INFLUENCE OF DIETARY PROTEIN ON THE EFFECT OF COUMAPHOS AND TRIFLUPROMAZINE INTERACTION IN SHEEP

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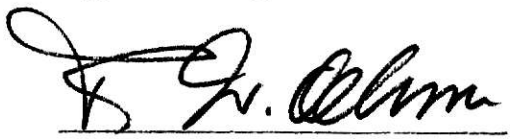

Major Professor

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To them I dedicate this thesis.

INTRODUCTION

Despite the fact that organic chemicals are increasingly being synthesized and extensively studied for their use in agricultural and medicinal practices, the biological behavior of many of these chemicals is not completely understood. The biological behavior of any given compound may have such wide variation that it is often difficult to predict its effect. More than one factor may modify this biological behavior. Such modification can be further complicated by the presence of another chemical which may possess similar pharmacological activity. The interaction which takes place may result in synergistic benefits, total or partial supression of therapeutic effects, or totally unexpected potentiation with adverse reactions.[1]

Synthetic organophosphorus compounds are chiefly used as pesticides and systemic insecticides and have provided many benefits to man and animals. However, their pharmacological activity is subjected to modification by several biological or extraneous factors which may potentiate their activity. Although laboratory studies provide information for safe therapeutic application of such chemicals, field experiences indicate that toxicity occurs even when the compounds are used as recommended. There is wide species variation reflecting variability in the biotransformation of these agricultural chemicals. Stress and malnutrition may further influence animal susceptibility.[2]

Phenothiazine and its derivatives are used in veterinary practice as antihelmintics and tranquilizers. Combination therapy with organophosphorus compounds and phenothiazine derivatives has produced additive therapeutic benefits. However, reports also indicate toxic potentiation of their effects, resulting in acute poisoning and death.[3]

To obtain an understanding of this biological phenomenon, an investigation was undertaken in sheep to study the interaction of the organophosphorus compound coumaphos and the phenothiazine derivative trifluprомazine. Since malnutrition is suggested as a contributing factor to toxicity, the influence of induced protein stress in modifying the host response was included in the investigation.

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INFLUENCE OF DIETARY PROTEIN ON THE EFFECT OF COUMAPHOS AND TRIFLUPROMAZINE INTERACTION IN SHEEP

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SUMMARY

Coumaphos[a] (C), 8 or 17 mg/kg body weight orally, and Triflupromazine HCl[b] (TFP), 1.1 mg/kg body weight IM, or

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[a] BAYMIX, Chemagro Division of Baychem Corporation, Kansas City, MO

[b] VETAME, Squibb, E. R. Squibb and Sons, New York, NY

physiological saline, were given to 8 groups of 5 sheep on low or normal protein ration. Onset of clinical signs mortality rate, mean survival time, necropsy lesions, plasma and erythrocyte cholinesterase (ChE) activity was monitored for each group. Observations suggested potentiation effect between the administered compounds. Inhibition of ChE activity was enhanced in groups receiving both drugs. Low dietary proteins adversely affected the development of clinical signs, the mortality rate, the mean survival of time and ChE activity. Recovery of ChE activity of TFP -treated animals was faster than in their respective controls, and animals on normal protein diet had faster ChE recovery than those fed the low protein diet. Inhibition of erythrocyte ChE found to be a better index of organophosphorus toxicity than plasma ChE.

Drug interaction from the concomitant use of different drugs has been of increasing interest during the past two decades. The result of such interaction may appear as additive response, suppression of desired therapeutic action, or unexpected potentiation that adversely affect the patient.[15] The interaction of organophosphorus compounds (OPC) with phenothiazine derivatives (PHE) has clinical and pharmacological importance.[12,14,19,21,31]

The inhibition of cholinesterase (ChE) activity with OPC has been observed in vitro and in vivo studies;[11,17,25,28] PHE are also reported to inhibit ChE activity.[7] Although PHE have been effectively used to treat OPC toxicities,[9,36] aggravation of OPC toxicity has resulted from the concomitant use of PHE in humans.[3] This effect was confirmed in rats, where concurrent administration of OPC and PHE resulted in higher mortality than when each compound given alone,[14] and also by in vitro study that demonstrated significant inhibition of ChE activity.[7,12] In contrast, potentiation was not observed when therapeutic doses of both compounds were tested in healthy calves.[31]

The clinical practice of concurrently using potentiating drugs may give rise to unforseen toxicity, and the combined use of OPC and PHE has produced potentiation and toxic effects in man and rats.[3,14] The subjection of OPC treated animals to stress may be associated with unpredictable toxic episodes.[6,8,18,24,30,32] Emaciation due to heavy parasitic infestation increases susceptibility to OPC toxicity.[6] This is not unexpected since ChE is a protein and in rats a dietary deficiency of protein causes emaciation and reduced ChE synthesis by the liver.[24] The present investigation was undertaken to evaluate the interaction of coumaphos (C), an organophosphorus insecticide and triflupromazine HCl (TFP), a phenothiazine tranquilizing agent, in sheep on low and normal protein ration.

MATERIALS AND METHODS

Animals - Forty 2-3 month-old clinically healthy female lambs of white suffolk and southdown breeding (mean body weight 28.7 ± 4.1 kg) were adjusted to a specially formulated low (7%) protein ration for 14 days. On the 15th day the lambs were individually weighed, and they were randomly divided into two groups of equal numbers. One group (low protein) was maintained on the 7% protein diet and the other groups (normal protein) was gradually switched to a 12% protein diet. Both groups were maintained on their respective diets throughout the period of experimentation. At the end of 28 additional days, each group was randomly subdivided into four groups of five lambs each and housed in individual pens. (One lamb in group V died before the treatment due to causes unrelated to the study.) Respective feeds and water were provided ad lib.

Experimental Procedure - The groups were treated as outlined in Table 1. Dosages of C and TFP were adjusted to current body weights. C was administered by balling gun as a single dose in a gelatin capsule. TFP or an equal volume of sterile physiological saline was administered IM in the gluteal muscle; a second injection of TFP or saline was repeated 24 hours later. The lambs were kept under constant observation for ten days, and they were examined daily for the remaining 15 weeks of the experiment.

Collection of Samples - Pretreatment CHE values of plasma (ChEP) and erythrocytes (ChER) were determined at weekly

intervals and on the day of treatment. Post-treatment determinations were conducted on samples collected at the same hour at 24 hour intervals for the first 10 days and at weekly intervals during the remaining 15 weeks.

Heparinized tubes[c] were used to collect 5 ml blood by jugular venipuncture. The samples were centrifuged immediately, and plasma and erythrocytes were separated. ChEP and ChER activity were determined by Michel's electrometric method[23] using different molar concentrations of acetylcholine iodide[d] substrate in separate phosphate buffer systems for the respective assays. The reaction was allowed to proceed for one hour at 24 C. Change in pH (ΔpH) was determined at the end of one hour with an expanded scale pH meter.[e] Values were recorded as percent of mean pretreatment ChEP and ChER activity.

Clinical Studies - The time of onset of clinical signs, and the nature of signs observed and the time of death was recorded for each lamb. Dead lambs were subjected to complete postmortem examination.

Statistical Evaluation - Statistical analysis utilized ChEP and ChER values for the last 15 weeks from the groups receiving the low C (8 mg/kg) treatment. Since the experimental situation was a randomized split-plot design, least square analysis was used for groups I, II, V and VI (low

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[c] Venoject. Kimble-Terumo Inc., Teledo, OH.

[d] Acetylcholine iodide; M.W. 271.1 Calbiochem, San Diego, CA.

[e] Corning pH meter, Model 10 Corning Scientific Instruments Medfield, MS.

C + TFP or saline). A level of $P \leq 0.05$ was considered significant for the different variables analyzed.

Analysis could not be applied to the data during the first 10 days because of high death losses in groups III, IV, VII and VIII (high C + TFP or saline). On these groups the clinical effects (mean times of toxicity onset, percent mortality, and mean survival times) were subjectively evaluated.

RESULTS

Clinical Signs - The onset of clinical signs varied in relation to the treatment received by each group (Table 2). Signs of toxicity were consistent and characteristic of ChE inhibition. Affected lambs exhibited restlessness, excessive salivation and lacrimation, respiratory distress, and abdominal palpitation. Frequent micturition and straining were noticed at the later stage of toxicity. Muscular twitchings and fasciculations, involving ventral abdomen, flank and lumbar regions, were seen as the clinical signs progressed. Tremors occurred in the hind quarters with over extension of limbs. Affected lambs became progressively weak and recumbent, and remained comatosed until death.

Groups I, II, III, IV (low protein) all showed signs of toxicity (Table 2). Group III (high C + TFP), showed signs of toxicity as early as 2 hours. The longest mean onset time (6 hours) was noticed in group I (low C + saline). The lambs most affected were in group VII (high C + TFP). One

lamb in group V and three in group VI did not show signs of toxicity. Other lambs in these two groups had mild signs which disappeared by the 3rd to 5th day.

Relapse of clinical signs were noticed in groups II and VI during the 5th and 7th day. Transient salivation occurred. Complete recovery was noticed in all affected lambs that survived by the 10th day, with the exception of one lamb in group I. This lamb remained weak and debilitated throughout the period of study. All others were clinically healthy.

Mortality and Survival Times - The mortality rate was directly related to the 15 mg C/kg, all but one lamb given this dose died regardless of the level of protein and TFP administered (Table 2).

In groups receiving low protein, the percent mortality was highest in groups III and IV (high C) (Table 2). Although the first death was noticed in group IV 4.5 hours post-treatment, the mean survival time of that group was 50.9 $\pm$ 14 hours as compared with 40.4 $\pm$ 13.6 hours in group III (Table 2). Only one lamb each from groups I and II died.

Mortality rates different from those seen in low protein groups were noticed in the high protein groups (Table 2). There were no deaths in groups V and VI (low C + TFP saline). In group VII (high C + TFP), the survival time varied from 4 to 36 hours, with a mean of 19.4 $\pm$ 5.2 hours. This is the shortest survival time of any of the groups. Four of the lambs in group VIII (high C + saline) died, but the survivor

remained clinically healthy throughout the study. The survival time in the group varied from 12 to 144 hours, with a mean of 66 ± 24.9 hours.

Necropsy Findings - Necropsy examination was conducted on all lambs dying during the study. Lesions were similar and consistent and were unrelated to various treatments and different dietary protein levels. Congestion and edema of trachea and lungs, with pulmonary emphysema were found. Petechial hemorrhage in the myocardium (Fig 1) were characteristic in all lambs with acute toxicity. Areas of ecchymosis on the omasal mucosa (Fig 2) and occasional abomasal and anterior duodenal congestion were seen. These lesions were noticed in groups II, III, VII and VIII. Liver and kidneys had varying degree of congestion, and occasional congestion of bladder mucosa was also present. One lamb in group II had small ulcerations in the intestinal mucosa. Another lamb in group VII had pleuropneumonia with thoracic adhesions. All lambs died of acute respiratory and myocardial failure.

Cholinesterase Activity During First 10 Days - During the first 10 days the ChE activity was markedly depressed in all lambs regardless of treatment. Only the extent of inhibition and the pattern of recovery varied among the groups in relation to the treatments.

The mean ChEP and ChER in low and normal protein groups are given in Tables 3 and 4 for the first 10 days of study. Although there was variation in the degree of ChEP inhibition

between low and normal protein groups, ChER inhibition did not reach zero activity at any time.

Cholinesterase activity of low protein groups - The ChEP activity in groups I, II, III and IV was inhibited to zero levels in a few lambs (Table 3). Groups III and IV (high C) showed more ChEP inhibition than groups I and II (low C) (Fig 3). The degree of inhibition in groups I and II were similar except for differences in the recovery of enzyme activity. Recovery of ChEP in group I (low C + saline) was first noticed on the 2nd day, whereas in group II (low C + TFP), recovery began on the 3rd day. By the end of 10th day 60% activity was restored in group II and 52% ChEP activity was present in group I. The pattern of ChEP varied in the two high C groups. There was a sudden drop to zero ChEP activity in group III (high C + TFP) resulting in deaths. ChEP inhibition was gradual in group IV (high C + saline) and continued until the 4th day when deaths occurred, even though ChEP activity was zero on the 3rd day.

The ChER activity in groups I, II, III and IV varied. Groups III (high C + TFP) and IV (high C + saline) had gradual inhibition to levels approximately 5% of the pretreatment ChER activity; deaths occurred on the 3rd day in group III. There was initial recovery of ChER in group IV on the 4th day, but all lambs died by the next day (Fig 4). In groups I (low C + saline) and II (low C + TFP) a similar pattern was noticed in ChER inhibition, but the degree of inhibition noted in groups III and IV was absent. Recovery to 35-40%

of pretreatment levels was noticed by the 4th day (Fig 4).

Cholinesterase Activity of normal protein groups - The ChEP activity in groups V, VI, VII and VIII never decreased to zero level, but stayed between 4-15% (Fig 5). The most severe effect was in VIII, although deaths occurred in group VII (high C + TFP), where mean ChEP activity was 6% on the first day at which time deaths occurred. The degree of ChEP inhibition was more in group V (low C + saline) than in group VI (low C + TFP); however, there was no difference between the two groups at the end of 10 days.

The degree of ChER inhibition in groups V, VI, VII and VIII was relatively less than in the low protein groups. ChER activity dropped to between 30-35% in groups V and VI (low C), but recovery occurred from the 2nd day. Group VI (low C + TFP) reached a maximum recovery by the 6th day. Groups VII and VIII (high C) had ChER activity that was less than 20% of pretreatment levels; at that time all lambs in group VII (high C + TFP) died. The one survivor in group VIII (high C + saline) had a slow ChER recovery (Fig 6).

Cholinesterase Activity During the Last 15 Weeks - Mean ChE activities for the surviving lambs in groups I, II, V and VI (low C + TFP or saline) are given in Tables 5 and 6. The recovery of the ChE activity in these groups is presented in Fig 7. There was a significant variation in all the four groups when ChER values were compared (Fig 7). Groups II and VI (low C + TFP) had a faster ($0.01>P>0.009$) ChER recovery than group I and V (low C + saline). The shortest time was

in group VI (normal protein, C + TFP); ChER returned to pretreatment levels in 22 days compared to 27 days in group II (low protein, C + TFP). A similar response was noticed in group V (normal protein, C + saline); 36 days were required compared to 64 days for group I (low protein, C + saline) (Fig 7).

No significant statistical difference in ChEP activity between groups was found in relation to protein levels and TFP or saline treatment.

Low protein groups - ChE response varied in relation to the administration of TFP or saline, and a wide variation was also noticed in ChE in individual subjects from week to week (Table 5). Although recovery occurred earlier in group II (C + TFP) than in group I (C + saline), there was no consistency in the response of ChEP as compared to ChER (Table 5).

The recovery of ChEP and ChER was more rapid in group II (C + TFP) than in group I (C + saline). Levels above pretreatment were reached by the 3rd week in group II, but required 4 weeks in group I. A marked difference between the ChER of groups I and II was seen at the end of 15 weeks. ChER levels at the end of study were as high as 140% of pretreatment in group II, but approximately 100% of pretreatment levels in group I (Table 5). In some instances, particularly in group II (C + TFP), lambs had ChEP levels as high as 340% above pretreatment values (Table 5). Such an elevation was also seen in lambs of normal protein group. Elevations of ChER activities much above pretreatment levels were noticed in both protein groups (Table 6).

Normal protein groups - ChEP in group V (C + saline) had levels lower than pretreatment, although it reached that level during the 7th and 9th week (Table 6). Groups VI (C + TFP) had a ChEP response that remained at levels above pretreatment from 4th to 15th week. ChER activity remained same in both groups except that group VI (C + TFP) returned to pretreatment levels on the 1st week, while group V (C + saline) required 3-4 weeks. The activity of ChER remained above pretreatment levels in both groups. The highest individual ChEP level reached was 261% in group VI (C + TFP) (Table 6).

DISCUSSION

Organophosphorus compounds (OPC) form phosphorylated complexes with ChE, which are not easily hydrolyzed.[11] Toxicity depends on the degree of ChE inhibition and death occurs as a result of acute respiratory failure.[17,28] Lambs dosed with C regardless of dosage, showed clinical muscarinic and nicotinic signs characteristic of OPC toxicity and 55% of them died due to ChE inhibition.

OPC usage has resulted in unpredictable adverse effects[6] indicating potentiation, when administered at recommended doses.[25] The chemical nature of OPC,[24] its bioavailability and biotransformation, lethal synthesis or detoxification[10,21,24] are important in the determination of potentiation. The extent of detoxification in the animal exposed to OPC[13,32] can be adversely modified by physiological stressors; such

as emaciation,[18,30] and malnutrition, (e.g., inadequate dietary proteins).[8]

The inhibition of ChE by PHE is reversible.[7,12] The combined use of C and TFP is beneficial due to its synergetic effect on internal parasites.[19,21] In the present study, OPC toxicity was produced with recommended doses of C. The degree of ChEP and ChER inhibition seen in low (7%) and normal (12%) protein level groups had significant variation.

Low protein level appear a contributing factor in causing the toxic effects produced in this study. The shortest time of onset of signs (3.2 hours) in low protein groups was in group III (high C + TFP), suggesting that potentiation between C and TFP occurred. The mean onset of clinical signs in group IV (high C + saline) was 5.2 hours, indicating the absence of TFP caused delay in onset of signs. A 1.2 hour difference in onset of signs between groups I and II (low C + TFP or saline) may be explained in the same way.

Although 100% mortality occurred in both groups III and IV (high C + TFP or saline), the difference in mean survival times is clinically evident (Table 2). Only one animal in each groups I and II died. There is no definite explanation as to why the group II (low C + TFP) lamb survived 84 hours while the group I (low C + saline) animal lived only 36 hours. TFP may stabilize the liver lysosomes[16] and thereby reduce the availability of esterases that normally detoxify the metabolite of C.[7]

The longer mean times required for development of clinical signs in the normal protein groups (Table 2) suggests that this dietary protein level reduced the severity of C toxicity.[8] Whereas animals receiving low protein diets and low C showed clinical signs. One lamb in group V (low C + saline) and three in group VI (low C + TFP), all on normal protein, did not develop any signs of toxicity. Those that did develop signs of toxicity had them after 6 hours following treatment (groups I and VI). None of the lambs from these two groups died during the study (Table 2).

In the animals receiving normal protein diets, those receiving high dosages of C developed clinical signs first and those also receiving TFP (group VII) had the shortest survival time of all (19.4 hours). Healthy animals maintained on adequate protein diet should have well developed detoxification enzyme systems.[8] However, biotransformation of C results in the formation of an oxidative metabolite, coroxon, by replacing P=S with P=0.[20,27] Coroxon is a more potent inhibitor of ChE than its parent compound. The presence of adequate enzymatic proteins to catalyse this reaction contributes to the severity of toxicity. Further, the presence of TFP, another ChE inhibitor, likely produced a potentiation[3,34] which resulted in the high mortality rate, short survival time in group VII.

The toxicity exhibited by C and C + TFP interaction suggests a distinct hazard if one of the other more toxic OPC were used in place of C. In those instances, judgement as to

dosage, the sensitive state of nutrition, and the potential effect of OPC-PHE synergism becomes of clinical importance.

The longer survival time of group VII (66 hours) and the presence of one surviver in this group suggests that normal protein levels result in decreased toxicity when potentiating substances are not present.

The inhibition of ChE appears a primary factor of C toxicity. Significant ChE inhibition was characteristic of all experimental groups, regardless of dietary protein levels and use of TFP or saline (Tables 3, 4; Fig 3, 4, 5, 6). The degree of inhibition was a noticable difference between various treatments. A similar group difference in ChEP and ChER inhibition was also apparent. There was lack of correlation between ChEP and ChER depression and mortality rates. Individual lambs in groups I, II, VI and VIII had 0% ChEP activity. These lambs completely recovered at the end of 10 days, and pretreatment levels of ChEP and ChER were reached by the 3rd and 4th week. ChER inhibition among survivors in groups V and VI never dropped below 30-35% of pretreatment levels. The more prominent drop in ChEP activity, often to 9% of pretreatment levels and then recovery, is in contrast to the less severe depression of ChER. This was a consistent trend and supports the observations of others[2,3,4,35] that ChER more closely reflects the physiological level of ChE than does ChEP.

Deaths occurred in low protein groups when ChEP activity was near 0% and ChER was 5%. In normal protein groups this inhibition resulted in 5% of ChEP and 20% ChER activity. This

discrepancy indicates lack of correlation between percent of ChE inhibition and mortality. Although complete inhibition of ChEP occurred in individual animals of groups I, II, V and VI (low C + TFP or saline), recovery took place without any fatalities. On the other hand, ChER activity below 5% of pretreatment levels appeared invariably fatal; this is in agreement with other workers.[11,28]

Since ChE, particularly ChER, has a definite physiologic role in OPC toxicities, its recovery becomes important from the clinical and therapeutic point of view. ChER recovery to pretreatment levels probably occurred by reactivation of the phosphorylated enzyme, new enzyme synthesis or both.[5,11,35] Recently phosphorylated ChE may undergo spontaneous hydrolysis,[11] which can be further inhibited by additional OPC or its metabolites. Aging causes stabilization of the phosphorylated enzyme making it more resistant to enzymatic degradation.[24,36] Spontaneous hydrolysis occurs most frequently soon after ChE phosphorylation, but as aging progresses, spontaneous recovery becomes slower and enzymatic degradation takes preference.[5,35] This is a constant feature with dialkyl OPC toxicity.[2]

Evaluation of ChE response during the first 10 days of the present study did not reveal specific differences in recovery between the low and normal protein groups. The ChEP response had only a slight variation at the end of the 10th day.

