Studies on Experimental Benzene Hexachloride Poisoning in Buffalo-calves.

THESIS

Submitted to the Faculty of Veterinary Science
RAJENDRA AGRICULTURAL UNIVERSITY
In partial fulfilment of the requirement for the Degree
of
MASTER OF VETERINARY SCIENCE
IN
MEDICINE

By

Murli Dhar Pandey

JUNIOR RESEARCH FELLOW, R. A. U., BIHAR
POST-GRADUATE DEPARTMENT OF MEDICINE

BIHAR VETERINARY COLLEGE
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DEDICATED TO PARENTS

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P A T N A

Dated, the 25th May, 1978.

This is to certify that the work embodied in this Thesis entitled "STUDIES ON EXPERIMENTAL BENZENE HEXACHLORIDE POISONING IN BUFFALO-CALVES" is the bonafide work of Dr. Murli Dhar Pandey and was carried out under my guidance and supervision.

25/5/78

(S. S. MISHRA)

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9/9/78.

C E R T I F I C A T E

Certified that the research work encorporated in this Thesis has not been published in part or in full in any of the journals.

M D Pandey
25/5/78

(M. D. PANDEY)

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ACKNOWLEDGEMENT

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(M. D. PANDEY)

C O N T E N T S

INTRODUCTION

INTRODUCTION

Man has evolved chemicals which could poison insects and other pests that injure or destroy his crops or livestock. In India, with the introduction of high yielding varieties of crops together with the monoculture, the control of pests has assumed a greater importance. Besides the crop management as well as the rapid growth of livestock farming in the country, it has become essential to control livestock pests which usually act as vectors of different diseases. Among the various methods of pest management, use of pesticide has become widespread.

The production of pesticides in India started in 1952 and informations available indicate that the consumption and production of pesticides have increased year after year (Singh and Bhatti, 1974). About 40,000 tons of technical grade pesticides are being consumed in agriculture and 10,000 tons in public health programmes and by the end of Fifth Plan consumption is expected to reach a level of eighty five thousand tons (Paharia, 1974).

Among the various pesticides used in our country, BHC constitutes the highest percentage (47,000 tons) Visweswariah et al. (1974).

Among the five isomers of Benzenehexachloride which was introduced in United Kingdom in the year 1942, only gamma isomer had the highest insecticidal activity. Thus the toxicity of BHC is proportional to its gamma isomer content.

The gamma isomer causes "grand mal" type of electro-encephalogram with bradycardia and increased blood pressure. The other symptoms are of neuromuscular type including excitement, accelerated respiration, trembling, blepharospasms, the twitching of the facial and cervical muscles, increased salivation, champing of the jaws, abnormal postures, rise of temperature followed by convulsions and death due to respiratory failure (McNamara, 1948; McEnerney, 1951 and Clarke and Clarke, 1967).

According to 1961 census, India has 24.24 million of breeding buffaloes giving 58% of milk against 51.01 million breeding cows producing 42% of total milk yield from cows and buffaloes. On the other hand, buffalo milk contains more fat than that of cow. It has also been noted that the productive life and lactation period of a buffalo is much longer and dry period is shorter in comparison to those of the cows. This shows that the buffaloes have better potentiality to meet the pressing challenge of supplementing diet of ill fed vegetarian Indians (Patrick John, 1969). Thus emphasis has been given in national breeding programmes for boostering buffalo population so as to usher in white revolution. Thus, the buffaloes will be a dominant species exposed to the hazards of pesticides.

The widespread use of insecticides and other pesticides has introduced a serious and novel health hazard to livestock. The buffaloes are inquisitive animals and apparently consume almost anything placed in their way. When they are kept accidentally on pasture sprayed with gamma BHC, the unfortunate beasts have no alternative but to eat or starve. Hazards of acute poisoning in

the farms also include the accidental ingestion of gamma BHC, inhalation while dusting or spraying, licking of emulsion or paste applied on the skin for the control of ectoparasites besides its absorption through intact skin (Jager, 1970).

Jager (1970) while reviewing the literature on dieldrin and its sister compounds concluded that the species difference plays an important role in causing various types of hepatic lesions.

Literature available on the poisoning of BHC in different species of animals indicate that most of the studies have been done in cattle, sheep and goat with least attention towards buffaloes and as such no specific antidotes for BHC poisoning could be introduced for them.

The present experimental study thus, was envisaged to study the symptomatology, biochemical, haematological, post-mortem and histopathological changes and to evolve antidotal treatment against gammexane poisoning in buffalo-calves.

The detailed knowledge about above mentioned changes can give some useful indications for the purpose of diagnosis of gammexane poisoning in these animals, particularly when considered in association with the history of a case and clinical observa - tions. On the other hand, specific antidotes evolved in regard to gammexane poisoning would help clinicians to treat the animals poisoned with gammexane.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Occurrence.

Lehman and Arnold (1948) reported that gamma BHC at the rate of 125 mg/kg body weight was the mean lethal dose for rats when given orally.

Karl and Loren (1950) used 0.06% dip of gamma BHC to control mites in 75 ewes and 15 lambs. He further reported that 10 out of 15 lambs died and 7 ewes gave birth to dead lambs.

Leighton et al. (1951) observed that Jersey cow can consume 5 gm of toxaphene a day without harmful effects. Larger quantities given for a period of 2 to 3 weeks caused severe toxicity or death.

Ramanujam and Gurumurti (1951) reported BHC poisoning when 4 ounce packet of 20% lindane wettable powder was made in emulsion and 2/3 of it was applied to the body of ongole cow and remaining to the body of its heifer aged about one and half year. The heifer showed symptoms of poisoning sooner whereas cow after 7 hours ofapplication.

Jolly (1952) has reviewed the method of applications, toxicity, metabolism, general toxic hazards and post-mortem findings of DDT and BHC in domestic animals.

Jolly (1954) studied the acute toxicity of dieldrin as a sheep dip for prevention of maggot fly strive. He assessed the acute oral LD 50 of dieldrin to sheep to be 50 to 75 mg/kg

body weight. He further reported that sheep survived a single dieldrin dipping at 0.5 to 1.0% without any ill effect.

Hamilton (1955) reported that out of 5 ponies which ate BHC contaminated bran, two died of poisoning.

Jerome (1958) studied endrin poisoning in cattle as a result of spray at 1:176 dilution. Out of 370 animals four calves suffered from fits within two hours and 45 cattle died after 2 hours and 20 minutes.

Moraillon (1958) reported the toxaphene poisoning in a flock of 35 ewes and 10 yearlings when 75 litres of a 2.5% emulsion of toxaphene had been discharged into the pastures from a faulty compressor. An ewe and a yearling died one day after the sheep were introduced to the contaminated pasture.

Pearson et al. (1958) reported an outbreak of aldrin poisoning in 107 suckling lambs as a result of misuse of insecticide concentrate containing aldrin causing death of 105 lambs.

Burgisser (1960) described BHC poisoning in one week old puppies applied to the bitch before whelping. He assumed that the bitch had excreted it in the milk.

Koudela (1961) reported BHC poisoning in young cattle when an unknown quantity of 10% BHC powder was accidentlly mixed with food. It was thought that the calves might had ingested about 10-50 gm/100 kg body weight of the powder.

Pethy and Moore (1964) described 85 to 125 mg of gamma BHC/kg body weight as LD 50 for acute toxicity when given orally.

Blood and Henderson (1968) have reported toxic oral dose of lindane to be 5, 25 and 25 mg/kg body weight for calves upto 2 weeks, cattle and sheep respectively. The maximum safe spray concentrations have been reported to be 0.025, 0.1, 1.00 and 0.5% for calves upto 2 weeks, cattle, sheep, pig and horses respectively. The oral toxic dose of BHC has been reported to be 1,000 mg/kg body weight in cattle, sheep, pig, goat and horses.

Kunhurt et al. (1969) have reported combined DDT and BHC poisoning in horses due to cummulative effects resulting from the use of food contaminated with small quantities of DDT and BHC for about 60 days. Out of 21 horses, 16 were reported to die or were slaughtered.

Radeleff (1970) reported that gamma BHC proved lethal for dairy calves under two weeks of age at a dosage of 5 mg/kg and higher for adult cattle at 25 mg/kg and higher when given orally. He further reported that the dairy calves under two weeks of age might be killed by sprays or dips containing as low as 0.05% of gamma isomer and adult cattle by 0.3%.

Bhaskar and Sreemannarayana (1973) reported a clinical case of endrin poisoning in a she-buffalo.

Sharma and Gautam (1973) studied the experimental endrin poisoning in 13 calves and observed that death occurred as a

result of grazing on grass sprayed with 0.2 to 0.4% and 9 after oral administration of 2 mg/lb body weight of pesticide.

Gautam and Sharma (1974) have reported accidental endrin poisoning in Hissar area as a result of grazing of the animals in recently sprayed fields or use of endrin containers or accidental mixing of the insecticides with food or water.

Jones (1974) reported no harmful effects when mature sheep, goat and cattle were treated repeatedly at 4 days interval with spray/dips of technical BHC containing 0.5 to 0.24% of gamma isomer. However, death in cattle was recorded with 0.75% of gamma isomer. It was further observed that single application of gamma BHC spray/dips to baby calves produced toxicity at 0.05% but not at 0.025% concentration.

McParland et al. (1974) observed BHC poisoning in cattle when a powder containing 19.1% gamma BHC was administered to eight cows, mistaking it for calcined magnesite. Cows which received 112 gm or more of powder was reported to die while those given 70 gm survived.

Ray et al. (1975) reported BHC poisoning in cattle dipped in an emulsified preparation of BHC (0.14% active ingredient) labelled for plant use. Out of 174 cattle, 18 were reported to be fatally poisoned.

Symptoms.

Radeleff (1948) observed the symptoms of ataxia, motor-blindness, circus movement, convulsion, bleating or groaning,

inappetence, billigerency, cyanosis before death and death in agony in cholordane poisoning in sheep and heifers.

McEnerney (1951) has reported an acute poisoning in calves following the feeding of meal contaminated with gamma BHC. The calves were bawling, trembling, salivating profusely, arching their neck sharply backward, staggering, falling and paddling with their legs within 45 minutes of feeding. Calves were reported to have died within 1 to 5 hours after eating contaminated meal.

Ramanujam and Gurumurti (1951) observed BHC poisoning in a cow and its calf. The calf was found in prostration, kicking and struggling but unable to get up from the ground, temperature being 102°F, excessive salivation, jaws tightly closed, eyes protruding wide and staring with pupils fully dilated, muscular twitchings especially at neck, hyperexcitability to stand, great pain and suffering. Before death calf was reported to fall prostrate on the ground, struggle violently and bellow very loudly. The cow showed the same symptoms as mentioned above and in addition, aimless walking, increased pulse rate and quick respiration were constantly observed.

