UNDATED

865

Name	:	Merphos
Chemical Name	:	Tributyl Phosphorotrithioite
Chemical Formula	:	$(CH_3CH_2CH_2CH_2S)_3P$
Molecular Weight	:	298.4
Specific Gravity	:	0.99-1.01
Refractive Index	:	1.54-1.55
Physical State	:	liquid
Color	:	colorless to pale yellow
Corrosive Action	:	non-corrosive
Solubility	:	Soluble in acetone, ethyl alcohol, benzene, kerosene, xylene, methylated naphthalenes. Insoluble in water.
Use	:	Cotton defoliant
Company	:	Virginia Carolina Chemical Corporation

Data Submitted

1. Chemical and physical data sheet
1. Acute Rat Oral — LD_{50} = 1272 mg/kg
2. Acute Rabbit Dermal — $LD_{50} \approx$ 7600 mg/kg irritant
3. Acute Eye (Rabbit) — mild irritation
4. Subacute Rat Feeding (90 days) — no effect level = <10 ppm
5. Subacute Dog Oral (90 days) — no effect level = >30 and <100 ppm
6. In Vitro Cholinesterase
7. Subacute Rat Feeding (112 days) — no effect level $\approx$ 2.0 ppm
8. Potentiation Study - (Rats)
9. Subacute Dog Potentiation Feeding (6 wks) sl potentiation noted.

Studies Needed

1. Acute Inhalation Study.
2. Antidote Study.
3. Analytical Methods.
4. Human Experience Data (lab and field)

MERPHOS

Acute Rat Oral

Groups of five male albino rats were tested per dosage level of 0.1, 0.215, 0.464, 1.0, 2.15, and 4.64 ml/kg of body weight. Material was administered as either undiluted or as a 1% or 10% volume/volume solutions in corn oil. Animals were fasted three to four hours prior to dosage.

Results

The oral LD_{50} equals 1272 mg/kg (with a range of 935-1727 mg/kg.) During the first 24 hours, animals at each level appeared depressed and showed labored respiration, while those at the four higher levels also showed preening and lacrimation. At 24 hours post treatment, the animals generally appear depressed and showed nasal discharge, lacrimation, labored respiration and diarrhea with light colored stools. Animals at the four highest dosage levels, showed hematuria and phonation upon handling. Prior to death, the animals generally showed unthriftiness and depressed righting and placement reflexes. Surviving animals recovered within three to five days.

Gross pathological findings

The animals that died showed hyperemic or hemorrhagic lungs, irritation of the gastro intestinal tract, congested kidneys and adrenals, and bloody urine in the urinary bladder. The intestines contained a yellowish-white fluid with the odor of the experimental material.

Acute Rabbit Dermal

Groups of four rabbits each were tested at dosage of 1.0, 2.15, 4.64,

and 10.0 ml/kg. Exposure was 24 hours.

Results

LD_{50} = approximately 7600 mg/kg. One half of the animals on the three lower dosage levels exhibited diarrhea from one to five days. The sole survivor of the 10 ml/kg level exhibited diarrhea commencing at 24 hours post treatment and continuing throughout the remainder of the observation period. This animal also showed signs of depression and lost 184 grams from its initial body weight. Two of the three animals on the 10 ml/kg which died showed extreme depression, unthriftiness, unsteadiness, labored respiration, slight sprawling of the limbs and depressed righting and placement reflexes approximately 24 hours prior to death.

The undiluted material produced mild to moderate degrees of dermal irritation, erythema, and slight edema. The majority of the animals showed atonia, and/or desquamation during the final two to four days of the observation period.

Autopsy finding of the one animal of the 10 ml/kg level which died showed hyperemic lung, small and pale appearing spleen, congestion of the mudullary portion of the kidney, irritated small intestines and secum.

Summary

From the aforementioned data it can be concluded that the test material is a definite skin irritant. Also, that it has adverse effects on the gastro intestinal tract. Also, the chemical is absorbed through the skin and does cause systemic toxicity as is pointed out by the hyperemic lungs and congestion of the kidney.

Acute Eye Study (Rabbit)

A single application of 0.05 ml of the undiluted material was placed in the left eye of a group of three albino rabbits. The treated eye was held closed for approximately 30 seconds after which an immediate reading was made.