The additional ChEP inhibition on day 2 in the groups receiving C + TFP probably resulted from the second TFP

injection. ChEP recovery appeared progressively consistent through 10th day (Fig 3, 5).

In contrast, ChER activity fluctuated, but recovery began on the 2nd day irrespective of protein levels. During this period there was intermittent fluctuation suggesting that the inhibition of ChER was still in progress. This possibly shows that C and its metabolites were still active or inhibition continues to persist long after the disappearance of C.[2] The therapeutic value of PHE thus becomes doubtful, since the probability of potentiation and further ChER inhibition was significant. Deaths due to OPC-PHE interaction were features of previous reports.[3,14] In the light of the enhancement of acute toxicity due to OPC-PHE interaction, it is interesting that in the present study recovery of ChER was more rapid in TFP treated groups than in those receiving saline ($0.01>P>0.009$).

The ChEP and ChER response during the last 15 weeks appear related to protein levels and TFP treatment (Fig 7). ChER in group VI (high C + TFP) returned to pretreatment levels in the shortest time suggesting the beneficial effects or normal protein diets and TFP exposure on later stages of ChE recovery. The highly significant values obtained for ChER in relation to dietary proteins, effect of TFP, and duration of enzyme recovery indicate the same.

ChEP and ChER activity during the last 15 weeks of the study increased to levels greater than pretreatment. In some individuals ChEP activity reached 300-350% of pretreatment

values. This has been observed with OPC toxicity[33] but was not discussed. The activity of ChE is subject to much variation and is influenced by factors such as sex, age, genetics, hormones, nutrition, season and time of day.[32] To minimize some of these variations samples were always collected at the same time of day in the present study. Influence of sex and age were limited by using female lambs of same age.

The ChE rebound phenomenon noticed in the last 15 weeks may be due to several factors. Stress induced by continued malnutrition might be a possible factor,[32] since low protein groups showed higher ChEP values than normal protein groups. The liver is the major source of ChEP, and increased ChEP activity reflects increased synthesis or increased release from the hepatic cells. However, there is no direct evidence to substantiate that C or its metabolites might induce synthesis of ChEP[23] and/or alter hepatic cell permeability. Further, the suggested stabilization of liver lysosomes by TFP,[16] should have suppressed release of ChEP. TFP may not even play any role in increasing ChEP activity, since in the present study similar ChEP response also occurred in lambs treated with TFP or saline.

The recovery of ChER during the 15 week period showed a linear return to normal, especially in normal protein groups. This response was not prominent in low protein groups and reinforces the relationship of diet to hemopoiesis and synthesis of new enzyme.[5,11,32,35]

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Fig 1 - Myocardial Hemorrhages. Extensive petechiae are seen around the coronary groove in a lamb treated with 8 mg coumaphos and 1.1 mg/kg triflupromazine maintained on low (7%) protein diet.

Fig 2 - Severe congestion of omasal mucosa in a lamb treated with 8 mg/kg coumaphos and 1.1 mg/kg triflupromazine, maintained on low (7%) protein diet.


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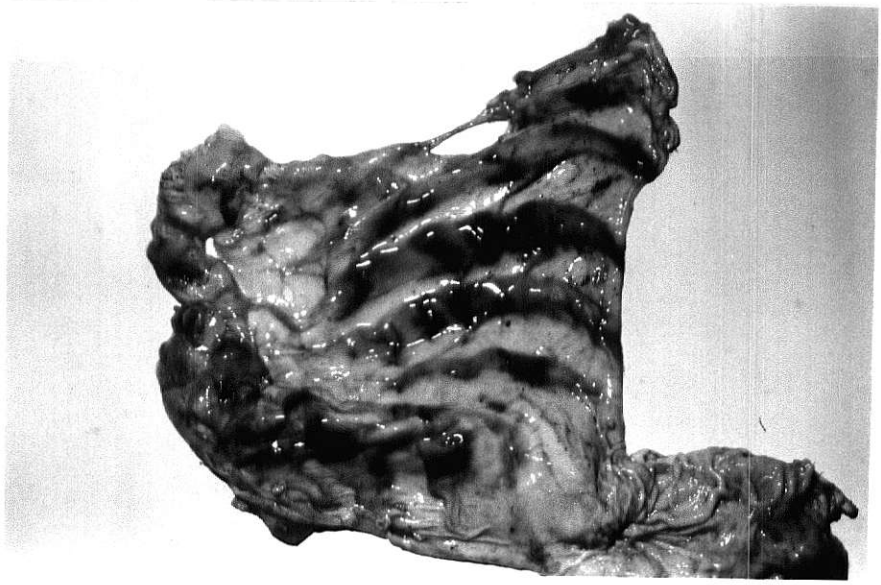

Fig 3 - Cholinesterase Plasma (ChEP) Activity in Lambs Maintained on Low (7%) Protein Diet, and Treated with Coumaphos (C) and Triflupromazine (TFP) or Saline.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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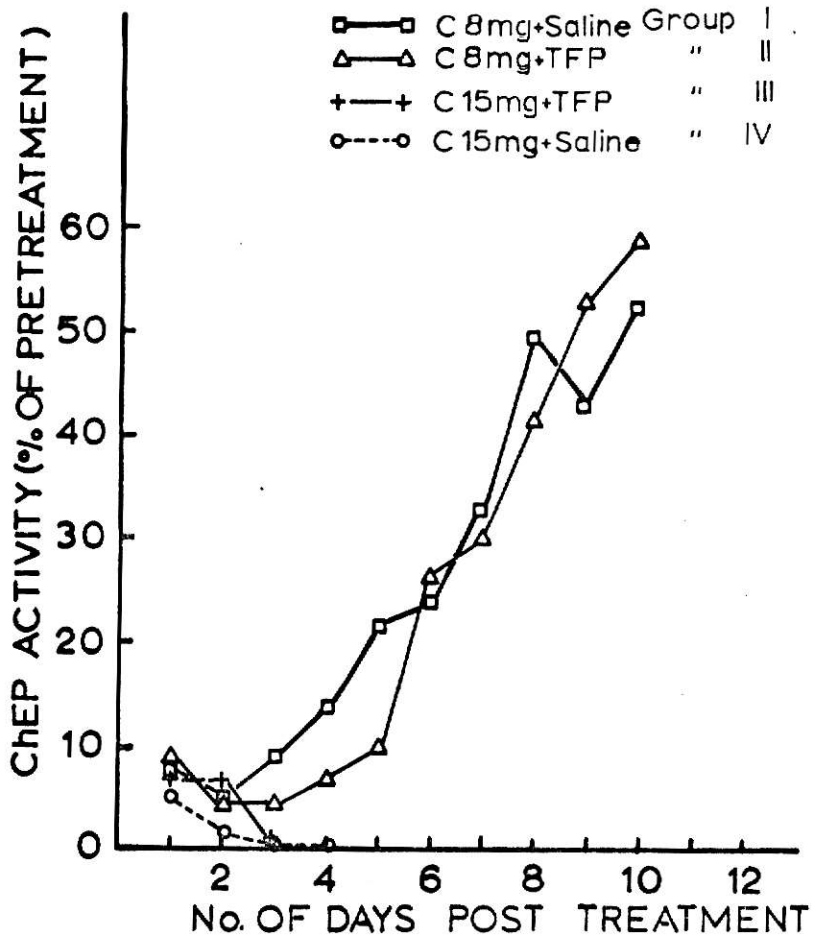

C 8mg+Saline Group I
C 8mg+TFP " II
C 15mg+TFP " III
C 15mg+Saline " IV
ChEP ACTIVITY (% OF PRETREATMENT)
0
10
20
30
40
50
60
2
4
6
8
10
12
No. OF DAYS POST TREATMENT

Fig 4 - Cholinesterase Erythrocyte (ChER) Activity in Lambs Maintained on Low (7%) Protein Diet, and Treated with Coumaphos (C) and Trifluopromazine (TFP) or Saline.

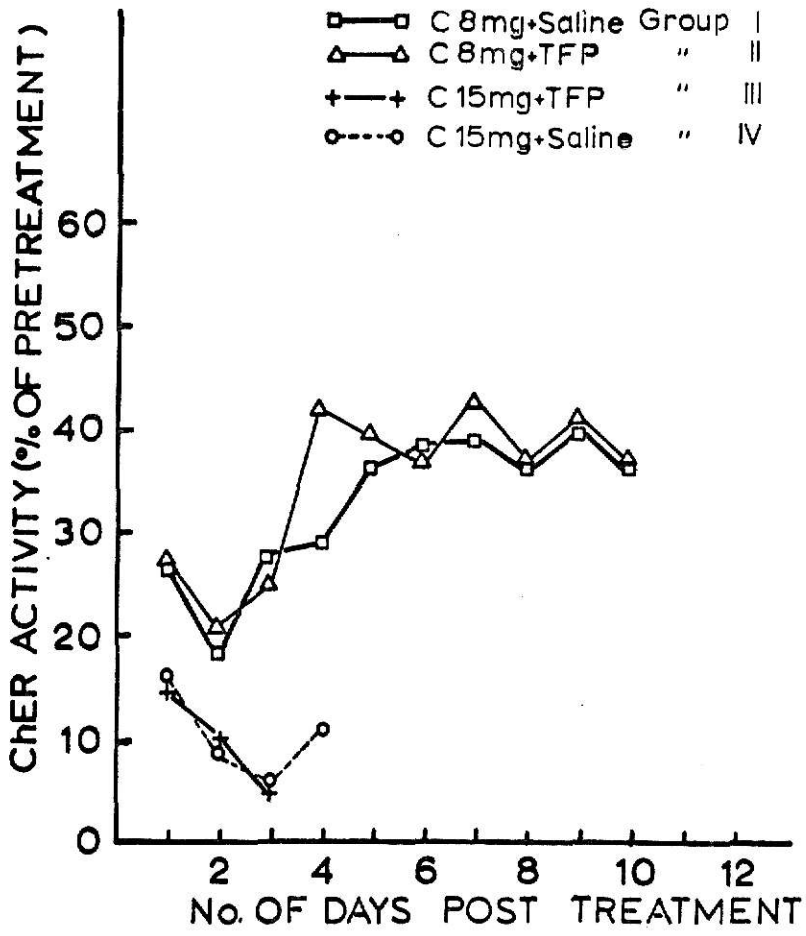

C 8mg+Saline Group I
C 8mg+TFP " II
C 15mg+TFP " III
C 15mg+Saline " IV
ChER ACTIVITY (% OF PRETREATMENT)
0
10
20
30
40
50
60
2
4
6
8
10
12
No. OF DAYS POST TREATMENT

Fig 5 - Cholinesterase Plasma (ChEP) Activity in Lambs Maintained on Normal (12%) Protein Diet, and Treated with Coumaphos (C) and Trifluopromazine (TFP) or Saline.

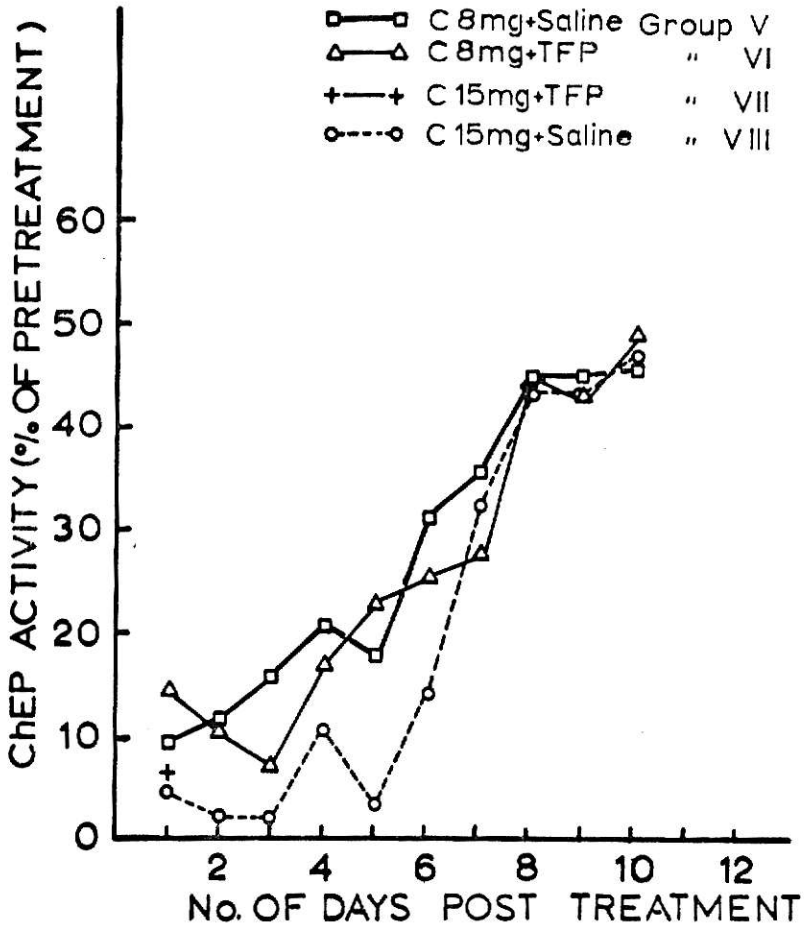

C 8mg+Saline Group V
C 8mg+TFP " VI
C 15mg+TFP " VII
C 15mg+Saline " VIII
ChEP ACTIVITY (% OF PRETREATMENT)
0
10
20
30
40
50
60
2
4
6
8
10
12
No. OF DAYS POST TREATMENT

Fig 6 - Cholinesterase Erythrocyte (ChER) Activity in Lambs Maintained on Normal (12%) Protein Diet, and Treated with Coumaphos (C) and Trifluopromazine (TFP) or Saline.

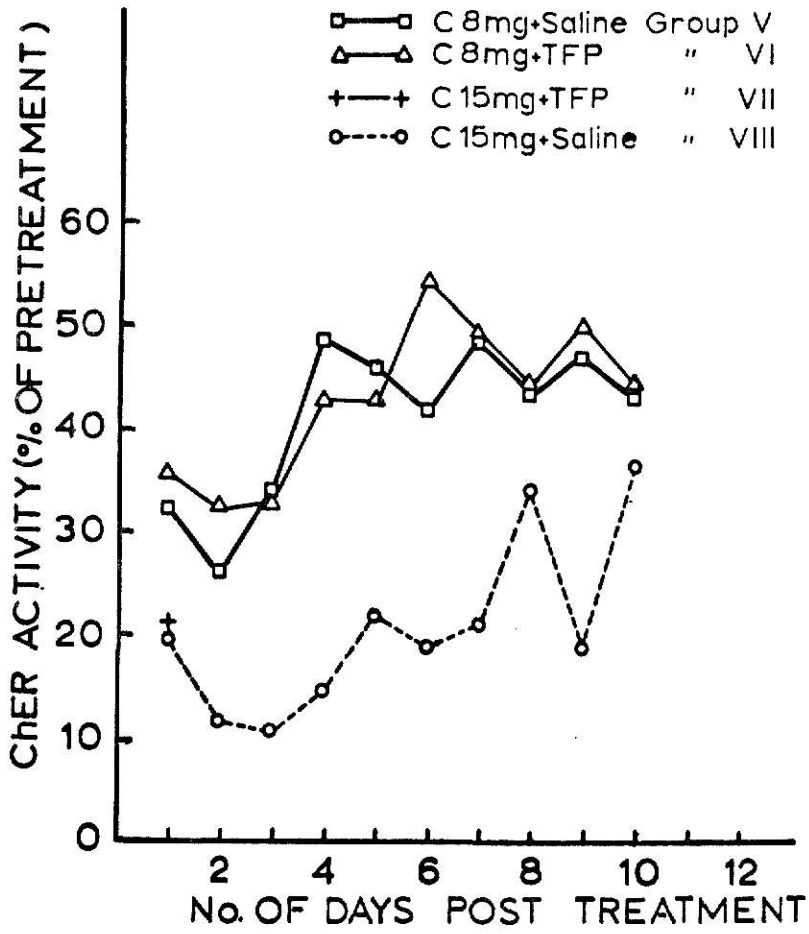

C 8mg+Saline Group V
C 8mg+TFP " VI
C 15mg+TFP " VII
C 15mg+Saline " VIII
ChER ACTIVITY (% OF PRETREATMENT)
0
10
20
30
40
50
60
2
4
6
8
10
12
No. OF DAYS POST TREATMENT

Fig 7 - Days Required for the Recovery of Cholinesterase Plasma (ChEP) and Erythrocytes (ChER) to Pre-treatment Levels in Lambs Maintained on Low (7%) and Normal (12%) Protein Diets, and Treated with Coumaphos (8 mg/kg) (C) and Triflupromazine (1.1 mg/kg) (TFP) or Saline.

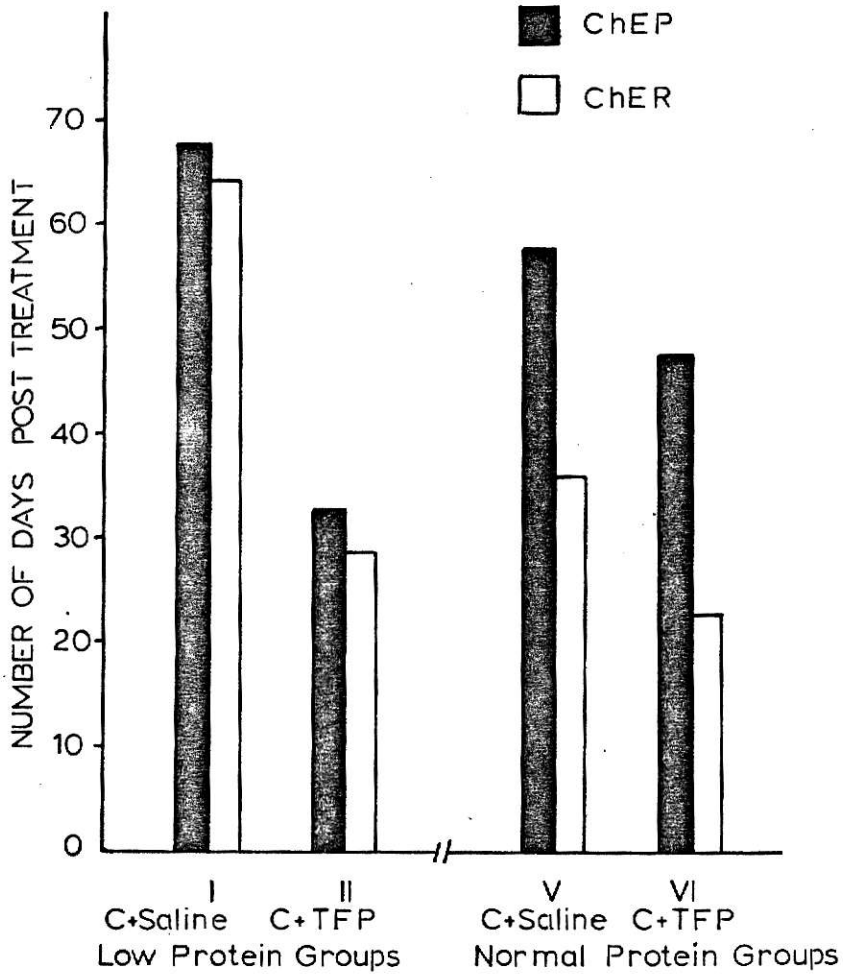

ChEP
ChER
NUMBER OF DAYS POST TREATMENT
0
10
20
30
40
50
60
70
I
C+Saline
II
C+TFP
Low Protein Groups
V
C+Saline
VI
C+TFP
Normal Protein Groups

TABLE 1 - Treatment Schedule of Lambs on Low (7%) or Normal (12%) Protein Diets and Receiving Coumaphos (C) and Triflupromazine (TFP) or Saline

Group	Treatment	
	Coumaphos (mg/kg)	Triflupromazine (mg/kg) or saline equal volume
Low protein diet		
I	8	saline
II	8	1.1
III	15	1.1
IV	15	saline
Normal protein diet		
V	8	saline
VI	8	1.1
VII	15	1.1
VIII	15	saline

TABLE 2 - Mean Time of Onset of Signs, Percent Mortality, and Mean Survival Times of Lambs on Low (7%) or Normal (12%) Protein Diets and Treated with Coumaphos (C)* Triflupromazine (TFP)** or Saline

Group	Treatment	Time of onset of signs (hrs ± SEM)	No. died/ group	Mortality (%)	Survival time (hrs ± SEM)
Low protein diet					
I	C 8 mg/kg + saline	6.0	1/5	20	36.0
II	C 8 mg/kg + TFP	4.8 (±0.2)	1/5	20	84.0
III	C 15 mg/kg + TFP	3.2 (±0.5)	5/5	100	40.4 (±14.0)
IV	C 15 mg/kg + saline	5.2 (±0.9)	5/5	100	50.9 (±14.0)
Normal protein diet					
V	C 8 mg/kg + saline	>6.0	0/4†	-	-
VI	C 8 mg/kg + TFP	>6.0	0/5	-	-
VII	C 15 mg/kg + TFP	5.0 (±0.6)	5/5	100	19.4 (± 5.2)
VIII	C 15 mg/kg + saline	>6.0	4/5	80	66.0 (±24.9)

* Coumaphos ** Triflupromazine, 1.1 mg/kg. † One lamb died of causes unrelated to the study.