Jolly (1954) studied the nervous symptoms manifested by hypersensitivity, convulsion and depression in the acute toxicity of dieldrin in sheep.

Hamilton (1955) observed tetanic spasm, great pain, sweating, laboured breathing, violence and colic in gammexane

poisoning in horses.

Jerome (1958) has reported endrin poisoning in cattle. The peracute symptoms were sudden throw up their heads as though startled, twitching of facial muscles and ears with rapid blinking of the eyes and jumping as though frightened. Death was reported within 30 seconds following respiratory and cardiac arrest. The observed symptoms in the acute cases were falling on the ground, spasmodic attempts to rise, tetanic convulsions, grinding of the teeth, excessive salivation, respiratory embarrassment with short convulsive inhalation and exhalations, muscular spasm, uncontrolled defecation and micturition and death within few minutes. In subacute cases, there was cyanosis and milder degree of signs as compared to peracute and acute cases.

Loomis (1966) described the symptoms of dieldrin poisoning in puppies as steady loss of weight, depression with immobility, intermittent periods of irritability followed by terminal brief epileptiform convulsion.

Radeleff (1970) studied the symptoms of chlorinated hydrocarbon insecticide poisoning in animals and observed apprehension and hypersensitiveness, blepharospasms and fasciculation of the facial and cervical muscles. Repeated or intermittent spasms and increase in flow of saliva followed by champing of jaws were recorded. The loss of co-ordination, aimless walking and abnormal postures were also recorded. Convulsions clonic to tonic in nature accompanied by periods of paddling movement, nystagmus, grinding of the teeth and

groaning or grunting were noticed. The convulsive seizures resulting in sharp rise of body temperature (114 to 116°F), dyspnoea and death followed by respiratory failure were observed.

In contrast to those active symptoms, in some cases depression, drowsiness, inappetence and reluctance to move about followed by emaciation and dehydration were reported to persist until death.

Singh and Thakur (1973) reported a clinical case of gammexane poisoning in one and half year old bull calf which showed the symptoms of excitability, salivation, grinding of the teeth, dyspnoea and severe convulsion.

Gautam and Sharma (1974) reported the symptoms of the cases of accidental endrin poisoning in animals with excitement, incoordination, muscular tremor and convulsion, excessive salivation, froth from the mouth, conjunctivitis rise of temperature and respiration, aimless walking as well as repeated convulsive seizures at certain intervals. In some cases, death occurred within a few hours of ingestion of the insecticides.

Singh et al. (1974) studied the symptoms of experimental endrin poisoning in buffalo-calves with dullness, depression, excessive salivation for a few minutes followed by hypersensitiveness and sharp response to external stimuli. Congestion of the eye-balls, profuse salivation, clamping of the jaws, grinding of the teeth, occasional grunting, protrusion of tongue, convulsions, incoordination and staggering gaits, frog-sitting position typical with stiffneck extended to lateral side and

paddling movement of legs in lying position were also recorded. The dyspnoea with accelerated respiratory movements and increased heart rate to about 3 to 4 times more than normal were reported.

Haematology.

Hothi and Kwatra (1972) studied the aldrin poisoning in experimental buffalo-calves and found appreciably low values for haemoglobin content, erythrocytic count and packed cell volume. There was an increase in the leucocytic count upto the 17th day of the study.

Malik et al. (1973) studied haematology in acute dieldrin poisoning in buffalo-calves and found an increase in leucocytic count particularly in the percentage of neutrophils without evoking any change in haemoglobin, haemotocrit and total erythrocytic count.

Sharma and Gautam (1973) observed a drop in haemoglobin, erythrocytic count and packed cell volume and increase of total number of leucocytes particularly lymphocytes in experimentally induced endrin poisoning in calves.

Biochemical analysis.

Blood glucose:

Huthway and Mallinson (1964) reported an accelerated rate of glycolysis in insecticide toxicity in experimental rats.

Malik et al. (1973) found that the blood glucose values

were significantly increased during the evaluation of therapeutic measures in acute dieldrin poisoning in buffalo - calves.

Giurgea et al. (1976) concluded that lindane interferes with carbohydrate and fat metabolism and with the hormonal regulation following intake of lindane in chicken.

Hurkat (1977) observed comparative study of dieldrin induced hepatotoxicity in rabbits and rats and concluded that with the administration of dieldrin, the glycogen content of liver cells diminished in both the animals. Much more marked loss of glycogen was reported in the terminal stage of the study in rabbit.

Singh et al. (1977) reported that the average glucose value of blood collected before administration of endrin and during convulsions induced by insecticide were 53.3 and 135.1 mg/100 ml of blood respectively.

Serum alkaline phosphatase:

Hurkat (1977) observed in histochemical study that in dieldrin treated rabbits the SAP activity was considerably increased. He further reported that with the fatty degeneration and necrosis, the alkaline phosphatase activity became more diffuse.

Post-mortem and histopathology.

Furman (1947) studied the toxicity of BHC in mammals and

reported haemorrhagic gastritis, duodenal enteritis and in some cases intestinal tract was found swollen containing gas and food

Karl and Loren (1950) studied the losses in lambs dipped in BHC and reported about post-mortem findings. They observed that the stomach and caecum were greatly distended with gas whereas intestine was moderately distended probably due to atony of the intestinal tract. Liver and lungs were observed to be hyperemic and in one liver there was mild fatty metamorphosis. Cerebrum was found to be essentially normal on microscopic examination.

Hamilton (1955) found haemorrhage in mesenteric lymphnodes and mesentery during post-mortem examination of 5 gammexane poisoned ponies. He also observed the diffuse area of inflammation on the intestinal wall, fibrinous exudate between stomach and diaphragm and presence of blood tinged fluid in the pericardial sac. Friability of the kidney and liver; and petechial haemorrhages on the myocardium were also observed in 5 autopoisoned ponies.

Adamic (1958) observed the degeneration of liver and kidney and lesions in ganglion cells in ducks and fowls experimentally poisoned with gammexane.

Jerome (1958) reported myocardial haemorrhages varying from petechiae to infarcts, acute swelling of the liver and spleen in endrin poisoning in cattle. However, in one case he found excess of dark coloured fluid in the pericardial sac.

Baxter (1959) studied the histopathology of aldrin poisoned lambs and reported; acute toxic hepatitis in which a well marked central necrosis undergoing repair by regeneration of liver cells and by replacement with fibrous tissues. Acute hephritis of renal tubule without indication of undergoing resolution were also recorded. Acute pulmonary congestion associated with respiratory failure were also observed by him.

Radeleff (1970) reported the cloudy swelling of most of the viscera with distinct blenching of the intestines due to high activity and coeval rise in body temperature. Small haemorrhages occurring atrandom through out the body but more consistently on heart were seen. The heart was found to be in systole with excessive pericardial fluid. The lung was reported to be heavily congested, dark in colour showing haemorrhages and oedema. In some cases blood tinged exudate was seen in the bronchioles with lobar or lobular involvement. The brain and spinal cord were usually congested and frequent oedematous with excess of crebrospinal fluid. In chronic cases, foregoing lesions, together with degenerative changes in the liver and kidney were reported.

Smith (1972) reported petechial and echymoses on and in the heart and also in many other places. Oedema and pulmonary congestion either localised or diffuse were observed in lungs. Nissler's degeneration and necrosis of the neurons, especially in the ganglia of the medulla, cerebellum and brain congestion in CNS and presence of increased cerebrospinal fluid were also

seen in animals poisoned with chlorinated hydrocarbon insecticide groups. In acute but in prolonged cases, toxic hepatitis and acute toxic tubular nephrosis were noted, centrilobular necrosis being especially prominant. Enteritis was noted in acute poisoning by oral route.

Sharma and Gautam (1973) found the gelatinization and liquifaction of the body fats especially in medullary portion of kidney alongwith degenerative changes in experimental dieldrin poisoning in calves.

Treatment.

Mc Namara and Krop (1948b) suggested the use of pentobarbital sodium and atropine sulphate to protect from CNS stimulation and bradycardia respectively produced by BHC poisoning.

Lehman and Arnold (1948) suggested the doses of phenobarbital sodium below the anaesthetic dose to control the tremors and convulsions of DDT poisoning.

Ramanujam and Gurumurti (1951) reported a case of BHC poisoning in cattle as a result of BHC emulsion application with successful treatment. The cow was thoroughly washed and given cold water to drink and on the following day magnesium sulphate, sodium chloride and pulv. zingiber were given as drench.

Jolly (1951) advocated the use of glucose saline in view of the liver damage associated with BHC poisoning.

Garner (1957) advocated the use of chloral hydrate and pentobarbitone to control convulsions together with calcium borogluconate and glucose saline I/V to avoid liver damage as well as saline purgative in chlorinated hydrocarbon poisoning.

Jerome (1958) successfully treated the subacute cases of endrine poisoning in cattle with pentobarbital sodium with sinan as well as chloral hydrate (7%) intravenously. The peracute and acute cases had been reported to have died before any medical aid could be given.

Radeleff (1970) reported the use of narcotic or anaesthetic agents such as chloral hydrate or barbiturates for as long as 24 hours and removal of toxicants from body. In dull, listless and unreactive poisoned animal, stimulants had been indicated. Calcium gluconate had been mentioned as an effective antidote. The cold water had been reported to aid in bringing the temperature down near normal.

Bhaskar and Sreemanarayana (1973) reported a clinical case of endrin poisoning in a she-buffalo and treated successfully with atropine sulphate (2 mg/hour I/M), chloral hydrus 10% (0.5 ml/kg body weight I/V), calcium borogluconate 25% (300 ml I/V) and dextrose 25% (500 ml I/V) in repeated doses.

Malik et al. (1973) studied the evaluation of therapeutic measures consisting of chloral hydrate (12%) + magnesium sulphate (6%) (0.5 ml/kg I/V), atropine sulphate (0.5 mg/kg),

glucose (1 ml/kg I/V), d-tubocurarine (0.025 mg/kg I/V) and calcium borogluconate in acute dieldrin poisoning in buffalo-calves. The measures used were reported to increase the survival period but could not save the animals.

Mc Parland et al. (1973) reported BHC poisoning in cattle and suggested that lower doses with calcium borogluconate and chloral hydrate might be of value.

Singh and Thakur (1973) reported a clinical case of gammexane poisoning in a buffalo calf with successful treatment with thiopental sodium 250 mg I/V, calcium borogluconate 100 ml I/V and chloral hydras 1 dram orally.

Sharma and Gautam (1973) studied the experimental endrin poisoning in 13 calves and treatment with chloral hydrate 10%, calcium borogluconate 10% and saline purgative was successful in four out of five cases.