Results

Test material produced a mild degree of eye irritation which was characterized by mild erythema, edema of the lids and vacuolization of the sclera and nictitating membrane. Each animal appeared normal about 24 hours.

Summary

From the aforementioned data, it appears as though the test material is a mild irritant to the eye. Adequate precaution should be listed on the label of this product.

Subacute Rat Feeding

Study No. 1

25 male and female rats were used per level of 0.0, 1.0 and 2.0 ppm. The 1 ppm and 2 ppm levels were increased to 10 and 50 ppm respectively on day 28.

Five male and five female rats from each group were sacrificed at 21, 47, 63, and 91 days for plasma, red blood cell, and brain cholinesterase activity determinations.

Results

None of the test animals exhibited gross signs of toxicity or abnormal behavior.

Body weights and food consumption values for the test rats were comparable to those of the control. Females of the 50 ppm showed liver weights and liver to body weight ratios significantly greater than the controls. This level also produced significant depression in plasma and red blood cells, cholinesterase activity. Brain cholinesterase activity was significantly depressed in the male rats after 95 days of feeding and in the females after 63 days of feeding.

Females of the 10 ppm exhibited significant inhibition of plasma and red blood cell cholinesterase activity only after 63 days of feeding. Brain cholinesterase activity was unaffected. The males of the level also showed red blood cell cholinesterase inhibition. From the aforementioned data it is generally concluded that the no-effect level is less than 10 ppm.

Study No. 2

The experimental conditions of this study are identical to those of Study No. 1 with the exception of dosage. 25 male and 25 female rats were tested per level of 100 and 500 ppm. The test material was excluded from the diet after 13 weeks of feeding for the 500 ppm level.

Five male and five female rats from each test group were sacrificed after 24 and 42 days of feeding for plasma, red blood cell, and brain cholinesterase determinations. Group V (100 ppm) was terminated at the 49th day of feeding and cholinesterase activity determined.

After 91 days of feeding, five male and five female rats from Group IV (Control) and 5 male and 4 female rats from Group VI (500 ppm) were

sacrificed for cholinesterase determinations.

The following tissues were taken for histological examination from three males and three females of Groups No. IV and VI. Thyroid, lung, heart, liver, spleen, kidney, adrenal, stomach, pancreas, large and small intestines, urinary bladder, gonads, and bone marrow.

At 91 days the surviving male and female rats from Group VI were placed on the basic laboratory diet and sacrificed after 32 additional days.

Results

No gross signs of toxicity or abnormal behavior were observed. Average body weights for the test animals were progressively lower than the control animals with those of group VI being considerably lower. The food consumption values for the test rats of both sex were fairly comparable to those of the corresponding control group. Cholinesterase activity determinations made at 91 days for the control and high level rats showed complete inhibition of plasma and red blood cell activity in both the male and female rats of the 500 ppm level, with brain cholinesterase activity also being markedly depressed.

The average liver body weight ratio for both sexes fed 500 ppm were significantly greater than those in the control groups. The average kidney body weight ratio for the female rats of the 500 ppm level were significantly greater than those of the control rats.

Gross autopsies of the rats fed 500 ppm revealed no pathological findings which could be associated with the ingestion of the test material.

Summary

It is apparent that the high level caused suppression of growth. The significant increase in liver and kidney body weight ratios may be a direct result of this depressed body weight. The gross autopsy findings are somewhat surprising as one would expect to find at least irritation of the gastro-intestinal tract plus some organic changes within the liver. The absence of these findings could be attributed to the animals being off the test material between 51 and 91 days at which time they were sacrificed. If this was so, then one may consider the findings in prior studies to be transit[illegible]

Study No. 3

The experimental conditions of this study are identical to those of Study 1 with the exception of dosage and time intervals. Two groups of 25 male and 25 female each were tested at the dosage levels of 0.0 and 20 ppm. The test material was excluded from the 20 ppm level after seven weeks of feeding.

