2-9-88

Spec. #: DC 9

MRID: 401745-27

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DATA EVALUATION REPORT

STUDY TYPE: Chronic Toxicity

TOX. CHEM. NO.: 660

MRID NO.: 401745-27

TEST MATERIAL: O,O-diethyl-S-(ethylthio) methyl phosphorodithioate

SYNONYMS: Phorate

STUDY NO.: 85015

SPONSOR: American Cyanamid Co.

TESTING FACILITY: Tegeris Laboratories, Inc.

TITLE OF REPORT: One-year Oral Toxicity Study in Purebred Beagle Dogs with AC 35,024.

AUTHORS: T. Shellenberger and A. Tegeris

REPORT ISSUED: April 20, 1987

CONCLUSIONS: The oral administration of phorate in capsules to male and female beagle dogs for 1 year at daily dosage levels of 5, 10, 50 and 250 ug/kg caused slight tremors in high dose males and females and marginal inhibition of body weight gain in high dose males. There were no treatment related mortalities.

Plasma cholinesterase was inhibited at 50 ug/kg/day in both sexes; RBC and brain cholinesterase were inhibited in both sexes at 250 ug/kg/day.

The other investigated parameters in this study, which included ophthalmic examination, food consumption, clinical chemistry, hematology, urinalysis, organ weights, gross pathology and histopathology, did not reveal any changes which would reflect phorate-induced toxicity.

The NOEL and LEL for systemic toxicity were 50 and 250 ug/kg/day, respectively. The LELs for cholinesterase inhibition were: plasma--50 ug/kg/day; RBC and brain--250 ug/kg/day. The corresponding NOELs were: plasma--10 ug/kg/day; RBC and brain--50 ug/kg/day.

Core classification: Minimum

Quality assurance: A statement was submitted with the study.

1/37

007010

Test material

Phorate technical (AC 35,024); Lot No. AC 4870-110; Purity = 92.1%. A stock solution was prepared in corn oil at a concentration of 25,000 ug/ml; aliquots of this solution were diluted in corn oil to prepare working solutions at concentrations of 500, 100 and 50 ug/ml.

Test animals

Purebred beagle dogs, which were 5-6 months of age at the initiation of the study, were used. Initial weights were: males- 6.7 to 10.4 kg; females 5.3 to 10.0 kg. The acclimation period was 4 weeks. During the pretest period, the dogs were weighed and randomized into 5 experimental groups. The control group consisted of 8 dogs/sex and the treated groups had 6/sex. Each animal was identified by ear tattoo number.

Methods

Test material stability

All working solutions were analyzed for the first 20 weeks; thereafter, analysis was performed with one dosing solution per week, selected via a computer-generated random number. All stock solutions were analyzed periodically.

Environmental parameters

The animals were housed in one room, individually, in stainless steel cages which were cleaned daily. Temperature and humidity in the rooms were monitored twice daily to assure a room temperature of 22 ± 2°C and humidity of 30-70%. Fluorescent lighting was provided on a 12-hour light/dark cycle.

Feed and water

Water was provided ad libitum via water bowls. Feed (Purina Certified Canine Chow, 5007 Meal) was provided for one hour each day; each lot of feed was recorded. The feed and water were monitored for contaminants. Feed consumption was measured daily and summarized on a weekly basis as the mean daily feed consumption of each dog for that week. Each animal was provided with 400 grams of feed for one hour; unconsumed feed was weighed and daily food consumption was calculated by difference.

Compound administration

Phorate was administered by capsule to each dosage group at a constant dose, based on body weights determined weekly, expressed as micrograms (ug) per kilogram (kg) of body weight per day. Capsules were prepared on a weekly basis and stored under refrigeration. The working solutions of test material were prepared at the previously noted concentrations so that 0.1 ml/kg body weight could be administered to each dog at each dosage level. The test protocol did not indicate if control dogs received solvent control (ie. corn oil only) capsules. Capsules were administered approximately 1 hour after each daily feeding period. The dosage groups were described as follows:

Group	Phorate dose level (ug/kg/day)
Control	0
Low dose	5
Mid-I dose	10
Mid-II dose	50
High dose	250

Clinical examination

All animals in the study were examined twice daily for overt signs of toxicity including mortality, general physical appearance, behavior, gait and excretory function. A more detailed examination was conducted weekly which included all of thr above as well as palpation for masses and examination of mucous membranes and external orifices.

Body weight

Body weights were determined during the pretest period (for randomization), on the day of test iniation prior to the first dose, weekly thereafter during the 52-week test period and immediately prior to termination.

Ophthalmologic examinations

Ophthalmologic examinations were performed on each dog by the consulting Veterinary Ophthalmologist during the pretest period, at 6 months and prior to terminal sacrifice.

Clinical laboratory studies

Clinical laboratory studies (hematology and clinical chemistry) were conducted on all dogs once during the pretest period, after 6 weeks, at 3 and 6 months and prior to terminal sacrifice. Blood samples were collected from dogs prior to the daily feeding and during the treatment period were obtained approximately 18 hours after the prior dose of the chemical. Control animals were bled concurrently with the dosed animals.

Clinical chemistry

The following clinical chemistry parameters were investigated:

Serum glutamic oxaloacetic transaminase (SGOT)
Serum glutamic pyruvic transaminase (SGPT)
Blood urea nitrogen (BUN)
Fasting blood sugar or glucose (FBS)
Total protein (TP)
Albumin (A)
Globulin (G)-calculated
A/G ratio-calculated
Total(TB) and Direct(DB) bilirubin- DB only if TB is elevated
Serum alkaline phosphatase (SAP)
Serum lactic dehydrogenase (LDH)
Serum creatinine (CREAT)
Calcium (CA)

Phosphate (PO_4)
Sodium (NA)
Potassium (K)
Chloride (CL)
Total cholesterol (CHOL)
Gamma glutamyl transpeptidase (GGTP)
Carbon Dioxide (CO_2)
Creatine phosphokinase(CPK)

Special assays for plasma and erythrocyte (RBC) cholinesterase (ChE) activities were conducted twice prior to initiation of the study and after 6 weeks, at 3 and 6 months and prior to terminal sacrifice. Brain cholinesterase activity in cerebrum and cerebellum was determined on each dog after the terminal sacrifice; brain samples were collected at necropsy and frozen until assayed. Mean RBC and plasma cholinesterase activities in treated animals were calculated relative to the mean pretest cholinesterase activities, whereas brain enzyme activities in treated animals were calculated relative to activity in concurrent controls.