Gautam and Sharma (1974) reported the clinical cases of endrin poisoning in animals and treated successfully with chloral hydras, calcium gluconate, saline purgative and fluid therapy in some of the cases.

Sathuraman (1977) reported a case of BHC poisoning in a heifer calf and treated successfully with atropine sulphate 15 mg S/C, coramine 2 cc, glucose solution 10% 500 cc S/C, saline solution 500 cc by stomach tube and calcium borogluconate 200 cc I/V.

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study was carried out on 23 apparently healthy male buffalo-calves of approximately same age and weight. They were maintained under similar nutritional and environmental conditions. They were divided randomly into 4 groups i.e. group I, group II, group III and group IV.

All the animals under-study were clinically examined before exposing them to gammexane so as to ensure that they were free from systemic and parasitic diseases.

To each buffalo-calf a single dose of gammexane 50% WDP (The Alkali and chemical corporation of India Ltd., Bombay) suspended in one litre of tap water was administered orally through a stomach tube at the rate of 100 mg/kg body weight.

Experimental design.

Group I:

It consisted of five calves. Symptoms were observed after the administration of gammexane. Post-mortem and histopathological changes in liver, kidney, lung, intestine, heart, spleen, brain and spinal cord were recorded after the death of gammexane poisoned calves.

Group II:

The group was divided into two subgroups.

Subgroup A :- It consisted of 5 animals. After the appearance of clinical symptoms of poisoning, the calves were subjected to treatment with the following drugs separately in the sequence mentioned below :

(i) Phenobarbital sodium (Gardenal sodium - M & B) :- It was administered at the rate of 13 mg/kg body weight intravenously.

(ii) Atropine sulphate (Bengal immunity Ltd.) :- It was administered at the rate of 0.25 mg/kg body weight. Half of the calculated dose was given intravenously and half intramuscularly.

(iii) Dextrose 20% (Mc Gaw Ravindra Laboratories) :- Irrespective of the body weight, it was administered at the rate of 450 ml/day in two divided doses intravenously.

The 2nd dose (one half of the first dose) of drugs (i) and (ii) was given when the clinical symptoms of poisoning reappeared. Later on only drug (i) (one half of the first dose) was given whenever needed.

Subgroup B :- It consisted of only one calf. The calf was treated as control for subgroup A and was allowed to die eventually without any treatment to judge the therapeutic efficacy of drugs administered in subgroup A.

Group III:

This group was divided into two subgroups.

Subgroup A:- It consisted of 5 calves. On the appearance of clinical symptoms of poisoning, calves were treated with following drugs separately in sequence given below:

(i) Chloral hydrate 12% and Magnesium sulphate 6% :- The solution was prepared in sterile distilled water and was administered at the dose of 0.75 ml/kg body weight. The total calculated dose was injected slowly through intravenous route.

(ii) Atropine sulphate (B.I) :- The dose and route of administration was same as in subgroup A of group II.

(iii) Dextrose 20% (Mc Gaw) :- The dose and route was same as in subgroup A of group II.

The 2nd dose (one half of the first dose) of drugs (i) and (ii) was given when the clinical symptoms of poisoning reappeared. Later on only drug (i) (one half of the first dose) was given whenever needed.

Subgroup B :- It consisted of only one calf which was control for subgroup A of group III. Rest was same as mentioned in B of group II.

Group IV:

This group was further divided into two subgroups.

Subgroup A :- It consisted of 5 animals. After the appearance of clinical symptoms of poisoning, calves were

treated with following drugs separately in the sequence given below:

(i) Diazepam (Calmpose - Ranbaxy) :- It was administered at the dose of 2.00 mg/kg body weight. Half of the calculated dose was injected slow intravenously and rest half intramuscularly.

(ii) Atropine sulphate (B.I) :- The dose and route of administration was same as in subgroup A of group II.

(iii) Calborol (M & B):- It was administered daily at the dose rate of 2 ml/kg body weight intravenously.

The 2nd dose (one half of the first dose) of drug (i) and (ii) was given when the clinical symptoms of poisoning reappeared. Later on, only drug (i) one half of the first dose) was given whenever needed.

Subgroup B :- It consisted of only one calf which was control for subgroup A of group IV. Rest was same as in subgroup B of group II.

The time interval between administration of gammexame and appearance of clinical symptoms of poisoning, administration of gammexane and death of the calves and survival period after the appearance of the clinical symptoms were also recorded.

In addition to above, each calf of all the groups were

subjected of poisoning and one week after treatment if calves survived.

Clinical examinations.

The following clinical observations were made:

(1) General condition of the calves.
(2) Pulse rate/minute.
(3) Respiration rate/minute.
(4) Rectal temperature in ^{o}F.

Haematological studies.

The anticoagulant solution (Heller and Paul, 1934) was dispersed in vials at the rate of 0.1 ml for each 1.0 ml of blood. The vials were kept in sterilizer at 60^{o}C to get it dried. Jugular blood was collected from each calf in the same vial. The following haematological studies were carried out.

Total erythocyte count (TEC):

It was determined by standard haemocytometer (Boddie, 1962). The Hayem's solution was used for the purpose.

Total leucocyte count (TLC):

It was determined by standard haemocytometer (Boddie, 1962). The two per cent acetic acid was used as diluting fluid.

Differential leucocyte count (DLC):

DLC was carried out as per the procedure described by

Boddie (1962). The Leishman's stain (Sarabhai chemicals) was used for the purpose.

Packed cell volume (PCV):

It was determined by the wintrobe haematocrit method (Schalm, 1967).

Haemoglobin (Hb%):

It was estimated by acid haematin method using Sahli's haemoglobinometer (Schalm, 1967).

Biochemical analysis;

In this serum alkaline phosphatase and blood glucose levels were estimated as follows:

Serum alkaline phosphatase (SAP):

The serum was separated and tests were carried out as soon as possible. The reading was recorded with the help of klett summersion photoelectric colorimeter. The method of determination of SAP was adopted as per methods given in the leaflet of SAP kit supplied by Bharat Laboratories, Bombay.

The method is based upon hydrolysis of phenylphosphate by alkaline phosphatase under defined conditions of time, temperature and pH, releasingphenol, which is estimated colorimetrically after successive additions of sodium bicarbonate, aminoantipyrine and potassium ferricyanide

solutions which adjust pH and develop reddish brown colour. The colour produced is compared to that of standard containing known amount of phenol.

Blood glucose:

Blood glucose was estimated using Folin-Wu tube and the reading was recorded with the help of klett summersion photo-electric colorimeter. The method of determination of blood glucose was adopted as per instructions method given in the leaflet of blood glucose kit supplied by Bharat Laboratories, Bombay.

The method used is based upon the principle that when protein free blood filtrate is heated with an alkaline copper solution, the sugar reacts in alkaline solution, being oxidised by cupric ion, which is simultaneously reduced to cuprous and precipiated as cuprous oxide, Cuprous oxide is dissolved by and reduces phosphomolybdic acid solution to a blue colour which is measured colorimetrically.

Urine examinations.

Urine samples were collected in clean test tubes and were examined for the presence of albumen and sugar.

Urine albumen:

Robert's test was carried out to detect the presence of albumen in urine samples (Ganti, 1971).

Urine sugar:

Benedict's qualitative test was carried out to detect the presence of sugar in the urine samples (Ganti, 1971).

Pathological examinations.

Post-mortem examination:

Post-mortem of poisoned calves were conducted as soon as possible after their death. The gross pathological changes were recorded.

Histopathological examination:

At the time of post-mortem, pieces of liver, kidney, lung, intestine, heart, spleen, cerebrum and spinal cord were collected and fixed in 10% formol saline solution. Tissues were embedded in paraffin. The routine process of washing, dehydration and clearing with cedarwood oil was followed. After microsectioning, the sections were deparaffinized and stained with haematoxylin and eosin.

RESULTS

RESULTS

In the present study a total number of 23 male buffalo-calves, approximately of the same age and weight were taken up and maintained under similar nutritional and environmental conditions. They were divided randomly into 4 groups.

Group I	(control)	--	5 buffalo-calves.
Group II		--	5 + 1 = 6 buffalo-calves.
Group III		--	5 + 1 = 6 buffalo-calves.
Group IV		--	5 + 1 = 6 buffalo-calves.

Group I acted as control group for the present experimental design. One calf of the group II to IV was kept as control for the respective groups. For the convenience of statistical analysis, above mentioned 3 control calves of the groups II to IV were shifted to the control group I, thus the total number of the calves of the control group became eight.

Before the administration of gammexane (BHC), clinical examination, haematological study, biochemical analysis and urine examination were carried out in all the groups.

The calves were weighed to calculate the actual dose of gammexane to be administered to produce acute poisoning. The calves of all the four groups under study were drenched with gammexane through a stomach tube at the dose rate of 100 mg/kg body weight, suspended in one litre of tap water.

Clinical symptoms.

Just after the administration of gammexane at the above dose rate, the calves looked dull and severely depressed with flow of saliva for a few minutes. Thereafter the symptoms mainly observed were like neuromuscular type. At the onset of the symptoms, the affected calves were hypersensitive and responded sharply to external stimuli. The calves were having tendency to be away from the groups. The congested conjunctiva, dilated pupil with bulging and rolling of eye ball (nystagmus) and muscular twitching especially of the neck portion were recorded (Fig. I). Concurrent with the appearance of muscular spasms, there was an increase in the flow of saliva, grinding of the teeth and champing of the jaws thereby producing froth that adhered to the lips and muzzle. The muscular spasms commencing from face and neck were observed to extend backward untill all the body musculatures were involved. As the action of toxicant grew more intense, the calves were increasingly agitated and lost coordination. The calves were unable to stand and suddenly fell on the ground with spasmodic attempts to rise but in vein. Abnormal posture like dog sitting position and keeping the head down between the forelegs was seen. Occasionally, there was grunting or groaning and protrusion of the tongue. The muscular twitching was soon followed by convulsion. The stiff and extended neck, muscular twitching through out the body; and convulsion used to force the calves to lie down on the ground. During recumbency, the calves used to struggle violently and showed paddling movement of the legs

(Fig. II). During convulsion involuntary defection, micturition and bloat were observed as constant signs. The respiratory embarrassment with short convulsive inhalation and exhalation were noticed. The recurrent convulsive seizures were observed to be present till death. As the seizures prolonged, dyspnoea was observed and death was followed by respiratory failure.

There was significant increase in pulse, respiration rate and body temperature (Table 2 & 2"A").

The calves were allowed to die eventually in natural course of poisoning. The mean survival period after the appearance of clinical symptoms was 6.10 ± 0.68 hours in the calves of group I.

Clinical examinations.

Before the administration of gammexane, all the calves under study were subjected to thorough clinical examinations. Following examinations, all the calves were found to be in normal health with good look and temperament having no wounds or the lesions of ectoparasites over the body.