After 21 and 42 days of feeding, five male and five female rats from each group were sacrificed for plasma, red blood cell and brain cholinesterase activity determinations. Sections of the thyroid, lung, heart, liver, spleen, kidney, adrenals, stomach, pancreas, large and small intestines, urinary bladder, gonads and bone marrow were taken from three male and three female rats of each group sacrificed at 42 days.

After 49 days the size of each group was reduced to five males and five females at which time the compound was excluded from the diet. These

animals were maintained for 25 more days on regular feed. At this time cholinesterase activity determinations were performed on each rat.

Results

Survival, physical appearance, behavior, and food consumption of the test rats were unaffected by ingestion of the test material.

Plasma and red blood cell cholinesterase activity was depressed in the test animal during the seven week feeding period. During the 25 day recovery period, the plasma and red blood cell cholinesterase activity returned to within normal limits. Brain cholinesterase activity was unaffected during the course of the entire study.

Average liver weight and liver to body weight ratio of the female rats were significantly greater than those of the control group after 21 days of feeding. The kidney to body weight ratio for the male and female rats were significantly greater than those of the control animals after 21 days of feeding. This relationship remained for the female rats only after 42 days of feeding.

Summary

The data of this study tends to indicate that there is at least a 100 fold difference between the no-effect level and the fatal dose. It should be noted that the reviewer does not indicate that 500 mg/kg is the fatal dose.

Subacute Dog Oral (90 days)

Groups of 2 male and 2 female mongrel dogs were tested per dosage of 0, 30, 100 and 300 ppm. These levels are equal to approximately 0.0, 0.75, 2.5, and 7.5 mg/kg/day respectively.

The test material was administered orally by capsule daily six days per week for a period of 90 days. Blood was extracted for cholinesterase determination on the 1st, 3rd, 7th, 15th, 31st, 63rd, and 91st days of the study. No gross autopsies were performed at the termination of the study.

Results

In general, the physical appearance, behavior, appetite and survival of the test dog was comparable to the control dogs. The 0.75 mg/kg/day level had no effect on plasma cholinesterase activity. The 2.5 mg/kg/day level caused a slight inhibition of the plasma cholinesterase activity. The 7.5 mg/kg/day level caused a significant inhibition of the plasma cholinesterase activity. There was no significant reduction in the red blood cell cholinesterase activity at any dosage level.

Summary

The aforementioned data tends to indicate that the no-effect level subacutely in dogs is greater than 30 ppm but less than 100 ppm.

In Vitro Cholinesterase Study

Cholinesterase assays were carried out manometrically using the experimental method described by Nachmansohn and Rothenberg. The substrate was acetylcholine bromide at a final concentration of 0.15M. Beef erythrocyte cholinesterase served as the enzyme source, and was stabilized by diluting the concentrated preparation in 1% gelatin solution.

Results

Month-old solutions of the compound were found to be considerably more

potent against cholinesterase than freshly prepared solutions. The rate of inactivation of the enzyme was greater for old than fresh solutions, using the time required to reach 50% inhibition as the criterion. Experiments designed to study the reversibility of the cholinesterase inhibition gave inconclusive results.

Attempts to demonstrate conversion of merphos by liver hemogenates to a more potent cholinesterase inhibitor were ambiguous. It seems possible that activation and destruction may occur simultaneously, but since fortification with co-enzyme was apparently not required for activation this process probably takes place by some mechanism other than those described for other types of organo-phosphorous compounds.

Subacute Rat Feeding (11 weeks)

25 males and 25 female rats were tested per level of 0, 2.0, 5.0, 750 and 1000 ppm. The 1000 ppm level was increased to 1250 ppm for weeks 4 to 6, then to 1500 ppm for weeks 7 to 9 and finally 2000 ppm for weeks 10 and 11.

Five male and five female rats from each group including the controls, were sacrificed after 22, 43 and 64 days of feeding for plasma, red blood cells, and brain cholinesterase activity determinations. Five males from group II, five males from group III, and five untreated adult male rats of the same strain were sacrificed after 76 days for plasma cholinesterase determination.

Results

The appearance and behavior of the rats in groups II (2.0 ppm), No. III

(5.0 ppm) and No. IV (750 ppm) were generally comparable to those of the control rats. The male and female rats in group No. V appeared thin and exhibited rough coats with hair standing erect. They also appeared excited when being handled and several animals exhibited very rapid respiration.

