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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Phorate; EPA Reg. No. 241-212.  
Caswell No.: 660.

TO: William Miller, PM# 16  
Registration Division, TS-767

THRU: Christine F. Chaisson, Ph.D. *C.F. Chaisson 6/14/84*  
Review Section IV Head  
Toxicology Branch  
Hazard Evaluation Division, TS-769

FROM: George Z. Ghali, Ph.D. *G. Ghali 6/14/84*  
Toxicology Branch  
Hazard Evaluation Division, TS-769

Registrant: American Cyanamid Co.  
Princeton, N.J.

Action Requested:

Review and evaluation of toxicology studies submitted to the Agency in response to the "Data Call In Notice" of February 5, 1981:

"24-Month Chronic Toxicity and Potential Carcinogenicity Study in Rats" - by P. Boughton et al.; LBI Project No. 20821; EPA Acces. Nos. 248778 and 248779.

"18-Month Chronic Toxicity and Potential Carcinogenicity Study in Mice" - by Allan G. Manus et al.; LBI Project No. 20820; EPA Acces. No. 248780.

"Teratology Study in Rats" - by L.A. Wilson et al. LBI Project No. 20819; EPA Acces. No. 248777.

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Conclusions and Recommendations:

The following studies were evaluated by Dynamac Corporation, and further evaluated by the Toxicology Branch and considered as follows:

a. Twenty-four month chronic toxicity and potential carcinogenicity in rats.

Under the conditions of this study, phorate was not oncogenic to rats fed diets containing 1, 3, and 6 ppm daily for 24 months. Chronic toxicity data revealed that treatment caused the following statistically significant cholinesterase inhibition: Plasma cholinesterase inhibition in males of the high dose group (45 percent) on month 12 and in all treated male groups on month 24 (39-64 percent); plasma cholinesterase inhibition in females of the high dose group on months 3, 6, 12, and 24 (59-77 percent) and in females of the medium dose group on month 12 and 24 (40-49 percent); RBC cholinesterase inhibition in females of the high dose group on month 24 (7 percent); brain cholinesterase inhibition in males of the high dose group (57 percent) and in females of the medium and high dose groups (45 percent). Because the cholinesterase data may be limited, and inhibition was noted at 1 ppm, a NOEL cannot be established and the apparent LEL is considered to be 1 ppm of phorate in the diet.

Core Classification: Core minimum for oncogenicity.  
Core supplementary for chronic toxicity.

b. Eighteen-month chronic toxicity and potential carcinogenicity in mice.

Phorate was non-oncogenic at dietary levels of up to and including 6 ppm when administered in the diet to Swiss albino (CD-1) mice for 18 months. There were no consistent toxic signs or any non-neoplastic pathological findings (gross or microscopic) related to test compound administration. The only effect noted was a slight decrease in weight gain in

females at 6 ppm Phorate during the first 25 weeks of the study. This slight decrease in body weight gain in addition cholinergic signs observed in this group is a good evidence that the maximum tolerated dose was at least approached. Further more, the oral LD<sub>50</sub> in rodents is not appreciably higher than the highest dose tested in this study. Based on the decrease in body weight gain, the LEL is 6 ppm and the NOEL 3 ppm.

Core Classification: Core minimum data.

c. Teratology study in rats.

Phorate was tested in Sprague-Dawley rats at three dose levels consisting of a vehicle control (corn oil), 0.125, 0.25, and 0.50 mg/kg.

The treatment resulted in maternal and embryotoxic changes at dosage levels of 0.50 mg/kg during day 6-15 of gestation in Sprague-Dawley rats. The compound did not produce any frank teratogenic (visceral or skeletal) changes in the fetuses of dams exposed to phorate at levels of 0.5 mg/kg/day or less. The enlargement of the heart, which was present in fetuses of dams exposed to the high dose (0.50mg/kg), is considered to be an embryotoxic change and not a true terata. Based on the observation of maternal toxicity and the incidence of enlarged fetal hearts the LEL in pregnant Sprague-Dawley rats orally administered phorate during organogenesis is 0.50 mg/kg and the NOEL is 0.25 mg/kg.

Core Classification: Guideline data.

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DATA EVALUATION

The following studies were reviewed by Dynamac Corporation.  
The data evaluation records are attached.

"24-Month Chronic Toxicity and Potential Carcinogenicity  
Study in Rats"- by P. Boughton et al.; LBI Project No.  
20821; EPA Acces. #248778 and 248779.

"18-Month Chronic Toxicity and Potential Carcinogenicity  
Study in Mice" - by Allan G. Manus et al.; LBI Project No.  
20820; EPA Acces. No. 248780.

" Teratology Study in Rats" - by L.A. Wilson et al. LBI  
Project No. 20819; EPA Acces. No. 248777.

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DATA EVALUATION RECORDS

(THIMET<sup>R</sup>, PHORATE)

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PHORATE

STUDY TYPE: 18-Month Potential Carcinogenicity Study in Mice.

CITATION: Goldsmith LA, Manus AG, and Craig DK. 1981. 18-month Chronic Toxicity and Potential Carcinogenicity Study in Mice. An unpublished report. (LBI NO. 20820) prepared by Litton Bionetics Inc. for American Cyanamid Co., Princeton, N.J.

ACCESSION NUMBER: 248777

MRID NUMBER: Not available.

LABORATORY: Litton Bionetics, Inc., Kensington, MD.

TEST MATERIAL: . Phorate technical, Thimet<sup>R</sup>. Purity: 91.7 percent minimum.

DATE: January 1981; addendum May 26, 1982.