Pulse, Respiration rate per minute and Temperature(^{o}F).

The mean pulse rate was markedly increased from 54.78 ± 1.04 to 113.04 ± 0.91. The maximum pulse rate was 114.40 ± 1.91 in group III whereas the minimum was 109.60 ± 2.16 in group II

in post-poisoning stage. There was no significant variation in pulse rate between groups either in pre or post-poisoning stage. However, highly significant increase in pulse rate was observed in post-poisoning stage over pre-poisoning one (Table 2, 2"A" and 2"B"). In calves which survived after treatment, the mean pulse rate was 73.00 ± 3.46. A very significant increase in pulse rate in survived cases was recorded over pre-poisoning one (Table 5 and 5 "A").

The mean respiration rate was markedly increased from 14.74 ± 0.40 to 52.39 ± 1.77. The maximum respiration rate was 59.40 ± 3.30 in group IV whereas minimum was 45.75 ± 2.52 in group I in post-poisoning stage. Highly significant increase in respiration rate was observed in post-poisoning stage over pre-poisoning one. However, no significant variation in respiration rate was observed between groups either in pre or post-poisoning stage (Table 2, 2"A" and 2 "B"). The respiration rate was 19.33 ± 0.88 in survived cases. A highly significant increased respiration rate in survived cases was recorded over pre-poisoning one. (Table 5 and 5 "A").

The mean temperature was 104.55 ± 0.17 varying from 104.10 ± 0.17 in group IV to 105.14 ± 0.25 in group III. No significant variation in temperature between groups was recorded either in pre or in post-poisoning stage. However, highly significant increase in temperature was recorded in post-poisoning stage to that of pre-poisoning one (Table 2, 2"A" and 2 "B").

The mean temperature was 99.35 ± 0.44 in survived cases. A highly significant decrease in temperature was observed in surviving cases over pre-poisoning stage (Table 5 and 5 "A").

Haematological studies.

The haematological studies were carried out in all the groups at pre and post-poisoning stages and on 7th day of treatment in survived cases (Table 1, 1 "A", 1 "B" 4 and 4"A").

Total erythrocyte count (TLC).

The mean total erythrocyte count in pre-poisoning stage was 6.81 ± 0.33 whereas 6.91 ± 0.30 in post-poisoning stage. There was no significant variation between groups either in pre or post-poisoning stage. Similarly no significant variation could be recorded between pre and post-poisoning stages. There was no significant variation in TEC also in survived cases on 7th day of treatment.

Total leucocyte count (TLC).

The mean total leucocyte count was 8.49 ± 0.05 in pre-poisoning stage whereas 11.40 ± 0.15 in post-poisoning stage. The maximum TLC was 11.59 ± 0.35 in group I whereas minimum was 10.99 ± 0.25 in group II in post-poisoning stage. A highly significant increase in TLC was recorded in post-poisoning stage to that of pre-poisoning one. However, no significant variation could be recorded between groups either in pre or post-poisoning stage.

In survived cases, the mean TLC was 14.45 ± 0.24 showing thereby a highly significant increase in TLC to that of pre-poisoning one.

Differential leucocyte count (DLC).

There was highly significant increase in number of neutrophil from 30.04 ± 0.56 to 46.57 ± 0.88 with corresponding highly significant decrease in number of lymphocyte, eosinophil and monocyte from 58.00 ± 0.65 to 49.07 ± 0.90, 2.83 ± 0.29 to 1.70 ± 0.17 and 3.91 ± 0.20 to 2.35 ± 0.22 respectively. However, no significant change could be recorded in number of basophil.

In survived cases only very significant increase in number of neutrophil was recorded on 7th day of treatment in comparison to pre-poisoning one. However, there was no signifficant variation in number of lymphocyte, eosinophil, basophil and monocyte.

Packed cell volume percentage (PCV%).

The mean PCV was 32.52 ± 0.55 and 32.61 ± 0.54 in pre and post-poisoning stages respectively. A very significant increase in PCV was recorded in post-poisoning stage over pre-poisoning one. However, no significant variation in PCV could be noted between groups either in pre or post-poisoning one.

In survived cases, no significant variation in PCV could be recorded on 7th day of treatment over pre-poisoning one. The mean PCV in survived cases was 33.42 ± 1.06.

Haemoglobin gm. percentage (Hb gm.%).

The mean Hb was 10.25 ± 0.63 and 10.13 ± 0.25 in pre and post-poisoning stages respectively. No significant variation could be recorded in Hb between groups either in pre or post-poisoning stage. Similarly no significant variation in Hb could be found in post-poisoning stage over that of pre-poisoning one.

In survived cases, there was very significant decrease in Hb on 7th day of treatment from 12.27 ± 0.33 to 10.00 ± 0.12.

Biochemical analysis.

The results of serum alkaline phosphatase and blood glucose level in all the four groups of calves have been presented in Table 3, 3 "A" and 3 "B".

Blood glucose mg%:

It was observed that the blood glucose levels were markedly increased from 58.08 ± 3.49 to 135.83 ± 4.35 in group I 60.20 ± 1.55 to 129.11 ± 15.73 in group II, 60.89 ± 2.30 to 135.91 ± 4.11 in group III and 60.00 ± 1.93 to 133.74 ± 5.13 in group IV after poisoning. The mean blood glucose level of all the groups in pre-poisoning stage was 59.58 ± 1.41 while that of post-poisoning stage was 133.93 ± 3.73. There was no significant variation between groups either in pre or post-poisoning stage. However, a highly significant increase in blood glucose level was recorded in post-poisoning stage over

pre-poisoning one.

In survived cases, the mean blood glucose level tended to come towards normal although there was still highly significant increase in blood glucose level in survived cases to that of pre-poisoning one (Table 6 and 6 "A").

Serum alkaline phosphatase (SAP) King-Armstrong units % :

It was observed that the SAP level was markedly increased from 14.34 ± 1.34 to 32.82 ± 2.02 in group I, 11.55 ± 1.57 to 28.93 ± 1.76 in group II, 16.27 ± 1.05 to 33.05 ± 1.90 in group III and 14.36 ± 2.61 to 29.00 ± 2.54 in group IV. The mean SAP level of all the groups in pre-poisoning stage was 14.16 ± 0.85 while that of post-poisoning stage was 31.20 ± 1.07. There was highly significant increase in SAP level in post-poisoning stage to that of pre-poisoning one. However, no significant variation could be recorded between groups either in pre or post-poisoning stage.

In survived cases, the SAP level tended to come towards normal although there was still very significant increase in mean SAP level in survived cases to that of pre-poisoning one (Table 6 and 6 "A").

Urine analysis.

Qualitative tests of collected urine samples of all the four groups of calves either in pre or post-poisoning stages were found negative for sugar and albumen.

Mean time interval.

The mean time intervals have been presented in Table 7 and 7 "A". The maximum time interval between administration of gammexane and appearance of clinical symptoms was 5.38 ± 0.41 hours in group II whereas minimum was 3.98 ± 0.41 hours in group I with an average value of 4.66 ± 0.22 hours for all the groups. The maximum time interval between administration of gammexane and death of the calves was 104.92 ± 12.33 hours in group IV and minimum was 10.08 ± 0.98 hours in group I with an average value of 62.96 ± 10.40 hours of all the groups. The maximum time interval between appearance of clinical symptoms and death of the calves was 99.69 ± 12.38 hours in group IV whereas minimum was 6.10 ± 0.68 hours in group I with an average value of 53.53 ± 1.68 hours.

There was very significant variation between groups in time interval between administration of gammexane and appearance of clinical symptoms. A highly significant variation was recorded between groups in time interval between administration of gammexane and death of the calves as well as appearance of clinical symptoms and death of the calves.

Pathological examinations.

Macroscopic changes:

On post-mortem examination of gammexane intoxicated buffalo-calves, the visible mucous membrane was found cyanotic. The body position of all the animals were found in opisthotonos condition. Dribling of saliva from the mouth wetted the surface

of ground under head and the frothy foam was present on lips in all the cases. The bloat was found in almost all the cases. The most significant changes were the congestion of liver, lung, kidney, intestine, brain, spinal cord and myocardium. The cut surface of the liver was found to be bulging in a few cases. The small intestine contained a considerable amount of mucous and the mucosa of intestine was congested. The tracheal tree was found to contain excess amount of frothy fluid. The lung, in addition to hyperemia, also showed excessive amount of serosinguinous fluid which came out on cutting the lung tissues. Pin-point haemorrhagic spots were descernible on the epicardial surfaces. In some cases, the heart was in systole but the clotted blood was found in the ventricles of the heart in almost all the cases. The brain and spinal cord did not show any significant abnormality by nacked eye inspection except congestion.

Microscopic changes:

Liver :- The normal architectural pattern of liver was found disturbed on microscopic examination. The sinusoidal space was increased and contained a large number of erythrocytes (Fig. III). The blood vessels of liver appeared dilated. The liver cells around the central vein showed coagulative necrosis and the hepatocytes of the periphery were showing varying degree of degenerative changes (Fig. IV). The nucleus was either absent or was pyknotic in many of the liver cells. The connective tissues in the portal tract showed tendency for proliferation but frank fibrosis could not be seen in any part of the sections of liver. Proliferation of the bile duct and cellular

infiltrations were absent in these cases.

Kidney :- On histopathological examination of kidney it was noted that the tissues alterations were mainly confined to the renal tubules. Some of the tubules showed almost complete desquamation of the lining epithelium (Fig. V) where as some of the tubules contained eosinophilic or homogenous mass in the lumen. At places, the epithelial cells were showing necrotic changes characterised by pyknosis or absence of the nuclei. Some of the epithelial cells of the tubules showed fatty degeneration. The glomerular tufts were swollen and occupied more than three fourth space of Bowman's capsule. The Bowman's capsule itself did not show any appreciable alterations. There was no cellular infiltrations in any part of the kidney. In some cases tubular nephrosis and proliferations of glomerular tuft were also observed (Fig. VI).

Lung :- This organ was affected adversly. Severe congestion, oedema and haemorrhages were observed in all the sections. In some sections, the distribution of oedematous fluid was focal whereas in others it was diffuse in nature. In one animal the eosin stained homogenous oedematous fluid was seen in the bronchioles, alveolar ducts and also in alveoli (Fig. VII). The peribronchiolar blood vessels as well as interalveolar blood vessels were engorged with erythrocytes (Fig. VIII). At some locations, focal haemorrhagic spots were also seen, although there was infiltrations of a few mono-nuclear cells but frank pneumonic lesions were not descernible.