No deaths occurred ~~at~~ in any of the control and test groups during the eleven week experiment.

The mean weekly body weights for the male test rats in groups No. II and III were comparable to the male control values. The females of these groups showed a slightly lower value than the corresponding control females. The overall body weight gain for the male and female test rats in groups IV and V ~~were~~ was significantly lower than the corresponding control animal. The food consumption for these rats ~~were~~ was also significantly lower than the corresponding controls.

Cholinesterase activity

Male and female rats in Groups No. IV and V consistently showed total plasma and red blood cell cholinesterase depression throughout the 64-day feeding period. Brain activity in these two groups was consistently inhibited 58 to 87%. The plasma cholinesterase activity was unaffected for groups II and III. Red blood cell cholinesterase activity was unaffected in group II; however, it was depressed at 43 days in the group III female rats. A borderline effect was also noted in the male rats of this group. Since no depression was noted at 64 days in either sex of group No. III, the 43-day values may be unreliable.

At 92 days, both male and female rats in group Nos. IV and V showed total plasma and red blood cell cholinesterase depression. The two methods utilized to determine the percent of plasma cholinesterase inhibition disagreed with each other more often than they agreed. These methods were the electrometric technique and the manometric technique. The testing laboratory concluded from the results that dosages of two and five ppm do not affect plasma, red blood cell, or brain cholinesterase activity. However, it does appear that the 5.0 ppm can and does exhibit some anti-cholinesterase action.

Gross Pathology

Thickening of the small intestinal walls and presence of catarrhal exudate in the lumen was observed at 22 days in both of the male and female rats in group Nos. IV and V, and at 43 days in several animals from each test group. At 64 days the incidence of this finding had decreased. At 92 days thickening of the intestinal wall was observed in ~~both~~ animals from both groups IV and V. Paleness or a chalky color of the adrenals was observed at 23 days, and in each subsequent sacrifice in a large number of females in groups number IV and V and in the males from group No. V which were sacrificed at 92 days. Pale adrenals in groups No. IV and V varied between 0 and 80%.

Microscopic Pathology

Adrenal sections from 10 rats of group No. V showed slight to severe vacuolation of the chord cells of the zona fasiculata in the adrenal cortex at 92 days. Ten adrenal sections from group IV, sacrificed

at 92 days, showed identical cell changes in five out of five male rats, while five ~~out~~ out of five female rats appeared to be within normal limits. It appears that the test material when fed to rats at dietary levels of 750 and 1000 and 2000 ppm has an untoward effect upon the adrenal cortex.

Examination of other tissues, previously listed, were found to be within normal limits.

Organ Weights

No significant differences were noted between the control rats and the rats in groups No. II (2.0 ppm) and No. III (5.0 ppm) except for significantly lower liver weights for the females in groups No. II at 92 days. It should be noted that the organ to body weight ratio for this group was within normal limits.

Male and female rats of group IV (750 ppm) were comparable to the controls at 92 days. At earlier intermittent sacrifices, both males and females at one time or another showed some significant findings. It appears evident that the animals were able to cope with the extra strain and finally overcome it.

At 92 days the male rats of group V showed lower terminal body weight, liver and kidney weight. The kidney to body weight ratios were shown to be higher than the corresponding control animals. The females of this group at 92 days showed lower kidney weights and higher liver and kidney to body weight ratios.

In the reviewer's opinion, these findings could well be attributed to the high concentration of the test material offered to the test animals

and also to the observed thickening of the intestinal wall which may not have allowed the proper absorption of nutrients. This opinion is backed up by the negative gross and microscopic findings with regard to sections of the liver and kidney from these animals.

Recovery Study

On the 101st day all remaining animals in the control and high level group were placed on the basal laboratory diet for a total of 12 days. Two males and three females in the recovery group had regained normal appearance and behavior on completion of the 12-day period. (The report does not indicate whether these animals were test or control). Both male and females gained weight during this period.

In the male rats where plasma and red blood cell activity was previously 100% inhibited, these values returned to 40 and 51% inhibition during this recovery phase. In the female rats, no control values were available, but by comparison of the pH/2 values of the female animals at 92 and 112 days, it is apparent that the recovery of a significant magnitude had taken place during the recovery phase.