PROTOCOL:

1. Four hundred 41-day-old Swiss Albino mice (CD-1) from the Charles River Breeding Laboratories were acclimated for 19 days prior to commencement of the study. The animals, 50 males (average weight 24.6 g) and 50 females (average weight 19.39) were randomly selected for each test group. Phorate was given in the diet at concentrations of 0, 1, 3 or 6 ppm for 78 weeks.
2. Test diets were prepared weekly, one week in advance of feeding test animals, to allow for analysis by the sponsor. Acetone was added to the appropriate amount of Phorate and this solution was added to a carrier, "Grit-O-Cobs", and mixed manually. This premix was allowed to stand at room temperature for 20 minutes to allow evaporation of the acetone. The premix was then added to Purina Lab Chow and blended for 20 min in a twin shell blender. Control diet was Purina Lab Chow. Diets were stored frozen and offered at 3 to 4 day intervals. Acidified water (pH 2.5) was offered ad libitum.
3. The animals were housed individually in hanging wire cages in a temperature (74°F) controlled room with 12-hour light/dark cycle.

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4. The animals were observed prior to initiation of the study and at least daily, thereafter. Daily observations included physical appearance, signs of toxicity, and mortality. Weekly detailed observations and palpations were conducted. Individual body weights were obtained prior to initiation, weekly for 13 weeks, every second week until the 25th week, and monthly thereafter.
5. All animals found dead or sacrificed moribund and all remaining animals sacrificed at 18 months were necropsied.
6. The following tissues from all animals that died or were sacrificed were preserved in 10 percent buffered formalin and were examined histopathologically:

Adrenal gland	Jejunum	Seminal vesicle
Aorta	Kidney	Skin
Gallbladder	Liver	Spinal cord
Urinary bladder	Lung	Spleen
Bone and marrow	Lymph node	Stomach
Brain	Mammary gland	Testis
Cecum	Peripheral nerve	Thymus
Ear	Ovary	Thyroid
Esophagus	Pancreas	Tongue
Eye	Parathyroid	Trachea
Heart	Pituitary	Uterus
Intestine, large	Prostate	Skeletal muscle
Duodenum	Salivary gland	Vagina
Ileum		

7. Statistical analysis, comparing dosed and control animals, was performed using the Dunnett's t-test. The basis of significance was a probability value of equal to or less than 0.05.

#### RESULTS:

Diet Analysis and Stability: Weekly feed samples were frozen and shipped to the sponsor, for analysis. With the exception of week 5, when for 3 days animals received 209-298 percent of the stated dose levels, the concentration of compound in the diet was within an acceptable range. The mean concentrations of Phorate in the diet at levels of 1, 3, and 6 ppm were  $0.956 \pm 0.167$ ,  $2.889 \pm 0.864$ , and  $5.750 \pm 1.047$  ppm, respectively.

The material was sufficiently stable in the feed when diets were prepared and used according to the schedule in the study procedures. Over an 8 day period, concentrations in diet at 4 levels between 2 and 8 ppm were  $83 \pm 3.3$  percent of the nominal concentration or 95.4 percent of the analyzed day-1 level.

General Observations: Observations were recorded weekly throughout the study; tissue masses were recorded and their sizes approximated. Cholinergic signs which cleared within 30 seconds (hyperactivity, tremors or muscle spasms, excessive salivation, occasional ataxia or convulsions)



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were prevalent, particularly between weeks 38-48, but occurred in controls as well as in dosed animals. There was no dose related trend and this reviewer concludes that these effects were not compound related. A low incidence of hair loss (1-2 animals/group/week) was also observed to occur randomly in control and test groups.

Mortality: There were no compound related mortalities at any dose level. Mortality was higher in females than in males; 66 to 74 percent of females in all groups survived 18 months and 78 to 90 percent of males in all groups survived 18 months. The distribution of mortality was as follows:

Table 1. Summary Mortality Data

Dose group (ppm)	Sex	Found dead or Moribund sacrificed		Accidental Deaths	No. for Final Sacrifice	Percent Survival
		Months 0 - 12	Months 12 - 18			
0	M	1	3	1	45	90
1	M	0	5	2	43	86
3	M	4	6	1	39	78
6	M	1	7	1	41	82
-----						
0	F	4	9	0	34	68
1	F	3	8	2	37	74
3	F	4	12	0(1)*	33	68
6	F	3	12	0(1)*	34	69

\*Mouse missing - escaped.

Body Weight and Food Consumption: There was a slight decrease ( $1.15 \pm 0.4$  g) in mean body weights of high dose females when compared to controls during the first 25 weeks of the study (Table 2). The mean weights were statistically lower in 12 of 19 measurements during the first 25 weeks of the study, but statistically lower only in 1 of 13 measurements from weeks 29 to 77. Although there were statistically significant variations in food consumption, there was no consistent positive or negative correlation with compound administration. In the first 25 weeks of the study, however there was a slight decrease in mean daily food consumption in 6 ppm females ( $4.96 \pm 0.55$  g) compared to controls ( $5.5 \pm 0.56$ g), which correlated with decreased weight gain.

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Table 2. Mean Body Weight Data in Grams

Dose Level (ppm)	WEEK							Percent Weight Gain	Weeks Statistically Significant
	Initial	6	13	25	37	53	65		
0	19.7	27.3	29.5	31.3	31.9	32.7	33.8	33.4	169.5
1	19.1	26.8	28.8	30.3	31.6	32.7	33.9	33.4	174.9
3	19.4	27.2	29.0	30.8	32.5	32.9	34.2	33.5	172.7
6	19.1	26.2*	28.3	29.7*	31.7	31.9	32.3	32.9	172.3

FEMALES

\* Statistically significant at p = 0.05.