Hematology

The following hematological parameters were determined on all dogs at the same intervals as the clinical chemistry determinations:

Total white blood count (WBC)
Total red blood cell count (RBC)
Hemoglobin (HGB)
Hematocrit (Hmct)
White blood cell differential count (WBC Diff)
Platelet count (Plat)
Red blood cell morphology
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Reticulocyte count (if signs of anemia are present)

Urinalysis

Urinalysis parameters were determined during the pretest period, after 6 weeks, at 3 and 6 months and prior to terminal necropsy. The following parameters were determined:

Color
Appearance
Specific gravity
pH
Qualitative tests for glucose
Ketones
Bilirubin
Urobilinogen
Protein
Occult blood
Microscopic examination of sediment after centrifugation

Necropsy

Organ weights and macroscopic examination

A mid-I dose male that was sacrificed moribund was necropsied for a complete gross examination and tissues were retained for subsequent histological examination. All surviving animals were sacrificed and necropsied over a 5-day period following completion of the test period. It was indicated that the gross necropsy was performed under the supervision of a Veterinary Pathologist. Organ weights and all gross lesions for each animal were recorded at necropsy. The following organs were weigned "fresh" from all survivors at terminal sacrifice for the calculation of mean organ weights, mean organ-to-body weight and mean organ-to-brain weight ratios:

Adrenals(combined weight)
Brain
Gonads(combined weight)
Heart
Kidneys(combined weight)
Liver
Lungs
Thyroids/parathyroids(combined weight)
Spleen
Thymus

Histologic examination

The following tissues from all dogs were taken at necropsy and retained in 10% neutral buffered formalin and examined microscopically:

Adrenals
Aorta
Bone and marrow (sternum)
Brain (fore, mid and hind sections)
Cecum
Colon
Duodenum
Epididymis
Esophagus
Eyes(both)
Gallbladder
Heart
Ileum
Jejunum
Kidneys(both)
Liver
Lungs with mainstem bronchi(left and right)
Lymph nodes(mediastinal and mesenteric)
Mammary gland
Optic nerve
Ovaries(both)
Pancreas
Parathyroids(both)
Pituitary
Prostate
Salivary gland
Sciatic nerve
Skeletal muscle
Skin(mammary area)
Spinal cord(cervical, thoracic and lumbar)
Spleen
Stomach
Testes(both)
Thymus
Thyroids(both)
Tongue
Trachea
Urinary bladder
Uterus(corpus and cervix)
Vagina
Tissues with gross lesions

This list of tissues includes all of those specified under Guideline 83-1.

007010

Results

The analytical results from GC analyses of the working solutions are summarized in the following table.

Concentrations of Phorate Working Solutions

Nominal concentration (ug/ml)	Analytical values	Mean ug/ml(SD)	Mean % of nominal
50	30.2--58.5	44.2(±8.5)	88.4
100	67.1--110.4	94.0(±11.2)	94.0
500	404--545	480(±37)	96.0
2500	2000--2720	2480(+198)	99.2

All phorate concentrations in the working solutions were consistently within 15% of nominals with the exception of the 50 ug/ml solution which ranged between 17 to 45% below nominal during weeks 2-11. Concentrations of phorate in the stock solutions (25,000 ug/ml) ranged from 94.3 to 141.5% of nominal during the study.

Food consumption

Food consumption was slightly depressed among low and high dose males, relative to controls, however, the differences were not consistent throughout the study, nor were they statistically significant. Additionally, there was no evidence of a dose-response relationship, therefore, the noted differences did not appear to be induced by treatment.

Clinical examination

Mortality

One male in the mid-I dose group was sacrificed moribund during study-week 24 (163 days on test). The investigator indicated that the animal appeared emaciated and exhibited swelling and edema in the neck and body areas as well as alopecia in most areas of the body. The cause of this lethargic condition was not readily apparent from the information provided.

Clinical signs

Alopecia was observed in 1/6 (high-dose) to 5/6 (mid-I and mid-II doses)males; this change was not observed in control males. Similar incidences of alopecia, which were also not dose-related, were observed among treated female groups, however, this observation was also noted for 3/8 control females. Therefore, alopecia can not be conclusively associated with treatment.

Soft feces were observed sporadically in every group among both sexes; the incidences were not dose-related.

Occasional vomiting was observed in high-dose males(3/6) as well as mid-I, mid-II and high dose females(1/6, 2/6 and 1/6, respectively). Apparently, this was induced by the ingestion of phorate since vomiting was not observed among the controls of either sex.

6

High dose males (1/6) and females (2/6) had slight tremors which, evidently, did not become more intense during the study. This effect was not observed in any other dosage group.

07010

Body weight

Slight inhibition of body weight gain was evident in high-dose males. The mean 52-week gain in this group was 37.9% compared to 49.6% for controls and the mean final group weight was 10.2% below that for controls (10.8 vs. 12.03 kg for test and controls, respectively); the initial mean weight for high-dose males was 2.6% below the control value (7.83 vs. 8.04 kg). Total mean weight gains among the treated group for both sexes were comparable to those for corresponding controls. None of the differences in body weights between treated and control groups were statistically significant (Dunnett's t test) for either sex throughout the study.

Ophthalmologic examinations

The ophthalmic examinations performed at 6 and 12 months did not reveal compound induced changes. Isolated incidences of retinal folds and the absence of tapetum were observed only in those animals in which these anomalies were seen during the pre-test examination.

Clinical laboratory studies

Cholinesterase determinations

The differences between pre-test and 12-month plasma cholinesterase levels for each group are included in the following table.

Comparison of Pre-test and 12-Month Plasma ChE Levels

	Mean ChE Levels(mU/ml)				
Dose(ug/kg/day)--	0	5	10	50	250
Males					
Pre-test--	2028.8	2054.2	1814.5	1929.7	1994.7
12-month--	1727.9	1661.7	1344.8	900.3	408.8
% change--	-14.8	-19.1	-25.9	-53.3	-79.5
Females					
Pre-test--	1891.1	2048.0	1858.3	1922.3	1861.5
12-month--	1964.0	1970.5	1560.5	1259.0	426.5
% change--	+3.9	-3.8	-16.0	-34.5	-77.1

The mean plasma ChE level in control males decreased during the study to a 12-month level that was 14.8% lower than the pre-test value. The decreased level in low-dose males was probably within the range of normal variability and a possible compound-related effect on the decrease observed in mid-I dose males is questionable. The 12-month ChE levels in mid-II and high dose females, which were substantially lower than respective pre-test levels, appear to be the result of compound-induced reductions.

Levels of erythrocyte and brain ChE, observed at the end of the study, are shown in the following tables.

