 ± 0.34	1 ± 0.14	22 ± 0.83	74 ± 0.32	4 ± 0.08	0	23 ± 0.82	75 ± 0.16	2 ± 0.28	0	22 ± 0.63	76 ± 0.34	1 ± 0.24	1 ± 1.08
2	4th	27± 0.85	72 ± 0.38	1 ± 0.18	0	23 ± 0.24	74 ± 0.30	2 ± 0.28	1 ± 0.18	27 ± 0.58	70 ± 0.84	3 ± 0.40	0	26 ± 0.34	72 ± 0.92	2 ± 0.22	0	26 ± 0.14	72± 0.88	2 ± 0.36	0
3	6th	30 ± 0.62	69 ± 0.82	1 ± 0.12	0	26 ± 0.84	73 ± 0.42	1 ± 0.48	0	28 ± 0.34	72 ± 0.66	0	0	29 ± 0.12	70 ± 0.18	1 ± 0.10	0	27 ± 0.36	72 ± 0.40	1 ± 0.24	0
4	8th	23 ± 0.74	74 ± 0.62	3 ± 0.34	0	24 ± 0.82	74 ± 0.23	2 ± 0.12	0	22 ± 0.76	74 ± 0.38	3 ± 0.14	1 ± 0.34	22 ± 0.88	74 ± 0.36	4 ± 0.08	0	24 ± 0.86	74 ± 0.14	2 ± 0.04	0
5	10th	21 ± 0.84	77 ± 0.36	2 ± 0.82	0	20 ± 0.88	78 ± 0.24	2 ± 0.34	0	21 ± 0.64	76 ± 0.34	3 ± 0.24	0	22 ± 0.34	76 ± 0.24	2 ± 0.18	0	21 ± 0.66	78 ± 0.37	1 ± 0.06	0
6	12th	26 ± 0.42	74 ± 0.28	0	0	23 ± 0.84	76 ± 0.54	1 ± 0.92	0	24 ± 0.24	74 ± 0.86	2 ± 0.42	0	25 ± 0.04	74 ± 0.64	1 ± 0.44	0	27 ± 0.14	72 ± 0.48	1 ± 0.04	0
7	End of Exp.	25 ± 0.18	72 ± 0.62	3 ± 0.29	0	26 ± 0.28	74 ± 0.42	0	0	27 ± 0.40	72 ± 0.62	1 ± 0.14	0	24 ± 0.44	74 ± 0.94	2 ± 0.36	0	26 ± 0.37	72 ± 0.54	2 ± 0.08	0

N - Neutrophil, L - Lymphocyte, E – Eosinophil, M – Monocyte

Table 5. Effect of dicofol on haemoglobin concentration (g %) in rats.

Sl. No.	Interval (week)	Group I (control)	Group II (50mg/kg)	Group III (75 mg/kg)	Group IV (100 ml/kg)	Group V (external application)
1	2nd	13.67 ± 0.67	14.83 ± 1.08	14.60 ± 1.14	13.63 ± 1.02	13.37 ± 0.37
2	4th	14.03 ± 0.95	14.33 ± 0.95	14.10 ± 0.84	14.43 ± 0.49	14.20 ± 0.37
3	6th	15.08 ± 0.22	14.88 ± 0.43	14.84 ± 0.60	15.08 ± 0.29	15.28 ± 0.37
4	8th	14.64 ± 0.29	14.92 ± 0.14	15.52 ± 0.36	14.24 ± 0.43	14.60 ± 0.35
5	10th	13.00 ± 0.56	14.40 ± 0.57	13.84 ± 0.29	13.44 ± 0.24	14.12 ± 0.32
6	12th	14.24 ± 0.29	14.84 ± 0.34	13.64 ± 0.57	14.80 ± 0.14	14.56 ± 0.43
7	End of Experiment	14.24 ± 0.60	15.00 ± 0.09	13.84 ± 0.94	13.68 ± 0.84	14.80 ± 0.66

Discussion

5. DISCUSSION

The objectives of the study included the assessment of effect of dicofol on growth, body weight, percentage of mortality and detection of dicofol in vital organs like liver, kidney and heart.

5.1 Incidence of Mortality

Mortality was seen in the treated groups. The percentage of mortality ranged from 5-10. Out of 20 rats each in Group II and Group IV, two died from each group during the period of experiment; while in Group III and Group V, one rat from each group was found dead. But no lesions could be detected in organs like liver, heart and kidney during post-mortem examination.

5.2 Clinical symptoms of toxicity

The clinical signs of toxicity of dicofol was evident in the experimental group of rats with oral administration. The onset of clinical signs were seen in the first week of study itself and progressed towards the end of experiment. Chlorinated

hydrocarbon pesticides show nervous phenomena like stiff gait and ataxia. In chronic toxicosis, decrease in weight gain and decreased appetite can occur (Hatch, 1982).