Gross Necropsy: There were no consistent gross lesions that were considered to be associated with compound administration. This reviewer checked data for all animals in the control and high dose male groups and determined that all recorded gross lesions had corresponding histopathologic evaluation unless the tissue was too severely autolyzed to evaluate. 003856

Histopathology:

Neoplastic: A summary of neoplastic lesions by organ, compiled by this reviewer, is presented in Table 3. With a couple of minor exceptions, this corresponds with summary data in the final report. Malignant lymphomas involving multiple organs and adenomas of the lungs were relatively common findings in both control and treated mice. Tumors of the liver were relatively common in both control and treated males. There does not appear to be a dose-related increase in any tumor.

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Table 3. Number of Animals with Tumors by Organ<sup>a</sup>

	Males				Females			
	0	1	3 ppm	6	0	1	3 ppm	6
Lung - Adenoma <sup>b</sup>	3	3	6	8	6	2	3	4
- Carcinoma	-	-	-	-	-	-	-	1
Hematopoietic - malignant lymphoma <sup>c</sup>	5(1)	1(1)	1(1)	-	9(8)	7(4)	5(2)	7(6)
- leukemia	1	-	-	-	-	4	1	-
Spleen - hemangiosarcoma	-	1	-	1	-	-	1	1
Lymph node - neoplasm NOS.	-	-	-	-	-	-	-	1
Liver - adenoma	3	2	2	4	-	-	-	-
- carcinoma	6	3	2	3	-	-	-	-
- hemangioma	1	-	-	-	-	-	-	-
- hemangiosarcoma	-	-	-	2	-	-	-	-
Jejunum - adenomatous polyp	-	-	-	-	-	-	1	-
Pituitary - adenoma	-	-	-	-	1	-	-	-
Adrenal - adenoma	1	-	-	-	-	-	-	1
- pheochromocytoma	-	-	-	1	-	-	-	-
Uterus - adenocarcinoma	-	-	-	-	-	-	1	-
- benign tumors	-	-	-	-	2	1	6	3
Ovaries - benign tumors	-	-	-	-	1	1	2	1
Kidneys - adenoma	-	-	-	1	-	-	-	-
Ear - mast cell tumor	-	1	-	-	-	-	-	-
Subcutaneous - leiomyosarcoma	-	-	-	-	-	1	-	-
Sternum - hemangioma	-	-	-	1	-	-	-	-
Abdominal - hemangiosarcoma	-	-	-	-	-	-	1	-
Tail - carcinoma	-	-	-	-	1	-	-	-
- sarcoma	-	-	1	-	-	-	1	-

<sup>a</sup> Tissues from 50 animals of each group were examined histopathologically, with the exception that tissues were examined from only 49 females of the 3 and 6 ppm groups.

<sup>b</sup> The average time to tumor was 73, 80, 75.5 and 78 weeks in males at 0, 1, 3, and 6 ppm respectively; 78.5, 77.5, 71.5 and 76.5 weeks for females at 0, 1, 3, and 6 ppm, respectively.

<sup>c</sup> The numbers in parenthesis are the number of animals with lymphomas at multiple sites.

Summary compilation of numbers of malignant and benign tumors and number of animals with tumors is presented in Table 4. Some minor discrepancies were found in the summary table of the final report. There was no apparent effect of dosing on the number of tumors in any organ, the total number of benign or malignant tumors, or the number of animals with tumors. 003856

Table 4. Summary of Malignant and Benign Tumors

Dose (ppm)	Males				Females			
	0	ppm			0	ppm		
	1	3	6		1	3	6	
Number of benign tumors	6	8	9	16	10	3	15	12
Animals with benign tumors	6	8	9	13	8	3	14	10
Number of malignant tumors <sup>a</sup>	11	4	4	4	10	11	7	8
Animals with malignant tumors	11	4	4	4	9	11	6	8
Total tumors	17	12	13	20	20	14	22	19
Number of animals with tumors	15	12	13	17	17	13	18	17
Animals with tumors at 2 sites	1	0	0	2	2	2	2	1
Animals with tumors at 3 sites	0	0	0	0	1	0	0	0
Animals with two different types of tumors in one organ	1	0	0	0	0	0	0	1
Animals necropsied	50	50	50	50	50	50	49	49

<sup>a</sup>There were no metastases of any tumors. Malignant lymphomas at multiple sites are tallied as one tumor.

Non-neoplastic: There were relatively high incidences of several non-tumor histopathologic findings, but there was no obvious differences in incidence or severity of lesions between control and dosed animals. Amyloidosis of multiple organs, particularly kidney, ileum, and adrenal, was a common finding. Lymphocytic inflammatory infiltration of the kidneys, liver, salivary glands, lungs, and bladder was seen at high incidence in both control and treated animals. Follicular and paraovarian cysts occurred in the ovaries of many control and dosed females. Myofibrosis of the sternum, consisting of aggregation of spindle shaped cells in the marrow cavity was relatively common in all groups of females but not found in males. Vacuolar lesions of the lens of the eye, presumed to be early stages of cataract formation, were found in many mice of all groups; although the incidence was higher in treated groups than control groups, there did not appear to be a dose-response relationship.

## DISCUSSION:

The study was adequately conducted according to the protocol indicated in the procedures section of the final report. Sufficient numbers of mice survived 18 months to evaluate oncogenicity. There was no increase in tumors in dosed animals compared to controls; however, there was no statistical analysis of histopathologic data. Tissues examined histopathologically were as specified in the protocol with the exception of a few tissues that were missing. Some tissues were too severely autolyzed to evaluate histologically, in particular eyes and intestines of several animals that died or were moribund sacrificed; but in the opinion of this reviewer, this did not compromise the histopathologic evaluation. Minor errors in summary tabulation of tumors in the final report were noted by this reviewer; however these do not alter the conclusions of the report. This reviewer agrees with the conclusion of the pathology report that "all histopathologic findings were considered incidental or part of the spontaneous disease process of mice ... [and] there was no obvious increase in the incidence of these lesions in dosed versus control mice." The study is less than Core Guideline for oncogenicity since there were no hematologic studies and no organ weights were recorded at necropsy; summary gross necropsy findings were not presented in the final report although gross observations were entered on individual animal pathology records.