It is concluded that cholinesterase depression produced by the test material is reversible in both male and female rats following withdrawal of the material.

Gross pathological findings of the 10 remaining animals is as follows: Thickening of the intestinal walls was observed in 3 males and 4 females of Group V. In the same group, pale, chalky-appearing adrenals were observed in four males and 2 females. These two findings appear to be the most persistent pathological changes.

The microscopic examination of the ten rats of Group V showed slight to

severe vacuolization of the chord cells of the zona fasiculata. This was observed in five out of five adrenal sections from the males and three out of five adrenal sections from the females.

Summary

It is apparent from the results of this test that the no-effect level of the test material lies somewhere between 2.0 and 5.0 ppm with the higher level definitely producing pathological changes.

Potentiation Study (Rats)

Merphos was given orally in combination with several other organic phosphates to determine if greater mortality was produced than that which would be expected. The other organic phosphates tested are as follows: phosdrin, parathion technical, OMPA technical, systox technical, methylparathion technical, guthion, EPN, trithion, delnav, ethion technical, sevin, diazinon, ronel (ET-57), malathion technical. The animals were fasted for a period of three to four hours prior to the dosage.

Results

The experimental toxicity of merphos plus malathion, plus methyl parathion, plus guthion were found to be 6.1, 2.6 and 2.2 times greater than the theoretical value, respectively. The theoretical values for the remaining combinations indicated mild potentiation with the exception of ethion which was found to be less toxic in combination.

Gross signs of systemic toxicity observed included varying degrees of depression, lacrimation, bloody discharge from the eyes, exophthalmos, excessive masticatory movement, labored respiration, salivation and

evidence of excessive urination and/or diarrhea, tremors, ataxia, sprawling of the limbs, and impaired righting, placement, and pain reflexes.

Summary

The reviewer reevaluated the data presented in this study as follows: The LD_{50} value of each combination was broken down to the quantity of each involved chemical that it represented. This quantity of the chemical was then located on the LD curve for that chemical. If the location on the LD curve was well below the exhibited LD_0, potentiation is present. If these values fall at the LD_0 or LD values greater than 0, little if any potentiation was noted. Following this method, the following results were obtained: Acute potentiation was exhibited by the combination merphos and malathion technical. Severe potentiation was noted in the combination of merphos plus ronel, guthion, and methylparathion. Moderate potentiation was noted with the combinations of merphos and diazinon, delnav, systox technical and parathion technical. The remaining combinations gave either slight or borderline potentiation.

Subacute Dog Potentiation Feeding Study (Six Weeks)

Two male and two female mongrel dogs were tested per level of 0, 2.0 merphos, 2.0 merphos plus 100 ppm malathion, 2.0 ppm merphos plus 5.0 ppm methylparathion, and 2.0 ppm merphos plus 5.0 ppm guthion. Plasma and red blood cell cholinesterase activity was determined on all dogs on the 2nd, 4th, 9th, 12th, 18th, 24th, 31st, and 43rd experimental days.

Results

The four control dogs in group I and test dogs in Groups II, III, IV, and V maintained normal appearance and behavior.

One dog in Group No. II and one dog in Group No. IV gave birth during the experimental period to normal, healthy pups.

In the group which received 2.0 ppm merphos alone, no significant plasma or red blood cell cholinesterase activity depression was observed in any animal, except one which showed significant plasma depression on day 43.

In the group which received 2.0 ppm merphos plus 100 ppm malathion, significant but minimal plasma and red blood cell cholinesterase depression was observed in three of the four animals.

In the group receiving 2.0 ppm merphos in combination with 5.0 ppm methyl-parathion, one animal showed significant but minimal red blood cell depression, and three animals showed significant but minimal plasma cholinesterase depression.

In the group receiving 2.0 ppm merphos plus 5.0 ppm guthion, plasma and red blood cell cholinesterase depression was noted in two of the four dogs.

Summary

The results of this study clearly indicate that a definite degree (small) of potentiation is present. The dosage level used for all the chemicals is considered to be well below their no-effect level.