Comparison of Pre-test and 12-Month Erythrocyte ChE Levels

	Mean ChE Levels (mU/ml)				
Dose(ug/kg/day)--	0	5	10	50	250
Males					
Pre-test--	3768.8	3570.0	3581.7	3706.7	3758.3
12-month--	3675.0	3703.3	3616.0	3523.3	2780.0
% change--	-2.5	+3.7	+1.0	-4.9	-26.0
Females					
Pre-test--	3636.2	3741.7	4018.3	3778.3	3726.7
12-month--	3552.5	3793.3	3873.3	3713.3	3026.7
% change--	-2.3	+1.4	-3.6	-1.7	-18.8

Apparent compound induced reduction in erythrocyte ChE was observed only in high-dose animals; this effect was marginal in females.

Twelve-Month Brain ChE Levels

	Mean ChE Levels (mU/g)				
Dose(ug/kg/day)--	0	5	10	50	250
Males					
Cerebrum--	714.1	685.4	717.4	652.1	404.2
Rel.to controls--	----	-4.0	+0.5	-8.7	-43.4
Cerebellum--	2564.1	2522.9	2778.5	2264.6	1175.0
Rel.to controls--	----	-1.6	+8.4	-11.7	-54.2
Females					
Cerebrum--	781.2	872.9	862.5	825.0	433.4
Rel.to controls--	----	+11.7	+10.4	+5.6	-44.5
Cerebellum--	2334.4	2887.5	1975.0	2314.6	1116.7
Rel.to controls--	----	+23.7	-15.4	-0.8	-52.2

The only obvious compound-induced reduction in brain ChE levels occurred in high dose males and females. These data show considerable fluctuation in ChE levels (both above and below control levels) in both sexes among the other dosage groups.

Clinical chemistry

Total plasma protein levels were lower at all intervals in both high dose males and females than in respective controls; the differences were statistically significant ($p<0.05$, Dunnett's t test) at all intervals in males and at 3 and 6

months in females. However, a dose-response trend was not evident for these differences and the terminal total protein levels were approximately 5% higher than respective pre-test values for both high dose males and females. The corresponding increases for controls were approximately 18 and 16% for males and females, respectively. A similar pattern of lower levels of albumin and globulin relative to controls, but not to pre-test values, was shown by the data.

Low plasma calcium levels relative to control values, were statistically significant in high dose males at 6 months, mid-II dose females at week 6, all female treatment groups at 6 months and high dose females at 12 months. Compared to pre-test values, terminal calcium levels in high dose dogs were approximately 15 and 16% lower, respectively, in males and females. However, terminal calcium levels in controls were also lower than pre-test values; approximately 13 and 10% for males and females, respectively.

Other statistically significant differences between test and control values were either sporadic, observed in only 1 sex and/or did not conform to a dose/response relationship.

Based on considerations indicated above, none of the noted differences between control and test clinical chemistry values appear to be biologically significant.

Hematology

The investigator indicated that the following differences between control and test values for the measured hematologic parameters were statistically significant: decreased mean corpuscular hemoglobin concentration in mid-I, mid-II and high dose males at 6 weeks, mid-II dose males at 3 months and all female treatment groups at 3 months; decreased mean corpuscular volume in high dose males at 12 months; decreased platelet count in low dose males at 12 months and increased mean corpuscular hemoglobin in low and high dose males at 12 months. Considering the absence of dose/response patterns, the sporadic nature of these differences and the limited degree of fluctuation observed for the noted parameters, there is not convincing evidence to indicate that treatment had an effect on the investigated hematologic parameters.

Urinalysis

There was a slight but consistent reduction in urine pH (6.0-7.0) in high dose males and a sporadic reduction (week 6 and month 12) in high dose females.

Urine color tended to be straw or amber among treated males and females only, however, a relationship to phorate dose-level was not apparent.

The changes observed in the urinalysis parameters have no obvious toxicological significance.

Necropsy data

Organ weights

A comparison of mean values for absolute organ weights and organ weights relative to body or brain weight did not reveal any obvious compound-related changes. Compared to respective control values, none of the differences for mean absolute or relative organ weights were statistically significant ($p<0.05$, Dunnett's t test) in any treatment group of either sex.

007010

Gross pathology

Treatment-related changes were not observed during gross necropsy. A summary table (T-4.11.2) as well as the individual animal data (page V-61) indicated that a mass was observed on the spleen of 1 high dose female(No. 504232), however, subsequent histologic examination evidently did not reveal a neoplastic lesion.

Microscopic pathology

Data from the histologic examination of noted tissues and organs did not demonstrate treatment-induced lesions (neoplastic or non-neoplastic) in either sex. The presence of a plasmacytoma, associated with the prescapular lymph node, and a skin fibroma (head region) were found in the mid-I dose male that was sacrificed moribund, however, these lesions were apparently incidental findings that were unrelated to treatment.

A summary table for the incidence of neoplastic microscopic findings for male dogs was omitted from the report.

Conclusions

The oral administration of phorate in capsules to male and female beagle dogs for 1 year at dosage levels of 5, 10, 50, and 250 ug/kg/day did not cause any mortalities. One 10 ug/kg male was killed moribund during week 24 of the study, however, the reason for the lethargic condition was not apparent.

Clinical signs which reflected compound-induced changes included slight tremors in some of the high dose males and females, sporadic vomiting among all treatment groups and slight inhibition of body weight gain in high dose males. None of the differences in mean body weights between treated and respective control groups were statistically significant.

Low plasma levels of total protein, albumin,, globulin and calcium in treated groups compared to corresponding control levels did not appear to be toxicologically significant. Although the respective differences were usually statistically significant, the concentration of each was typically higher than the corresponding pre-test level.

Other clinical chemistry and hematological parameters were similar in control and treated animals at all dosage levels.

There was a slight but consistent reduction in urine pH in high dose males; this occurred sporadically in females. These observations have no obvious toxicological significance.

Plasma cholinesterase was clearly inhibited at 50 ug/kg/day in both males and females while erythrocyte and brain cholinesterase were inhibited at 250 ug/kg/day in both sexes.

The administration of phorate had no apparent effect, at any dose level, on organ weights which were presented as absolute weights, weights relative to body weight and weights relative to brain weight.

Gross and histologic examination of guideline organs and tissues from all animals did not reveal any lesions that could be clearly associated with treatment.

10

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The registrant should submit a summary table for the incidence of neoplastic microscopic findings for male dogs, as previously requested (Memorandum, Swentzel, TS-769C, to Edwards, TS-767C, January 27, 1988).

The LEL for the systemic toxicity of phorate in this study, based on body tremors in males and females and inhibited body weight gain in males, was 250 ug/kg/day; the NOEL for systemic toxicity was 50 ug/kg/day.

The LELs for cholinesterase inhibition were: plasma= 50 ug/kg/day; RBC and brain= 250 ug/kg/day. The corresponding NOELs were: plasma= 10 ug/kg/day; RBC and brain= 50 ug/kg/day.