TABLE 3 - Post-Treatment Plasma (ChEP) and Erythrocyte (ChER) Cholinesterase Activity (% ± SEM; Pre-treatment = 100%) in Lambs Maintained on Low (7%) Protein Diet, and Treated with Coumaphos (C) and Triflupromazine (TFP) or Saline

No of Days	Group I* C 8 mg/kg + Saline		Group II** C 8 mg/kg + TFP 1.1 mg/kg		Group III† C 15 mg/kg + TFP 1.1 mg/kg		Group IV‡ C 15 mg/kg + Saline	
	ChEP	ChER	ChEP	ChER	ChEP	ChER	ChEP	ChER
1	7.3 ± 3.0 (0.0 - 19.4)	26.8 ± 3.8 (19.7 - 42.9)	8.9 ± 1.6 (4.5 - 11.0)	27.0 ± 1.9 (21.1 - 32.9)	6.8 ± 0.6 (4.4 - 8.9)	14.5 ± 1.8 (11.5 - 22.2)	5.0 ± 2.2 (0.0 - 13.3)	15.8 ± 1.5 (11.0 - 20.2)
2	5.0 ± 1.8 (0.0 - 9.7)	17.7 ± 2.5 (13.9 - 26.1)	4.7 ± 1.3 (0.0 - 7.8)	20.3 ± 2.1 (13.8 - 28.3)	6.5	9.5	1.7 ± 1.2 (0.0 - 3.3)	8.4 (8.3 - 8.4)
3	8.6 ± 2.6 (3.3 - 16.1)	27.3 ± 4.6 (19.2 - 42.9)	4.6 ± 1.6 (0.0 - 11.0)	24.3 ± 4.9 (8.6 - 41.1)	0.0	4.8	0.0	5.1
4	13.3 ± 2.9 (6.5 - 21.2)	28.4 ± 5.2 (13.0 - 30.3)	6.8 ± 2.8 (4.5 - 18.3)	41.3 ± 9.7 (24.6 - 73.9)	- -	- -	0.0	10.1
5	21.0 ± 3.8 (13.0 - 30.3)	35.7 ± 3.4 (26.9 - 43.3)	9.4 ± 5.7 (4.5 - 18.5)	38.9 ± 5.0 (24.6 - 52.2)	- -	- -	- -	- -
6	23.2 ± 3.4 (13.8 - 30.3)	37.7 ± 4.3 (30.3 - 52.2)	26.0 ± 4.5 (15.5 - 40.3)	36.6 ± 2.6 (32.6 - 50.0)	- -	- -	- -	- -
7	32.0 ± 2.5 (25.8 - 39.4)	38.1 ± 5.2 (24.7 - 54.0)	29.3 ± 1.9 (23.3 - 33.3)	42.1 ± 4.9 (29.8 - 56.0)	- -	- -	- -	- -
8	49.1 ± 9.7 (32.6 - 81.8)	35.5 ± 2.5 (32.6 - 41.0)	41.1 ± 4.0 (36.3 - 51.9)	36.3 ± 3.9 (29.8 - 47.8)	- -	- -	- -	- -
9	42.6 ± 4.6 (32.6 - 57.6)	39.0 ± 2.5 (33.5 - 45.9)	52.3 ± 6.9 (31.0 - 66.7)	40.1 ± 2.4 (35.1 - 47.8)	- -	- -	- -	- -
10	51.4 ± 2.1 (45.6 - 57.6)	35.5 ± 1.6 (32.6 - 41.0)	58.0 ± 10.3 (38.9 - 92.6)	36.3 ± 2.3 (28.8 - 47.8)	- -	- -	- -	- -

N = Number of animals in the group.
* N=5 on day 1, 4 from day 2 onwards.
** N=5 on day 1-4, 4 from day 5 onwards.
† N=5 on day 1, 1 on days 2 and 3.
‡ N=5 on day 1, 2 on day 2, 1 on days 3 and 4.

TABLE 4 - Post-Treatment Plasma (ChEP) and Erythrocyte (ChER) Cholinesterase Activity (%± SEM; Pre-treatment = 100%) in Lambs Maintained on Normal (12%) Protein Diet, and Treated with Coumaphos (C) and Triflupromazine (TFP) or Saline

No of Days	Group V* C 8 mg/kg + Saline		Group VI** C 8 mg/kg + TFP 1.1 mg/kg		Group VII† C 15 mg/kg + TFP 1.1 mg/kg		Group VIII‡ C 15 mg/kg + Saline	
	ChEP	ChER	ChEP	ChER	ChEP	ChER	ChEP	ChER
1	9.7 ± 1.2 (7.5 - 13.2)	31.9 ± 4.3 (19.7 - 41.0)	14.6 ± 3.7 (5.9 - 28.4)	35.2 ± 7.7 (20.9 - 67.0)	6.3 ± 3.6 (0.0 - 17.4)	20.4 ± 5.9 (10.3 - 39.7)	4.8 ± 2.4 (0.0 - 10.8)	19.3 ± 1.7 (15.0 - 24.5)
2	12.0 ± 4.4 (3.8 - 26.5)	25.6 ± 6.2 (13.1 - 46.3)	10.7 ± 4.4 (0.0 - 28.4)	32.1 ± 6.2 (14.4 - 53.4)	- -	- -	2.3 ± 0.9 (0.0 - 3.6)	11.6 ± 3.1 (6.5 - 18.9)
3	15.9 ± 4.4 (7.8 - 30.9)	33.5 ± 6.1 (18.0 - 50.0)	7.3 ± 3.1 (0.0 - 18.2)	32.8 ± 8.4 (12.0 - 57.6)	- -	- -	1.6 ± 0.9 (0.0 - 3.6)	10.4 ± 2.0 (7.5 - 13.2)
4	21.1 ± 5.9 (11.3 - 34.5)	48.1 ± 6.7 (31.2 - 63.0)	17.0 ± 6.6 (0.0 - 37.8)	42.4 ± 7.8 (16.0 - (67.0)	- -	- -	10.8 ± 7.7 (0.0 - 21.7)	14.1 ± 2.0 (11.3 - 17.0)
5	18.0 ± 5.7 (0.0 - 31.0)	45.6 ± 7.5 (32.8 - 68.5)	23.0 ± 6.0 (2.9 - 44.2)	42.2 ± 8.0 (48.1 - 65.6)	- -	- -	3.6 ± 2.6 (0.0 - 7.2)	21.7 ± 8.7 (9.4 - 34.0)
6	31.7 ± 1.8 (26.3 - 35.1)	41.3 ± 4.2 (32.8 - 53.7)	25.5 ± 4.7 (14.2 - 44.2)	54.0 ± 8.5 (17.7 - 64.1)	- -	- -	14.5	18.9
7	35.8 ± 3.1 (27.3 - 37.9)	48.0 ± 7.1 (33.3 - 70.4)	27.9 ± 4.7 (17.7 - 45.5)	49.2 ± 8.6 (61.8 - 72.3)	- -	- -	32.5	20.8
8	45.2 ± 1.6 (41.2 - 48.5)	42.8 ± 6.6 (27.9 - 63.9)	44.6 ± 4.3 (29.4 - 60.0)	44.4 ± 7.8 (14.4 - 71.0)	- -	- -	43.4	34.0
9	45.2 ± 2.7 (37.5 - 51.7)	46.9 ± 3.2 (37.7 - 55.6)	43.3 ± 4.4 (26.5 - 56.8)	49.6 ± 8.9 (14.4 - 64.3)	- -	- -	43.4	18.9
10	46.1 ± 2.6 (37.5 - 51.7)	42.8 ± 6.6 (27.9 - 63.9)	49.0 ± 3.4 (50.0 - 72.6)	44.4 ± 7.8 (14.4 - 64.3)	- -	- -	47.0	35.9

N = Number of animals in the group. * N=4 animals on all days. ** N=5 on all days. † N=5 on day 1 only; all died.
‡ N= 4 on day 1, 3 on day 2, 2 on days 3-4, and 1 rest of the period.

TABLE 5 - Post-Treatment Plasma (ChEP) and Erythrocyte (ChER) Cholinesterase Activity (% ± SEM; Pre-treatment = 100%) in Lambs Maintained on Low (7%) Protein Diet, and Treated with Coumaphos (C) and Triflupromazine (TFP) or Saline During 15 Weeks Following 10 Days Observation

No of Weeks	Group I * C 8 mg/kg + Saline		Group II ** C 8 mg/kg + TFP 1.1 mg/kg		Group III ** C 15 mg/kg + TFP 1.1 mg/kg		Group IV ** C 15 mg/kg + Saline	
	ChEP	ChER	ChEP	ChER	ChEP	ChER	ChEP	ChER
1	55.0 ± 3.3 (46.2 - 63.6)	59.6 ± 8.4 (41.6 - 83.9)	79.9 ± 12.5 (53.7 - 114.8)	90.2 ± 12.9 (57.9 - 121.3)	- -	- -	- -	- -
2	75.7 ± 5.6 (58.1 - 87.7)	45.6 ± 6.2 (25.8 - 59.6)	97.7 ± 12.5 (69.8 - 137.0)	60.1 ± 7.8 (49.3 - 87.0)	- -	- -	- -	- -
3	68.9 ± 6.5 (51.5 - 87.7)	80.7 ± 7.3 (58.4 - 94.2)	102.6 ± 28.3 (62.7 - 200.0)	128.5 ± 22.7 (84.2 - 201.4)	- -	- -	- -	- -
4	104.8 ± 8.4 (93.9 - 133.9)	101.7 ± 8.2 (76.4 - 117.4)	141.4 ± 31.3 (90.5 - 248.2)	157.3 ± 26.6 (102.6 - 240.4)	- -	- -	- -	- -
5	99.8 ± 10.8 (74.2 - 129.3)	50.2 ± 5.1 (33.7 - 59.6)	83.7 ± 7.5 (67.3 - 98.8)	59.5 ± 5.1 (47.4 - 69.9)	- -	- -	- -	- -
6	79.9 ± 11.8 (58.7 - 120.0)	54.6 ± 4.0 (47.2 - 68.0)	151.4 ± 44.5 (76.8 - 296.3)	86.5 ± 10.3 (57.5 - 108.7)	- -	- -	- -	- -
7	114.3 ± 27.1 (78.3 - 129.3)	70.5 ± 8.3 (43.8 - 89.5)	159.3 ± 55.1 (72.4 - 348.1)	99.4 ± 8.4 (79.3 - 125.4)	- -	- -	- -	- -
8	94.9 ± 10.1 (78.3 - 129.3)	113.4 ± 7.9 (88.8 - 132.6)	89.1 ± 9.3 (64.7 - 117.0)	141.6 ± 11.1 (119.3 - 171.7)	- -	- -	- -	- -
9	122.0 ± 12.3 (87.9 - 157.0)	138.2 ± 15.0 (88.8 - 162.2)	105.2 ± 10.1 (85.2 - 134.4)	165.8 ± 3.8 (157.0 - 173.9)	- -	- -	- -	- -
10	110.7 ± 20.1 (74.2 - 176.1)	77.8 ± 4.9 (65.2 - 91.5)	148.0 ± 49.7 (69.8 - 318.5)	112.6 ± 9.0 (85.5 - 130.4)	- -	- -	- -	- -
11	100.1 ± 27.6 (54.8 - 192.4)	69.8 ± 6.8 (49.4 - 87.2)	114.3 ± 37.9 (56.9 - 244.4)	111.6 ± 10.0 (88.6 - 141.8)	- -	- -	- -	- -
12	150.6 ± 49.2 (63.6 - 303.3)	95.4 ± 11.7 (58.4 - 122.1)	129.5 ± 47.4 (62.0 - 292.6)	118.5 ± 5.2 (108.8 - 135.6)	- -	- -	- -	- -
13	140.5 ± 36.0 (71.0 - 244.6)	107.7 ± 5.7 (92.1 - 122.1)	127.9 ± 33.1 (75.0 - 240.7)	128.6 ± 10.8 (94.8 - 152.0)	- -	- -	- -	- -
14	128.0 ± 27.0 (74.2 - 202.2)	105.9 ± 13.0 (66.7 - 130.8)	114.9 ± 22.9 (75.0 - 192.6)	141.0 ± 5.3 (130.6 - 156.2)	- -	- -	- -	- -
15	94.8 ± 16.2 (63.6 - 140.2)	101.9 ± 10.8 (69.7 - 127.3)	90.0 ± 13.1 (67.3 - 133.3)	139.0 ± 5.9) (126.3 - 156.2)	- -	- -	- -	- -

(N) = Number of animals in the group. * N=4 in each group. ** N=All died.

Maintained on Normal (12%) Protein Diet, and Treated with Coumaphos (C) and Trifluoromazine (TFP) or Saline During 15 Weeks Following 10 days Observation

No of Weeks	Group V * C 8 mg/kg + Saline		Group VI ** C 8 mg/kg + TFP 1.1 mg/kg		Group VII † C 15 mg/kg + TFP 1.1 mg/kg		Group VIII ‡ C 15 mg/kg + Saline	
	ChEP	ChER	ChEP	ChER	ChEP	ChER	ChEP	ChER
1	62.8 ± 6.5 (44.1 - 77.9)	81.9 ± 5.8 (73.8 - 101.6)	64.8 ± 3.8 (50.0 - 72.6)	100.9 ± 9.1) (70.6 - 116.8)	- -	- -	65.1	73.6
2	86.2 ± 2.9 (79.4 - 89.7)	61.0 ± 3.1 (50.8 - 66.7)	82.9 ± 4.1 (67.8 - 91.0)	62.4 ± 8.1 (27.3 - 80.4)	- -	- -	101.2	26.4
3	80.0 ± 6.4 (69.0 - 101.5)	97.9 ± 4.7 (88.5 - 113.0)	85.2 ± 11.8 (63.6 - 135.3)	117.0 ± 3.4 (107.1 - 128.4)	- -	- -	72.8	96.2
4	97.9 ± 1.8 (75.9 - 103.5)	114.4 ± 7.0 (100.0 - 136.1)	129.1 ± 29.9 (80.9 - 261.8)	150.5 ± 15.1 (124.0 - 198.9)	- -	- -	122.9	120.8
5	86.7 ± 5.7 (75.9 - 105.2)	60.5 ± 3.2 (51.9 - 68.8)	109.1 ± 16.7 (78.8 - 182.4)	61.6 ± 3.4 (48.1 - 70.0)	- -	- -	97.6	41.5
6	82.6 ± 1.2 (79.4 - 86.2)	73.3 ± 4.7 (60.7 - 85.2)	118.1 ± 24.8 (70.8 - 220.6)	78.0 ± 6.5 (62.6 - 104.0)	- -	- -	65.1	56.6
7	100.4 ± 5.2 (89.7 - 116.9)	82.4 ± 8.3 (66.7 - 106.6)	123.2 ± 21.3 (90.9 - 217.7)	88.6 ± 3.5 (80.8 - 98.5)	- -	- -	119.3	64.2
8	87.1 ± 3.2 (79.3 - 97.0)	96.0 ± 2.3 (90.2 - 101.9)	116.5 ± 21.0 (78.8 - 191.2)	100.2 ± 3.9 (86.6 - 109.9)	- -	- -	155.4	77.4
9	101.3 ± 8.9 (75.9 - 120.8)	97.7 ± 4.2 (90.2 - 105.6)	152.9 ± 24.6 (106.1 - 226.5)	102.6 ± 8.5 (76.3 - 124.0)	- -	- -	126.5	83.0
10	85.0 ± 2.5 (79.3 - 92.6)	89.7 ± 3.6 (82.0 - 98.4)	148.6 ± 34.6 (77.5 - 247.5)	106.5 ± 7.3 (88.4 - 130.5)	- -	- -	130.1	77.4
11	60.7 ± 2.9 (55.2 - 70.2)	92.5 ± 4.7 (78.7 - 104.7)	120.6 ± 22.7 (72.7 - 193.2)	99.6 ± 6.0 (77.7 - 112.2)	- -	- -	94.0	67.9
12	88.4 ± 6.4 (78.7 0 110.3)	103.3 ± 2.1 (98.4 - 109.8)	119.6 ± 16.9 (84.3 - 169.5)	113.1 ± 6.6 (88.4 - 132.8)	- -	- -	101.2	94.3
13	95.0 ± 2.4 (88.2 - 101.3)	114.4 ± 2.0 (109.4 - 118.5)	137.8 ± 8.7 (111.2 - 159.3)	128.1 ± 14.3 (88.4 - 183.2)	- -	- -	144.6	98.1
14	91.3 ± 0.8 (89.6 - 93.1)	119.1 ± 3.5 (109.4 - 127.8)	109.2 ± 6.8 (87.9 - 128.8)	132.5 ± 4.7 (116.5 - 145.1)	- -	- -	115.7	109.1
15	90.4 ± 2.1 (86.2 - 97.3)	115.5 ± 4.7 (108.2 - 131.5)	98.3 ± 3.8 (84.9 - 108.5)	134.1 ± 4.7 (119.2 - 148.3)	- -	- -	94.0	100.0

(N) is the no. of animals in the group. * N=4 in each group. ** N=5 in each group. † N=none survived ‡ N=one survived.

APPENDIX I

GROUP AND INDIVIDUAL DATA

Experimental Design and Treatments Adopted in the Study, Influence of Dietary Protein on the Effect of Coumaphos (C) and Triflupromazine (TFP) Interaction in Sheep. N = Five animals per group.

	C 8 mg/kg		C 15 mg/kg	
Low (7%) Protein	I Saline	II 1.1 mg/kg TFP	III 1.1 mg/kg TFP	IV Saline
Normal (12%) Protein	V Saline	VI 1.1 mg/kg TFP	VII 1.1 mg/kg TFP	VIII Saline

Fig 1 - Individual Survival Time (hrs) of Lambs Maintained on Low (7%) and Normal (12%) Protein Diets, and Treated with Coumaphos (C) and Triflupromazine (TFP) or Saline

Low Protein Groups	Mortality
I C 8 mg/kg + saline	1/5
II C 8 mg/kg + TFP 1.1 mg/kg	1/5
III C 15 mg/kg + TFP 1.1 mg/kg	5/5
IV C 15 mg/kg + saline	5/5
Normal Protein Groups	
V C 8 mg/kg + saline	0/4
VI C 8 mg/kg + TFP 1.1 mg/kg	0/5
VII C 15 mg/kg + TFP 1.1 mg/kg	5/5
VIII C 15 mg/kg + saline	4/5

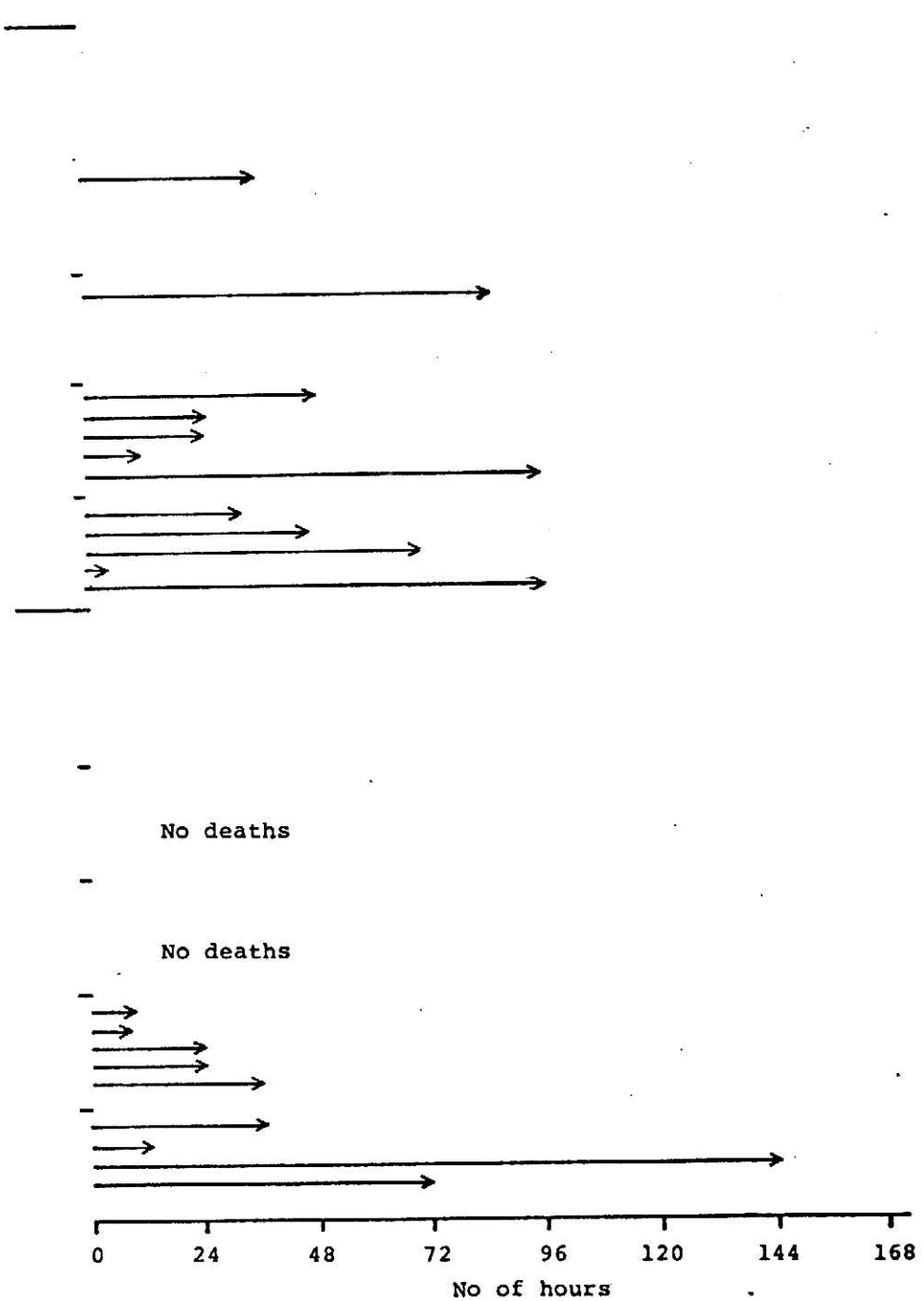


Fig 2 - Least Square Means of Cholinesterase Plasma (ChEP) and Erythrocytes (ChER) Activity in Lambs Maintained on Low (7%) Protein Diet, and Treated with Coumaphos (8 mg/kg) (C) and Triflupromazine (1.1 mg/kg) (TFP) or Saline (during last 15 weeks following 10 days post-treatment observation)