Intestine :- The tips of the villi were almost observed to be nacked. The epithelial cells of the villi could be seen only in the deeper parts of the mucosa. The lamina propria was infiltrated with a large number of mononuclear cells (Fig. IX). The muscularis mucosa was least affected. The submucosa was found thickned due to infiltrations of mononuclear cells. There was also a mild proliferation of connective tissue in the submucosa. The muscularis layer was also infiltrated with a few mononuclear cells. No significant change could be noted in the serosa.

Spleen :-The malpighian corpusles of the spleen were deplated of lymphocytes. Almost all the lymphoid follicles were showing a washed out appearance (Fig. X). The germinal epithelium of lymphoid follicles were also devoid of lymphoblasts. The capsule of the spleen and the trabeculae did not show any appreciable amount of tissue alterations.

Heart :-The sections of the myocardium showed congestion of the blood vessels and haemorrhage in the interfibrilar space (Fig. XI). There was also focal degeneration of muscle fibres. However, cellular infiltrations were not a feature in any part of the myocardium.

Brain and spinal cord :- In contrast to the marked nervous symptoms shown by the gammexane intoxicated buffalo-calves, only slight histopathological changes were found in all sections of the brain and spinal cord. The neurons of the grey matter were looking almost normal and there was no signs of satellitosis and

neuronophagia in any part of the sections. However, in the white matter there was an indication of demyelination of the wellerian degeneration as judged by the vacant space around the axon trunk.

Treatment.

The result of the treatment of acute gammexane poisoning in buffalo-calves has been shown in Table 8.

Group II:

The calves of this group were treated with phenobarbital sodium, atropine sulphate and dextrose (20%). Though the calves of this group were given four repeated treatments when the clinical symptoms reappeared, only one calf could survive out of five. It was further observed that the mean time interval between administration of gammexane and death of the calves was 97.81 ± 9.26 hours and the mean time interval between appearance of clinical symptoms and death of the calves was 92.75 ± 8.98 hours.

Group III:

The calves of this group were treated with magnesium sulphate (12%), chloral hydrate (6%), atropine sulphate and dextrose (20%). It was found that none of the calves could be saved with the above set of drugs even after giving five repeated treatments. It was observed that the mean time interval between administration of gammexane and death of the

calves was 94.48 ± 8.97 hours and the mean time interval between appearance of clinical symptoms and death of the calves was 70.34 ± 14.64 hours.

Group IV:

The calves of this group were treated with diazepam, atropine sulphate and calborol. Out of five only two calves could survive after treatment. The calves of this group received three repeated treatments whenever the clinical symptoms reappeared. The mean time interval between administration of gammexane and death of the calves was 104.92 ± 12.33 hours and the mean time interval between appearance of clinical symptoms and death of the calves was 99.69 ± 12.38.

When the therapeutic efficacy of the drugs were judged on the basis of above observations and findings, it was concluded that the set consisting of diazepam, atropine sulphate and calborol was of value and more efficacious as an antidote whereas phenobarbital sodium, atropine sulphate and dextrose being intermediary in action and magnesim sulphate, chloral hydrate, atropine sulphate and dextrose was ineffective in acute gammexane poisoning in buffalo-calves.

Abbreviations used in statistical analysis:

Pre	:	Pre-poisoning stage
Post	:	Post-poisoning stage
Post T.	:	Post treatment (survived cases)
NS	:	Non-significant
+	:	Significant at 5% level of significance ($P < 0.05$)
++	:	Significant at 1% level of significance ($P < 0.01$)

Group	No. observations
I	8
II	5
III	5
IV	5
Total	23

Table 2

Showing clinical observations in pre and post-poisoning stages in gammexane poisoned buffalo-calves.

Group	No. of observation	Mean ± S.E.					
		Pulse rate		Resp. rate		Temperature °F	
		Pre	Post	Pre	Post	Pre	Post
I	8	54.63 ±2.80	114.00 ± 1.51	13.87 ±0.61	45.75 ±2.52	100.99 ±0.18	104.59 ±0.35
II	5	50.20 ±3.34	109.60 ±2.16	14.20 ±0.49	50.20 ±0.86	101.36 ±0.16	104.36 ±0.21
III	5	57.40 ±3.41	114.40 ±1.91	15.40 ±0.81	58.20 ±3.29	101.34 ±0.26	105.14 ±0.25
IV	5	57.00 ±2.70	113.60 ±1.54	16.00 ±1.14	59.40 ±3.30	101.34 ±0.44	104.10 ±0.17
Total	23	54.78 ±1.04	113.04 ±0.91	14.74 ±0.40	52.39 ±1.77	101.22 ±0.13	104.55 ±0.17

Table 2 "A"

Analysis of varation table showing the effect of gammexane poisoning in between groups either in pre or post-poisoning stages

Sources of variation	df.	M.S.					
		Pulse rate		Resp. rate		Temp. °F	
		Pre	Post	Pre	Post	Pre	Post
Between groups	3	54.68NS	25.79NS	5.85NS	263.73NS	0.23NS	0.98NS
Error	19	54.84	17.98	3.41	42.33	0.42	0.51
Total	22						

Table 2 "B"

Analysis of variation showing effect of gammexane poisoning on clinical observations in between periods in buffalo-calves.

Sources of variation	df.	M.S.		
		Pulse rate	Respiration rate	Temperature °F
Between periods	1	39034.78 ++	16303.39++	127.55++
Error	44	36.93	38.13	0.45
Total	45			

Table 3

Showing SAP and blood glucose in pre and post-poisoning stages in gammexane poisoned buffalo-calves.

Group	No. of observation	Mean ± S.E.			
		SAP		Blood glucose	
		Pre	Post	Pre	Post
Group I	8	14.34 ±1.34	32.82 ±2.02	58.08 ±3,49	135.83 ±4.35
Group II	5	11.55 ±1.57	28.93 ±1.76	60.20 ±1.55	129.11 ±15.73
Group III	5	16.27 ±1.05	33.05 ±1.90	60.89 ±2.30	135.91 ±4.11
Group IV	5	14.36 ±2.61	29.00 ±2.54	60.00 ±1.93	133.74 ±5.13
Total	23	14.16 ±0.85	31.20 ±1.07	59.58 ±1.41	133,93 ±3.73

Table 3 "A"

Analysis of variation showing effect of gammexane poisoning on SAP and Blood glucose in between groups either in pre or post-poisoning stage.

Source of variation	df.	M.S.			
		SAP		Blood glucose	
		Pre	Post	Pre	Post
Between groups.	3	18.99^{NS}	29.31^{NS}	9.88^{NS}	54.99^{NS}
Error	19	16.15	25.85	51.75	361.73
Total	22				

Table 3 "B"

Analysis of variation showing effect of gammexane poisoning on SAP and Blood glucose in between periods in buffalo-calves.

Source of variation	df.	M.S.	
		SAP	Blood glucose
Between period	1	3339.33^{++}	63569.63^{++}
Error	44	21.43	182.97
Total	45		

Table 5

Showing clinical observations in pre-poisoning stage and survived cases.

Group	No. of observation	Mean ± S.E.					
		Pulse rate		Respiration rate		Temperature °F	
		Pre	Post T	Pre	Post T.	Pre	Post T.
Survived cases.	3	49.00 ±2.65	73.00 ±3.46	14.00 ±0.58	19.33 ±0.88	101.50 ±0.06	99.33 ±0.44

Table 5 "A"

Analysis of variation of clinical observations between pre-poisoning stage and survived cases.

Sources of variation	df.	M.S.		
		Pulse rate	Respiration rate	Temperature °F
Between groups	1	864.00^{+}	42.66^{++}	7.04^{++}
Error	4	28.50	1.67	0.29
Total	5			

Table 6

Showing SAP and Blood glucose in pre-poisoning survived cases.

Group	No. of observation	Mean + S.E.			
		SAP		Blooc glucose	
		Pre	Post T.	Pre	Post T.
Survived cases.	3	11.19 ±1.88	20.32 ±1.31	55.82 ±1.64	83.66 ±5.51

Table 6 "A"

Analysis of variation of SAP and Blood glucose between pre-poisoning stage and survived cases.

Sources of variation	df.	& M.S.	
		SAP	Blood glucose
Between groups	1	124.85^{+}	1162.03^{++}
Error	4	7.89	49.56
Total	5		

Table 7

Showing different time intervals in different groups of gammexane poisoning.

Group	Mean time interval betwen adm.of gammexane & appearance of symptoms.		Mean time interval between adm. of gammexane and death of calves.		Mean time interval between adm. apperance of clinical symptoms and death of calves.	
	n	Mean ± S.E.	n	Mean ± S.E.	n	Mean ± S.E.
Group I	8	3.98 ± 0.41	8	10.08 ± 0.98	8	6.10 ± 0.68
Group II	5	5.38 ± 0.41	4	97.81 ± 9.26	4	92.75 ± 8.98
Group III	5	4.35 ± 0.39	5	94.48 ± 8.97	5	70.34 ±14.64
Group IV	5	5.35 ± 0.10	3	104.92 ±12.33	3	99.69 ±12.38
Total	23	4.66 ± 0.22	20	62.96 ±10.40	20	53.53 ± 1.68

Table 7 "A"

Showing analysis of variation between different time intervals during gammexane poisoning.

Sources of variation	Mean time interval between adm. of gammexane & appearance of symptoms		Mean time interval between adm.of gammexane & death of calves.		Mean time interval between appearance of symptoms & death of calves.	
	df.	M.S.	df.	M.S.	df.	M.S.
Between groups	3	3.06^{+}	3	13492.47^{++}	3	10650.73^{++}
Error	19	0.84	16	225.20	16	387.38
Total	22		19		19	

Table 8

Showing the survived cases of buffalo-calves after different antidotal therapy in acute gammexane poisoning.

Group	Treatment	No.of treated buffalo-calves	No.of survived buffalo-calves.
I	No treatment	-	-
II	(1) Phenobarbital sodium (2) Atropine sulphate (3) Dextrose	5	1
III	(1) Chloral hydrate and magnesium sulphate (2) Atropine sulphate (3) Dextrose	5	Nil
IV	(1) Diazepam (2) Atropine sulphate (3) Calborol	5	2

Fig. I : Showing dilated pupil and froth adhered to the muzzle in acute gammexane poisoning in buffalo-calves.

Fig. II : Showing protrusion of the tongue, stiff and extended neck and convulsion in acute gammexane poisoning in buffalo-calves.

Fig. 3 : Section of Liver of a buffalo-calf showing congestion of sinusoid.