Guideline No. 83-3
Species: Rabbit
DP#: 0
MRID: 401745-28

Reviewed by: K. Clark Swentzel K. Clark Swentzel 2/25/88
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Secondary reviewer: Marcia van Gemert, Ph.D. M. van Gemert 3/23/88
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DATA EVALUATION REPORT

STUDY TYPE: Teratogenicity:Rabbits TOX. CHEM. NO.: 660

MRID NO.: 401745-28

TEST MATERIAL: O,O-diethyl-S-(ethylthio) methyl phosphorodithioate

SYNONYMS: Phorate

STUDY NUMBER(S): 86-3039

SPONSOR: American Cyanamid Co.

TESTING FACILITY: Bio/dynamics, Inc.

TITLE OF REPORT: A Teratology Study with Phorate in Rabbits

AUTHOR(S): R. Schroeder

REPORT ISSUED: April 20, 1987

CONCLUSIONS: Phorate, dissolved in corn oil, was administered via oral gavage to groups of 20 pregnant New Zealand white rabbits on days 6-18 of gestation at dosage levels of 0.15, 0.50, 0.90 and 1.2 mg/kg/day. The animals were sacrificed on Day 30 of gestation to examine the fetuses.

Maternal toxicity was evident at the 0.50, 0.90 and 1.2 mg/kg/day dosage levels, based on body weight loss during treatment and compound-induced mortality.

Noted malformations: open eyes and curved scapula in one high-dose litter and angulated hyoid arch(es) in a small number of fetuses in the 0.9 and 1.2 mg/kg/day dosage groups, appeared to be secondary effects of maternal toxicity.

Treatment had no apparent effect on any of the investigated reproductive parameters. None of the other skeletal or visceral malformations/variations, observed in treated fetuses, were indicative of developmental toxicity.

The LOEL and NOEL for maternal toxicity in this study, based on mortality and body weight loss during treatment, are 0.5 and 0.15 mg/kg/day, respectively. The NOEL for developmental toxicity is 1.2 mg/kg/day, the highest dosage of phorate administered.

Classification: core-minimum

Quality assurance: A statement was submitted with the study.

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Materials and Methods

Test material

Phorate: Lot No. AC4870-110; 92.1% active ingredient; described as a clear, slightly yellow liquid.

Dosing solutions were prepared fresh weekly one week prior to use, to provide time for analysis. The stock solution, with a phorate concentration of 6.5 mg/ml, was prepared in corn oil; adjustment was made to compensate for the concentration of active ingredient in the test sample. Aliquots of the stock solution were used to prepare the dosing solutions, which were stored under refrigeration. Samples of the stock solutions and weekly dosing solutions were sent to the sponsor for analysis.

Animals

New Zealand white rabbits; females were 4 to 5 months of age and males were 1 to 2 years old. The animals were housed individually, except during mating, in suspended stainless cages and acclimated for 29 days. Twenty mated females were assigned to each group based on Day 0 mean group body weights; each female was identified by a number engraved on an ear tag as well as with a cage number.

Environmental conditions

The room temperature was maintained between 58 and 72°F and the relative humidity between 53 and 85% during the study. A 12 hour light/dark cycle was provided.

Food and water

Food and water were provided ad libitum. The feed was Purina High-Fiber Rabbit Chow #5326; tap water was delivered by an automatic watering system.

Mating

Each female was placed in a male's cage. One to two hours after coitus was observed, the female was placed in the cage of a second male. When coitus was observed with the second male, the female was considered mated and returned to her cage. The day on which coitus was observed with both males was considered Day 0 of gestation.

Compound administration

The test material was administered via gastric intubation on Days 6 to 18, inclusive, of gestation. The controls were given the vehicle (corn oil) alone. The dosage volume was 2 ml/kg for all groups.

The female rabbits (100) were divided into the following dosage groups:

Group No.	Dose Level (mg/kg/day)
I	0
II	0.15
III	0.50
IV	0.90
V	1.20

Evaluations

Maternal

In-life

Physical observations

Each female was examined twice daily for signs of pharmacologic or toxicologic effects and mortality. In addition, each female was given a detailed physical exam on Days 0, 6, 12, 18, 24 and 30 of gestation. On Days 6, 9, 12, 15 and 18 these detailed physical examinations were given after all animals were dosed for that day.

Body weights

Individual weights were recorded on Days 0, 6, 12, 15, 18, 24 and 30 of gestation.

Postmortem

Gross examinations

Gross postmortem examinations were performed on all mated rabbits. It was indicated that only abnormal tissues were saved in 10% neutral buffered formalin. Females showing signs of abortion (passing of placental/fetal tissue prior to Day 26 of gestation or later) were killed via intravenous injection of sodium pentobarbital in the marginal ear vein on the day such evidence was observed. Reproductive systems were examined. All remaining females were sacrificed on Day 30 of gestation.

Reproductive system

The intact uterus (ovaries attached) was removed from the abdominal cavity, weighed and the number and location of the following were recorded for each uterine horn:

live fetuses

dead fetuses (no evidence of tissue degeneration)

late resorptions (recoginzable dead fetus undergoing degeneration regardless of size)

early resorptions (evidence of implantation but no recognizable fetus)

implantation sites

The ovaries were dissected free from the uterus and evaluated for the presence of corpora lutea.

When no uterine implants were grossly apparent, the uterus was stained with ammonium sulfide. If no foci were visualized, the female was considered not pregnant.

Fetal

Prior to scheduled termination

Fetuses/delivered pups recovered during the Day 26-30 gestation interval were evaluated for external malformations, eviscerated and processed for staining of the skeletal structures with Alizarin Red S. These stained specimens were evaluated for skeletal malformations only. Only grossly abnormal fetuses obtained earlier than Day 26 were saved (10% formalin) for possible future examination.

Scheduled termination

All fetuses were given a gross examination for malformations of the external form, including examination for palatal defects. Subsequently, each fetus was weighed and tagged individually for identification.

Soft tissue examination

All fetuses were evaluated for soft tissue malformations and variations using a microdissection procedure (Staples) and the sex was noted from the internal examination. Following this procedure, the viscera were removed from the thoracic and abdominal cavities and discarded. The eviscerated fetus was then skinned. As the skin of the head was removed, the eyes were evaluated grossly for obvious malformations. The brain was evaluated by making a transverse cut with a razor blade parallel and just posterior to the frontal-parietal suture and through the cerebral hemispheres. The micro-dissection procedure was performed under a dissecting microscope (10-20X) and magnification lens (2.0X). Eyes and brain observations were performed grossly.

Skeletal examination

The eviscerated/skinned fetal specimens were processed for staining of the ossified skeletal structures using the Alizarin Red S staining procedure of Crary

as modified by the investigator (Bio/dynamics). Fetal skeletal specimens were evaluated under a 2.0X magnification lens.

Late resorptions

Late resorptions were weighed, examined grossly for external malformations (size permitting) and discarded.