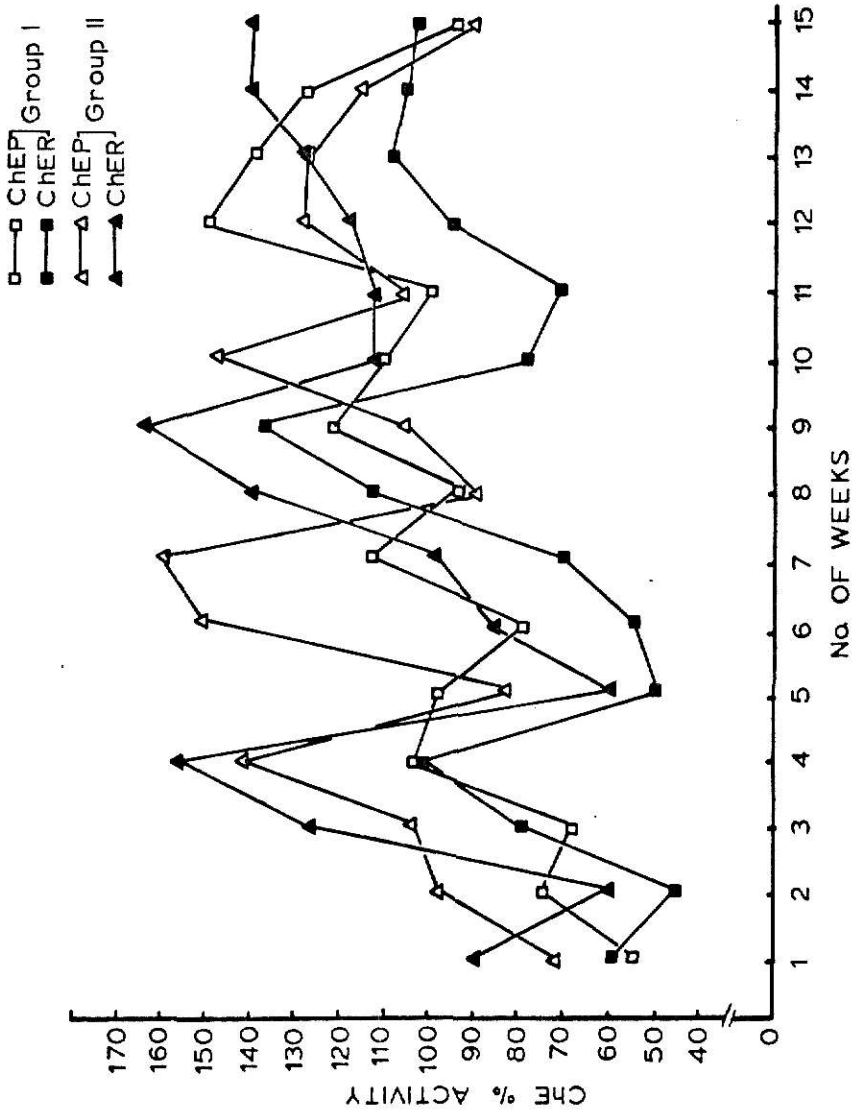
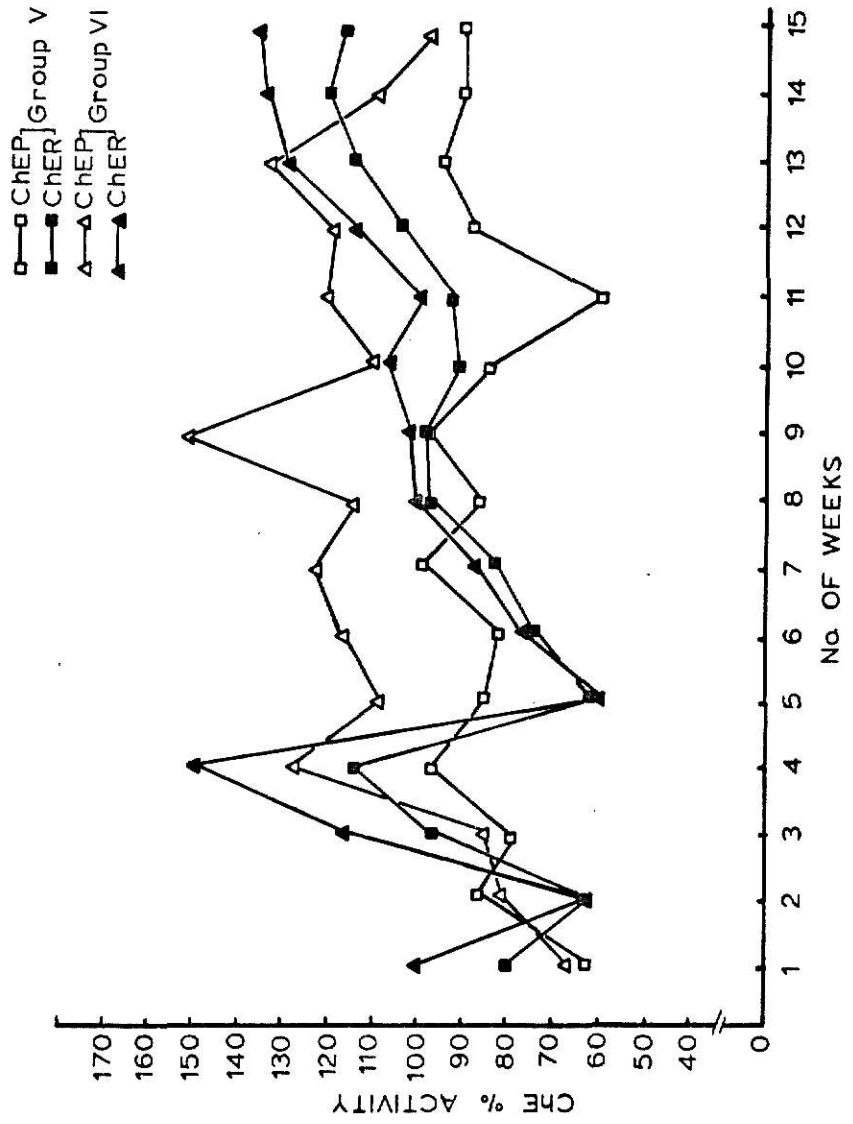

ChEP Group I
ChER
ChEP Group II
ChER
ChE % ACTIVITY
170
160
150
140
130
120
110
100
90
80
70
60
50
40
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
No OF WEEKS

Fig 3 - Least Square Means of Cholinesterase Plasma (ChEP) and Erythrocytes (ChER) Activity in Lambs Maintained on Normal (12%) Protein Diet, and Treated with Coumaphos (8 mg/kg) (C) and Trifluprom azine (1.1 mg/kg) (TFP) or Saline (during last 15 weeks following 10 days post-treatment observation).


ChEP
ChER
Group V
ChEP
ChER
Group VI
ChE % ACTIVITY
170
160
150
140
130
120
110
100
90
80
70
60
50
40
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
No. OF WEEKS

GROUP NO: I

No. of lambs: 5

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: Saline equal volume

Mean initial body weight: 28.7 kg
Mean final body weight: 36.5 kg
No lambs survived: 4

Mean ChE Activity (±SEM) as ΔpH

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.36(±0.1)	0.59(±0.2)
- 7d	0.27(±0.1)	0.62(±0.2)
0d	0.24(±0.1)	0.54(±0.2)
Mean ChE = 100%	0.29(±0.1)	0.58(±0.2)

Mean onset time: 6 hours
Mean survival time: 36 hours (one lamb)
Percent mortality: 20

Clinical: Dyspnea, salivation, lacrimation, muscular fasciculation twitches and tremors. Arching of back and straining abdomen. Frequent micturition and diarrhea.

10 days post-treatment

1	7.3(±3.0)	26.8(±1.2)
2	5.0(±1.8)	17.7(±4.7)
3	8.6(±2.6)	27.3(±4.4)
4	13.3(±2.9)	28.4(±5.9)
5	20.7(±3.8)	35.7(±5.7)
6	23.2(±3.6)	37.7(±1.8)
7	31.8(±2.5)	38.1(±3.1)
8	49.1(±9.7)	35.5(±1.6)
9	42.6(±4.6)	39.0(±2.7)
0	51.4(±2.1)	35.5(±2.6)

Necropsy: Trachae and lungs congested, edematous. Myocardial petichae. Intestines and rectum congested. Kidney congested with petichae on bladder mucosa.

15 weeks following 10 days

1	55.0(±3.3)	59.6(±6.5)
2	75.7(±5.6)	45.6(±2.9)
3	68.9(±6.5)	80.7(±6.4)
4	104.8(±8.4)	101.7(±1.8)
5	99.8(±10.8)	50.2(±5.7)
6	79.9(±11.8)	54.6(±1.2)
7	114.3(±27.1)	70.5(±5.2)
8	94.9(±10.1)	113.4(±3.2)
9	122.9(±12.3)	138.2(±8.9)
0	110.7(±20.1)	77.8(±2.5)
1	100.1(±27.6)	69.8(±2.9)
2	150.6(±49.2)	95.4(±6.4)
3	140.5(±36.0)	107.7(±2.4)
4	128.0(±27.0)	105.9(±0.8)
5	94.8(±16.2)	101.9(±2.1)

ChE response:

ChEP

Lowest:	5.0 (day 2)
Return to pre-treatment level:	104.8 (week 4)
Highest:	150.6 (week 12)

ChER

Lowest:	17.7 (day 2)
Return to pre-treatment level:	101.7 (week 4)
Highest:	138.2 (week 9)

LAMB NO: 4

GROUP NO: I

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Trifluprom azine dose: saline equal volume

Initial body weight: 26.4 kg
Final body weight: - -
Outcome: died

ChE Activity			Observations
Pretreatment (as Δ pH)			Onset time: 6 hours Survival time: 36 hours
Time	ChEP	ChER	
- 14d	0.35	0.58	Clinical: Dyspnea, salivation, lacrimation , fasciculations and twitchings. Frequent micturition. Straining of the abdomen, diarrhea. Prolapse of the rectum. Recumbant.
- 7d	0.28	0.62	
0	0.23	0.63	
Mean ChE = 100%	0.29(±0.1)	0.61(±0.2)	
10 days post-treatment			
1	0.0	19.7	
2	†	†	Necropsy: Trachea and lungs congested edematous. Myocardial petichae. Intestines and rectum congested. Congested kidney, bladder showed petichae.
15 weeks following 10 days			ChE response:

ChEP

Lowest: 0.0 (day 1)
Return to pre-treatment level: †
Highest: †

ChER

Lowest: 19.7 (day 1)
Return to pre-treatment level: †
Highest: †

† Data not available due to death of the animal.

LAMB NO: 5

GROUP NO: I

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 31.82 kg
Final body weight: 44.55 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.39	0.58
- 7d	0.31	0.57
0	0.29	0.53
Mean ChE = 100%	0.33(±0.1)	0.56(±0.2)

10 days post-treatment

1	9.1	21.3
2	7.0	14.6
3	10.5	24.7
4	21.2	32.8
5	30.3	27.0
6	30.3	30.3
7	39.4	24.7
8	81.8	32.6
9	57.6	45.9
0	57.6	32.6

15 weeks following 10 days

1	63.6	41.6
2	81.8	25.8
3	51.5	58.4
4	93.9	76.4
5	84.8	33.7
6	69.7	47.2
7	90.7	43.8
8	84.9	88.8
9	87.9	88.8
0	81.8	65.2
1	60.6	49.4
2	63.6	58.4
3	75.8	92.1
4	78.9	62.7
5	63.6	69.7

Onset time: 6 hours
Survival time: survived

Clinical: Dyspnea, salivation, lacrimation. Muscular fasciculations and twitchings. Signs continued after 24 hours Animal remained dull and recumbant. Gradual recovery by 8th day. Normal by the 10th day.

Necropsy: - -

ChE response:

ChEP

Lowest:	9 (day 2)
Return to pre-treatment level:	-
Highest:	93.9* (week 4)

ChER

Lowest:	14.6 (day 2)
Return to pre-treatment level:	-
Highest:	92.1* (week 13)

* Animal did not reach pretreatment level during the study.

LAMB NO: 7

GROUP NO: I

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 25.91 kg
Final body weight: 26.82 kg
Outcome: Alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.29	0.64
- 7d	0.18	0.68
0	0.18	0.55
Mean ChE = 100%	0.22(±0.1)	0.62(±0.2)

10 days post-treatment

1	4.6	27.3
2	0.0	16.0
3	4.6	22.5
4	9.2	36.9
5	13.9	43.3
6	13.9	35.3
7	29.4	36.9
8	36.9	33.7
9	41.6	41.7
0	50.8	33.7

15 weeks following 10 days

1	46.2	46.8
2	87.7	46.5
3	87.7	77.0
4	133.9	97.9
5	124.3	49.7
6	120.0	52.9
7	207.8	73.8
8	129.3	118.7
9	157.0	139.6
0	110.8	91.5
1	92.3	73.8
2	170.8	105.9
3	170.8	113.9
4	157.0	120.3
5	110.8	113.9

Onset time: 6 hours
Survival time: survived

Clinical: Dyspnea, salivation lacrimation, muscular fasciculation and twitchings. Straining of abdomen. Remained recumbant slow recovery. Remained under weight and weak till the end of the experiment.

Necropsy: - -

ChE response:

ChEP

Lowest:	0.0 (day 2)
Return to pre-treatment level:	133.9 (week 4)
Highest	207.8

ChER

Lowest:	16.0 (day 2)
Return to pre-treatment level:	118.7 (week 8)
Highest:	139.6 (week 9)

LAMB NO: 12

GROUP NO: I

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: Saline equal volume

Initial body weight: 27.73 kg
Final body weight: 32.73 kg
Outcome: Alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.38	0.61
- 7d	0.29	0.66
0	0.25	0.45
Mean ChE = 100%	0.31(±0.1)	0.57(±0.2)

Onset time: 6 hours
Survival time: survived

Clinical: Onset of signs similar with dyspnea, salivation, lacrimation, muscular twitchings. Straining of abdomen, frequent micturition and diarrhea. Remained recumbant for 5 days - recovery gradual.

10 days post-treatment

1	3.3	22.7
2	3.3	14.0
3	3.3	19.2
4	6.5	10.5
5	12.9	31.4
6	19.6	33.6
7	32.6	36.6
8	32.6	34.9
9	32.6	34.9
0	45.7	34.9

Necropsy: - -

15 weeks following 10 days

1	58.7	66.3
2	75.0	50.6
3	65.2	94.2
4	94.6	115.1
5	110.9	57.6
6	58.7	68.0
7	75.0	75.0
8	78.3	132.6
9	127.2	162.2
0	176.1	82.0
1	192.4	87.2
2	303.3	122.1
3	244.6	122.1
4	202.2	130.8
5	140.3	127.3

ChE response:

ChEP

Lowest:	3.3 (day 1)
Return to pre-treatment level:	110.9 (week 5)
Highest:	303.3 (week 12)

ChER

Lowest:	10.5 (day 4)
Return to pre-treatment level:	115.1 (week 4)
Highest:	162.2 (week 9)

LAMB NO: 14

GROUP NO: I

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 30.45 kg
Final body weight: 41.82 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.40	0.54
- 7d	0.28	0.55
0	0.25	0.52
Mean ChE = 100%	0.31(±0.1)	0.52(±0.2)

Onset time: 6 hours
Survival time: survived

Clinical: Dyspnea, salivation, lacrimation, muscular twitchings, frequent micturition and diarrhea. Signs less severe. First four days showed signs - gradual recovery by 9th day.

10 days post-treatment

1	19.4	42.9
2	9.7	26.1
3	16.1	42.9
4	16.1	33.5
5	25.8	41.0
6	29.0	52.2
7	25.8	54.0
8	45.2	41.0
9	38.7	33.5
0	51.6	41.0

Necropsy: - -

15 weeks following 10 days

1	51.6	83.9
2	58.1	59.6
3	71.0	93.2
4	96.8	117.4
5	74.2	59.6
6	71.0	50.3
7	83.9	89.5
8	87.1	113.7
9	119.4	162.1
0	74.2	72.7
1	54.8	69.0
2	64.5	95.0
3	71.0	102.5
4	74.2	110.0
5	64.5	96.9

ChE response:

ChEP

Lowest:	9.7 (day 2)
Return to pre-treatment level:	119.4 (week 9)
Highest	119.4 (week 9)

ChER

Lowest:	26.1 (day 2)
Return to pre-treatment level:	117.4 (week 4)
Highest:	162.1 (week 9)

GROUP NO: II

No. of lambs: 5

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Mean initial body weight: 28.9 kg
Mean final body weight: 38.5 kg
No lambs survived: 4

Mean ChE Activity (±SEM) as ΔpH

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.34(±0.1)	0.55(±0.2)
- 7d	0.28(±0.1)	0.56(±0.2)
0d	0.22(±0.1)	0.53(±0.2)

Mean ChE = 100%

10 days post-treatment

1	8.9(±1.6)	27.0(±1.9)
2	4.7(±1.2)	20.3(±2.1)
3	4.4(±1.6)	24.3(±4.9)
4	6.8(±2.8)	41.3(±9.6)
5	19.4(±5.8)	38.9(±5.0)
6	26.0(±4.5)	36.6(±2.6)
7	29.3(±1.9)	42.1(±4.9)
8	41.1(±4.0)	36.3(±3.9)
9	52.3(±6.9)	40.1(±2.4)
0	58.0(±10.3)	36.3(±2.6)

15 weeks following 10 days

1	71.9(±12.5)	90.2(±12.9)
2	97.9(±12.5)	60.1(±7.8)
3	102.6(±28.3)	128.5(±22.7)
4	141.4(±31.3)	157.3(±26.6)
5	83.7(±7.5)	59.5(±5.1)
6	151.4(±44.5)	86.5(±10.3)
7	159.3(±55.1)	99.4(±8.4)
8	89.1(±9.3)	141.5(±11.1)
9	105.2(±10.1)	165.8(±3.8)
0	148.0(±49.7)	112.6(±9.0)
1	114.3(±37.9)	111.6(±10.0)
2	129.5(±47.4)	118.5(±5.2)
3	127.9(±33.1)	128.6(±10.8)
4	114.9(±22.9)	141.0(±5.3)
5	90.0(±13.1)	139.1(±5.9)

Mean onset time: 4.8 hours
Mean survival time: 84 hours (one lamb)
Percent mortality: 20

Clinical: Animals became dull half hour after treatment and remained so for 4 - 6 hours. Dyspnea, salivation, lacrimation, muscular twitches and tremors. Frequent micturition and diarrhea were seen. One animal became comatosed and died on the 4th day.

Necropsy: Trachea and lungs congested and edematous. Very few myocardial petichae. Prominent ecchymatic patches on the mucosa of omasum and duodenum. G.I. mucosa thickened and ulcerated. Kidney slightly congested.

ChE response:

ChEP

Lowest:	4.4 (day 3)
Return to pre-treatment level:	102.6 (week 3)
Highest:	159.3 (week 7)

ChER

Lowest:	20.3 (day 2)
Return to pre-treatment level:	128.5 (week 3)
Highest:	165.8 (week 9)

LAMB NO: 6

GROUP NO: II

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 32.73 kg
Final body weight: 35.91 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.33	0.46
- 7d	0.30	0.59
0	0.18	0.41
Mean ChE = 100%	0.27(±0.1)	0.49(±0.2)

Onset time: 4 hours
Survival time: survived

Clinical: Became dull half hour after treatment, remained so until hour 4. Classical clinical signs appeared and persisted until 10th day, gradually disappeared by 17th day.