H. & E. x 400.

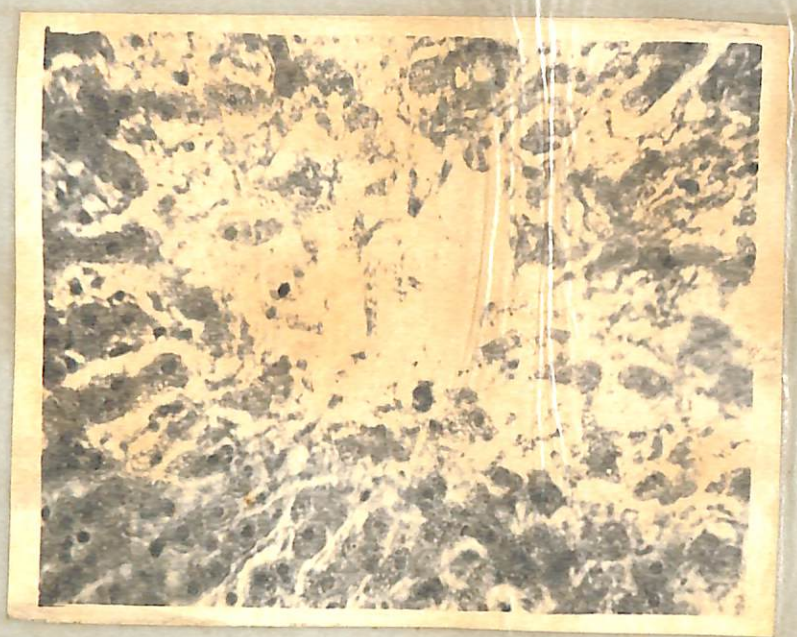

Fig. IV : Section of liver of a buffalo calf showing centrilobular necrosis.

H. & E. x 400.

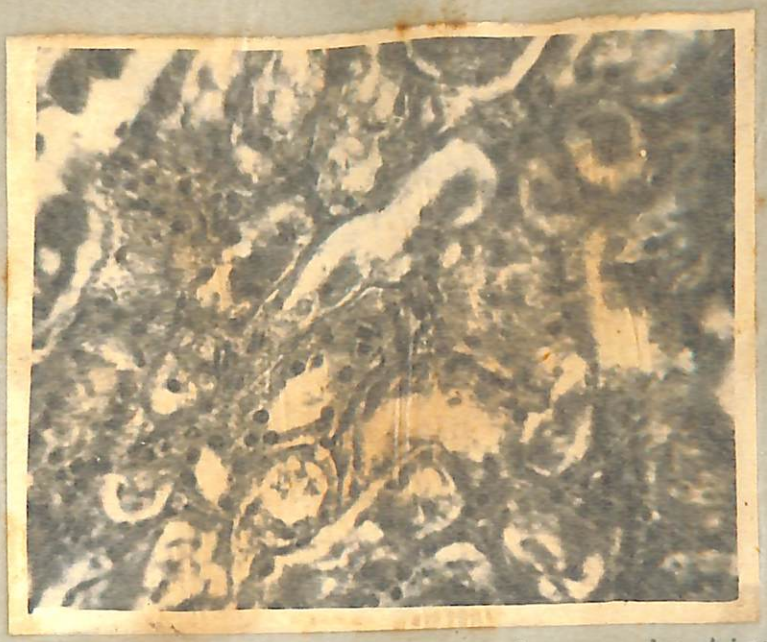

Fig. V : Section of kidney showing desquamination of tubular epithelium.

H. & E. x 400.

Fig. VI : Section of kidney of a buffalo-calf showing tubular nephrosis and proliferation of glomerular tuft.

H. & E. x 400.

Fig. VII : Section of lung of a buffalo-calf showing diffused distribution of oedematous fluid in alveoli and bronchi.

H. & E. x 100.

Fig. VIII : Section of lung showing severe congestion of blood vessels.

H. & E. x 100.

Fig. IX : Section of intestine showing loss of lining epithelial cells and infiltration of mononuclear cells in lamina propria.

H.& E. x 400.

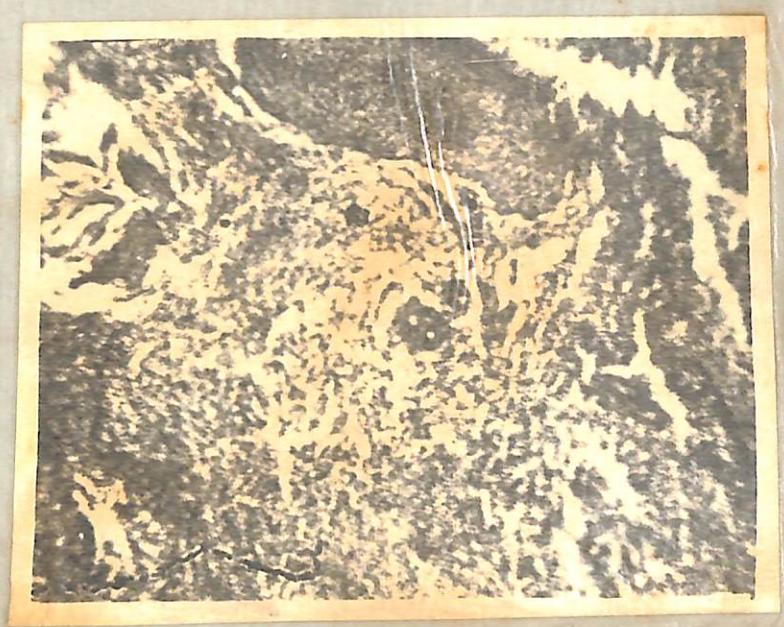

Fig. X : Section of spleen showing depletion of lymphocyte in lymphoid follicles.

H.& E. x 100.

Fig. XI : Section of heart showing congestion of the myocardial blood vessels.

H.& E. x440

DISCUSSION

DISCUSSION

Lehman (1948) reported that gamma BHC at the rate of 125 mg/kg body weight was the mean lethal dose for rats when given orally. Petty and Moore (1964) described 85 to 125 mg of gamma BHC/kg body weight as LD 50 for acute toxicity when given orally. Blood and Henderson (1969) have reported the toxic oral dose of lindane to be 25 mg/kg body weight and to that of BHC to be 1000 mg/kg body weight in cattle. Radeleff (1970) reported that gamma BHC proved lethal for adult cattle at 25 mg/kg body weight and higher when given orally. However, no specific toxic dose of gammexane in buffalo-calves could be found from the available literature.

Thus, with a view to assess the toxic oral dose of gammexane to produce acute toxic symptoms in buffalo-calves, several attempts were made by increasing the dose of gammexane from 25 mg/kg body weight to 100 mg/kg body weight. It was further observed that even 75 mg/kg body weight could not produce acute toxicity and as such 100 mg/kg body weight was found to be the optimum dose level to produce acute toxicity in buffalo-calves of all the groups during the present investigation.

In the present study, the calves were drenched with gammexane through a stomach tube at the dose rate of 100 mg/kg body weight suspended in one litre of tap water. After the administration of gammexane, the calves looked dull and severely

depressed with flow of saliva for a few minutes. Later on, there was hypersensitiveness, well marked sharp response to external stimuli, congestion of conjunctiva, dilated pupil with nystagmus, excessive salivation champing of the jaws and grinding of the teeth thereby producing froth. Grunting or groaning and protrusion of tongue were also observed. Muscular spasms through out the body, falling on the ground with spasmodic attempts of rising, dog sitting position and convulsions with violent struggle and paddling movements of legs were also observed. There was increase in pulse and respiration rate alongwith rise in body temperature. There was respiratory embarrassment with short convulsive inhalation and exhalations and as the seizures prolonged, dyspnea was observed resulting into death following respiratory failure. Similar symptoms were also observed in different animals due to poisoning with BHC (McEnerney, 1951); Ramanujam and Gurumurti, 1951) and Allsup and Warton, 1957), endrin (Eknathrao, 1966);(Jerome, 1958) and Singh et al., 1974) as well as chlorinated hydrocarbons (Radeleff, 1970). However, Radeleff (1970) described the depression, drowsiness, reluctance to move and oblivious to surroundings instead of active and violent manifestations in some cases of chlorinated hydrocarbon poisoning in animals. The present findings were not in agreement with that of Radeleff (1970). Larger doses of DDT disturbed the central function in the cord and brain stem (Bromiley and Bard, 1949). Jones (1974) described that the primary systemic effects of DDT were disturbances of the CNS characterized by hyperexcitability, convulsion and

paralysis primarily acting on cerebellum and the higher motor cortex whereas gammexane stimulated the CNS due to gamma isomer. The present findings of clinical symptoms are in agreement with the Bromiley and Bard (1949) and Jones (1974). The observed clinical symptoms might be due to CNS stimulation.

The signs of various shorts of outcries exhibited by the poisoned calves might be the manifestations of pain and sufferings (Ramanujam and Gurumurit, 1951) and nervous derangement (Jones, 1974).

Clinical observation.

Pulse rate:

The mean pulse rate was 113.04 ± 0.91 in post-poisoning stage. The pulse rate was not found to differ significantly between groups either in pre or post-poisoning stage. A very sharp highly significant increase in mean pulse rate was observed in post-poisoning stage in comparison to pre-poisoning one. On 7th day observation in survived cases, the mean pulse rate was 73.00 ± 3.46 which tended to come to near normal.

The present findings are in agreement with Radeleff (1970) who observed increase in pulse rate in chlorinated hydrocarbon poisoning. Ramanujam and Gurumurti (1951) and Hamilton (1955) have reported great pain and suffering in BHC poisoning in cattle and horses respectively. During the present study of gammexane poisoning, increase in pulse rate might be due to great pain and suffering as pain causes a reflex acceleration of the pulse rate (Boddie, 1970).

On 7th day observation in survived cases, the pulse rate showed downward trend towards normal value.

Respiration rate:

The mean respiration was 52.39 ± 1.77 in post-poisoning stage. The respiration between groups was found non significant in both pre and post-poisoning stages. However, highly significant increase in mean respiration was observed in post-poisoning stage over pre-poisoning one.

Similar increase in respiration rate was observed in cattle due to poisoning with BHC (Ramanujam and Gurumurti, 1951) and endrin (Sharma and Gautam, 1973) as well as (Singh et al., 1974). In survived cases the respiration rate showed downward tendency towards normal on 7th day.

Temperature:

The mean temperature of all the groups was 104.55 ± 0.17 in post-poisoning stage. The temperature between groups either in pre or post-poisoning stage turned out to be non-significant. However, highly significant increase in temperature was observed in post-poisoning stage over the pre-poisoning one.

The rise in body temperature might be due to muscular tremors and repeated convulsive seizures (Radeleff, 1970). Similar rise in temperature was also observed in cattle due to poisoning with BHC (Ramanujam and Gurumurti, 1951). Radeleff (1970) also observed a sharp rise of body temperature (114-116°F. However, a sharp rise of body temperature

was not recorded during present study and this might be due to the lesser degree of convulsive seizures and muscular tremors.