Statistical analyses: See Appendix

Results

Test material stability

Analyses showed that the mean phorate concentration in the stock solution for the duration of the study was 97% of the nominal concentration while that for the dosing solutions was 94-96% of the respective nominal levels.

Maternal mortality

The unscheduled deaths on this study are tabulated below:

Maternal Mortalities during Gestation

Group No.	Animal No.	Day of Gestation (No. of days dosed)	Mode of Death*
I	1506	13(8)	1
II	2504	14(9)	1
III	3503	19(13)	1
IV	4504	18(12)	1
	4520	18(12)	1
V	5502	15(13)	1
	5503	23(13)	2
	5504	18(12)	1
	5505	19(13)	1
	5507	14(8)	1
	5509	18(12)	1
	5510	18(12)	1
	5513	17(11)	1
	5514	28(13)	3
	5518	15(9)	1

* 1: spontaneous
2: sacrificed-aborted
3: sacrificed-premature delivery

The animals in Groups I and II (1506 and 2504) died soon after dosing, therefore, it is the investigator's opinion that these deaths should be attributed to intubation error.

The Group III female that died (3503) had lost weight during the treatment period. Since the investigator found no evidence of an intubation injury, the death of this animal was considered to be treatment-related. Based on similar evidence, the Group IV deaths also appear to be treatment-related. Likewise, the spontaneous deaths in Group V (3) were evidently related to treatment.

Pregnancy rate

All of the females that died spontaneously were pregnant at the time of death.

Pregnancy Rates (%)

Group No.:	I	II	III	IV	V
	90	90	95	85	100

Maternal body weights

Mean maternal body weight gains during gestation (Days 0-30) were as follows:

Mean Maternal Body Weight Gain

Group No.:	I	II	III	IV	V
Mean wt.(g):	491	467	334	348	468
% Gain:	14.2	13.8	9.8	10.2	13.8

These data indicate that maternal bodyweight gain was inhibited in Groups III and IV. An examination of the individual data revealed that there were 2 outliers in each of these groups; 2 females in Group III had slight weight losses (33 and 21g) while 2 females in Group IV had small weight gains (95 and 138g). Excluding these outliers increases the mean bodyweight gains to 11.5 and 11.7% for Group III and IV, respectively. Although the intergroup differences in body weight gain were not remarkable, the differences noted in Groups III and IV may be treatment-related. It is possible that inhibited body weight gain was not indicated by these data in Group V because those females which were adversely affected by treatment did not live until Day 30 of gestation. For example, the mean body weight in Group V at Day 18 of gestation was 9.4% lower ($p<0.05$, ANOVA) than the control value (control=3729g; Group V=3377g), however, this mean weight included data from females which subsequently died. The difference in mean body weight between Groups I and V at termination was only -2.7% (Group I =3958g; Group V =3850g).

Intergroup comparisons of mean maternal body weights on the last day of treatment (Day 18) showed that treatment caused slight weight losses which followed a dose trend.

Mean maternal body weights at Day 18

Group No.:	I	II	III	IV	V
(g)--	3729	3622	3525	3561	3377
Rel. to control (% diff.)	----	-2.9	-5.5	-4.5	-9.4
Day 18 vs Day 6 (% diff.)	+3.2	+2.8	-0.6	-0.3	-4.6

These data show relatively small differences, in comparison to controls, for the mean body weights in Groups II, III, and IV on the last day of treatment. The difference for Group V was more pronounced. Differences relative to pre-treatment weights were also more pronounced in high-dose females.

The mean corrected Day 30 body weights (body weight minus gravid uterus weight) followed a relative pattern similar to that for the mean body weight gains shown above.

Corrected Day 30 Maternal Body Weights

Group No:	I	II	III	IV	V
Mean wt.(g)	3392	3301	3234	3272	3317
Relative to control (% diff.)	.---	-2.7	-4.7	-3.5	-2.2

Although treatment appeared to have a slight adverse effect on terminal maternal body weight gain in Groups III and IV, the intergroup differences in carcass weights did not appear to be toxicologically significant. Treatment caused body weight loss during the treatment period, particularly in Group V females, however, this effect was not remarkable over the entire gestation period.

Maternal food consumption

Mean food consumption values were lower in treated groups compared to the control value, however, the differences were neither remarkable nor dosage-related.

Mean Maternal Food consumption (Gestation Days 3-30)

Group No.:	I (g/day)	II	III	IV	V
	48.5	45.5	42.0	43.8	43.9

Sporadic decreases in mean food consumption values were noted for Group V near the end of the treatment period (Days 15, 18 and 19 of gestation), however, only the difference at Day 18 was statistically significant ($p<0.05$, ANOVA).

Clinical observations during gestation

The investigator did not note any treatment-related clinical signs for females in Groups II-IV. Several Group V females were observed with stained skin/fur in the ano-genital area during the treatment period, however, this is a common observation in dying animals.

92 18

Reproduction data

Abortion and premature delivery

One abortion and 1 premature delivery were observed in Group V. One female aborted on Day 23; 4 placentae were found in the cage pan and 8 uterine implantation scars were found. The premature delivery occurred on Day 28 of gestation; 8 intact fetuses and 1 partially cannibalized fetus were found in the cage pan and 9 uterine implantation scars were noted.

The investigator's historical control data for 18 groups from 17 studies showed the following:

Abortion Index: mean = 3.5%; aborted pregnancies were seen in 33.3% of all groups; in these groups, the incidence ranged from 4.8 to 33.3%.

Premature Delivery Index: mean = 7.0%; premature delivery was seen in 66.7% of all groups; in these groups, the incidence ranged from 4.5 to 19.0%.

Considering the incidences of abortion and premature delivery in Group V (10% each) in comparison to the investigator's historical data, it is difficult to clearly associate these findings with treatment, especially in a group in which the incidence of maternal mortalities was 40%.

Corpora lutea count

Mean corpora lutea counts were comparable between groups.

Implantation sites

Mean implantation site counts were also comparable between groups.

Pre-implantation losses

The mean number of pre-implantation losses per group were:

Group No.:	I	II	III	IV	V
	0.111* ±0.138	0.063 ±0.153	0.083 ±0.122	0.123 ±0.223	0.104 ±0.136

* The investigator calculated this value to be 0.082 ±0.097.

A treatment-related incidence was not evident from these data.

Resorptions

Treatment did not affect the number of resorptions/number of implants or the proportion of litters with resorptions.

Viable fetuses and sex ratio

The mean number of viable fetuses were comparable between all groups. No dead fetuses were found in any group at the termination of the study.

93 19

Treatment had no apparent effect on the fetal sex ratio.

Fetal body weight

Mean fetal body weights were slightly lower than corresponding controls in both males and females in all treated groups, however, the Group V weight (combined sexes) was only 3.6% lower than the control value and apparently does not reflect a toxic effect.