10 days post-treatment

1	14.8	32.9
2	3.7	18.5
3	3.7	41.1
4	7.4	30.8
5	18.5	37.0
6	25.9	34.9
7	33.3	37.0
8	51.9	28.8
9	66.7	37.0
0	92.6	28.8

Necropsy: - -

15 weeks following 10 days

1	114.8	121.3
2	137.0	49.3
3	200.0	201.4
4	248.2	240.4
5	70.4	69.9
6	296.3	102.8
7	348.2	125.4
8	85.2	154.1
9	85.2	172.6
0	318.5	127.4
1	244.4	141.8
2	292.6	135.6
3	240.7	152.1
4	192.6	156.2
3	133.3	156.2

ChE response:

ChEP

Lowest:	3.7 (day 2)
Return to pre-treatment level:	114.8 (week 1)
Highest	348.2 (week 7)

ChER

Lowest:	18.5 (day 2)
Return to pre-treatment level:	121.3 (week 1)
Highest:	240.4 (week 4)

LAMB NO: 15

GROUP NO: II

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 27.73
Final body weight: --
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.37	0.61
- 7d	0.29	0.50
0	0.26	0.63
Mean ChE = 100%	0.31(±0.1)	0.58(±0.2)

10 days post-treatment

1.	6.5	25.9
2	0.0	13.8
3	0.0	8.6
4	†	†

Onset time: 4-6 hours
Survival time: 84 hours

Clinical: Became dull and inactive ½ hour after treatment. Severe dyspnea, salivation, lacrimation, muscular twitching. Frequent micturition and diarrhea, became comatosal on 3rd day. 4th day he was found dead.

Necropsy: Trachea and lungs congested and edematous. Very few myocardial petichae. Prominent ecchymotic patches on the mucosa of omasum and duodenum. G.I. mucosa thickened with ulceration. Kidneys slightly congested.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	0.0 (day 2)
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	8.6 (day 3)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animal.

LAMB NO: 23

GROUP NO: II

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 28.64 kg
Final body weight: 34.55 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.25	0.55
- 7d	0.25	0.58
0	0.17	0.58
Mean ChE = 100%	0.22(±0.2)	0.57(±0.2)

10 days post-treatment

1	4.5	21.1
2	4.5	19.3
3	4.5	17.5
4	4.5	24.6
5	4.5	24.6
6	22.4	33.3
7	31.4	29.8
8	44.8	29.8
9	49.3	35.1
0	49.3	29.8

15 weeks following 10 days

1	53.7	57.9
2	85.1	52.6
3	62.7	84.2
4	120.9	121.1
5	98.5	47.4
6	152.3	77.2
7	103.0	93.0
8	89.6	119.3
9	134.4	159.7
0	94.0	107.0
1	71.7	115.8
2	71.7	112.3
3	89.6	126.3
4	89.6	131.6
5	71.7	126.3

Onset time: 4 - 6 hours
Survival time: survived

Clinical: Became dull ½ hour after treatment. Dyspnea, salivation, lacrimation. Muscular twitchings and fasciculation. Frequent micturition and diarrhea. Recovered and had a relapse for 2 days. Recovered gradually then on.

Necropsy: - -

ChE response:

ChEP

Lowest:	4.5 (day 1)
Return to pre-treatment level:	120.9 (week 4)
Highest:	152.3 (week 6)

ChER

Lowest:	17.5 (day 3)
Return to pre-treatment level:	121.1 (week 4)
Highest:	159.7 (week 10)

LAMB NO: 26

GROUP NO: II

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Trifluprom azine dose: 1.1 mg/kg

Initial body weight: 27.27 kg
Final body weight: 42.73 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.32	0.63
- 7d	0.25	0.69
0	0.25	0.61
Mean ChE = 100%	0.27(±0.1)	0.64(±0.2)

10 days post-treatment

1	11.0	24.9
2	7.3	21.8
3	11.0	28.0
4	18.3	35.8
5	36.6	42.0
6	40.3	32.6
7	29.3	56.0
8	36.6	38.9
9	62.2	40.4
0	51.2	38.9

15 weeks following 10 days

1	62.2	73.1
2	98.8	51.3
3	80.5	97.9
4	106.1	102.6
5	98.8	51.3
6	76.8	57.5
7	113.4	79.3
8	117.1	121.3
9	113.4	157.0
0	109.8	85.5
1	84.2	88.6
2	91.5	108.8
3	106.1	94.8
4	102.5	130.6
5	87.8	130.6

Onset time: 4 - 6 hours
Survival time: survived

Clinical: Animal became dull ½ hour after dosage. Dyspneas, salivation, lacrimation, muscular twitchings, frequent micturition and diarrhea. Abdominal palpitation and straining. Recovery started on 4th day and was normal in 28 days.

Necropsy: - -

ChE response:

ChEP

Lowest:	7.3 (day 2)
Return to pre-treatment level:	106.1 (week 4)
Highest:	117.1 (week 8)

ChER

Lowest:	21.8 (day 2)
Return to pre-treatment level:	102.6 (week 4)
Highest:	157.0 (week 9)

LAMB NO: 40

GROUP NO: II

Dietary protein level: Low (7%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 28.18 kg
Final body weight: 40.91 kg
Outcome: alive

ChE Activity

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.43	0.52
- 7d	0.31	0.44
0	0.26	0.42
Mean ChE = 100%	0.33(±0.1)	0.46(±0.2)

10 days post-treatment

1	7.8	30.4
2	7.8	28.3
3	2.6	26.1
4	7.8	73.9
5	18.1	52.2
6	15.5	50.0
7	23.3	45.7
8	31.0	47.8
9	31.0	47.8
0	38.8	47.8

15 weeks following 10 days

1	56.9	108.7
2	69.8	87.0
3	67.3	130.4
4	90.5	165.2
5	67.3	70.0
6	80.2	108.7
7	72.4	100.0
8	64.7	171.7
9	88.0	173.9
0	69.8	130.4
1	56.9	100.0
2	62.1	117.4
3	75.0	141.3
4	75.0	145.7
5	67.3	143.5

Observations

Onset time: 4 - 6 hours
Survival time: survived

Clinical: Animal became dull after ½ hour following treatment. Dyspnea, salivation, lacrimation, muscular twitching and fasciculation, frequent micturition and diarrhea. Improved on 4th day, gradual recovery to normal on 8th day.

Necropsy: - -

ChE response:

ChEP

Lowest:	2.6 (day 3)
Return to pre-treatment level:	>90.5 (week 4)
Highest	>90.5 (week 4)

ChER

Lowest:	26.1 (day 3)
Return to pre-treatment level:	108.7 (week 1)
Highest:	173.9 (week 9)

GROUP NO: III

No. of lambs: 5

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: 1.1 mg/kg

Mean initial body weight: 28.8 kg
Mean final body weight: -
No lambs survived: none

Mean ChE Activity (±SEM) as ΔpH

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.34(±0.1)	0.50(±0.2)
- 7d	0.30(±0.1)	0.59(±0.2)
0d	0.22(±0.1)	0.53(±0.2)
Mean ChE = 100%	0.29(±0.1)	0.54(±0.2)

10 days post-treatment

1	6.8(±0.6)	14.5(±1.8)
2	6.5	9.5
3	0.0	4.8
4	†	†

15 weeks following 10 days

Observations

Mean onset time: 3.2 hours
Mean survival time: 40.4 hours
Percent mortality: 100

Clinical: Dull and inactive in 15-30 minutes after treatment. Salivation 2-4 hours. Dyspnea, lacrimation, musclar tremors, frequent micturition and diarrhea. Comatosed before death. One animal in this group was treated with atropine 1/M. Reversing of clinical effects but died next day.

Necropsy: Trachea and lungs congested and edematous. Emphysema. Myocardial petichae and hemorrhages. Duodenum congested and hemorrhagic. Liver, kidneys congested. Bladder occasional congestion.

ChE response:

ChEP

Lowest:	0.0 (day 3)
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	4.8 (day 3)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of animals.

LAMB NO: 21

GROUP NO: III

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 30.91 kg
Final body weight: --
Outcome: died

ChE Activity

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.31	0.53
- 7d	0.31	0.54
0	0.22	0.60
Mean ChE = 100%	0.28(±0.1)	0.56(±0.2)
10 days post-treatment		
1	7.1	14.3
2	†	†

15 weeks following 10 days

Observations

Onset time: < 4 hours
Survival time: 48 hours

Clinical: Dull and inactive in 15 minutes after treatment. Salivation within 4 hours. Dyspnea, lacrimation, muscular twitches, frequent micturition and diarrhea. Remained same for 24 hours. 6 ml atropine 1M, 8 hours after treatment stopped salivation and dyspnea . The lamb was alert, died next day.

Necropsy: Trachea congested contained frothy material, lungs edematous, congested and emphysematous. Kidney and bladder congested. Duodenum showed congestion and hemorrhage.

ChE response:

ChEP

Lowest:	7.1 (day 1)
Return to pre-treatment level:	†
Highest	†

ChER

Lowest:	14.3 (day 1)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animal.

LAMB NO: 31

GROUP NO: III

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Trifluprom azine dose: 1.1 mg/kg

Initial body weight: 29.09 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.33	0.40
- 7d	0.26	0.41
0	0.25	0.47
Mean ChE = 100%	0.28(±0.1)	0.43(±0.2)

10 days post-treatment

1	7.1	11.7
2	†	†

Onset time: < 4 hours
Survival time: 24 hours

Clinical: Dull and inactive within 15 minutes of treatment. Severe dyspnea, salivation, lacrimation muscular twitchings, fasciculations, frequent micturition and diarrhea. Became very weak and comatosed before death.

Necropsy: Severe edema and congestion of trachea and lungs. Emphysema in lungs. Kidney and bladder highly congested. Intestine congested.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	7.1 (day 1)
Return to pre-treatment level:	†
Highest	†

ChER

Lowest:	11.7 (day 1)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animal.

LAMB NO: 32

GROUP NO: III

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Trifluprom azine dose: 1.1 mg/kg

Initial body weight: 27.73 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.45	0.53
- 7d	0.36	0.60
0	0.20	0.44
Mean ChE = 100%	0.34(±0.1)	0.52(±0.2)
10 days post-treatment		
1	8.9	11.5
2	†	†

Onset time: < 4 hours
Survival time: 24 hours

Clinical: Became dull and inactive in 15 minutes. Dyspnea, severe salivation, lacrimation, muscular tremors and twitches. Severe diarrhea and frequent micturition. Stretched hind limbs and arched back. Gradually became worse and died.

Necropsy: Severe edema and congestion in trachea and lungs. Emphysematous areas in lungs. Intestine and kidneys congested. Bladder showed areas of congestion.

15 weeks following 10 days

ChE response:

ChEP

Lowest: 8.9 (day 1)
Return to pre-treatment level: †
Highest: †

ChER

Lowest: 11.5 (day 1)
Return to pre-treatment level: †
Highest: †

† Data not available due to death of the animal.

LAMB NO: 35

GROUP NO: III

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 29.55 kg
Final body weight: ---
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.24	0.44
- 7d	0.25	0.67
0	0.19	0.54
Mean ChE = 100%	0.23(±0.1)	0.55(±0.2)

10 days post-treatment

1	4.4	12.7
2	†	†

Onset time: 2 hours
Survival time: 10 hours

Clinical: Depressed and inactive, with severe dyspnea, salivation, lacrimation, muscular twitchings and tremors, with frequent micturition and diarrhea. Became recumbant 8 hours following treatment and comatosed before death.

Necropsy: Severe edema and congestion of trachea. Lungs did not show any prominent lesions. Myocardial petichae. Petichae on duodenal serosa. Ecchymotic and hemorrhagic kidneys. Bladder hemorrhagic streaks.

15 weeks following 10 days

ChE response:

ChEP

Lowest: 4.4 (day 1)
Return to pre-treatment level: †
Highest: †

ChER

Lowest: 12.7 (day 1)
Return to pre-treatment level: †
Highest: †

† Data not available due to death of the animal.

LAMB NO: 39

GROUP NO: III

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Trifluprομazine dose: 1.1 mg/kg

Initial body weight: 26.36 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.37	0.59
- 7d	0.32	0.72
0	0.23	0.58
Mean ChE = 100%	0.31(±0.1)	0.63(±0.2)

10 days post-treatment

1	6.5	22.2
2	6.5	9.5
3	0.0	4.8
4	†	†

Onset time: 2 hours
Survival time: 96 hours

Clinical: Severe dyspnea, salivation and lacrimation within 2 hours. Severe diarrhea and frequent micturition. Muscular twitchings and tremors. Animal was very weak and remained so for 3 days.

Necropsy: Trachea showed peticheal hemorrhages, lungs less edematous and congested. Liver congested. Kidneys pale. Bladder showed no hemorrhage. Duodenal mucosa had had a few hemorrhagic spots.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	0.0 (day 3)
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	4.8 (day 3)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animal.

GROUP NO: IV

No. of lambs: 5

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: Saline equal volume

Mean initial body weight: 29.5 kg
Mean final body weight: - -
No lambs survived: none

Mean ChE Activity (±SEM) as ΔpH

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.38(±0.1)	0.55(±0.2)
- 7d	0.28(±0.1)	0.55(±0.2)
0d	0.27(±0.1)	0.52(±0.2)
Mean ChE = 100%	0.31(±0.1)	0.54(±0.2)

Mean onset time: 5.2 hours
Mean survival time: 50.9
Percent mortality: 100

Clinical: Severe dyspnea, salivation, lacrimation, fasciculations and tremors. Frequent micturition and diarrhea. Terminal recumbancy and coma.

10 days post-treatment

1	5.0(±2.2)	15.8(±1.5)
2	1.7	8.4
3	0.0	5.1
4	0.0	10.1
5	†	†

Necropsy: Trachea and lungs edematous and congested. Emphysema. Myocardial hemorrhages. Gastro intestinal congestion and severe hemorrhages. Occasional bladder congestion.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	0.0 (day 3)
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	5.1 (day 3)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animals.

LAMB NO: 8

GROUP NO: IV

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Trifluprom azine dose: Saline equal volume

Initial body weight: 30.81 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.37	0.62
- 7d	0.21	0.71
0	0.18	0.43
Mean ChE = 100%	0.25(±0.1)	0.59(±0.2)

Onset time: 4 hours
Survival time: 34 hours

Clinical: Severe dyspnea, salivation, lacrimation, fasciculation and tremors. Frequent micturition and diarrhea. Terminal recumbancy and coma.

10 days post-treatment

1	7.9	13.6
2	†	†

Necropsy: Tracheal edema and congestion. Lungs did not show evidence of congestion and emphysema. Bladder congested. Pharyngeal lymph nodes edematous and enlarged.

15 weeks following 10 days

ChE response:

ChEP

Lowest: 7.9 (day 1)
Return to pre-treatment level: †
Highest: †

ChER

Lowest: 13.6 (day 1)
Return to pre-treatment level: †
Highest: †

† Data not available due to death of the animal.

LAMB NO: 11

GROUP NO: IV

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Trifluprομazine dose: saline equal volume

Initial body weight: 32.73 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.35	0.48
- 7d	0.28	0.42
0	0.17	0.46
Mean ChE = 100%	0.27(±0.1)	0.45(±0.2)

10 days post-treatment

1	3.7	11.0
2	†	†

Onset time: 7 hours
Survival time: 48 hours

Clinical: Severe dyspnea, salivation, lacrimation, muscular fasciculations and tremors. Frequent micturition and diarrhea Became progressively weak and died in the night.

Necropsy: Trachea and lungs severely edematious and congested. Emphysema of lungs was prominent. Myocardial petichae. Kidney and liver pale. Bladder highly congested.

15 weeks following 10 days

ChE response:

ChEP

Lowest: 3.7 (day 1)
Return to pre-treatment level: †
Highest: †

ChER

Lowest: 11.0 (day 1)
Return to pre-treatment level: †
Highest: †

† Data not available due to death of the animal.

LAMB NO: 13

GROUP NO: IV

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 25.91 kg
Final body weight: --
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.34	0.59
- 7d	0.25	0.60
0	0.23	0.61
Mean ChE = 100%	0.27(±0.1)	0.60(±0.2)

10 days post-treatment

1	0.0	15.0
2	0.0	8.3
3	†	†

Onset time: 7 hours
Survival time: 42 hours

Clinical: Severe dyspnea, salivation, lacrimation. Muscular twitchings and tremors. Frequent micturition and diarrhea, straining. Became very weak and died.

Necropsy: Trachea and lungs edematous and congested. Kidneys congested, bladder showed hemorrhage omasal ecchymosis on the mucosa. Areas of hemorrhage on the intestinal mucosa.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	0.0 (day 1)
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	8.3 (day 2)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animal.

LAMB NO: 17

GROUP NO: IV

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 29.55 kg
Final body weight: --
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.44	0.46
- 7d	0.38	0.41
0	0.52	0.55
Mean ChE = 100%	0.45(±0.2)	0.47(±0.2)

10 days post-treatment

1	13.1	19.0
2	†	†

Onset time: 2 hours
Survival time: 4.30 hours

Clinical: Severe frothy salivation. Dyspnea and lacrimation. Curved back and tucked in abdomen. Muscular tremors all over the hind limbs. Attempted to micturate and died suddenly.

Necropsy: Lungs consolidated with evidence of pneumonia showed fibrous adhesions to the thoracic wall. Myocardial petichae. Pericardium contained serofibrinous exudate.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	13.1 (day 1)
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	19.0 (day 1)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animal.

LAMB NO: 28

GROUP NO: IV

Dietary protein level: Low (7%)
Coumaphos dose: 15 mg/kg
Trifluprom azine dose: saline equal volume

Initial body weight: 28.18 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.38	0.62
- 7d	0.29	0.62
0	0.24	0.54
Mean ChE = 100%	0.30(±0.1)	0.59(±0.2)

10 days post-treatment

1	0.0	20.2
2	3.3	8.4
3	0.0	5.1
4	0.0	10.1
5	†	†

Onset time: < 6 hours
Survival time: 96 hours

Clinical: Marked dyspnea, severe salivation and lacrimation. Muscular twitches and tremors. Straining, frequent micturition and diarrhea. Animal remained recumbant until death.

Necropsy: Trachea and lungs edematous and congested. Bladder had streaks of hemorrhage. Hemorrhages on duodenum and jejunum.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	0.0 (day 1)
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	5.1 (day 1)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animal.

GROUP NO: V

No. of lambs: 5

Dietary protein level: normal (12%)
Coumaphos dose: 8 mg/kg
Trifluprazine dose: saline equal volume

Mean initial body weight: 27.9 kg
Mean final body weight: 43.6 kg
No lambs survived: 5

Mean ChE Activity (±SEM) as ΔpH

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.31(±0.1)	0.62(±0.2)
- 7d	0.25(±0.1)	0.60(±0.2)
0d	0.22(±0.1)	0.59(±0.2)
Mean ChE = 100%	0.26(±0.1)	0.60(±0.2)

10 days post-treatment

1	9.7(±1.2)	31.9(±4.3)
2	12.0(±4.7)	25.6(±6.2)
3	15.9(±4.4)	33.5(±6.1)
4	21.1(±5.9)	48.1(±6.7)
5	18.0(±5.7)	45.6(±7.5)
6	31.7(±1.8)	41.3(±4.2)
7	35.8(±3.1)	48.0(±7.0)
8	45.2(±1.6)	42.8(±6.6)
9	45.2(±2.7)	46.9(±3.2)
0	46.1(±2.6)	42.8(±6.6)

15 weeks following 10 days

1	62.8(±6.5)	81.9(±5.8)
2	86.2(±2.9)	61.0(±3.1)
3	80.0(±6.4)	97.6(±4.7)
4	97.9(±1.8)	114.4(±7.0)
5	86.7(±5.7)	60.5(±3.2)
6	82.6(±1.2)	73.3(±4.7)
7	100.4(±5.2)	82.4(±8.3)
8	87.1(±3.2)	96.0(±2.3)
9	101.3(±8.9)	97.7(±4.2)
0	85.0(±2.5)	89.7(±3.6)
1	60.7(±2.9)	92.5(±4.7)
2	88.4(±6.4)	103.3(±2.1)
3	95.0(±2.4)	114.4(±2.0)
4	91.4(±0.8)	119.1(±3.5)
5	90.4(±2.1)	115.5(±4.7)

Mean onset time: > 6 hours
Mean survival time: --
Percent mortality: 0

Clinical: No clinical signs in one animal, others showed dullness and inactivity. Moderate salivation and occasional respiratory distress. Remained normal rest of the study.