In survived cases the temperature was 99.33 ± 0.44 on 7th day of treatment. This subnormal temperature might be due to the weakness in the survived cases.

Haematological studies.

Total erythrocyte count (TEC):

There was no significant variation in TEC between groups and within periods of pre and post-poisoning stages as well as in survived cases.

A drop in erythrocyte count was recorded in cattle due to poisoning with aldrin (Hothi and Kwatra, 1972) and endrin (Sharma and Gautam, 1973). The present finding is in agreement with Malik et al. (1973) who found no change in TEC. The unchanged level of TEC might be due to the insufficient time of toxicants (gammexane) to cause extensive damage of erythrocytes as the interval between administration of gammexane and appearance of the clinical symptoms was only 4.66 ± 0.25 hours. Similarly in the survived cases, there was no significant differences in TEC on 7th day of treatment.

Total leucocyte count (TLC):

The TLC between groups either in pre or post-poisoning stage revealed no significant difference. However, a highly significant increase in TLC in post-poisoning stage to that of

pre-poisoning one was recorded. In survived cases the TLC was still higher to that of pre-poisoning stage.

Similar increase in TLC was observed due to poisoning with aldrin (Hothi and Kwatra, 1972) and dieldrin (Malik et al.,1973) as well as endrin (Sharma and Gautam, 1973). The above findings are also in agreement with Wintrobe (1967) who suggested leucocytosis in a variety of toxic conditions.

Differential leucocyte count (DLC):

No significant difference in number of neutrophil, lymphocyte, eosinophil, basophil and monocyte content between different groups either in pre or post-poisoning stage was observed. However, a highly significant increase in number of neutrophil with corresponding significant decrease in lymphocyte,eosinophil and monocyte were recorded in post-poisoning stage over the pre-poisoning one. Here also there was no significant difference in basophil count. On 7th day of treatment the survived cases showed no significant difference except in neutrophil which was significantly higher in comparison to pre-poisoning stage. The present findings are in agreement with Malik et al. (1973) who observed increase in number of neutrophil in acute dieldrin poisoning. Schalm (1967) stated that under conditions of stress the adrenal cortical secretion depressed the number of circulating eosinophil and lymphocyte and lead to an elevation in number of circulating neutrophil. In the present study, the number of neutrophil was elevated with corresponding decrease in eosinophil and lymphocyte. This might be due to the stress factor caused by the gammexane poisoning. However, the significant decrease in

monocytes could not be correlated due to unavailibility of literature and it needs further study. An increase in number of lymphocytes was observed in poisoning with aldrin (Hothi and Kwatra, 1972) and endrin (Sharma and Gautam, 1973). However, during the present study no relative increase of lymphocytes were detected. This might be due to single toxic dose of gammexane given to calves and as such in such condition the lymphoid organs might not have got sufficient time for the production of increased lymphocytes. If the gammexane could be given in small but repeated doses, it might have stimulated the bone marrow to produce a large number of leucocytic cells (Florey, 1970).

In the survived cases the neutrophils content was singificantly high which showed a tendency to come to normal value.

Packed cell volume (PCV):

The mean PCV also showed no significant difference between groups either in pre or post-poisoning stage . However, significant increase in mean PCV was recorded in post-poisoning stage over pre-poisoning one.

A decrease in PCV was observed in cattle due to poisoning with aldrin (Hothi and Kwatra, 1972) and endrin (Sharma and Gautam, 1973). However, Malik et al. (1973) observed no significant change in PCV during acute dieldrin poisoning in buffalo-calves. In the present study, the increase in PCV might be due to haemoconcentration associated with dehydration (Boddie, 1970), but TEC and Hb did not reveal the condition

like haemoconcentration associated with dehydration. However, the significant rise in PCV could not be substantiated due to unavailibility of literature and as such it needs further study.

On 7th day of treatment in survived cases there was no significant variation in PCV showing thereby the normal condition.

Haemoglobin (Hb):

There was no significant difference in Hb between groups either in pre or post-poisoning stage . No significant difference in Hb between pre and post-poisoning stage could be recorded in the present study.

The present finding is in agreement with Malik et al. (1973) who observed no change in haemoglobin in acute dieldrin poisoning. However, a marked decrease in Hb was recorded in poisoning with aldrin (Hothi and Kwatra, 1972) and endrin (Sharma and Gautam, 1973). This decrease in Hb might be due to chronic condition but during the present study, non significant change in Hb might be due to acute gammexane poisoning in calves.

On 7th day of treatment in survived cases there was significant decrease in Hb and that might be due to the losses caused by the gammexane.

Biochemical estimation.

Blood glucose:

A highly significant increase in mean blood glucose level was recorded in post-poisoning stage as compared to pre-poisoning one. However, no significant differences were observed between

groups of pre and post-poisoning stages. Significant rise in blood glucose level was observed in animals due to poisoning with insecticides (Huthway and Mallinson, 1964), dieldrin (Malik et al., 1973) and endrin (Singh et al., 1974). Hurkat (1977) observed the comparative study of dieldrin induced hepatotoxicity in rabbits and rats and concluded that with the administration of dieldrin there was much more marked loss of glycogen content of liver cells in the terminal stage of the study in rabbits. During the present investigation, the main symptoms of gammexane toxicity were convulsion, dyspnoea, anoxia and muscular tremors and these active symptoms might have caused significant rise in blood glucose level (Coles, 1968). The present increase in blood glucose level might be also due to accelerated rate of glycolysis in gammexane poisoning to meet the increase requirements of energy (Huthway and Mallinson, 1864).

In survived cases, there was significant increase in blood glucose level to that of pre-poisoning stage. However, the blood glucose level in survived cases tended to come to near normal. This might be due to the absence of symptoms like convulsions, anoxia and muscular tremors (Coles, 1968). On the other hand, the blood glucose level in the survived cases was still raised in comparison to pre-poisoning stage and this might be due to the continuing glycolysis to meet the energy requirements by the calves as on that day they were observed week and emaciated (Huthway and Mallinson, 1964).

Serum alkaline phosphatase (SAP):

A highly significant increase in SAP value was recorded in post-poisoning stage as compared to pre-poisoning one. However, no significant difference could be recorded between groups of pre and post-poisoning stages.

In the present study, the SAP value recorded in pre-poisoning stage is in agreement with Allcroft and Folley (1941) and Garner (1952).

There is not much literature on the SAP activity in cases of gammexane poisoning. However, elevated level of SAP was recorded in billiary obstructions (Armstrong, 1934) and Stevenson and Wilson, 1963), acute necrosis of liver (Hurbut, 1959), cellular wall dissolution in necrosis (Cornelius, 1964) and any disease process of spleen, liver, kidney, intestinal mucosa or bone (Ward, 1966). Hurkat (1977) observed in histo-chemical study that in dieldrin treated rabbits the SAP activity was considerably increased. He further reported that with the fatty degeneration and necrosis, the SAP activity became more diffuse. On the basis of above literature, it can be correlated that an increase in SAP activity was due to necrosis of the liver, damage to the cellular wall of kidney and intestinal mucosa caused by gammexane toxicity.

In survived cases there was significant variation in SAP activity between pre-poisoning stage to that of survived cases. However, the SAP activity of the survived cases tended to come to near normal. The SAP activity coming to near normal might

be due to the occurrance of regeneration and healing process of the damaged organs caused by gammexane toxicity(Hurbut , 1959).

Mean time interval.

There was very significant variation between groups in time interval between administration of gammexane and appearance of clinical symptoms but highly significant variation was recorded in time interval between administration of gammexane and death of the calves as well as appearance of clinical symptoms and death of the calves. That significant variation might be due to the therapeutic efficacy of the drugs administered during the present study of gammexane poisoning.

Post-mortem and histochemical examinations.

There is every likelihood of herbivores to be the victims of insecticide poisoning as a result of either ingesting freshly sprayed crops or being accidentally expossed to it. The gross and microscopic changes can give some useful indications for the purpose of diagnosis of poisoning in these animals, particularly when considered in association with the history of a case and clinical observations. For this reason post-mortem and histopathological examinations were carried out in gammexane poisoned buffalo-calves. For the purpose in the calves of group I acute poisoning was produced by tube administration with gammexane and as such the calves showed symptoms of acute poisoning and succumbed to the toxicity after a lapse of 10.08 ± 0.98 hours after the gammexane administration.

On post-mortem examination the most significant changes

were the congestion of liver, lung, kidney, intestine and myocardium. The cut surfaces of the liver were found to be bulging and the intestine contained excess frothy fluid. The lung also showed excess amount of sero-singuinous fluid. Haemorrhagic spots were descernible on the epicardial surface of the heart. These findings are in agreement with the Radeleff (1948, 1949 and 1970), Jerome (1950), Smith (1972) and Karl and Loren (1950). However, no specific gross lesions could be detected in brain and spinal cord tissues.

On histopathological examination in the present study, congestion and centrilobular necrosis in liver, nephrosis and congestion in kidney, mild hyperaemia and haemorrhages in the myocardium, and oedema as well as congestion in the lungs were found to be the constant lesions of gammexane poisoning.These findings are in agreement with those of Radeleff (1948), Karl and Loren (1950), Kitselman (1953), Adamic (1958), Loomis (1966) and Smith (1972). However, only slight histopathological changes were found in sections of brain and spinal cord of the poisoned animals in contrast to the marked nervous symptoms observed probably due to the acute death in very short time. This finding is in agreement with Karl and Loren (1950) and Runnells et al. (1965).

It is well known that any poison on ingestion would go to deudenum from where it reaches the liver via portal vein. Liver is a detoxifying organ. If the concentration of the poison is low, it is completely converted into non toxic substances by the hepatocytes. However, if the intensity of

the poison is high, the hepatocytes get themselves damaged. The poison which escapes to the systemic circulation finally excreted from the body through the kidney. In the kidney again the poison is likely to cause damage to the tubular epithelium since it stays there for a reasonable time. In the present experiment also coagulative necrosis around the central vein of liver and tubular nephrosis might have been caused by the gammexane. The cellular infiltration was not pronounced either in the liver or in kidney because the interval between administration of gammexane and death was quite short. If these poisoned calves could have survived for a longer duration only then cellular infiltration might have developed.

The small intestine showed considerable amount of damage due to the poison. A similar observation was made by Furman (1947), Radeleff (1948) and Hamilton (1955). On administration through stomach tube gammexane might have remained in contact with the intestinal epithelium and as such excess production of mucous and other signs of reaction to poison like vascular hyperaemia and infiltration of mononuclear cells were noticed. If the calves could have been survived for a longer duration, they might have exhibited shooting diarrhoea due to irritation of the intestinal mucosa. However,during present study, haemorrhagic gastritis and duodenal enteritis could not be observed as stated by Furman (1947).