At the end of two months of study, the rats belonging to experimental groups were even reluctant to take the feed. Chlorinated hydrocarbons have a high affinity of deposition in body fat. The release of pesticide from body fat strongly results in aggravating the symptoms of toxicity. Neurotoxicity to chlorinated hydrocarbons is mainly due to alteration in motor function and changes in the ontogeny of sensorimotor reflexes (Evangelista and Duffard, 1996). The rats showed the symptoms of organochlorine compound toxicity like gasping and convulsion towards death (Lorgue *et al.*, 1996).

In Kerala, there were reports of toxicity of dicofol in animals due to consumption of dicofol contaminated coconut tree leaves, plantain leaves etc. These toxicity signs were same those of chlorinated hydrocarbons.

5.3 Dermal toxicity

The treated group with external application of dicofol showed moderate skin irritation and alopecia. Dicofol penetrate

the rat skin with relative ease (Matsumara, 1976). The Material Safety Data Sheet (Rohm and Hass Company, 1999) cautions that repeated skin exposure of dicofol causes moderate skin irritation. Repeated irritation and scratching by the animal lead to hair loss at the end of experiment.

5.4 Assessment of body weight

The body weight of rats of both the control and treated groups at weekly intervals showed a decrease in the mean body weight of rats belonging to the experimental groups with oral administration of dicofol. The decrease in body weight is due to the decrease in feed consumption. From the third week of study, a decline in their body weight was noticed. The mean body weight of rats of Group II, Group III and Group IV were significantly different from that of the control group from sixth week of study.

Even though, Group V (0.1 per cent dicofol, external application) did not show any decrease in their mean body weight during the period of experiment, the values showed significant difference ($p<0.05$) from that of control from tenth week of experiment.

The assessment of body weight confirms that dicofol causes decreased appetite which results in reduced weight gain.

5.5 Detection of dicofol

The detection of dicofol in tissues of liver, heart and kidney was done by thin layer chromatography. High concentration of dicofol was detected in liver than heart and kidney of the experimental groups of rats. The intensity of concentration of dicofol in tissues depends on the dose schedule of administration of dicofol.

In Group II, only the liver tissue showed the presence of dicofol, while in Group III dicofol was clearly detected in liver and kidney and only slight presence of dicofol was there in heart. The presence of dicofol was clearly detected in the tissues of liver, heart and kidney of rats of Group V.

The presence of dicofol in organs like liver, kidney and heart indicate toxicity to these organs. This may lead to impairment of vital functions of the body leading to death. DDT and its analogues can inhibit mitochondrial respiration (Ohyama *et al.*, 1982). As the dose of dicofol increases, its ability to deposit

in the body fat will be high. Hence chronic toxicity will be more. Dicofol is capable of accumulating in tissues as residues even at sublethal concentration under chronic exposure (Surendranath and Rao, 1991).

A very small amount of dicofol was also detected in liver tissues of rats of Group V. This may be due to constant licking of dicofol sprayed to the body. Continuous intake of dicofol from the skin lead to its metabolism in liver and subsequent presence of dicofol and its metabolites can be detected in liver tissues.

The detection of the residues of dicofol in liver, kidney and heart confirms that it is a potent toxic chemical to these organs.

5.6 Post-mortem findings

The severity of both the gross and microscopic lesions increased directly with the dose of dicofol. All the experimental groups of rats showed lesions in liver, heart and kidney. Necrotic changes were observed on both the visceral and parietal surfaces of liver. Reports of electron microscopic studies (Muminov *et al.*, 1990) of cochlea of rats in chronic dicofol poisoning displayed golgi

body hyperplasia, mitochondrial swelling, perivascular edema etc. In this study, sections of the liver showed a dose dependant variation in the intensities of cytoplasmic vacuolation in the hepatocytes of the experimental animals belonging to the different groups, besides periportal mononuclear infiltration and focal areas of hepatocyte necrosis.

Experiments conducted by Kostka *et al.* (1996) in rats with DDT, organochlorine pesticide which is a structural analogue of dicofol revealed cytoplasmic vacuolation and focal necrosis of the hepatocytes. But in this study hepatomegaly was not evident probably because the dose of dicofol administered was not sufficient enough to trigger a regenerative response in the hepatocytes. Also this finding was in discordance with O' Brien's report (1974) that short or long term dietary exposure of dicofol in rats causes hepatomegaly. Moreover the degenerative changes in the hepatocytes and renal tubules observed in this study was corroborative of the findings in the experimental trials undertaken in chicken with DDT by Ramalingam (1987).

Echymosis was observed in liver tissue of rats of Group V. This may be due to constant intake of dicofol by licking which could lead to the metabolism of dicofol in liver tissues. Continuous licking lead to the entry of a high amount of dicofol to

liver tissue for detoxification and that could be the reason for echymosis.

5.7 Haematological parameters

5.7.1 Total erythrocyte count

In the present study, the number of erythrocytes in the rats of all the experimental groups was within the normal range of 7-10 millions/ mm^3 (Hrapkiewicz *et al.*, 1998). There was no significant difference in value when compared with that of the control group.