External malformations

The only noted external malformations were omphalocele in 1 Group III fetus and open eyes (bilateral) in 3 fetuses from 1 Group V litter (litter size = 3). An examination of the maternal data for the female (No. 5512) which had this litter revealed maternal toxicity: body-weight loss was observed during treatment Days 6-9, 12-15 and 15-18; the total weight loss during treatment (days 6-18) was 778g; food consumption was also relatively low. Additionally this female had 3 pre-implantation losses and 5 early resorptions.

The incidence of open eyes in Group V (3.5%) was considerably higher than the reported 0.20% (5/2524) historical incidence among controls in the investigator's laboratory, however, all of the control fetuses observed with this anomaly (5) were from one litter. The investigator cited a report (Palmer, A.K., 1972. Sporadic malformations in Laboratory Animals and Their Influence on Drug Testing. Advances in Experimental Medicine and Biology. Vol. 27: 45-60) which indicated that the distribution of open eye can be clonic (litter-based). The data in the current study is consistent with this conclusion. Considering the extent of maternal toxicity that was noted for female No. 5512, it can not be concluded with any confidence that the open fetal eyes were a direct result of treatment.

External variations

There was no evidence of treatment-related changes; distended abdomen was observed in 1 Group II fetus.

Visceral malformations and variations

The sporadic anomalies noted by the investigator were not associated with the dosage levels of administered phorate.

Skeletal malformations

The only noteworthy malformations which occurred at higher incidences in treated versus control fetuses were angulated hyoid arches and curved scapulae.

Incidences of Noted Skeletal Malformations

Group No:	I	II	III	IV	V
1/ Hyoid arch(es) angulated					
Fetal incidence: N(%)	3(2.1)	2(1.4)	2(1.4)	8(7.1)	4(4.7)
Litter incidence: N(%)	3(17.6)	2(11.9)	2(11.1)	6(40.0)	4(40.0)
2/ Scapula curved					
Fetal incidence: N(%)	0	0	0	0	3(3.5)
Litter incidence: N(%)	0	0	0	0	1(10.0)

Mean fetal body weights were slightly low among treated groups, relative to controls, however, the differences did not appear to be biologically significant.

Two noteworthy malformations were observed in all of the fetuses (3) in one 1.2 mg/kg/day litter: open eyes and curved scapula, however, the dam exhibited significant signs of maternal toxicity during gestation. Additionally, a slightly increased incidence of angulated hyoid arch(es) was observed at the 0.9 and 1.2 mg/kg/day dosage levels, however, this anomaly also appeared to be secondary to maternal toxicity.

There were no other skeletal or visceral malformations/variations in fetuses from treatment groups which were indicative of developmental toxicity.

The LOEL and NOEL for maternal toxicity in this study, based on mortality and body weight loss during treatment, are 0.5 and 0.15 mg/kg/day, respectively.

The NOEL for developmental toxicity was 1.2 mg/kg/day, the highest dosage of phorate administered.

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APPENDIX

Pharate For Review

Page _____ is not included in this copy.

Pages 23 through 27 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product inert impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

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_____ Information about a pending registration action.

✓ FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Guideline no. 83-
Species : Mouse
MRID : 00092853
G.G. 6-
OCTCP

Three Generation Reproduction in Mice

FICHE/MASTER ID 00092853

Morici, I.J.; Shaffer, C.B.; Ribelin, W.E.; et al. (1965) Thimet (R) Systemic Insecticide: Successive Generation Studies with Mice: Report No. 65-136. (Unpublished study received on unknown date under PP0378; submitted by American Cyanamid Co., Princeton, N.J.; CDL: 092662-A)

Test Chemical:

Phorate Technical

Protocol:

Four groups of male and female Albino mice (CF1 strain) were assigned to diets containing 0 ppm (control), 0.6 ppm, 1.5 ppm or 3.0 ppm of Phorate (Thimet). Animals were approximately one month of age and acclimatized for one week before testing. Diet was prepared weekly. For mating purposes, 8 mating groups, each consisting of one male and 2 females, were set up for each dietary level. Some of the subsequent matings had fewer females since a few dams were lost. Mating groups were housed together until it was obvious that females were pregnant or a maximum of 18 days had passed. Males were rotated within their specific group about midway through this interval. After separation from the males, females were housed individually in nesting cages.

The study was performed according to the following chart.

F_0 generation

- F_{1a} (discard)
- F_{1b} generation
  - F_{2a} (discard)
  - F_{2b} generation
    - F_{3a} (discard)
    - F_{3b} generation

~~102~~
28

About 10 days after the last pups from the first mating were weaned, animals were remated for production of their second litters.

Litters were not reduced in size. Surviving pups were counted as soon after birth as possible, and at 5 and 21 days after birth. At weaning (21 days), the body weight and sex of each pup was recorded. Each litter, including stillborn pups and those which died before weaning, was examined for abnormal or deformed members.

F_0 animlas were received from a supplier and mated at random. In all matings of subsequent litters, records were kept of sires and dams to avoid sibling matings. Animals were selected at random from the F_{1b} and F_{2b} litters for subsequent matings. Insofar as possible, a female was taken from each litter and a male was selected from alternate litters. In those instances where less than 16 litters were cast, additional animals were selected to bring the total number up to 16 females and 8 males.

In addition to these general comments relative to all litters, observation specific to given mating were:

F_0 Generation

6 September 1963 - Started on Test. 4 weeks old.
25 October 1963 - First mating. 11 weeks old.
8 January 1964 - Second mating. 22 weeks old.
Food consumption measured to time of first mating.
Body weights recorded periodically and before each mating.

F_{1b} Generation

10 April 1964 - First mating. 12-14 weeks old.
4 January 1965 - Second mating. 23-25 weeks old.
Body weights recorded periodically and before each mating.

F_{2b} Generation

19 October 1964 - First mating. 12-14 weeks old.
4 January 1965 - Second mating. 23-25 weeks old.
Body weights recorded periodically and before each mating.

Gross Autopsy and Microscopic Observations. Autopsies and tissue examinations were conducted as follows:

F_0 - Parent generation - discarded without autopsy.

F_{1a} - First generation, first litters - intact animals observed for gross defects, discarded without autopsy.

F_{1b} - First generation, second litters - intact animals observed for gross defects, discarded without autopsy.

F_{2a} - Second generation, first litters - intact animals observed for gross defects, discarded without autopsy.