In the present experiment lungs showed focal to diffuse congestion and oedema. However, pneumonic changes were not pronounced in any part of the lungs. This is in agreement with

the findings of Hendrick (1969) and Smith et al. (1972).

These types of tissue alterations in the lungs were responsible for causing respiratory trouble to the calves and finally might have caused death of the calves due to anoxia. It is reasonable to assume that the lung changes and myocardial damage might have played a significant role in causing acute death of the calves.

Urine analysis.

The qualitative tests for the presence of sugar and albumen in urine collected from poisoned calves were found negative. However, Sharma and Gautam (1973) reported that urine contained albumen in experimental endrin poisoning in calves. The present finding could not be substantiated for want of literature and it needs further study.

Efficacy of therapeutic measures.

The therapeutic efficacy of the drugs have been shown in Table 8.

Group II:

Out of five calves treated with phenobarbital sodium, atropine sulphate and dextrose, only one calf could be saved. However, the calves were given four repeated treatments. It was observed that the mean survival period after the appearance of clinical symptoms was 92.75 $\pm$ 8.98 hours.

Phenobarbitone, a long acting barbiturate derivative

depresses the central nervous system where the higher centres becomes less responsive to afferent stimuli(Drill, 1958). Goodman and Gilman (1970) too, suggested that the phenobarbital had a selective anticonvulsant effect and useful in the symptomatic therapy of grandmal epilepsy including convulsive seizures. Various workers have tried different barbiturate derivatives in combination with other drugs. McNamara and Krop (1948b) suggested the use of penotobarbital sodium and atropine sulphate to protect the seizures from CNS stimulation and bradycardia respectively produced by BHC poisoning. Lehman(1949) suggested the doses of phenobarbital sodium below the anaesthetic dose to control the tremors and convulsions of DDT poisoning. Garner (1957) advocated the use of chloral hydrate and pentobarbitone to control convulsions. Jerome (1958) successfully treated the subacute case of endrine poisoning in cattle with pentobarbital sodium with sinan as well as chloral hydrate (7%) intravenously. Singh and Thakur (1973) successfully treated a clinical case of gammexane poisoning in buffalo-calves with thiopental sodium intravenously. In most of the above cases mainly short and ultra-short acting barbiturates have been selected to couneract the muscular tremors and convulsive seizures, but in the present study, phenobarbital, a long acting barbiturate has been put to trial on the basis of long lived anticonvulsant to hypnotic action (Goodman and Gilman, 1970).

Atropine sulphate counteracts the symptoms like incoordination of skeletal muscles, excessive salivation, bradycardia and dilates the bronchioles (Goodman and Gilman, 1970 and Jone, 1966).

As administration of dextrose saves the poisoned animals from liver damage, dehydration and acts as detoxifying agent (Jolly, 1951) advocated the use of glucose saline to counteract liver damage associated with BHC poisoning. Garner (1957) advocated the use of calcium borogluconate together with glucose salive I/V to avoid liver damage in chlorinated hydrocarbon poisoning.

In the present study too, phenobarbital, atropine sulphate and dextrose counteracted the convulsive seizures, muscular tremors, bradycardia, excessive salivation and liver damage. These facts are in agreement with the above workers.

Group III:

The calves of this group were treated with chloral hydrate and magnesium sulphate, atropine sulphate and dextrose. The calves of this group were given five repeated treatment. However, none of the calves could be saved. It was further observed that the mean survival period after the appearance of clinical symptoms was 70.34 $\pm$ 14.64.

In the mixture of chloral hydrate and magnesium sulphate, chloral hydrate produces the bulk of CNS depression whereas magnesium sulphate provides a more rapid onset of CNS depression, aids in over-coming reflex excitement and produces a distinct curariform effect upon the skeletal musculature(Jones, 1966). Several workers have successfully tried chloral hydrate and magnesium sulphate either alone or in form of mixture in chlorinated hydrocarbon poisoning. Garner (1957) advocated the use of chloral hydrate and pentobarbitone to control convulsions

together with calcium borogluconate and glucose saline (I/V) to avoid liver damage in chlorinated hydrocarbon poisoning. Jerome (1958) successfully treated the subacute cases of endrin poisoning in cattle with pent-obarbitone sodium with sinan as well as chloral hydrate intravenously. Radeleff (1970) reported the use of narcotic or anaesthetic agents such as chloral hydrate or the barbiturates for as long as 24 hours. Bhaskar and Sreemanarayana (1973) treated successfully a clinical case of endrin poisoning in a she-buffalo with atropine sulphate, chloral hydrus (0.5 ml/kg body weight) calcium borogluconate and dextrose (I/V) in repeated doses. Malik et al. (1973) studied the evaluation of therapeutic measures consisting of chlor.- mag. combination, atropine sulphate, glucose, d-tubocurarine and calcium borogluconate in acute dieldrin poisoning in buffalo-calves. The measures used were reported to increase the survival period but could not save the animals. Sharma and Gautam (1973) studied the experimental endrin poisoning in 13 calves and treatment with chloral hydrate and calcium borogluconate intravenously with saline purgative and treatment was successful in four out of five cases. Gautam and Sharma (1974) reported the clinical cases of endrin poisoning in animals and treated successfully with chloral hydras, calcium gluconate, saline purgative and fluid therapy in some of the cases.

Thus, the mixture of chloral hydrate and magnesium sulphate was able to combat the convulsions, CNS stimulation and aided in overcoming the reflex excitement alongwith producing curariform effect upon the skeletal muscles.

Among the above combination of drugs atropine sulphate controlled bradycardia and excessive salivation whereas dextrose might have helped in avoiding liver damage.

Group IV:

Diazepam, atropine sulphate and calborol were tried to overcome the clinical symptoms of poisoning. Three repeated treatments were given to calves of this group in which only one could be saved out of five calves. It was further observed that the mean survival period after the appearance of clinical symptoms was 99.69 ± 12.38.

Diazepam is a widely used benzodiazepine derivative which possesses anticonvulsant, muscle relaxant and tranquilizing properties (Zbinden and Randall, 1967). For the emergency treatment of certain convulsive disorder and particularly for status epilepticus, it might prove to be a useful drug as an intravenous agent but respiratory tract hypersecretion and lingual obstruction to respiration due to muscular relaxation were the most undesirable side effects (Goodman and Gilman, 1970). Studies indicated that it is toxic only at high levels over extended periods of time (Lumb and Jones, 1973). Diazepam probably acts by depressing the limbic system (Brown and Dundee 1968) and relieves muscular spasm and its use to induce anaesthesia (1-2 mg/kg and upto 5 mg/kg in fit patients) may be indicated in socked patients as it causes neither hypotension nor respiratory depression (Lee and Akinson, 1968). Garner (1957) recommended calcium borogluconate together with glucose

saline to avoid liver damage. He further suggested that calcium might act by neutrilizing the effects of the rise in serum potasium which was said to occur before the onset of convulsions. Mc Parland et al. (1973) reported BHC poisoning in cattle and suggested that the lower dose with calcium borogluconate and chloral hydrate might be of value. Calcium borogluconate alongwith other drugs were also tried successfully in different species due to poisoning with endrin (Bhaskar and Sreemanarayana, 1973), (Sharma and Gautam, 1973), (Gautam and Sharma, 1974), gammexane (Singh and Thakur, 1973) and BHC (Sathuraman, 1977). However, Malik et al. (1973) reported that therapeutic measures consisting of chlor.-mag., atropine sulphate, glucose, d-tubocurarine and calcium borogluconate could not save the animals but only increased the survival period in acute dieldrin poisoning.

In the present study, diazepam counteracted the recurrent convulsions and completely relieved muscular spasms (tremors). However, the undiserable side effects like respiratory tract hyposecretion and lingual obstruction to respiration due to muscular relaxation and bradycardia were checked by atropine sulphate (Kumar and Thurmon, 19). Calborol might have avoided liver damage and helped in overcoming the convulsions completely probably by neutrilizing the effects of the rise in serum potasium level which was said to occur before the onset of convulsions (Garner, 1957).

Conclusion of drug trials.

On the basis of survival period after the appearance of clinical symptoms, number of treatment required to counteract the symptoms and the number of survivals in different groups it can be concluded that the drug combination of calmpose, atropine sulphate and calborol administered in group IV proved more efficacious than the other drug combinations. This was followed by the drugs of group II in which phenobarbitone, atropine sulphate and dextrose were used. The least efficacy was noted with the drugs of group III in which chloral hydrate, magnesium sulphate, atropine sulphate and dextrose were used in experimental cases of gammexane poisoning.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

In the present study 23 buffalo-calves were drenched through stomach tube with gammexane (BHC) at the dose rate of 100 mg/kg body weight to produce acute toxicity.

The clinical symptoms appeared in 4.66 ± 0.22 hours in all calves. After the administration of gammexane there was dullness and severe depression with flow of saliva for a few minutes.

As the course of toxicity advanced, the calves were hypersensitive and responded sharply to external stimuli, congestion of conjunctiva, dilatation of pupil, bulging and rolling of eye balls, profuse salivation, champing of the jaws and grinding of the teeth thereby producing froth, protrusion of the tongue, occasional groaning or grunting, muscular twitching through out the body, falling on the ground with spasmodic attempts to rise were observed. Abnormal posture like a dog sitting position as touching the sternum to the ground and keeping the head down between the forelegs was recorded.

Involuntary defecation, micturition and bloat during convulsion were constantly observed. Recurrent convulsive seizures, paddling movements, kicking and violent struggle were the common accomponishments. As the seizures prolonged, dyspnoea was marked resulting into death following respiratory failure.

A highly significant increase in pulse, respiration rate and temperature was recorded after poisoning.

Urine did not contain sugar or albumen after poisoning.

There was significant increase in TLC particularly neutrophils and PCV without evoking any change in TEC and Hb%.

There was highly significant increase in blood glucose and serum alkaline phosphatase level.

The common gross lesions were congestion of liver, lung, kidney, intestine, myocardium, brain and spinal cord. The cut surfaces of liver was found to be bulging. The intestine contained mucous. In addition to hyperaemia, lung showed excessive amount of sero-singuinous fluid which came out on cutting the lung tissues. Pin-point haemorrhagic spots were descernible on epicardial surface.

On histopathological examination, congestion and centri-lobular necrosis in liver, congestion and necrosis in kidney, mild hyperaemia and haemorrhages in myocardium and oedema as well as congestion in lungs were found to be the consistant lesions of gammexane poisoning.

A combination of diazepam, atropine sulphate and calborol gave the best result, phenobarbitone, atropine sulphate and dextrose being intermediary in action whereas chloral hydrate, magnesium sulphate, atropine sulphate and dextrose were found in-effective in gammexane poisoning in buffalo-calves.

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