Necropsy: --

ChE response:

ChEP

Lowest:	9.7 (day 1)
Return to pre-treatment level:	100.4 (week 7)
Highest:	101.3 (week 9)

ChER

Lowest:	25.6 (day 2)
Return to pre-treatment level:	114.4 (week 4)
Highest:	119.1 (week 14)

LAMB NO: 1

GROUP NO: V

Dietary protein level: Normal (12%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 25 kg
Final body weight: 36.36 kg
Outcome: alive

ChE Activity			Observations
Pretreatment (as Δ pH)			Onset time: > 6 hours Survival time: survived
Time	ChEP	ChER	
- 14d	0.37	0.61	Clinical: Dullness and inactive. Moderate salivation. No prominent respiratory distress on the second day. Remained normal rest of the study.
- 7d	0.27	0.56	
0	0.23	0.66	
Mean ChE = 100%	0.29(±0.1)	0.61(±0.2)	
10 days post-treatment			
1	10.3	41.0	
2	13.8	13.1	Necropsy: - -
3	13.8	39.3	
4	34.5	59.0	
5	31.0	42.6	
6	34.5	44.3	
7	37.9	49.2	
8	48.3	63.9	
9	51.7	49.2	
0	51.7	63.9	
15 weeks following 10 days			ChE response:
1	69.0	101.6	ChEP
2	89.7	61.6	Lowest: 10.3 (day 1)
3	69.0	96.7	Return to pre-treatment level: 103.5 (week 4)
4	103.5	136.1	Highest: 103.5 (week 4)
5	75.9	63.9	
6	82.8	78.7	ChER
7	90.0	106.6	Lowest: 13.1 (day 2)
8	79.3	90.2	Return to pre-treatment level: 101.6 (week 1)
9	75.9	101.6	Highest: 136.1 (week 4)
0	79.3	98.4	
1	55.2	78.7	
2	82.8	109.8	
3	96.6	118.0	
4	93.1	116.4	
5	89.7	111.5	

LAMB NO: 2

GROUP NO: V

Dietary protein level: Normal (12%)
Coumaphos dose: 8 mg/kg
Trifluprομazine dose: saline equal volume

Initial body weight: 27.27 kg
Final body weight: 44.09 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.32	0.65
- 7d	0.25	0.64
0	0.28	0.63
Mean ChE = 100%	0.27(±0.1)	0.64(±0.2)

Onset time: > 6 hours
Survival time: survived

Clinical: Dullness and inactive. Moderate salivation, no prominent respiratory distress. Remained normal rest of the period.

10 days post-treatment

1	7.5	28.2
2	3.8	21.9
3	11.3	26.6
4	11.3	39.1
5	18.8	40.6
6	26.3	34.4
7	33.7	39.1
8	41.2	40.6
9	37.5	45.3
0	37.5	40.6

Necropsy: - -

15 weeks following 10 days

1	60.0	78.1
2	93.7	60.9
3	71.2	92.2
4	93.7	104.7
5	79.4	68.8
6	79.4	68.8
7	101.5	89.1
8	86.2	98.4
9	93.7	100.0
0	82.5	95.3
1	60.0	104.7
2	78.7	103.1
3	93.7	109.4
4	90.0	109.4
5	86.2	110.9

ChE response:

ChEP

Lowest:	3.8 (day 2)
Return to pre-treatment level:	101.5 (week 7)
Highest:	101.5 (week 7)

ChER

Lowest:	21.9 (day 2)
Return to pre-treatment level:	104.7 (week 4)
Highest:	110.9 (week 15)

LAMB NO: 20

GROUP NO: V

Dietary protein level: Normal (12%)
Coumaphos dose: 8 mg/kg
Trifluprομazine dose: saline equal volume

Initial body weight: 29.09 kg
Final body weight: 49.09 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.25	0.61
- 7d	0.22	0.51
0	0.21	0.50
Mean ChE = 100%	0.23(±0.1)	0.54(±0.2)

Onset time: no clinical signs
Survival time: survived

Clinical: No clinical signs first two days. Dull and inactive on 3rd day. Transitary. Back to normal on 4th day. Remained so till the end of the study.

10 days post-treatment

1	13.2	38.9
2	26.5	46.3
3	30.9	50.0
4	30.9	63.0
5	22.1	68.5
6	30.9	53.7
7	44.1	70.4
8	48.5	38.9
9	48.5	55.6
0	48.5	38.9

Necropsy: - -

15 weeks following 10 days

1	44.1	74.1
2	79.4	66.7
3	101.5	113.0
4	97.0	116.7
5	79.4	51.9
6	79.4	85.2
7	101.5	66.7
8	97.0	101.9
9	114.7	105.6
0	92.6	83.3
1	57.3	96.3
2	110.3	101.9
3	88.2	118.5
4	92.6	127.8
5	88.2	131.3

ChE response:

ChEP

Lowest:	13.2 (day 1)
Return to pre-treatment level:	101.5 (week 3)
Highest:	114.7 (week 9)

ChER

Lowest:	38.9 (day 1)
Return to pre-treatment level:	113.0 (week 3)
Highest:	131.3 (week 15)

LAMB NO: 22

GROUP NO: V

Dietary protein level: Normal (12%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 28.18 kg
Final body weight: 45.45 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.29	0.59
- 7d	0.26	0.67
0	0.22	0.57
Mean ChE = 100%	0.27(±0.1)	0.61(±0.2)

10 days post-treatment

1	7.8	19.7
2	3.9	21.3
3	7.8	18.0
4	11.7	31.2
5	0.0	32.8
6	35.1	32.8
7	27.3	33.3
8	42.9	27.9
9	42.9	37.0
0	46.8	27.9

15 weeks following 10 days

1	77.9	73.8
2	81.8	50.8
3	77.9	88.5
4	97.4	100.0
5	105.2	57.4
6	81.8	60.7
7	116.9	67.2
8	85.7	93.4
9	120.8	83.6
0	85.7	93.4
1	70.2	90.2
2	81.8	98.4
3	101.3	111.5
4	89.6	123.0
5	97.3	108.2

Onset time: > 6 hours
Survival time: survived

Clinical: Very mild salivation and muscular tremors in the hind quarters. Only animal in the group with recognizable clinical signs. Remained dull during the 10 day observation period. Normal at the end of study.

Necropsy: - -

ChE response:

ChEP

Lowest:	0.0 (day 5)
Return to pre-treatment level:	105.2 (week 5)
Highest:	120.8 (week 9)

ChER

Lowest:	18.0 (day 3)
Return to pre-treatment level:	100.0 (week 4)
Highest:	123.0 (week 14)

LAMB NO: 25

GROUP NO: V

Dietary protein level: Normal (12%)
Coumaphos dose: -
Triflupromazine dose: -

Initial body weight: 30.00 kg
Final body weight: -
Outcome: died before experiment

ChE Activity

Observations

Pretreatment (as Δ pH)

Time ChEP ChER

- 14d
- 7d
0

Mean ChE
= 100%

10 days post-treatment

Onset time:
Survival time:

Clinical:

Necropsy:

15 weeks following 10 days

ChE response:

ChEP

Lowest:
Return to pre-treatment level:
Highest

ChER

Lowest:
Return to pre-treatment level:
Highest:

GROUP NO: VI

No. of lambs: 5

Dietary protein level: normal (12%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Mean initial body weight: 28.2 kg
Mean final body weight: 40.9 kg
No lambs survived: 5

Mean ChE Activity (±SEM) as ΔpH

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.38(±0.1)	0.61(±0.2)
- 7d	0.29(±0.1)	0.61(±0.2)
0d	0.24(±0.1)	0.55(±0.2)
Mean ChE = 100%	0.30(±0.1)	0.59(±0.2)

10 days post-treatment

1	14.6(±3.7)	35.2(±7.7)
2	10.7(±4.4)	32.1(±6.2)
3	7.0(±3.1)	32.8(±8.4)
4	17.0(±6.6)	42.4(±7.8)
5	23.0(±6.0)	42.2(±8.0)
6	25.5(±4.5)	54.0(±8.5)
7	27.9(±4.7)	49.2(±8.6)
8	44.6(±4.3)	44.4(±7.8)
9	43.3(±4.4)	49.6(±8.9)
0	49.0(±3.4)	44.0(±7.8)

15 weeks following 10 days

1	64.8(±3.8)	100.9(±9.1)
2	82.9(±4.1)	62.4(±8.1)
3	85.2(±11.8)	117.0(±3.4)
4	129.1(±29.9)	150.5(±15.1)
5	109.1(±16.7)	61.6(±3.5)
6	118.1(±24.8)	78.0(±6.5)
7	123.2(±21.3)	88.6(±3.5)
8	116.5(±21.0)	100.2(±3.9)
9	152.9(±24.6)	102.6(±8.5)
0	148.6(±34.6)	106.5(±7.3)
1	120.6(±22.7)	99.5(±6.0)
2	119.6(±16.9)	113.1(±6.6)
3	137.8(±8.7)	128.1(±14.3)
4	109.2(±6.8)	132.5(±4.7)
5	98.3(±3.8)	134.1(±4.7)

Mean onset time: > 6 hours
Mean survival time: - -
Percent mortality: 0

Clinical: Signs noticed only in two animals. Rest did not show any signs at all. Very scanty salivation, slight inactiveness. Remained apparently normal till the end of experiment.

Necropsy: - -

ChE response:

ChEP

Lowest:	7.0 (day 3)
Return to pre-treatment level:	129.1 (week 4)
Highest:	152.9 (week 9)

ChER

Lowest:	32.1 (day 2)
Return to pre-treatment level:	100.9 (week 1)
Highest:	150.5 (week 4)

LAMB NO: 3

GROUP NO: VI

Dietary protein level: Normal (12%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 32.27 kg
Final body weight: 42.27 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.46	0.46
- 7d	0.27	0.42
0	0.26	0.43
Mean ChE = 100%	0.33(±0.1)	0.44(±0.2)

10 days post-treatment

1	15.2	36.6
2	12.1	34.4
3	18.2	50.4
4	30.3	52.7
5	27.3	48.1
6	21.2	64.1
7	45.5	61.8
8	45.5	52.7
9	45.5	71.0
0	51.5	52.7

15 weeks following 10 days

1	69.7	116.8
2	90.9	68.7
3	63.6	114.5
4	103.0	183.2
5	78.8	68.7
6	84.9	82.4
7	90.9	98.5
8	78.8	109.9
9	106.1	112.2
0	81.8	130.5
1	72.7	112.2
2	87.9	132.8
3	136.4	183.2
4	87.9	142.2
5	84.9	139.7

Onset time: no clinical signs
Survival time: survived

Clinical: No clinical signs except transient inactiveness. Remained normal throughout the study.

Necropsy: - -

ChE response:

ChEP

Lowest:	12.1 (day 2)
Return to pre-treatment level:	103.0 (week 4)
Highest:	136.4 (day 2)

ChER

Lowest:	34.4 (day 2)
Return to pre-treatment level:	116.8 (week 1)
Highest:	183.2 (week 4)

LAMB NO: 19

GROUP NO: VI

Dietary protein level: Normal (12%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 28.64 kg
Final body weight: 38.64 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.35	0.52
- 7d	0.30	0.49
0	0.24	0.49
Mean ChE = 100%	0.30(±0.1)	0.50(±0.2)

10 days post-treatment

1	6.7	20.0
2	0.0	20.0
3	6.7	12.0
4	3.4	32.0
5	16.9	30.0
6	20.2	60.0
7	23.6	38.0
8	43.8	36.0
9	47.2	44.9
0	47.2	36.0

15 weeks following 10 days

1	70.8	118.0
2	91.0	68.0
3	77.5	112.0
4	80.9	124.0
5	91.0	70.0
6	70.8	104.0
7	97.7	84.0
8	80.9	94.0
9	111.2	124.0
0	77.5	100.0
1	87.6	90.0
2	84.3	118.0
3	111.2	110.0
4	104.5	142.0
5	97.7	138.0

Onset time: > 6 hours
Survival time: survived

Clinical: Moderate salivation, slight respiratory distress and muscular tremors. Animal was normal by 6th day. Remained apparently normal till the end of study.

Necropsy: - -

ChE response:

ChEP

Lowest:	0.0 (day 2)
Return to pre-treatment level:	111.2 (week 9)
Highest:	111.2 (week 9)

ChER

Lowest:	12.0 (day 3)
Return to pre-treatment level:	118.0 (week 1)
Highest:	142.0 (week 14)

LAMB NO: 29

GROUP NO: VI

Dietary protein level: Normal (12%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 25.91 kg
Final body weight: 38.64 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.43	0.69
- 7d	0.33	0.61
0	0.26	0.57
Mean ChE = 100%	0.34(±0.1)	0.62(±0.2)

10 days post-treatment

1	5.9	20.9
2	2.9	14.4
3	0.0	12.8
4	0.0	16.0
5	2.9	14.4
6	14.7	17.7
7	17.7	17.7
8	29.4	14.4
9	26.5	14.4
0	35.3	14.4

15 weeks following 10 days

1	50.0	70.6
2	76.5	27.3
3	135.3	128.4
4	261.8	198.9
5	182.4	48.1
6	220.6	62.6
7	217.7	81.8
8	191.2	86.6
9	226.5	83.4
0	238.2	93.1
1	170.6	109.1
2	161.8	107.5
3	144.1	120.3
4	120.6	125.1
5	105.9	125.1

Onset time: > 6 hours
Survival time: survived

Clinical: Moderate salivation, slight respiratory distress and muscular tremors. Transitary signs during first 10 days of observation. Recovered completely and remained normal until end of the experiment.

Necropsy: - -

ChE response:

ChEP

Lowest:	0.0 (day 3)
Return to pre-treatment level:	135.3 (week 3)
Highest:	238.2 (week 19)

ChER

Lowest:	12.8 (day 3)
Return to pre-treatment level:	128.4 (week 3)
Highest:	198.9 (week 4)

LAMB NO: 30

GROUP NO: VI

Dietary protein level: Normal (12%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 26.82 kg
Final body weight: 46.36 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.38	0.81
- 7d	0.28	0.75
0	0.29	0.68
Mean ChE = 100%	0.32 (±0.1)	0.75 (±0.2)

Onset time: no clinical signs
Survival time: survived

Clinical: No change in behavior. Remained apparently normal Throughout the study.

10 days post-treatment

1	28.4	67.0
2	28.4	53.6
3	0.0	57.6
4	37.9	67.0
5	44.2	65.6
6	44.2	52.2
7	18.9	72.3
8	60.0	64.3
9	56.8	68.3
0	56.8	64.3

Necropsy: - -

15 weeks following 10 days

1	72.6	116.5
2	88.4	80.4
3	85.3	123.2
4	97.9	117.9
5	91.6	60.3
6	82.1	68.3
7	101.0	97.8
8	78.9	103.1
9	107.4	76.3
0	97.9	88.4
1	78.9	77.7
2	94.7	88.4
3	120.0	88.4
4	104.2	116.5
5	94.7	119.2

ChE response:

ChEP

Lowest:	0.0 (day 3)
Return to pre-treatment level:	101.0 (week 7)
Highest:	120.0 (week 13)

ChER

Lowest:	52.2 (day 6)
Return to pre-treatment level:	116.5 (week 1)
Highest:	123.2 (week 3)

LAMB NO: 37

GROUP NO: VI

Dietary protein level: Normal (12%)
Coumaphos dose: 8 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 30.45 kg
Final body weight: 38.64 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.30	0.59
- 7d	0.25	0.72
0	0.17	0.59
Mean ChE = 100%	0.24 (±0.1)	0.63 (±0.2)

Onset time: no clinical signs
Survival time: survived

Clinical: No change in behavior. Remained apparently normal throughout the study.

10 days post-treatment

1	17.0	31.3
2	10.2	37.9
3	10.2	31.3
4	13.6	44.5
5	23.7	52.7
6	27.1	39.6
7	33.9	56.0
8	44.1	54.4
9	40.7	42.9
0	54.2	54.4

Necropsy: - -

15 weeks following 10 days

1	61.0	82.4
2	67.8	67.6
3	64.4	107.1
4	101.7	128.6
5	101.7	61.0
6	132.2	72.5
7	108.5	80.8
8	152.5	107.1
9	213.6	117.0
0	247.5	120.3
1	193.2	108.8
2	169.5	118.7
3	159.3	138.5
4	128.8	145.1
5	108.5	148.3

ChE response:

ChEP

Lowest:	10.2 (day 2)
Return to pre-treatment level:	101.7 (week 4)
Highest:	247.5 (week 10)

ChER

Lowest:	31.3 (day 1)
Return to pre-treatment level:	107.1 (week 3)
Highest:	148.3 (week 15)

GROUP NO: VII

No. of lambs: 5

Dietary protein level: normal (12%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: 1.1 mg/kg

Mean initial body weight: 27.0
Mean final body weight: - -
No lambs survived: none

Mean ChE Activity (±SEM) as ΔpH

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.31(±0.1)	0.50(±0.2)
- 7d	0.31(±0.1)	0.62(±0.2)
0d	0.21(±0.1)	0.55(±0.2)
Mean ChE = 100%	0.28(±0.1)	0.56(±0.2)

10 days post-treatment

1	6.3(±3.6)	20.4(±5.9)
2	†	†

Mean onset time: 5 hours
Mean survival time: 19.4 hours
Percent mortality: 100%

Clinical: Severe dyspnea, salivation, lacrimation, muscular, tremors and twitching. Frequent micturition, straining of abdomen and diarrhea. Terminal recumbancy and coma.

Necropsy: Trachea and lungs congested, edematous. Emphysema in lungs. Occasional omasal, and duodenal hemorrhages. Liver, kidney and bladder markedly congested..

15 weeks following 10 days

ChE response:

ChEP

Lowest: 6.3 (day 1)
Return to pre-treatment level: †
Highest: †

ChER

Lowest: 20.4 (day 1)
Return to pre-treatment level: †
Highest: †

† Data not available due to death of the animals.

LAMB NO: 10

GROUP NO: VII

Dietary protein level: Normal (12%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 29.55 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.31	0.51
- 7d	0.24	0.64
0	0.18	0.54
Mean ChE = 100%	0.24(±0.1)	0.56(0.2)

10 days post-treatment

1	†	†

Onset time: 6 hours
Survival time: 9 hours

Clinical: Severe dyspnea, salivation, lacrimation, muscular tremors and twitching. Frequent micturition, straining of abdomen and diarrhea. Prostration, recumbancy and terminal coma.

Necropsy: Trachea and lungs severe edema and congestion. Areas of emphysema in lungs. Duodenum hemorrhagic. Kidney and bladder markedly congested.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	†
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	†
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animal.

LAMB NO: 16

GROUP NO: VII

Dietary protein level: Normal (12%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 25.45 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.34	0.48
- 7d	0.54	0.62
0	0.33	0.68
Mean ChE = 100%	0.40(±0.2)	0.59(±0.2)

10 days post-treatment

1	17.4*	11.8*
2	†	†

Onset time: 3 hours
Survival time: 4 hours

Clinical: Severe dyspnea, salivation, lacrimation, muscular tremors and twitching. Frequent micturition and diarrhea. Convulsive movements before death.

Necropsy: lungs and trachea edematous and congested. Severe myocardial hemorrhages. Duodenum and jejunum highly congested.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	†
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	†
Return to pre-treatment level:	†
Highest:	†

* Sampling done just before death.

† Data not available due to death of the animal.

LAMB NO: 27

GROUP NO: VII

Dietary protein level: Normal (12%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 27.73 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.26	0.50
- 7d	0.25	0.66
0	0.17	0.37
Mean ChE = 100%	0.23(±0.1)	0.51(±0.2)

10 days post-treatment

1	0.0	39.7
2	†	†

Onset time: 4 hours
Survival time: 24 hours

Clinical: Frothy salivation, severe dyspnea, lacrimation. Stiff gait, stretched out hind quarters. Stretching and arching of back. Frequent micturition and diarrhea.

Necropsy: Trachea and lungs edematous and congested. Kidneys pale, bladder congested. Ecchymatic patches on the duodenum.

15 weeks following 10 days

ChE response:

ChEP

Lowest: 0.0 (day 1)
Return to pre-treatment level: †
Highest: †

ChER

Lowest: 39.7 (day 1)
Return to pre-treatment level: †
Highest: †

† Data not available due to death of the animal.

LAMB NO: 33

GROUP NO: VII

Dietary protein level: Normal (12%)
Coumaphos dose: 15 mg/kg
Trifluprομazine dose: 1.1 mg/kg

Initial body weight: 25.45 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.29	0.55
- 7d	0.27	0.65
0	0.19	0.54
Mean ChE = 100%	0.25(±0.1)	0.58(±0.2)

10 days post-treatment

1	8.0	10.3
2	†	†

Onset time: > 6 hours
Survival time: 24 hours

Clinical: Dullness began 6 hours after treatment. Frothy salivation, dyspnea and lacrimation. Severe muscular twitching and tremors. Stretched out hind quarters and arching back. Frequent micturition and diarrhea.

Necropsy: Trachea and lungs edematous and congested. Severe myocardial hemorrhages. Kidneys congested.

15 weeks following 10 days

ChE response:

ChEP

Lowest: 8.0 (day 1)
Return to pre-treatment level: †
Highest: †

ChER

Lowest: 10.3 (day 1)
Return to pre-treatment level: †
Highest: †

† Data not available due to death of the animal.