The final report did not state the rationale for dose selection. Evidence that the highest dose tested was at or near the MTD was a slight decrease in mean weight gain in females in the first 25 weeks of the study at 6 ppm Phorate. This decrease was significant in 11/13 weighings; the average decrease in weight compared to controls was  $1.15 \pm 0.4$  g. The high-dose females may have adapted since weight gain was normal throughout the remainder of the study. More convincing evidence that the MTD was used would have been a decrease in plasma cholinesterase activity, however cholinesterase determinations were not required by the protocol.

There were no consistent clinical signs that were compound related. However, there were cholinergic signs in all groups of mice, particularly notable between weeks 38-48. For example at week 38, 6 control males and 1, 2, and 9 males at 1, 3, and 6 ppm Phorate had transient cholinergic signs. The fact that these signs were present at all in controls might suggest that the control diets were contaminated with Phorate, a demonstrated cholinesterase inhibitor. However, there were no measurements of blood cholinesterase activity to support or reject the supposition of contaminated diets.

## CONCLUSIONS:

Phorate was non-oncogenic at levels of up to 6 ppm when administered orally via the diet to Swiss albino (CD-1) mice in a 18-month study. There were no consistent toxic signs or any non-neoplastic pathologic

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findings (gross or microscopic) related to test compound administration. The only effect noted was a slight decrease in weight gain in females at 6 ppm Phcrate during the first 25 weeks of the study. Based on this finding the LEL is 5 ppm and the NOEL 3 ppm.

CORE CLASSIFICATION: Core minimum data.

PHORATE  
(Thimet)

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STUDY TYPE: Twenty-Four Month Chronic Toxicity and Carcinogenicity Study  
in Rats.

CITATION: Goldsmith LA, Manus AG, Craig DK. 1981. Twenty-Four Month  
Chronic Toxicity and Potential Carcinogenicity Study in Rats. Unpublished  
report.

ACCESSION NUMBER: 248778 & 248779

MRID NUMBER:

SPONSOR: American Cyanamid Company, Princeton, NJ.

LABORATORY: Litton Bionetics, Inc., Kensington, MD.

TEST MATERIAL: Phorate (Thimet), Technical (91.5 percent minimum purity).

PROTOCOL:

1. Phorate (Thimet), Technical (91.5 percent minimum purity), Lot  
W-70513-4844-2, was used.
2. Groups of 50 male and 50 female Charles River rats [CRL: COBS CD  
(SD) BR] with an average weight of 135 and 121 g respectively,  
received diets containing 0, 1, 3, and 6 ppm of test material for a  
period of 2 years. The following parameters were investigated:

<u>Test Parameter</u>	<u>When Performed</u>
1. Clinical observations (mortality, morbidity)	Daily
2. Detailed observations, and palpation	Weekly
3. Animal body weight determina- tions	Weekly for 12 weeks, biweekly for weeks 12-26, and monthly thereafter
4. Animal food consumption determinations	Weekly for 12 weeks, biweekly for weeks 12-26, and monthly thereafter



5. Cholinesterase Inhibition <sup>a</sup>	Prior to treatment and on months
Plasma and RBC	3, 6, 12, and 24 of the study
Brain	Month 24
6. Hematology <sup>a,b</sup>	Months 6, 12, and 24 of the study
7. Urinalysis <sup>a,c</sup>	Months 6, 12, and 24 of the study
8. Clinical chemistry <sup>a,d</sup>	Months 6, 12, and 24 of the study
9. Terminal Sacrifice	Month 24 of the study
10. Gross necropsy <sup>e</sup>	Month 24 of the study

<sup>a</sup> Determined in 5 male and 5 female animals per group. The same animals were used to determine a specific parameter throughout the study, except if a selected animal died.

<sup>b</sup> Parameters measured included hematocrit, hemoglobin, erythrocyte count total and differential leukocyte counts and platelet count. The animals were fasted overnight prior to bleeding. However, data for the differential leukocyte and platelet counts were not presented in the results section.

<sup>c</sup> Parameters measured included: appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, and microscopic examination for sediments.

<sup>d</sup> Blood chemistry parameters included: serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, glucose, blood urea nitrogen, gamma-glutamyl transpeptidase. Analysis was conducted by means of an SMA-18 automated analyzer.

<sup>e</sup> All animals that died or were sacrificed were subjected to a complete necropsy. It was stated in the final report that "a specimen of the tissues listed below was preserved in 10 percent buffered formalin for possible future histopathological evaluation."

Adrenal glands	Aorta	Bone	Bone marrow
Brain	Ileum	Duodenum	Esophagus
Eyes/optic nerve	Lymph node, Me.	Jejunum	Kidney
Lymph node, Ma.	Pancreas	Pituitary	Mammary gland
Nerve, sciatic	Skeletal muscle	Skin	Prostate
Salivary glands	Stomach	Thyroid gland	Spinal cord
Spleen	Harderian gland	Cecum	Trachea
Urinary bladder	Testes	Liver	Heart
Thymus	Parathyroid	Vagina	Ear
Tongue	Ovary		Seminal vesicle
Uterus			

11. Organ weights<sup>f</sup>

Month 24 of the study

12. Histopathology<sup>g</sup>

Month 24 of the study

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<sup>f</sup> At necropsy, the following organ weights from 10 animals/group were recorded: liver, kidneys, heart, testes, brain, pituitary, spleen, ovaries, adrenals.