F_{2b} - Seocnd generation, second litters - 16 female and 8 male mice from each group were selected to produce the F_{3a} and F_{3b} generations. After a gross examination at weaning, the remaining F_{2b} pups were discarded without autopsy. When the F_{3b} pups were weaned at approximately 21 days after birth, survivors of these 24 parent animals for each group were anesthetized with chloroform, exsanguinated, autopsied, and liver, kidneys, and gonads were weighed. No microscopy was performed. Autopsies were in the following sequence: male parents, females failing to produce young or whose young had died before weaning, and females raising litters to 21 days.

F_{3a} - Third generation, first litters - intact animals observed for gross defects, discarded without autopsy.

F_{3b} - Third generation, second litters - pups were raised to 21 days, sexed, weighed and killed by placing in a jar containing vapor of chloroform. Immediately after death, the smallest or least healthy appearing male and female of each litter were autopsied and portions of the following organs were fixed with 10% neutral buffered formalin for histologic processing: skeletal muscle and femur, spleen, mesenteric lymph node, pancreas, liver with gall bladder, stomach, ileum, colon, urinary bladder, gonads, prostate or uterus, adrenals, kidneys, thyroids (and parathyroid if included), trachea, esophagus, lung, heart, brain.

The remaining animals in the litter were separated by sex and autopsied as completely as was possible without damaging any bones. They were then skinned, eviscerated, and trimmed of extraneous tissue. The carcass was fixed in 70% ethanol, dehydrated in acetone, cleared in 10% potassium hydroxide, and the bone structure was stained with saturated alizarin solution. The bony skeleton was then examined under an illuminated 3x magnifying lens.

Several litters, or individuals within litters, failed to reach 21 days of age. These pups were autopsied and their skeletons were processed whenever the animals were found in a pre-autolytic state or before having being eaten by the mother.

All animals (F_0 through F_3) of all levels, which died before weaning, were examined. Examination, without autopsy, was performed by the scientific assistants who conducted day-to-day operations in the performance of the successive generation study. Frequently, meaningful examinations were prevented by the rapid autolysis of mouse tissue.

The criteria proposed by Oser and Oser (J. Nutrition, 60: 489-505, 1956) were used to evaluate the results of this study.

Results:

General Observations. The author stated that the overall appearance and behavior of test and control animals was good throughout the feeding period. During the first 2 weeks after the start of the test, several 1.5-ppm and control animals, along with a few of the 0.6-ppm group of the F_0 generation became sick, and a control male died. Consequently, all sick-looking animals were replaced, i.e., 4 males and 1 female from the 1.5-ppm group, 3 males and 2 females from the control group, and 1 male and 1 female from the 0.6-ppm group. At various times throughout the study, some animals had soft feces, wet-bottoms, loss of fur, or sores on their bodies. These conditions occurred among test and control animals without any specific distribution as to feeding level. Except for these instances, the overall condition of the animals was good.

Body Weight. According to the author, no statistical evaluation of the data was undertaken. In general, however, mean weight of each group was comparable to that of its controls of the corresponding sex. There also was a relatively close agreement in body weight between each group at the start of each of the successive matings.

Food Intake. Food intake was measured only for the F_0 generation prior to the initial mating. Since animals were housed in pairs, no measure of individual consumption was obtained. Mean food intake and mean dosage of Phorate for each group, calculated for a 3-week period during this time, is summarized below:

Dietary level (ppm)	Males		Females	
	Mean food intake (g/day)	Mean dosage (mg/kg/day)	Mean food intake (g/day)	Mean dosage (mg/kg/day)
0	4.7	-	4.6	-
0.6	5.2	0.12	4.7	0.13
1.5	5.0	0.30	5.2	0.33
3.0	4.8	0.54	4.6	0.64

Food intake was not affected by addition of Phorate to the diets. In fact, the mean intake for each test group was slightly higher than that of control animals of the corresponding sex. Since the ratios between the dosages for each dietary level were essentially the same as the ratios between the dietary concentrations, this provides further evidence that the addition of Phorate to the diets did not affect food consumption.

Reproduction and Lactation.

a. F_0 Generation. In the first mating, the 1.5-ppm group had lowered Viability and Lactation Indices, and the 3.0-ppm group had a lowered Lactation Index. In the second mating, the 0.6-ppm group had a decrease in the Fertility Index. Since all other indices for test F_0 animals equalled or exceeded those of the controls, and since the decreases observed in the first mating did not recur in the second mating, it is concluded that feeding of a diet containing as much as 3.0 ppm of Phorate had no adverse effect on the reproduction and lactation performance of F_0 generation animals.

Mean body weight at weaning of male pups from Phorate-fed animals in the first mating was lower than that of the controls. However, since mean body weight of Phorate-derived females in both matings, and that of males in the second mating exceeded that of pups from control animals, it is concluded that diets containing 3.0 ppm, or less, of Phorate had no adverse effect on body weight at weaning.

The total number of pups weaned by the 1.5-ppm and 3.0-ppm groups in the first mating was less than that of the controls. In the second mating, all test groups weaned more pups than the controls. Therefore, it is concluded that diets containing as much as 3.0 ppm of Phorate had no adverse effect on the number of pups washed.

Among controls, one had a closed eye at weaning. In the 1.5-ppm group, 2 animals had both eyes closed at weaning, while the 3.0-ppm group had one animal with a closed eye and a stillborn pup which was deformed and had only a bud for one forelimb.

b. F_{1b} Generation. Only 14 females are indicated for the second mating of the 3.0-ppm group. Excluded were one female which was found dead the day after having been mated for the second time and another which suffocated after having cast a live litter. Since the pups of this latter animal were destroyed, she has been excluded from the calculations.

The results of both matings show that control animals had Fertility or Gestation Indices of 100% in 3 of 4 instances. Among test animals, the same indices were 100% in 9 of 12 instances. Failure to establish pregnancies in one 0.6-ppm animal and in another from the 3.0-ppm group, and the inability of a control and a 3.0-ppm group female to cast live litters, account for the 4 times that these indices fell below 100. However, since all animals in the 1.5-ppm group conceived and cast live litters, it is concluded that this level was without effect on the fertility of mice. Viability was slightly reduced for 0.6-ppm in both matings and in the 1.5-ppm group for the second mating. Since viability of the 1.5-ppm group exceeded that of the controls in the second mating, and since viability in the 3.0-ppm group in both matings exceeded that of the controls, it is concluded that 3.0-ppm of Phorate did not effect viability. The Lactation Index for the 3.0-ppm group in both matings fell below that of the controls. Therefore, 1.5 ppm again becomes the level at which no adverse effect was noted.

Mean body weight at weaning of Phorate-fed pups exceeded that of the controls except for 3 instances: 1.5-ppm males in the first mating and 0.6-ppm and 1.5-ppm females in the second mating. Since the decreases were slight, and since they did not occur in any particular pattern, it is judged that feeding of Phorate had no adverse effect on body weight of pups at weaning.

