ATOCHEM
NORTH AMERICA
elf aquitaine

Contains No CBI

ATOCHEM NORTH AMERICA, INC.
900 First Avenue, P.O. Box 1536, King of Prussia, Pennsylvania 19406-0018, (215) 337-6500

October 22, 1992

(A)

8EHQ-92-12588

88920010772

CERTIFIED MAIL

RETURN RECEIPT REQUESTED

Document Processing Center (TS-790)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M St., S.W.
Washington, D.C. 20460

Attn: Section 8(e) Coordinator (CAP Agreement)

RE: Report Submitted Pursuant to the TSCA Section 8(e) Compliance Audit Program

CAP Identification Number: 8ECAP-0026

Dear Sir/Madam:

Pursuant to the Toxic Substances Control Act (TSCA) Section 8(e) Compliance Audit Program and the Agreement for TSCA Section 8(e) Compliance Audit Program (CAP Agreement) executed by Atochem North America, Inc. (Atochem) and the Environmental Protection Agency (EPA), Atochem is submitting the enclosed series of studies on Triphenylphosphine and Triphenylphosphine Oxide to the EPA. These studies do not involve effects in humans.

Nothing in this letter or the enclosed studies is considered confidential business information of Atochem.

The enclosed studies provide information on the two related chemicals, triphenylphosphine and triphenylphosphine oxide. The CAS numbers are 603-35-0 and 791-28-6, respectively.

The titles of the enclosed studies are summarized as follows:

2/9/93

Triphenylphosphine Oxide - CAS # 791-28-6

Study Title and Report Date

1. Acute Toxicity of Triphenylphosphine to Dogs - Preliminary Report. Bio/dynamics Inc. 12/11/1978. Laboratory Reference # Unknown.

2. A Seven Week Neurotoxicity Study in Dogs With Triphenylphosphine Oxide. Bio/dynamics Inc. 6/29/1979. Laboratory Reference # 79-2379.

3. A Four Week Neurotoxicity Study in Dogs With Triphenylphosphine and Triphenylphosphine Oxide. Bio/dynamics Inc. 3/1/1979. Laboratory reference # 78-2317.

Triphenylphosphine - CAS # 603-35-0

Study Title and Report Date

1. An Acute Oral Toxicity Study in Hens. Bio/dynamics Inc. 6/22/1978. Laboratory Reference # 5083-78.

2. Acute Oral Toxicity Study in Rats. Bio/dynamics Inc. 6/30/1980. Laboratory Reference # 6218-80.

3. A Four Week Inhalation Study of Triphenylphosphine in Dogs. Bio/dynamics Inc. 12/31/1980. Laboratory Reference # 79-7349.

4. A Seven Week Neurotoxicity Study With Triphenylphosphine in Dogs. Bio/dynamics Inc. 8/22/1980. Laboratory Reference # 79-2445.

5. A Four Week Neurotoxicity Study in Dogs With Triphenylphosphine and Triphenylphosphine Oxide. Bio/dynamics Inc. 3/1/1979. Laboratory Reference # 78-2317.

6. A Four Week Oral Toxicity Study of Triphenylphosphine in the Dog. Bio/dynamics Inc. 12/17/1982. laboratory Reference # 81-7534A.

7. A Four Week Inhalation Toxicity Study of Triphenylphosphine in The Dog. Bio/dynamics Inc. 12/17/1982. Laboratory Reference # 81-7534B. Together With Additional Pathology Diagnoses By J.B. Cavanagh (Univ. London), Experimental Pathology Laboratories Inc. (Virginia, USA) and R.F. McConnell (Flemington, NJ).

Each of the chemicals in a variety of species indicates some potential to cause central nervous system dysfunction, in some cases with evidence of morphological alterations of the spinal cord. The effects have been elicited by both the oral and inhalation routes. In isolation, the evidence for these effects is equivocal, but review of the studies in total is sufficient to merit submission.

Atochem previously submitted TSCA Section 8(e) notices on triphenylphosphine and triphenylphosphine oxide. The following are the EPA Document Control Numbers of Atochem's previous TSCA Section 8(e) submissions on the subject chemicals:

8EHQ-1177-0015
8EHQ-0978-0015
8EHQ-1283-0015
8EHQ-0379-0015
8EHQ-0278-0055

Further questions regarding this submission may be directed to me at 215 337-6892.

Sincerely,

C.H. Farr, PhD, DABT
Manager, Product Safety
and Toxicology

Enclosures

Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO.: 81-7534A

A FOUR WEEK ORAL TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN THE DOG

Submitted to: M&T Chemicals
Rahway, N.J. 07065

Attention: Mr. Arthur Sheldon

Date: May 31, 1982

Revised Date: December 17, 1982

TABLE OF CONTENTS

I. INTRODUCTION 1

II. MATERIALS
A. Test Material 1
B. Test Animals 2
C. Selection 3
D. Animal Identification 3

III. METHODS
A. In-Life 3
Experimental Design 3
Husbandry 4
Test Material Administration 4
Animal Observations and Body Weights 4
Analysis of TPP in Corn Oil 4
B. Post Mortem 5