<sup>g</sup> It was stated in the pathology report that complete histopathologic examinations were conducted on all animals, but the tissues examined were not listed.

Body weight and food consumption data were analyzed statistically by Dunnett's t-test, and cholinesterase levels, clinical chemistry, and hematology data were analyzed by the Wilcoxin Rank Sum test.

## RESULTS:

### CLINICAL OBSERVATIONS

No apparent compound-related clinical signs were observed, except for tremors mainly in females following over-dosing during week 9 of the study. The final disposition of individual animals is listed in Table 1. Approximately 50 percent of the animals in each group, including the controls, survived to the end of the study, except for the females receiving the high dose (6 ppm), where 18/50 (36 percent) survived.

### BODY WEIGHTS

Normal body weight gains were observed in all male and female groups except for the female group receiving the highest dose, which exhibited a statistically significant reduction (7-24 percent) in body weights compared to the control group ( $<0.05$ ), during the periods of week 1-26 and 74-102 of the study. In addition, a gradual decrease in mean body weights (about 18 percent) of all male groups was noted during weeks 74-102 of the study.

### FOOD CONSUMPTION AND TEST MATERIAL INTAKE

There was no significant difference in food consumption between the treated and control groups of both sexes, except for the female group receiving 6 ppm of test material. Animals in this group consumed more food than the control animals during weeks 10-16, 30-42, and 70-86 of the study.

Table 1. Final Disposition of Rats Treated with Phorate

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Group/Sex	Found Dead	Moribound Sacrifice	Terminal Sacrifice
<b>MALE</b>			
Control <sup>a</sup>	14	7	29
1 ppm	15	8	27
3 ppm	16	7	27
6 ppm	20	6	24
-----			
<b>FEMALE</b>			
Control	9	13	28
1 ppm	14	12	24
3 ppm <sup>a</sup>	8	7	34
6 ppm	13	19	18

<sup>a</sup> In addition one animal died accidentally.

## HEMATOLOGY

There were no apparent treatment-related effects although some statistically significant differences were noted between the control animals and those receiving the high dose on month 12 of the study. These differences indicated significantly lower WBC in males and significantly lower RBC, hemoglobin and hematocrit in females.

However, since hematologic parameters were determined from only 5 animals per group and there was in general, a wide variability in results obtained for both individual and group data, the significance of these data may be limited. It was also stated in the final report that "platelets were evaluated at 6, 12, and 24 months with no instances of inadequate platelets reported" but no data were provided. Differential leukocyte counts were not determined.

## CLINICAL CHEMISTRY

There were no statistically significant or dose-related differences in the parameters tested. However, the significance of these data may be limited since a few parameters were determined (see Protocol) from only 5 animals per group and there was in general a wide variability between individual as well as group data.

## URINALYSIS

There were no apparent treatment related changes in any of the parameters determined. High protein levels in urine were detected in some groups during the study, but these were considered to be due to the presence of high bacterial levels in the urine samples. However, the significance of these data may be limited since the parameters were determined in only 5 animals per group.

## CHOLINESTERASE INHIBITION

Cholinesterase activity was determined in specimens from only 5 animals per group, however, the following treatment-related effects were noted. Plasma cholinesterase activity in males and females increased with age as indicated by a gradual increase during the course of the study. (Table 2). However, treatment-related inhibition of cholinesterase activity in males, compared to concurrent controls, was noted on months 12 (45 percent inhibition) in animals receiving the high dose, and on month 24 in all treated groups (39, 49, and 64 percent inhibition at the low, medium, and high doses, respectively). Similarly inhibition in treated females was noted on months 3, 6, 12, and 24 in animals receiving the high dose (59-77 percent inhibition) and on months 12 and 24 (40-49 percent inhibition) in animals receiving 3 ppm, whereas no inhibition was observed at the lowest dose level of 1 ppm (Table 2).

Table 2. Plasma, Erythrocyte, and Brain Cholinesterase Activity (mU/ml) in Rats Treated With Phorate

Group/Sex	Day -1	Plasma				RBC	Brain <sup>b</sup>
		Month 3	Month 6	Month 12	Month 24		
<b>Male</b>							
Control	427	470	490	730	1637	1205	2528
1 ppm	436	424	569	647	999 <sup>a</sup>	1365	1790
3 ppm	497	533	672	608	838 <sup>a</sup>	1402	2597
6 ppm	433	414	463	403 <sup>a</sup>	595 <sup>a</sup>	1033	1603 <sup>a</sup>
<b>Female</b>							
Control	546	1417	1933	2033	1892	1193	2995
1 ppm	556	1433	2009	2023	1786	1390	2726
3 ppm	527	1083	1205	1037 <sup>a</sup>	1137 <sup>a</sup>	1181	1649 <sup>a</sup>
6 ppm	532	585 <sup>a</sup>	631 <sup>a</sup>	466 <sup>a</sup>	725 <sup>a</sup>	1107 <sup>a</sup>	1498 <sup>a</sup>

<sup>a</sup> p < 0.05 compared to concurrent controls.

<sup>b</sup> Units not specified.

There were no significant differences in RBC cholinesterase activity in treated males and females as compared to controls except for female animals receiving high dose on month 24 (Table 2). The results also indicated a possible increase in activity in some groups on months 3 and 6 of the study, followed by a statistically significant decrease in activity in all male (53 percent) and female (52 percent) groups on month 24 as compared to the pretest activity.

Brain cholinesterase activity on month 24 (sacrifice) indicated a treatment related decrease in activity in males receiving 6 ppm (37 percent) and females receiving 3 (35 percent) and 6 ppm (41 percent) of test material (Table 2).