In both matings, the number of 3.0-ppm pups weaned was less than that of the controls. Since the number of pups weaned at 1.5-ppm exceeded that of the controls, it is concluded that dietary levels of 1.5-ppm, or less, of Phorate had no adverse effect on the number of pups weaned.

A small number of assorted defects were seen in the 0.6-ppm and 1.5-ppm groups. None were observed in the 3.0-ppm group, while a control pup had a missing eye and another had both eyes closed at weaning.

c. F_{2b} Generation. The author stated thaty a control female died at the start of the first mating while housed with the other female and the male. During the first mating, a 1.5-ppm female was found suffocated, and her pups also were dead, 7 days after she cast her litter. In the second mating, female from the 3.0-ppm dietary level died a few days after giving birth to a live litter. Her pups were destroyed. These 3 females have been excluded from the tabulations.

The data for these 2 matings show more variability that data obtained in earlier matings. The Fertility Index of all test groups in the second mating was below that of the controls. However, there is no suggestion of a dose-response effect. The Gestation Index was not affected by the test diets, since all but one of the established pregnancies resulted in the birth of live pups. The exception occurred in the lowest dietary level during the first mating, and it did not recur in the second mating While the viability of 0.6-ppm pups was decreased in both matings, the immediate post-natal survival of 1.5-ppm and 3.0-ppm pups equalled or exceeded that of the controls. The lactation performance of the 0.6-ppm group also fell below that of the controls. However, females from the 2 higher levels exceeded the controls in this respect during the first mating. Even though the Lactation Indices of the 1.5-ppm and 3.0-ppm groups during the second mating were above 90%, they were less than that of the controls. The amount of the decrease from control values was less than the amount of which lactation performance of these 2 groups exceeded that of the controls during the first mating. Since the failure of one female from the 3.0-ppm group to conceive accounted for the decreased Fertility Index of this group, and since the performance of the 3.0-ppm group otherwise was comparable to or greater than that of the controls, it is concluded that ingestion of diets containing up to 3.0-ppm of Phorate had no adverse effect on reproduction and lactation performance of mice during this final phase of the study.

Mean body weight of 3.0-ppm males was only slightly lower than that of the controls. While 3.0-ppm females also weighed less than their controls, the mean weight of this high level group was greater than that of pups from lower feeding levels. Since there is no suggestion of a dose-response relationship, it is concluded that overall there was no adverse effect on the mean weight at weaning of pups generated in the third cycle of this study.

The total number of pups weaned during these 2 matings was reduced at the 0.6-ppm and 1.5-ppm dietary levels. Controls and 3.0-ppm group animals weaned almost identical numbers of pups. Therefore, it is concluded that a diet containing 3.0-ppm of Phorate had no deleterious effect on the number of pups weaned.

The most common abnormalities noted in these pups was an ophthalmitis which caused swelling and closing of a number of eyes, often unilaterally. This occurred in all groups including the controls. Other spontaneous lesions were also observed, but with no dose effect relationship.

In order to get a comprehensive picture of the performance of the animals during all 6 mating cycles, the data were combined as shown below. These figures are based on the more than 90 matings conducted at each dietary level during this study.

Dietary level (ppm)	Pups per litter Born Alive	Weaned	F.I.	G.I.	V.I.	L.I.
0	10.1	7.8	100	99	87	89
0.6	9.8	6.9	95	99	80	88
1.5	10.2	7.9	96	100	88	89
3.0	10.1	7.5	98	99	90	93

The poorest overall performance, was that of the 0.6-ppm group. The 1.5-ppm and 3.0-ppm groups had an overall reproduction performance which compares very favorably with that of the controls. The only suggestion of an adverse effect would be the slight decrease in the Lactation Index observed for the 3.0-ppm group.

To get an overall assessment of pup weights, the mean weight of each sex from all litters at each dietary level of Phorate was calculated as follows:

Dietary level (ppm)	Mean weaning weight (g) Males	Females
0	8.0	7.7
0.6	8.2	8.0
1.5	7.7	7.9
3.0	8.6	8.4

It is evident that feeding of Phorate had no adverse effect on weight of pups at weaning.

Organ Weights at Autopsy. The weights of livers, gonads and kidneys were determined at autopsy for F_{2b} animals which had been mated to produce the F_3 litters. Mean weights of these organs, as percentages of body weight, were compared by the method of Dunnett (J. Amer. Statist. Assoc., 50: 1096-1121, 1955) using 2-sided comparisons at the 95% probability level.

Dietary level (ppm)	Sex	Relative organ weight		
		Liver	Gonad	Kidney
0	M	5.79	0.69	2.24
0.6	M	5.64	0.75	2.08
1.5	M	5.43	0.61	1.82*
3.0	M	5.48	0.70	1.89
0	F	6.25	0.12	1.65
0.6	F	6.98*	0.11	1.81
1.5	F	6.82	0.11	1.67
3.0	F	6.22	0.093	1.64

*Value differs significantly from control values ($P \leq .05$)

As seen in the above tabulation, mean liver weight of 0.6-ppm females was moderately increased and mean kidney weight of 1.5-ppm and 3.0-ppm males was slightly decreased. The decrease in kidney weight seen to be treatment-related.

Gross Autopsy and Microscopic Findings. Results of autopsies on the F_{2b} generation showed no consistent lesions associated with either male or female reproductive tracts were encountered. The only change which could be treatment-related might be the occurrence of discolored ("clay-colored") livers in 5 of 16 high level females. However, the presence of this subjectively noted change did not appear to impair either the animals' health or reproductive ability. Furthermore, the microscopic examination of the F_{3b} livers revealed nothing singificant.

Autopsy and microscopic examinations of F_{3b} were done on 11, 3, 9, and 10 litters available at weaning from the control, 0.6 ppm, 1.5 ppm, and 3.0 ppm groups respectively. The age of the animals that autopsy varied from 21 to 24 days except in some cases when animals died earlier. According to the author, no consistent abnormalities were seen either grossly or microscopically.

Conclusion:

Lactation indexes in six, and viability indexes in two, out of eighteen possible instances (in each case), are noticeable low for various levels of test compound though not consistently dose-related. This suggests that Phorate has a somewhat deleterious effect on the nursing animals most marked at the 3 ppm level.

Since cholinesterase determination is lacking here, one can not directly correlate reproductive performance with possible effect on enzyme activity.

Five of sixteen female mice of the parental F_{2b} generation showed clay-colored livers at autopsy. However microscopic studies in F_{3b} pups showed no pathological changes in the liver.

Conservatively, a no-observed effect level (NOEL) for reproductive effects is considered to be 1.5 ppm based on the viability and lactation indexes.

Core Classification:

Core minimum data.

P.S. This study was originally reviewed by Dr. M. Quaife 1966. The study is re-evaluated and the review re-written using parts of the registrant's report.

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37