LAMB NO: 36

GROUP NO: VII

Dietary protein level: Normal (12%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: 1.1 mg/kg

Initial body weight: 26.82 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.34	0.47
- 7d	0.25	0.53
0	0.20	0.38
Mean ChE = 100%	0.26(±0.1)	0.46(±0.2)

10 days post-treatment

1	0.0	19.6
2	†	†

Onset time: > 6 hours
Survival time: 36 hours

Clinical: Severe frothy salivation and lacrimation, dyspnea. With severe muscular twitches and tremors. Frequent micturition and diarrhea. Recumbant and comatosed.

Necropsy: Trachea and lungs showed severe edema and congestion. Areas of emphysema in lungs. Omasum showed severe hemorrhagic areas on the mucosal folds. Kidneys pale.

15 weeks following 10 days

ChE response:

ChEP

Lowest: 0.0 (day 1)
Return to pre-treatment level: †
Highest: †

ChER

Lowest: 19.6 (day 1)
Return to pre-treatment level: †
Highest: †

† Data not available due to death of the animal.

GROUP NO: VIII

No. of lambs: 5

Dietary protein level: normal (12%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: Saline equal volume

Mean initial body weight: 30.3 kg
Mean final body weight: (46.36 kg)*
No lambs survived: one

Mean ChE Activity (±SEM) as ΔpH

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.36(±0.1)	0.53(±0.2)
- 7d	0.25(±0.2)	0.59(±0.2)
0d	0.24(±0.1)	0.48(±0.2)
Mean ChE = 100%	0.28(±0.1)	0.53(±0.2)

10 days post-treatment

1	4.8(±2.4)	19.3(±1.7)
2	2.3(±0.9)	11.6(±3.1)
3	1.6(±0.9)	10.4(±2.6)
4	10.8(±7.7)	14.1(±2.0)
5	3.6(±2.6)	21.7(±8.7)
6*	14.5	18.9
7	32.5	20.8
8	43.4	34.0
9	43.4	18.9
0	47.0	35.9

15 weeks following 10 days

1	65.1	73.6
2	101.2	26.4
3	72.8	96.2
4	122.9	120.8
5	97.6	41.5
6	65.1	56.6
7	119.3	64.2
8	155.4	77.4
9	126.5	83.0
0	130.1	77.4
1	94.0	67.9
2	101.2	94.3
3	144.6	98.1
4	115.7	109.1
5	94.0	100.0

Mean onset time: 6 hours
Mean survival time: 66 hours
Percent mortality: 80

Clinical: Severe dyspnea, salivation, lacrimation, muscular fasciculation twitching and stiff gait. Abdominal straining. Occasional convulsion seen. Terminal prostration and coma.

Necropsy: Trachea and lungs edematous. and congested. Occasional emphysema and myocardial petichae. Omasal and duodenal congestion and hemorrhage. Bladder congested.

ChE response:

ChEP

Lowest:	1.6	(day 3)
Return to pre-treatment level:	101.2	(week 2)*
Highest:	155.4	(week 8)*

ChER

Lowest:	10.4	(day 3)
Return to pre-treatment level:	120.8	(week 4)*
Highest:	120.8	(week 4)*

* Values of only one survivor for the remaining period.

LAMB NO: 9

GROUP NO: VIII

Dietary protein level: Normal (12%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 32.27 kg
Final body weight: 46.36 kg
Outcome: alive

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.39	0.53
- 7d	0.23	0.58
0	0.21	0.48
Mean ChE = 100%	0.28(±0.1)	0.53(±0.2)

10 days post-treatment

1	10.8	24.5
2	3.1	18.9
3	0.0	13.2
4	21.7	17.0
5	7.2	34.0
6	14.5	18.9
7	32.5	20.8
8	43.4	34.0
9	43.4	18.9
0	47.0	35.9

15 weeks following 10 days

1	65.1	73.6
2	101.2	26.4
3	72.8	96.2
4	122.9	120.8
5	97.6	41.5
6	65.1	56.6
7	119.3	64.2
8	155.4	77.4
9	126.4	83.0
0	130.1	77.4
1	94.0	67.9
2	101.2	94.3
3	144.6	98.1
4	115.7	109.4
5	94.0	100.0

Onset time: > 6 hours
Survival time: survived

Clinical: Severe dyspnea, salivation, lacrimation, muscular fasciculation and twitchings. Stiff gait. Abdominal straining. Frequent micturition and diarrhea. Continued to show signs intermittently during the first 10 days. Gradually became normal and survived till the end of study.

Necropsy: - -

ChE response:

ChEP

Lowest:	0.0 (day 3)
Return to pre-treatment level:	101.2 (week 2)
Highest:	155.4 (week 2)

ChER

Lowest:	13.2 (day 3)
Return to pre-treatment level:	120.8 (week 4)
Highest:	120.8 (week 4)

LAMB NO: 18

GROUP NO: VIII

Dietary protein level: Normal (12%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 32.73 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.34	0.48
- 7d	0.16	0.49
0	0.21	0.53
Mean ChE = 100%	0.42(±0.1)	0.50(±0.2)

10 days post-treatment

1	8.5	18.0
2	†	†

Onset time: > 6 hours
Survival time: 36 hours

Clinical: Severe dyspnea, salivation, lacrimation, muscular fasciculation, and twitching. Abdominal straining. Frequent micturition and diarrhea. Became moribund and comatosed before death.

Necropsy: Trachea and lungs edematous and congested. Myocardial petichae. Kidneys and liver pale. Bladder highly congested.

15 weeks following 10 days

ChE response:

ChEP

Lowest: 8.5 (day 1)
Return to pre-treatment level: †
Highest: †

ChER

Lowest: 18.0 (day 1)
Return to pre-treatment level: †
Highest: †

† Data not available due to death of the animal.

LAMB NO: 24

GROUP NO: VIII

Dietary protein level: Normal (12%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 32.73 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.35	0.51
- 7d	0.28	0.58
0	0.27	0.41
Mean ChE = 100%	0.30(±0.1)	0.50(±0.2)

Onset time: > 6 hours
Survival time: 12 hours

Clinical: Severe dyspnea, salivation and lacrimation. Muscular tremors and twitching. Abdominal straining, frequent micturition and diarrhea. Convulsions present.

10 days post-treatment

1 † †

Necropsy: Trachea and lungs highly edemtous and congested. Emphysema prominent. Kidneys congested.

15 weeks following 10 days

ChE response:

ChEP

Lowest: †
Return to pre-treatment level:
Highest:

ChER

Lowest: †
Return to pre-treatment level:
Highest:

† Data not available due to death of the animal.

LAMB NO: 34

GROUP NO: VIII

Dietary protein level: Normal (12%)
Coumaphos dose: 15 mg/kg
Trifluprομazine dose: saline equal volume

Initial body weight: 26.82 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.38	0.52
- 7d	0.26	0.60
0	0.28	0.48
Mean ChE = 100%	0.31(±0.1)	0.53(±0.2)

Onset time: > 6 hours
Survival time: 144 hours

Clinical: Following excessive frothy salivation and dyspnea, she became prostrated, continued to remain the same for the next three days and died on the 5th day.

10 days post-treatment

1	0.0	15.0
2	3.3	9.4
3	3.3	7.5
4	0.0	11.3
5	0.0	9.4
6	†	†

Necropsy: Trachea and lungs highly congested. Emphysema was not prominent. Kidneys pale. Omasum and anterior duodenum had prominent hemorrhages.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	0.0 (day 1)
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	7.5 (day 3)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animal.

LAMB NO: 38

GROUP NO: VII

Dietary protein level: Normal (12%)
Coumaphos dose: 15 mg/kg
Triflupromazine dose: saline equal volume

Initial body weight: 26.82 kg
Final body weight: - -
Outcome: died

ChE Activity

Observations

Pretreatment (as Δ pH)

Time	ChEP	ChER
- 14d	0.33	0.61
- 7d	0.30	0.71
0	0.28	0.48
Mean ChE = 100%	0.31(±0.1)	0.60(±0.2)

Onset time: > 6 hours
Survival time: 72 hours

Clinical: Excessive salivation, dyspnea. The next day condition became worse, prostration, comatosed before death.

10 days post-treatment

1	0.0	19.6
2	0.0	6.5
3	†	†

Necropsy: Trachea and lungs congested with less prominent edemas Myocardium had few petichae. Omasum and duodenum showed hemorrhages.

15 weeks following 10 days

ChE response:

ChEP

Lowest:	0.0 (day 1)
Return to pre-treatment level:	†
Highest:	†

ChER

Lowest:	6.5 (day 2)
Return to pre-treatment level:	†
Highest:	†

† Data not available due to death of the animal.

APPENDIX II
LITERATURE REVIEW

Organophosphorus compounds (OPC) are an important group of chemicals because of their use as pesticides and systemic insecticides. Their ability to degrade in the environment, unlike the chlorinated hydrocarbons, and their behavior in systemic translocation in animal and plants, make them unique.[19,41,43]

Although these properties were noticed as early as 1936, results of the investigations were not published until World War II came to an end. Since then many OPC have been synthesized and their biological behavior has been studied with particular interest.[43,56] The insecticidal properties of various OPC differ from one another, and the effectiveness of each compound against a specific insect population also differs.[41] The insecticidal properties of OPC are attributed to their biological effects as inhibitors of cholinesterase (ChE).[28,35] OPC also have a wide variety of uses in animal and human medicine.[56,61] Recently, they have been studied for their use as fungicides, herbicides, and growth-regulating compounds.[19]

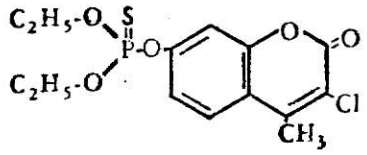
The multiple benefits and uses of OPC have been acclaimed highly beneficial to man and animals, and "it would certainly not be an exaggeration to claim OPC to be the most fruitful class of chemical compounds that man has found in his efforts to secure his physical existance."[19]

Years of research has yielded many synthetic OPC. The majority of the OPC are phosphoric esters used in agricultural and pharmacological practices,[19,28,41] and they belong to

to various groups of derivatives like phosphates, vinyl phosphates, phosphorothionates, phosphoroflurates, and phosphoroamidates. Some example compounds are parathion, malathion, coumaphous, their oxidative derivatives paroxon, maloxon and coroxon, chlorfenves, dichlorvos, mevinphos, tabun, diisopropylfluorophosphate (DFP), diazinen, and ecothiophate.[19,28,43,56]

THE ORGANOPHOSPHORUS COMPOUND COUMAPHOS

Coumaphos is a dialkyl aryl phosphorothionate (O, O-di ethyl, O-(3 chloro-4 methyl umbilliferone) phosphorothioate). It has the structure:



It is a colorless to tan-colored crystal with a molecular weight of 363. It has a melting point of 90-92 C, is practically insoluble in water, but is slightly soluble in acetone, chloroform and corn oil. Coumaphos, originally designated Bayer 21/199, is commercially available as asuntel, co-ral, muscotox and resitox.[35,46,56]

Uses

Coumaphos is an effective systemic organophosphorus insecticide that is administered orally or applied topically.[46]

It is available commercially in the form of an emulsifiable concentrate or as a wetable powder with an inert base.[46] Its effective therapeutic use has been studied in cattle,[31,32,46,49,56] sheep,[33,46,56] goats,[51,52] swine, horses, dogs and poultry.[35,46] It is specifically recommended in the control of cattle grubs[31] and is used as a topical dressing, spray or dip.[46] Using 0.25-0.5% suspensions, coumaphos was well tolerated by cattle although ChE activity was significantly inhibited. Dusting cattle with 0.5 - 5% coumaphos was effective against insects without inhibiting ChE activity. Topical and oral administration of coumaphos has been found to be effective against encysted larval forms of Hypoderma spp.[46]

The utility of coumaphos was further determined against internal parasites, especially nematodes of sheep.[33,35,37] It is highly effective against Hemonchus spp, Trichostrongylus spp, Cooperia spp and Œsophagastomum columbianum. Two to 2.5 mg/kg was well tolerated with maximum therapeutic benefit.[33,37] A dose of 10-15 mg/kg produced transient signs of toxicity without mortality. Doses from 15-25 mg/kg always produced acute toxicity with a high mortality. Repeated oral dosing with 2-4 mg/kg of coumaphos for six consecutive days did not produce clinical toxicity, although ChE was significantly reduced.[33,51] Doses of 8 mg/kg or less appeared well tolerated and were recommended for therapeutic use.[9,33,46]

In combination with other anthelmetic agents, like phenothiazine (PHE), the efficacy of coumaphos therapy was found enhanced in sheep[33,37] and in calves.[49] A dose of

2.0 mg/kg coumaphos with 200 mg/kg PHE gave excellent results in controlling sheep nematodes.[33] The use of 3.45 mg/kg coumaphos and 260 mg/kg PHE was well tolerated by sheep for therapeutic purposes.[24] A similar effect was noticed in cattle using 0.12% of coumaphos dermal pour-on and 60 gm/PHE calf orally.[49]

Toxicity

Coumaphos toxicity may be expected when there is accidental exposure, error in formulating the dose, or impaired animal health at the time of dosing.[46] Toxicity has been reported even at recommended doses.[31,42] The margin of safety for coumaphos appears to be narrow, and sheep are more susceptible than other domestic animals.[46]

Toxicity due to coumaphos is typical of that produced by OPCs.[9,31,46] Coumaphos forms an irreversible complex with ChE. The phosphorylated ChE undergoes stabilization with time (ageing) and is not easily hydrolysed.[2,3,7,16,28,31,32,41,42,43,44,50] Normally, acetylcholine (ACh) is released by the cholinergic nerve endings in response to a nerve impulse and is hydrolysed by ChE in the neuromuscular junction; hence, further conduction of the nerve impulse is halted. Inhibition of ChE by coumaphos leads to ACh accumulation and consequent continuous stimulation and excitation of the muscle or effector organ. The continued and increased accumulation of ACh becomes a prime factor in coumaphos toxicity. The effects are especially noticed at the neuromuscular junctions of skeletal

muscles and parasympathetic nerve endings where normal physiological activity of ChE is most important.[28,41,43,46,56,61]

Hence, the major clinical signs of coumaphos toxicity are salivation, lacrimation, violent contractions of the gastrointestinal tract and bladder, bronchiectasis, and twitchings and paralysis of skeletal muscles due to parasympathetic effects on smooth muscles and secretory glands. Hyperexcitibility, convulsions, and paralysis of the respiratory center are seen. Death is due mainly to respiratory failure and asphysia.[28,31,32]

The route of administration can modify the toxicity of coumaphos.[30,38,46] Usually topical and oral administration are chosen for therapeutic purposes, although other routes of administration have been tried. Coumaphos appears to be more toxic when given orally than when administered dermally.[30,35] The difference is probably due to the slower rate of absorption through the skins[30,34,44,47] and, secondly, due to the time taken for its metabolic activation to ceroxon in the liver.[15,39,44,47]

Repeated sprayings of cattle with 0.25-0.5% coumaphos suspensions did not produce signs of toxicity, although ChE activity was significantly inhibited. Sheep responded similarly but had decreased weight gains.[46] Signs of toxicity with increased inhibition of ChE were noticed when sheep were repeatedly dipped in 0.5% suspensions of coumaphos. Emaciated ewes in fleece appeared more affected than shorn sheep. Signs of toxicity were noticed in all the sheep treated and 50% mortality occurred after 3 dippings.[46]

Oral administration of 12 mg/kg of coumaphos did not appear toxic to calves of 1-2 weeks old; however, 30 mg/kg produced clinical signs with 25% mortality. In calves 6-12 months of age, 15-20 mg/kg did not produce significant effects but doses higher than 20 mg/kg caused moderate toxicity. Mortality occurred when calves were treated with 50 mg coumaphos/kg. Feeding calves 0.5-1.0 mg coumaphos/kg for 90 consecutive days did not produce signs of toxicity.[46]

In sheep, oral administration of coumaphos with doses of 15, 25, 30, 40 or 50 mg/kg resulted in high mortality.[46] The percent mortality in each group was proportional to the dosage, and 100% mortality was seen in sheep receiving 50 mg/kg.

Parenteral administration to cattle 2.5-15 mg coumaphos/kg, with various adjuvents like diethyl succinate, propylene glycol, peanut oil or saline, did not produce significant clinical effects except for mild poisoning with diethyl succinate. Intravenous injections of coumaphos 1-3 mg/kg in calves and adult cattle indicated no effect.[46]

Tests to determine the approximate MLD and LD_{50}s using different concentrations of wetable powders given orally or solutions of coumaphos polyethylene glycol given intraperitoneally (IP) to laboratory animals have indicated wide variation and unpredictibile results.[37] However, mice appeared more susceptible than rats, the former having an LD_{50} of 23.5 mg/kg compared to 150 mg coumaphos/kg in rats given chemical IP.[44] Female rats were more susceptible (oral LD_{50}= 16 mg/kg) than males (41 mg/kg).[43]

The concurrent presence of OPC[36] or other drugs, like PHE,[25] modify coumaphos toxicity due to potentiation of ChE inhibition.[10,11,15] Stress, known to enhance toxicity in cattle,[31,32,42] may also affect sheep. Inadequate dietary protein was shown to increase mortality in rats,[11] and emaciation associated with heavy parasitic infestation[31,32,46] may also contribute to increased toxicity from coumaphos.

Clinical Signs

Depending on the dosage given clinical signs may appear as early as 2 hours following oral administration, but in some cases signs may not appear until 7-8 hours after dosing. In ruminants slower and continued absorption is expected, because of the bulk of ingesta in the rumen with delayed onset of clinical signs and prolonged effects of toxicity.[23] Such a delay is also noticed following topical application. The delayed onset of clinical signs may be related to the time needed for metabolic activation of coumaphos to its more toxic oxidation product.[15,37,39]

Following oral administration of coumaphos depression occurs in sheep within 30 minutes. Excessive salivation and lacrimation are followed by increased respiratory rate. Dyspnea and abdominal palpitation occurs as a result of cholinergic paralysis of the diaphragm. Muscular signs begin as fasciculations and twitchings. Tremors of the hind limbs with incoordination are seen. Diarrhea and frequent micturition develop as a result of parasympathetic stimulation.

Abdominal straining, arching of the back and stretching of limbs occur as the toxicity progresses. Prostration and coma or convulsions occur as terminal events. Death is caused by respiratory failure due to paralysis of the respiratory muscles, although central depression of the respiratory center may also be involved. Depression of the respiratory center may result from ChE inhibition in the cerebrospinal fluid or brain itself as suggested for other OPC toxicity.[3,9,29,31,35,41,42,46,48,56]

Relapses of clinical signs have been noticed following periods of recovery.[23,31,42,46] The relapsing episodes may be related to spontaneous hydrolysis of phosphorylated ChE with subsequent inhibition of the reactivated ChE or inhibition of newly synthesized enzyme by actively circulating coumaphos or its metabolites.[7,16,41] Recovery depends on the dose, the degree of ChE inhibition and the relationship between biological absorption and excretion of coumaphos and its metabolites. Repeated daily dosing of cattle with 0.5-1.0 mg coumaphos/kg body weight in feed for 90 days did not produce toxicity but the whole blood ChE activity was reduced to 40% of normal. This was suggested as indicating the development of tolerance to continued low levels of ChE.[46]

The uncomplicated clinical course of coumaphos toxicity usually lasts 18-24 hours, but may occasionally be prolonged for several days.

Biochemical Effects

ChE inhibition appears the only significant biochemical alteration that occurs with coumaphos toxicosis. Much of the information on ChE inhibition following coumaphos exposure is reported for erythrocyte or whole blood, since erythrocyte ChE (ChER) activity is thought to be a better indication of OPC toxicity than plasma ChE (ChEP).[1,3,5,7,16,51,59] The level of ChER inhibition is closely related to the degree of toxicity because ChER (which is the true ChE mainly responsible for hydrolysis of ACh in the neuromuscular junctions) is concentrated in the erythrocytes and nerve terminals, whereas ChEP (which is the pseudo ChE and non-specific) is found in plasma, liver, glial cells and also in the central nervous system.[50] Peripherally inhibited ChEP can easily be reactivated and is rapidly replaced by synthesis in the liver.[7,16] However, ChER is complexed by OPC in an irreversible reaction and reactivation is not possible; synthesis of ChER is also closely associated with hemopoisis.[7,16,28,51,58,59]

Studies to evaluate other biochemical effects of coumaphos in sheep have not revealed significant alterations in SGOT or serum isocitrate dehydrogenase. This indicates absence of significant damage at the cellular level.[51] However, in cattle elevation of transaminase activity has been reported.[63] When studying dichlorvos, another OPC, in dogs significant alterations in the levels of SGOT, SGPT, serum alkaline phosphatase and B-glucurinidase were not observed, indicating little biological effect from this compound.[55]

Necropsy Lesions

Animals dying of coumaphos toxicity do not have diagnostic gross lesions and the lesions most frequently noticed with the other OPC toxicities are also not pathognomonic. Depending on the duration of illness, the carcass may appear emaciated and dehydrated. Small hemorrhages may be randomly seen throughout the body and the severe subcutaneous hemorrhages noticed in acute OPC toxicity of cattle[46] are also found in sheep.[31,46] Severe edema, congestion and hemorrhages are present in the trachea and lungs. Emphysema of the lungs is usually seen. There may be diffuse epicardial and endocardial hemorrhages.[31,46] Coumaphos has produced severe gastrointestinal lesions.[31] Hemorrhages and congestion of the esophagus and extending to other parts of gastrointestinal tract have been reported. Spleen, liver and kidneys show congestion. Severe congestion of bladder is occassionally noticed.