#### GROSS NECROPSY

There was no treatment-related increase in the number of masses or tumors in both sexes. Isolated gross lesions were noted in several organs, however, no consistent patterns were observed that were considered to be treatment-related.

#### ORGAN WEIGHTS

No statistically significant differences in organ weights were noted between control and treated animals. Organ weights expressed as percent of body weight for the females receiving the high dose indicated an increase for adrenals, brain, heart, liver, and spleen ratios when compared to control animals. However, this increase correlates with the decrease in the body weight of these animals. The findings that organ to brain weight ratios did not increase in treated animals corroborates this conclusion.

#### HISTOPATHOLOGY

Neoplastic lesions included mammary fibroadenomas and adenomas which are common in female rats together with a few mammary adenocarcinomas ranging from 0-2 in male and female groups. Pituitary adenomas were relatively common in all groups, ranging from 15-19 in male and 26-39 in female groups. None of these lesions were dose-dependent.

Several non-neoplastic lesions were also noted such as degeneration of kidney or nephropathy, cellular alteration and hemorrhagic cysts in the adrenal glands; bile ductule hyperplasia and cholangiofibrosis of the liver; epidermal inclusion cysts; tubules and nests of cells in the thymus; polyangitis most commonly in the testes and in mesenteric vessels; and suppurative or chronic inflammation of the prostate. However, none of the above mentioned lesions was treatment related and all were considered to be incidental or part of spontaneous disease complexes of rats. The only lesion that may have been treatment related, was chronic inflammation and epithelial hyperplasia in the forestomach

(Table 3). However, the pathologist concluded that these lesions were relatively common incidental findings and considered to be due to random variation or at most irritation of the forestomach. Chi-square analysis of the data by this reviewer based on the number of animals with lesion(s) were not significant.

#### DISCUSSION:

The oncogenic potential and chronic effects of phorate to male and female Charles River rats fed diets containing 0, 1, 3, or 6 ppm of test material for a period of 24-months, was studied. The survival rate at the end of the study was approximately 50 percent in all groups (including the controls), except for females receiving the highest dose, where only 18/50 (36 percent) animals survived. Normal body weight gains and food consumption were observed in all male and female groups except for the females receiving the highest dose. These females exhibited a treatment-related reduction in body weight compared to the controls ( $<0.05$ ) during weeks 1-26 and 74-102. No explanation is given for the decrease in the body weight of all the male animals, including controls, during the last 6 months of the study. Limited hematology, blood chemistry, and urine analyses conducted with only 5 animals per group instead of the required 8-10 animals, on months 6, 12, and 24 of the study did not reveal any treatment-related changes. However, differential leukocyte counts were apparently not performed and results of platelet counts were not presented in the final report. The blood chemistry parameters determined, included only blood urea nitrogen, glucose, gamma glutamyl transpeptidase, serum glutamic-oxaloacetic transaminase, and serum glutamic pyruvic transaminase. Consequently, possible effects, may have been missed, and the statistical and biological significance of these findings may be limited.

Plasma cholinesterase inhibition was noted on month 12 in males of the highest dose group (45 percent) and in males of all treated groups on month 24 (39-64 percent). Females were more susceptible with the high dose group exhibiting plasma cholinesterase inhibition on months 3, 6, 12, and 24 (59-77 percent), and the medium dose group exhibiting inhibition on months 12 and 24 (40-49 percent) of the study. Inhibition was not detected in females receiving the low dose. Statistically significant brain cholinesterase inhibition (about 37 percent) was noted in males receiving the high dose and females receiving the medium and high doses (35-40 percent). No significant differences in erythrocyte cholinesterase activity were noted in treated males and females as compared to controls, except in the high dose females on month 24. Cholinesterase activity was also determined in only 5 animals and the biological significance of these data may be limited. However, phorate is a known cholinesterase inhibitor and the results indicated statistically significant inhibition ( $<0.05$ ) in plasma, RBC, and brain cholinesterase activity which was dose-dependent and/or time-related. These results and pharmacotoxic signs observed mainly in females on week 9 as a result of accidental overdosing indicate that the females were more susceptible than the males; yet plasma cholinesterase inhibition was

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Table 3. Number of Animals with Non-tumor Lesions in the Forestomach

Group/Sex	Number of Animals <sup>a</sup>	Lesion		
		Inflammation	Hyperplasia	Other
<b>MALES</b>				
Control	7/50	4	6	1
1 ppm	6/48	3	4	2
3 ppm	10/49	4	9	1
6 ppm	14/50	10	13	2
-----				
<b>FEMALES</b>				
Control	5/46	0	5	2
1 ppm	6/49	6	5	0
3 ppm	2/49	0	1	1
6 ppm	11/48	3	9	5

<sup>a</sup>Total number of individual animals with single or multiple lesions/total animals examined.



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noted in all 3 dose levels in treated male groups on month 24, and only the medium and high doses in treated female groups. Consequently, a NOEL cannot be established, and the apparent LEL is 1 ppm of phorate in the diet.

Necropsy and organ weight determination did not reveal any treatment-related findings. Histologic findings of chronic inflammation and epithelial hyperplasia in the forestomach of both male and female animals receiving the high dose suggests that these findings may have been treatment-related, but data were not statistically significant.

It is concluded that the study was adequately conducted, except for the deficiencies noted for hematology, blood chemistry and cholinesterase inhibition. In addition, The rationale for selecting the dose levels for this study was apparently based on a 14-day pilot study, although a 90-day study may have provided more useful information. Consequently, the chronic toxicity testing phase of this study is classified as core supplementary, and the oncogenic testing phase is classified as core minimum.