5.7.2 Total leucocyte count

There was no significant difference in the leucocyte counts among control and experimental groups. All the values were within the normal range of 6000-1700 numbers/mm^3 (Hrapkiewicz *et al.*, 1998).

5.7.3 Differential leucocyte count

In the present study, the counts of neutrophils, lymphocytes, eosinophils and monocytes were within the normal

range as described by Hrapkiewicz *et al.*, 1998. The values were also in accordance with that of control group.

5.7.4 Haemoglobin concentration

Haemoglobin values of experimental groups of rats showed no significant difference between them and the control samples. All the values were in the normal range of 11-18g % (Hrapkiewicz *et al.*, 1998).

The assessment of haematological parameters in the present study reveals that there is no change from normal haematological values on chronic exposure to dicofol. Dicofol may not affect the haemopoetic system at these dose levels.

The present study leads to the following conclusions:

1. Dicofol could be detected in vital organs like liver, kidney and heart in all the treated groups.

2. The percentage of mortality in the experimental group ranged from 5-10.

3. Clinical signs of toxicity of dicofol was evident in the experimental group of rats with oral administration.

4. Dermal toxicity of dicofol was noticed on external application.

5. Prolonged administration of dicofol causes a decrease in the body weight of the animal.

6. Histopathological lesions of toxicity of dicofol was seen in liver, heart and kidney.

7. The haematological parameters revealed no definite change from the normal values.

The LD_{50} of dicofol with oral administration in rats is 1495 mg/kg (Budavari, 1989). The information gathered by the present study suggests that dicofol at a dose level of 50mg/kg daily for ninety days is toxic to rats.

Summary

6. SUMMARY

A study was conducted to assess the toxicity of dicofol in rats.

The experiment was done in ninety adult rats weighing 150-200g of either sex. The rats were included in five groups as the control group (Group I) consisting of 10 rats of either sex and the experimental groups (Group II, Group III, Group IV and Group V) consisting of 20 rats of either sex, in each group.

Dicofol was administered orally, once daily for three months, to Group II, Group III and Group IV at the rate of 50 mg/kg, 75mg/kg and 100mg/kg respectively. The rats of Group V were sprayed with 0.1 per cent dicofol, once daily for three months.

The clinical signs of toxicity of dicofol was evident in the experimental group of rats with oral administration. The percentage of mortality ranged from 5-10 in the experimental group of rats. The experimental group with external application of dicofol showed moderate skin irritation and alopecia.

The rats of all the experimental groups showed a decrease in body weight. The loss of appetite resulted in reduced weight gain.

Dicofol was detected in tissues of liver, heart and kidney by thin layer chromatography. The intensity of concentration depends on the dose schedule of administration of dicofol. High concentration of dicofol was detected in the liver tissue of the experimental Group IV. The presence of dicofol clearly in liver, heart and kidney indicate toxicity of dicofol to these organs.

Post-mortem findings indicated the severity of toxicity of dicofol to liver, heart and kidney. The severity of gross and histopathological lesions increased directly with the dose schedule of administration of dicofol. Necrotic changes were observed in the liver. The hepatocytes showed diffuse cytoplasmic vacuolation. The tubular epithelial cells of kidney exhibited degenerative changes. Congestion of the coronary blood vessels and extravasation of erythrocytes were evident in the heart tissue.

The haematological parameters like total erythrocyte count, total leucocyte count, differential leucocyte count and

haemoglobin concentration showed no variation from the normal values.

From the experiment, it was concluded that dicofol is a potent toxic to vital organs. The study revealed a note of caution to the extensive use of dicofol as insecticide.

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DICOFOL TOXICITY IN RATS

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ABSTRACT OF THE THESIS

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ABSTRACT

An experiment was conducted to assess the toxicity of dicofol in rats. A group of ninety adult rats weighing 150-200g of either sex were included in five groups as the control groups consisting of 10 rats of either sex and the experimental groups consisting of 20 rats of either sex, in each group.

Dicofol was administered orally, once daily for three months to the experimental Groups II, III and IV at the dose rate of 50 mg/kg, 75 mg/kg and 100mg/kg respectively. Group V was sprayed 0.1 per cent dicofol, once daily for three months.

The main items of observation included clinical symptoms of dicofol toxicity and assessment of body weight. The clinical signs were evident in the experimental groups. Dicofol caused skin irritation when applied externally. The percentage of mortality was 5-10 in the experimental groups. The rats of experimental groups showed a marked loss of appetite which resulted in decrease in body weight.

The detection of dicofol by thin layer chromatography in liver, heart and kidney tissues showed their presence in these organs. This indicated the toxicity of dicofol to these organs.

Necropsy study conducted revealed both gross and microscopic lesions in the tissues of liver, heart and kidney. The haematological parameters showed no variation in values from that of the normal.

The study revealed a note of caution to the wide spread use of dicofol as insecticide.