Physiopathology and Metabolism

The toxic nature of OPC in general is due primarily to their inhibitory effect on ChE. Many OPC insecticides are biotransformed by enzymatic activation to metabolites which produce an increased inhibitory effect on ChE. Coumaphos has been shown to be practically inactive *in vitro*[50,51] and is relatively low toxic to mammals in its original form. The activation of malathion and parathion *in vitro* to their oxidation products, which are more potent than the parent compounds, suggests a similar mechanism for coumaphos. *In vitro*

studies with rat liver microsomes showed that coumaphos is converted to its oxidation product, coroxon, by changing P=S to P=O.[15] A similar biotransformation has been recognized in mammalian systems.[31,32,37,44,47]

After absorption coumaphos undergoes metabolic activation and detoxification before it is excreted. The toxicity of coumaphos, therefore, can be accounted for by differences in the balance between activation and detoxification mechanisms in vivo.[39] The liver microsomal enzymes play an important role in determining the rate at which coumaphos is activated or detoxified.[15]

The metabolic pathway of coumaphos has been studied using ^{32}P-labelled coumaphos.[31,34,44] Coumaphos is converted into various metabolites depending on the route of administration;[30] de-ethylated coumaphos, coroxon, and the phosphorothioic, diethyl phosphoric and phosphoric acids. The main events of coumaphos metabolism can be summarised as activation, phosphorylation of ChE, detoxification by hydrolysis and dealkylation and excretion.[19,41,43]

The principal site of initial activation is the liver. The enzyme systems that catalyze this reaction belong to a group of NADPH dependent mixed-function oxidases of hepatic microsomes.[15] Recent evidence suggests that activation may also take place in tissues other than liver.[45] The activated metabolite appears to bind specifically to acetyl-ChE. However, non-specific binding to other enzymes or receptor sites has been suggested without any adverse implications.[39]

Hydrolysis represents enzymatic detoxification catalysed by aliesterases with or without prior oxidation of coumaphos. The rate at which the hydrolysis is catalysed and the availability of enzymes in the liver determines the degree of toxicity.[31,41,43]

Further dealkylation will produce de-ethylated coumaphos or coroxon. Tissue and plasma aliesterases producing hydrolysis of carboxy linkages has been suggested as another pathway of detoxification.[39]

The absorption, metabolic degradation and excretion of coumaphos and its metabolites depend on the route of administration.[38] Dermal application appears to result in slower absorption and excretion when compared with subcutaneous and oral dosing. Coumaphos and its metabolites appeared in urine with 14 days of oral, 18 days of subcutaneous and 28 days of dermal application.[38]

Lower levels of radioactivity was noticed in blood, urine and feces following dermal application of ^{32}P-labelled coumaphos when compared to similar oral administration.[30] Many of the tissues, and particularly fat, retain coumaphos metabolites.[34] Coumaphos or coroxon have been reported in the milk of cow and goat following dermal application of coumaphos.[34]

Initially most of the products that appear in the urine are the hydrolytic products of coumaphos. Although coroxon was found in the first six hours, the metabolites that appear later on were products of hydrolysis. They are excreted as phosphoric acid (35%), diethyl phosphoric acid (17%), diethyl

phosphorothioic acid (29%), and de-ethylated coumaphos and coroxon (18%) respectively,[34,44]

Diagnosis and Treatment

The extensive use of OPC insecticides and pesticides in agricultural and therapeutic practices results in accidental exposures with fatal toxicities.[27] It is imperative that the condition is diagnosed early for effective therapy. Clinical signs of toxicity characteristic of OPC, and the laboratory screening for blood ChE activity are useful in the early diagnosis of toxicity.[1,3,9] Several methods are in use for ChE assay.[1] Usually electrometric, titrimetric, colorimetric and visual color matching are employed for field or laboratory screening. Colorimetric and visual color-matching techniques provide information adequate for clinical and toxicological laboratories. Quantitative ChE assays by the electrometric and titrimetric methods are more accurate.[1]

Effective therapeutic measures based on experience with OPC toxicoses in man have been adopted in animals.[2,32,35,42,61] Standard treatment of OPC toxicosis is the administration of atropine and pyridine 2-aldoxime (2-PAM). Atropine acts by blocking the periphral cholinergic receptors to acetylcholine. 2- PAM acts as a nucleophilic ChE reactivator by attracting the OPC from the active sites of ChE.[35,58,61] Since OPC irreversibly phosphorylate ChE and aging stabilizes the complex by structural modification, the effectiveness of 2-PAM

as a nucleophilic reactivator of ChE is hindered by delay in the therapy.[58] However, spontaneous hydrolysis of the OPC-ChE complex[26] may permit limited reactivation by 2-PAM. The competitive nature of 2-PAM is useful if treatment is attempted shortly after OPC exposure.[27,35]

Initially PHE tranquillizers, such as chlorpromazine, was used in the treatment of human OPC toxicosis with some apparent benefits.[61] However, potentiation of PHE with OPC, resulting in fatal outcomes has discouraged the use of PHE tranquillizers for the treatment of OPC toxicities.[4]

Attempts to treat OPC toxicoses in animals have not always been successful, particularly in ruminants.[23,24] Clinical signs of ruminants appear late and are more prolonged than in other animals. Subsequently the duration of treatment and the chances of clinical sign reccurance are much greater. This is attributed to continued absorption of the toxicant from the ruminal ingesta.[24]

To overcome these prolonged toxic effects it was suggested that the amount of toxicant in the rumen be reduced by absorption onto an inert substance, by gastric lavage, or by surgical evacuation. Gastric lavage or surgical evacuation by rumenotomy may not be practicable under field situation. Hence, activated charcoal was found an appropriate and useful inert absorbant. An aqueous slurry of activated charcoal (approximately 120-250 gms) given by a stomach tube after atropine (0.5 mg/kg) and 2-PAM (20 mg/kg) administration,

gave beneficial effects in sheep.[24] This treatment may have to be repeated. A similar course of treatment can be made effectively in coumaphos poisoning.[24]

PHENOTHIAZINE AND ITS DERIVATIVES

Phenothiazine and its derivatives are a widely used group of therapeutic agents. Their pharmacological action is varied and diversified.[26] While the parent compound is an effective antihelmintic agent,[20,35] its derivatives are mainly used as tranquilizers in human and veterinary medicine.[8,62]

Although phenothiazine was first synthesized in 1885,[20] its biological affect as a mosquito larvicide was not recognized until 1936.[36] Subsequently, a variety of phenothiazine compounds (PHE) were extensively studied for their insecticide, larvicide, anthelmintic, and tranquilizing activity in both agricultural and medicinal practices. The studies relating to phenothiazine's anthelmintic properties are extensive and its efficacy has been reported in several domestic species, especially ruminants.[20]

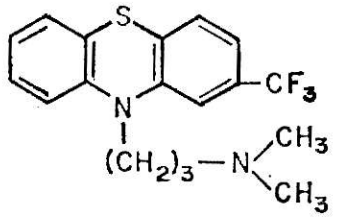
Most of the PHE commercially available are used as tranquilizers and neuroleptic agents in human medicine.[8,62] Some of the PHE tranquilizers are: chlorpromazine, mepazine, promazine, perphenazine, propiopromazine, trifluperazine, and triflupromazine.[8] Of these compounds, chlorpromazine is the most exclusively used and studied, although triflupromazine possess twice the tranquillizing effect of chlorpromazine on a mg/mg basis.[8,17] In addition to their tranquilizing activity, some of the PHE derivatives also exhibit antihistaminic and anticholinesterase activity.[62]

In the present review, PHE is discussed with a special emphasis on the biological effects of penothiazine and trifluprom azine.

Phenothiazine is thiodiphenylamine; it has the structure:

It is a pale lemon colored powder with a very low water solubility. Phenothiazine is relatively stable when dry, but undergoes spontaneous oxidation under moist conditions.[8]

Trifluprom azine has a phenothiazine nucleus with groupings at the 2 and 19 positions. Chemically it is 10-(3-dimethylamino-2-methyl propyl-2-triflurom ethyl-phenothiazine) and has the following structure:[8]



Uses

As an anthelmintic in ruminants phenothiazine has shown marked efficacy in the elimination of Hemonchus contortus, Oesophagostomum spp, Ostertagia spp, Trichostrongylus spp, and Chabertia ovina.[20] It is used in horses against Strongylus spp. Although its spectrum of anthelmintic activity is wide, it is least effective against ascarids and hookworms. Because of this, and its high toxicity, phenothiazine is not used in dogs.[35]

Phenothiazine's use as an anthelmintic presents a practical problem due to its insolubility in water and the fact that a relatively large quantity of the drug is required for therapeutic effect. Hence, a micronized purified form of phenothiazine is used. Continuous phenothiazine feeding by mixing in feed and salt mixture was found impractical for grazing animals. The dosage of phenothiazine varies between species. Approximate dosages for normal healthy animals are: 10-25 gms for sheep and goats; 10 gms/100 lb. (45 kg) body weight in cattle; up to 40-50 gms for horses; and 2 gm/kg body weight in swine. Young and debilitated pigs are highly susceptible to phenothiazine toxicity.[35]

Trifluprομazine, like chlorpromazine, is used in animals for its pharmacological action as an antiemitic, as a central nervous system depressant, and to enhance the effects of other anesthetics.[8,17] Its clinical use in dogs and horses is to quiet nervous animals for safe handling. Trifluprομazine is administered at the rate of 5 mg/100 lb. (45 kg) body weight for cattle, 10-15 mg/100 lb. (45 kg) body weight for horses, 1.1 mg/kg body weight for sheep, 40-60 mg/100 lb. (45 kg) body weight for swine, 1-4 mg/kg body weight in dogs, and 4-8 mg/kg body weight for cats by parenteral injections.[17]

Toxicity

Even with recommended therapeutic levels toxicity is produced with the use of PHE. Horses, humans and dogs are the most susceptible species and swine are less often affected.

Ruminants are relatively resistant, although cattle are more susceptible than sheep. Acute toxicities are noticed in animals that are weak, anemic and debilitated. Hepatic dysfunction contributes to the susceptibility. High doses have resulted in ataxia and paralysis due to inhibitory effect on ChE.[9]

Clinical Signs

Several toxic syndromes are associated with PHE toxicoses. Hemolytic anemia and photosensitization are the major clinical effects. Nervous signs with paralysis and ataxia are often noticed in swine. Abortions may occur in animals dosed late in pregnancy.[9,20]

Hemolytic anemia producing jaundice, hemoglobinuria, and death have been reported in horses and dogs,[9,20] and occasionally in human beings.[62] The hemolysis has been attributed to the presence of impurities, such as diphenylamine.[5] Although phenothiazine itself is not hemolytic, *in vitro* studies suggest that phenothiazine in the presence of lysolecithin produces accelerated hemolysis.[13] PHE, such as chlorpromazine, has been shown in the course of their metabolism to exert inhibitory effect on many enzymes, including glucose-6-phosphate dehydrogenase.[26] It is suggested that inhibition of glucose-6-phosphate dehydrogenase increases the fragility of the erythrocyte cell wall with resulting hemolysis.[6] The occurrance of Heinz bodies is thought to be

a significant feature of phenothiazine toxicity, but this may also be seen in other toxicities due to hemolytic agents.[53]

Phenothiazine itself is a photodynamic agent and its interaction with sun light results in acute dermatitis. In most animals phenothiazine photosensitization occurs as a result of biotransformation to its oxidative metabolite, phenothiazine sulfoxide.[12] Unless this compound is readily detoxified by the liver, it is released into the peripheral circulation. When exposed to bright sun light such animals show severe photosensitization. Non-pigmented areas on the skin of cattle and pigs, and the head and ear tips of sheep, are the most affected sites.[9]

Keratitis is one of the significant clinical features of phenothiazine toxicity. In cattle, sheep, and goats a diffuse bilateral corneal opacity is seen following phenothiazine therapy. Experimentally in cattle the condition is reproduced within 24-48 hours after dosing with phenothiazine.[12] Corneal opacity and keratitis are due to the excretion of unaltered phenothiazine sulfoxide in the tears and also to its localization in the aqueous humor.[12]

Neuromuscular paralysis noticed in swine as result of phenothiazine dosing may be related to its anticholinesterase activity.[57]

Necropsy lesions

The necropsy lesions usually seen in phenothiazine toxicities are varied and non-specific. Gross lesions

frequently encountered are related to the clinical outcome of the toxicity. Hemolytic cases show icterus and congestion of organs mainly due to extensive hemolysis. Liver and kidneys may occasionally show congestion. Other organs may appear pale depending on the extent of anemia. Lesions in photosensitized animals may appear confined to the skin, with subcutaneous edema and bilateral corneal opacity and keratitis. Urine and milk from affected animals often turn red when exposed to atmospheric air due to the formation of phenothiazone and thional from the oxidation of leucophenothiazone and leucothional, which are colorless compounds.[9,20]

Metabolism

The metabolism of phenothiazine and its derivatives is complicated.[12] Marked variations are seen in the metabolism of PHE tranquilizers. A similar variation occurs in different species of animals.[60]

After ingestion phenothiazine undergoes biotransformation in the intestine to its oxidative metabolite phenothiazine sulfoxide. Further metabolism may occur by liver enzymes. Large doses of phenothiazine or pre-existing hepatic injury interferes with detoxification and circulating phenothiazine sulfoxide causes photosensitization. The degree of susceptibility is determined by the efficacy of detoxification in the liver. Sheep appear more resistant because of greater efficiency in converting phenothiazine sulfoxide to leucophenothiazone and leucothional. Leucophenothiazone and

leucothional are colorless and are excreted mainly in the urine and feces, which will subsequently be changed into phenothiazone and thional by further oxidation in the atmosphere. These are red or pink in color.[12]

The PHE tranquillizers have a similar metabolic pathway to that of phenothiazine, and further metabolism and excretion depends on the substituent groups. Five common metabolic pathways of these drugs occur. These include, oxidation of the sulphur atom, oxidation of the terminal nitrogen, demethylation at the terminal dimethyl-amino group, and hydroxylation of phenothiazine nucleus followed by conjugation with glucuronic acid. As many as 30 metabolites are obtained from promazine. Following intramuscular administration of promazine in the horse, 11% was excreted in 96 hours, glucuronic acid conjugates predominate, their ratio to unconjugated metabolites being 5:1.[60]

Following absorption from the intestine, large quantities of the PHE tranquillizers are bound to plasma and other nonspecific sites.[26]

Diagnosis and Treatment

A history of PHE administration and the clinical signs of photosensitization and hemoglobinuria will help in the diagnosis of PHE or phenothiazine toxicity.[9] The presence of bilateral corneal opacity and keratitis with corneal ulcers will aid in the diagnosis.[12]

Symptomatic treatment to alleviate the signs of toxicity has been employed, including sheltering the susceptible animals from direct sunlight soon after phenothiazine therapy. In the event of hemoglobinuria and severe anemia blood transfusions may provide temporary relief.

Since there is no specific treatment, efforts should be aimed at prevention. Animals that are debilitated and weak should not be dosed with the recommended doses of phenothiazine; instead the dosage may be divided and given for several days in order to prevent any untoward reaction.

INTERACTION OF ORGANOPHOSPHORUS COMPOUNDS AND PHENOTHIAZINE DERIVATIVES

The *in vivo* effects of concurrently using two compounds with similar pharmacological action may result in adverse interactions. Such interactions may appear as suppression of the desired therapeutic action, additive effect or unexpected potentiation.

Phenothiazine compounds (PHE) were tested in combination with atropine and other drugs in the treatment of organophosphorus compound (OPC) toxicoses.[14,21,22,61] It was known that PHE form a reversible complex with ChE competing with acetylcholine (ACh) or any other anti-cholinesterase drug.[61] By introducing PHE as a competitive inhibitor, it was expected that the toxic effects of OPC could be reduced.[61] This hypothesis was tested *in vitro* studies using plasma ChE from rat, human and horse, and also studying ChE from rat brain.[18] These studies showed that PHE inhibited plasma ChE and rat brain ChE, whereas, antihistamine agents competitively inhibited only plasma ChE.[18]

On the otherhand, because of its neuroleptic action PHE could possibly effectively supress the effect of ACh in the continued conduction of nerve stimuli at the neuromuscular junction.[14] Thus, PHE were tested alone and in combination with other drugs for prophylactic or therapeutic use in OPC toxicosis.[14] Therapeutic effectiveness was noticed in

parathion poisoning,[21] and led to the use of several PHE derivatives in many types of OPC toxicities.[14,22,61]

Although on more than one occasion PHE were found highly beneficial in such treatment, the use of PHE as OPC antagonists has been a subject of controversy.[61] Further studies showed that a mixture of atropine and PHE was more effective in delaying deaths in guinea pigs after poisoning with parathion and malathion than did treatment with atropine or the PHE, chlorpromazine, alone.[22]

Shortly, however, potentiation of OPC toxicity was noticed when repeated treatment with promazine resulted in patient death.[4] A similar potentiation and high mortality was noticed in parathion poisoned rats treated with PHE chlorpromazine and/or atropine.[25] It is suggested that this potentiation occurred due to inhibition of residual ChE.[4] PHE derivatives might also have bound to tissues and occupied non-specific binding sites to which OPC might attach.[26] This would allow OPC to inhibit more ChE and enhance toxicity.

Systemic OPC also used in combination with PHE to produce synergistic effect against internal parasites in sheep.[33,37] In calves, similar therapy has been demonstrated using topical application of OPC in combination with the oral administration of PHE.[49] There was no toxicity observed, indicating the absence of potentiation when both the compounds were used in recommended doses.[49]

It is possible that other factors, like stress and nutritional status, can modify susceptibility for potentiated toxicity.[11,31,42] Differences in susceptibility was noticed due to sex when rats were concurrently treated with PHE and OPC. Female rats were more susceptible than male rats and had a greatly increased mortality rate with short survival times.[25]

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INFLUENCE OF DIETARY PROTEIN ON THE EFFECT OF COUMAPHOS AND TRIFLUPROMAZINE INTERACTION IN SHEEP

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AN ABSTRACT OF A MASTER'S THESIS

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The therapeutic value of simultaneous administration of organophosphorus compounds and phenothiazine derivatives has been controversial. Although phenothiazine derivatives were initially used in treating organophosphorus toxicities, adverse potentiation was subsequently reported. Unpredictable adverse reactions with the use of organophosphorus compounds indicate species and individual variability, and factors such as stress, heavy parasitism, and malnutrition further aggravate potential toxicity. To investigate this chemical interaction the effects of simultaneously administering an organophosphorus compound and a phenothiazine derivative to lambs with induced protein stress were studied.

Coumaphos, a systemic organophosphorus compound (8 mg/kg or 15 mg/kg body weight orally), and trifluprom azine HCl, a phenothiazine tranquilizer (1.1 mg/kg body weight intramuscularly), or an equal volume of saline were given to eight groups of 5 lambs each on low (7%) or normal (12%) protein diets. Animals were kept under close observation for 10 days and then observed for an additional 15 weeks. Clinical signs, mortality rates, survival times and plasma and erythrocyte cholinesterase activity were monitored for each group. Necropsy examinations were conducted on all lambs that died during the experimental period.

Lambs on both protein levels that received 15 mg coumaphos/ kg body weight died. Doses of 8 mg coumaphos/kg body weight were well tolerated. None of the lambs on normal protein diets that received 8 mg coumaphos/kg body weight died, and

3/5 lambs did not show clinical signs of toxicity; the detectable signs in the remaining were mild and transient. Significant differences in the severity of clinical signs were not apparent between groups that received the tranquilizer and those that did not. A greater inhibition of cholinesterase activity in groups receiving both the drugs suggested potentiation of anticholinesterase effects. Low dietary proteins adversely affected the development of clinical signs, the mortality rate, the mean survival time and cholinesterase activity. Recovery of cholinesterase activity in tranquilizer-treated animals was faster than in those that received saline. Lambs on normal protein rations had a faster cholinesterase recovery than those fed low protein diets. Inhibition of erythrocyte cholinesterase correlated more directly with toxicity than did plasma cholinesterase inhibition.

The dose levels of coumaphos used were too critical for accurate statistical evaluation. High mortality in the 15 mg coumaphos/kg level with a short survival time interfered with the study of cholinesterase response. Dose levels between 8 and 15 mg coumaphos/kg body weight appear more suitable for studying the cholinesterase response. At least 10 lambs/group may be warranted in future studies. Since deficiency of protein appears related to the synthesis of cholinesterase, evaluation of cholinesterase activity in relation to total plasma proteins may provide additional information as to the mechanisms involved in the anticholinesterase effects of organophosphorus compounds and phenothiazine derivatives.

Studies with radioisotope labelled organophosphorus compounds and phenothiazine derivatives may offer an additional research tool for investigating the nature of the cholinesterase-drug complexes and could reveal the nature of their inhibitory effects.

The treatment of protein stressed animals with organophosphorus compounds and phenothiazine therapeutic agents should be viewed with caution. Evaluation of the subjects' physiological status prior to the treatment can avert undesirable and unpredictable toxic potentiation.