#### CONCLUSIONS:

Under the experimental conditions of this study, phorate was not oncogenic to rats fed diets containing 1, 3, and 6 ppm daily for 24 months. Chronic toxicity data revealed that treatment caused the following statistically significant cholinesterase inhibition: Plasma cholinesterase inhibition in males of the high dose group (45 percent) on month 12 and in all treated male groups on month 24 (39-64 percent); plasma cholinesterase inhibition in females of the high dose group on months 3, 6, 12, and 24 (59-77 percent) and in females of the medium dose group on month 12 and 24 (40-49 percent); RBC cholinesterase inhibition in females of the high dose group on month 24 (7 percent); brain cholinesterase inhibition in males of the high dose group (57 percent) and in females of the medium and high dose groups (45-50 percent). Because the cholinesterase data maybe limited, and inhibition was noted at 1 ppm a NOEL cannot be established and the apparent LEL is 1 ppm of phorate in the diet.

CORE CLASSIFICATION: Core minimum for oncogenicity testing. Core supplementary for chronic toxicity testing.

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THIMET<sup>R</sup>, PHORATE

Study Type: Teratology Study in Rats.

Accession Number: 248777

MRID Number: Not given.

Sponsor: American Cyanamid Company, P.O. Box 400, Princeton, New Jersey 08540.

Contracting Laboratory: Litton Bionetics, Inc. 5516 Nicholson Lane, Kensington, Maryland 20795.

Date: May 1978 (revised March 1979).

Test Material: Phorate, technical. Purity was 91.6 percent. Lot No. W-70513-4844.

PROTOCOL:

1. Ten gallons of the test substance, phorate, technical were received from American Cyanamid on August 19, 1977. The sample was 91.6 percent phorate and was Lot Number W-70513-4844.
2. The test animals were CRL:COBS CD(SD)BR rats. The rats were received from Charles River Breeding Laboratories, Inc., Portage, Michigan. An unspecified number of sexually mature male and female rats were bred to yield the 100 inseminated female rats utilized in the study. There were four test groups consisting of 25 female rats each. The female rats were 11 weeks of age at the initial dosing and averaged 236 g at day 0 of gestation.
3. The test substance was administered daily from day 6 to day 15 of gestation by oral intubation. Each animal received a dose volume of 8.33 ml/kg with the dose volume based on the day 6 of gestation body weight. The phorate dosages used were 0.125, 0.25, and 0.50 mg/kg. A concurrent control group received the vehicle, corn oil.
4. The female rats were observed daily for general appearance, behavior, and condition. Body weights were obtained on days 0, 6, 15, and 20 of gestation. Food consumption was measured during periods 0-6, 6-15, and 15-20 of gestation. On day 20 of gestation, the females were sacrificed with chloroform and the visceral and thoracic organs examined. The gravid uterus was removed, opened, and examined for the number and distribution of total implantation sites, resorptions

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sites, live and dead fetuses. The fetuses were individually weighed and examined externally. One third of the fetuses in each litter were fixed in Bouin's solution and examined for visceral abnormalities. The remaining fetuses in each litter were prepared and stained with Alizarin Red and examined for skeletal abnormalities.

5. The litter was used as the basic sampling unit for statistical analysis and the level of significance was established at the 5 percent probability level. Maternal body weights, food consumption, and mean litter fetal body weights were analyzed using Dunnett's t-test. All ratios were analyzed with a 2x2 contingency table with Yate's correction. The number of resorptions, dead fetuses, and abnormal fetuses per litter were analyzed utilizing Wilcoxon's Rank Sum.

## RESULTS

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## CLINICAL OBSERVATIONS

Oral administration of phorate produced a significant increase ( $p < 0.05$ ) in the number of mortalities among the high dose females. The number of deaths per group is presented below:

	Dose (mg/kg)			
	0	0.125	0.25	0.50
Deaths (n=25)	0	1	0	7

One high-dose female displayed convulsions, a swollen tongue, and decreased body temperature prior to death on day 11 of gestation. These appear to be the only treatment-related observations. Other clinical observations noted through gestation appeared at comparable incidence rates in each of the treatment groups. These clinical observations included urine stains, red stains around the eyes and/or nose, rales, inguinal swelling, axillary swelling, and soft stool. These observations are not uncommon in Sprague-Dawley rats and were not considered treatment related.

## MATERNAL BODY WEIGHTS AND FOOD CONSUMPTION

Mean maternal body weights and food consumption are presented in Table 1. The mean body weights and food consumption of all phorate groups were comparable to the vehicle controls at all observation periods.

## REPRODUCTION INDICES

An examination of the following parameters indicated no treatment-related effects: a) fertility index, b) live litters, c) number of implantation sites, d) number of resorptions, e) number of dead fetuses, f) number of live fetuses, g) live fetus/implantation site ratio, and h) mean fetal body weight (see Table 1). No evidence of a compound-related effect on reproduction was observed.

## FETAL EXAMINATIONS

External examination of the fetuses indicated a comparable distribution of subcutaneous hematomas between the treatment groups. All fetuses in control litter 4650 and high-dose litter 4721 were below 2 grams. Twin

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Table 1. Summary of Body Weight, Food Consumption, and Reproductive Data

	Dose (mg/kg)			
	<u>0</u>	<u>0.125</u>	<u>0.25</u>	<u>0.50</u>
Mean day 0 gestation body weight (g)	235	241	234	233
Mean day 6 gestation body weight (g)	261	269	262	261
Mean day 15 gestation body weight (g)	284	299	294	290
Mean day 20 gestation body weight (g)	342	366	362	354
Day 0-6 gestation mean daily food consumption (g)	21	21	21	21
Day 6-15 gestation mean daily food consumption (g)	15	16	16	17
Day 15-20 gestation mean daily food consumption (g)	23	21	25	26
Mean implantation sites/litter	13.6	13.3	13.4	13.1
Mean resorptions/litter	1.3	1.3	0.8	1.3
Mean live fetuses/litter	12.8	12.5	12.6	11.8
Ratio of live fetuses/implantation sites	0.90	0.90	0.94	0.90
Mean fetal weight (g)	4.0	4.1	3.9	3.9

cojoined fetuses were observed in low-dose litter 4669. The fetuses were connected ventrally from the area of the umbilicus to the right lateral surface of the levorotated heads. Unilateral microphthalmia was observed on one head while bilateral anophthalmia, micrognathia, and misplaced ear buds were observed on the other head.

Visceral examination of the fetuses detected a number of fetuses with discolored livers. The incidence of this observation was not dose related but appeared to correlate to the weight of the individual fetuses. The discolored livers appeared generally in large fetuses and may have been indicative of imperfect fixation. A significant increase ( $p < 0.05$ ) in the number of high-dose fetuses with enlarged hearts was observed. The incidence of enlarged hearts is as follows:

	Dose (mg/kg)			
	<u>0</u>	<u>0.125</u>	<u>0.25</u>	<u>0.50</u>
Number of fetuses with enlarged hearts	0/115	2/116	1/123	7/86
Number of litters with affected fetuses	0/23	1/23	1/23	3/18

No other visceral malformations were reported.

The number of fetuses and litters with unusual skeletal variations were comparable between the groups and is presented below:

	Dose (mg/kg)			
	<u>0</u>	<u>0.125</u>	<u>0.25</u>	<u>0.50</u>
Fetuses with unusual skeletal variations	15	12	6	12
Fetuses examined	179	172	179	126
Litters with affected fetuses	5	4	5	5
Litters examined	23	23	23	18

With the exceptions of the two litters (control litter 4650 and high-dose litter 4721) that contained abnormally small fetuses and the cojoined fetuses in low-dose litter 4669, the skeletal variations reported consisted of delays in ossification, the presence of a 14th rib(s), and

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non-fused vertebral centra. Litters 4650 and 4721 contained fetuses with major retardation of bone ossification but no indication of misshapen or missing bones. The cojoined fetuses in litter 4669 had major and extensive malformations of the skeleton. These malformations consisted of malpositioned skull bones, ribs, and clavicles. Other bones including the pubes and mandibles showed no evidence of ossification.

#### NECROPSY

No remarkable or treatment related macroscopic lesions were detected at necropsy.

#### DISCUSSIONS:

Two deficiencies were noted in the conduct of this study. Neither of the deficiencies are considered serious enough to adversely affect the conduct of the study. The first deficiency was the failure to record the number and distribution of corpora lutea. This parameter is important in determining if a unusually high or low number of implantation sites in a treatment group is related to the test article or biological variation due to an unusually high or low number of ovum released (corpora lutea). The second deficiency was the administration of the dosage solutions based only on the day 6 of gestation body weight. Sprague-Dawley rats normally produce a 10 percent increase in body weight between days 6 and 15 of gestation. A readjustment of the dose volumes administered during the period of dosing would have reflected the gains in maternal body weight.

Treatment with phorate produced maternal deaths in the high dose animals. No indications of maternal toxicity were observed at 0.125 and 0.25 mg/kg.

No treatment-related effects on maternal body weight, food consumption, or reproduction capabilities of the dams were observed in any of the test animals.

Exposure to phorate during organogenesis produced an increase in the incidence of enlarged hearts in the high-dose fetuses. The report concluded that this effect was not a true teratogenic effect, but rather a physiologic effect resulting from the anticholinesterase activity of phorate. The report concluded that the increased acetylcholine that would be present produced excessive stimulation of the myocardium with ensuing enlargement. This reviewer concurs with this conclusion. No treatment related skeletal effects were observed. The occurrence of one pair of cojoined fetuses at 0.125 mg/kg, and of one litter of abnormally small fetuses in each of the control and high dose groups, is not believed to be treatment related. Based on the data reported, phorate does not appear to be teratogenic, but does appear to be indirectly embryotoxic as it affects myocardial development. No teratogenic events were seen in this study at the highest dose level tested, 0.50 mg/kg; however, embryotoxicity was observed at 0.50 mg/kg.

No evidence of any phorate produced adverse macroscopic lesions were observed in this study.

CONCLUSIONS:

A teratology study with phorate was conducted in Sprague-Dawley rats. Three dose levels consisting of a vehicle control (corn oil), 0.125, 0.25, and 0.50 mg/kg were studied. Each dose group initially consisted of a minimum of 23 pregnant rats. The females were orally dosed daily from day 6 to day 15 of gestation and sacrificed on day 20 of gestation. The uterus was excised and the contents examined. The fetuses were weighed and examined for external, visceral, and skeletal abnormalities.

The test compound, phorate, elicits maternal and embryotoxic changes at exposures of 0.50 mg/kg during day 6-15 of gestation in Sprague-Dawley rats. The compound does not produce any frank teratogenic (visceral or skeletal) changes in the fetuses exposed to phorate at levels of 0.50 mg/kg/day or less. The enlargement of the heart, which was present in fetuses of dams exposed to the high dose (0.50 mg/kg), is considered to be an embryotoxic change and not a true terata. Based on the observation of maternal toxicity and the incidence of enlarged fetal hearts the LEL in pregnant Sprague-Dawley rats orally administered phorate during organogenesis is 0.50 mg/kg and the NOEL is 0.25 mg/kg.

CORE CLASSIFICATION: Guideline data.