IV. RESULTS AND DISCUSSION
A. Analysis of TPP in Corn Oil Results 6
B. Mortality 6
C. Physical Observations 6
D. Neurological Examinations 6
E. Body Weights 7
F. Macroscopic Pathology 7
G. Microscopic Pathology 7

V. CONCLUSION 8

TABLES

1. Physical Examinations 9
2. Neurological Examinations 14
3. Individual Body Weights 30
4. Animal Termination History 31

APPENDICES

A. Methodology and References A-1
B. Analytical Methodology and Results B-1
C. Pathology Report C-1
D. Personnel D-1

I. INTRODUCTION

This study, conducted for M&T Chemicals, was designed to evaluate effects produced by oral gavage to Triphenyl Phosphine (TPP) when administered to male and female beagle dogs at dose levels of 1.0, 5.0, 10.0, and 20.0 milligrams per kilogram (mg/kg). Treated groups received 20 doses over four weeks. In addition, two dogs from the 1.0, 5.0, and 20.0 mg/kg groups received two doses only and were not treated during the remainder of the four week period (recovery). A control group, consisting of two dogs, was treated in the same manner, except they received twenty doses of the vehicle corn oil. At termination, necropsy and a histopathological examination of the spinal cord and sciatic nerve was performed. This study was conducted at Bio/dynamics, Inc., Mettlers Road, East Millstone, New Jersey 08873. However, the histopathology was performed by Robert F. McConnell, D.V.M., P.A.; Consulting Pathology Services; R.D.7, Wells Road, Flemington, N.J. 08852. All raw data and specimens will be stored in the archives of Bio/dynamics, Inc.

II. MATERIALS

A. Test Material:	Triphenyl Phosphine
Supplier:	M&T Chemicals Rahway, N.J. 07065
Description:	Cream colored white granular powder
Lot Number:	PNVAH-6KFA
Active Ingredient:	94.9%
Amount Received:	Gross weight - 3.325 kg Estimated net weight - 2.5 kg
Date Received:	December 2, 1981
Expiration Date:	December, 1982
Storage Information:	60 - 85°F

II. MATERIALS (Cont.)

A. Test Material (cont.)

Vehicle Control Material: Mazola® Corn Oil
The same batch of Mazola® corn oil was used throughout all groups.

B. Test Animals: Dog

Strain: Beagle

Number of Animals: 16 (8 males, 8 females)

Supplier: Marshall Research Animals, Inc.
North Rose, New York 14516

Date Received: September 29, 1981

Age at Receipt: 4-5 months

Age at Initiation of Dosing: 6-7 months

Weight at Initiation of Dosing (kilograms):

	Mean	Range
Males:	11.2	9.6 - 12.4
Females:	9.3	8.4 - 10.5

Immunization: Against Distemper, hepatitis, and leptospirosis by supplier. Treatment for rabies performed at Bio/dynamics, Inc. upon receipt of animals.

Parasites: Fecal examination conducted during pretest on all animals at Bio/dynamics, Inc. during the equilibration period. All results were negative.

II. MATERIALS (Cont.)

C. Selection: More animals were purchased and equilibrated than were required for the study. Animals considered unsuitable for the study on the basis of pretest physical examinations were eliminated prior to initiation of dosing to the test substance (Methodology and References, Appendix A). Animals were sorted into groups on a random basis from those animals considered suitable for study purposes.

D. Animal Identification: Each dog was assigned a unique Bio/dynamics, Inc. identification number which appeared on an ear tag and on the animal's cage. In addition, each animal bore an ear tattoo with a USDA number.

III. METHODS

A. In-Life

EXPERIMENTAL DESIGN:

Group	Concentration	Number of Treatment		Number of Animals	
		Two[1,2]	Twenty[1,3]	Necropsy	Histopathology
I	Corn Oil Control	0	2	2	2
II	1.0 mg/kg	2	2	4	4
III	5.0 mg/kg	2	2	4	4
IV	10.0 mg/kg	0	2	2	2
V	20.0 mg/kg	2	2	4	4
	Totals:	6	10	16	16

[1]Evenly divided by sex.
[2]Dosed for two days, sacrificed 30 days after last dose.
[3]Due to intervening holidays (Christmas and New Years), animals were dosed over a five week period 2-5 days/week, and sacrificed one day after the last dose.

III. METHODS (Cont.)

A. In-Life (cont.)

Husbandry:

Equilibration Period:	Minimum 4 weeks
Housing:	Individually in metal grid cages.
Food:	Standard laboratory diet (Wayne Bite-Size®), approximately 400 g/day.
Water:	City tap water (Elizabethtown Water Company) with automatic watering system ad libitum.
Lights:	12 hours on, 12 hours off (animals dosed during lights "on" period).

B. Test Material Administration:

Route:	Oral via gastric intubation
Method:	Appropriate amounts of the test substance were dissolved in Mazola® corn oil. Dosing solution were prepared daily. Individual doses were adjusted on the basis of the most recent weekly body weight data.
Dosing Volume:	1 ml/kg across all groups.
Sampling:	10 ml samples were taken from each dosing solution at least once a week for analytical verification of dosing solution concentrations (Appendix B).

Animal Observations and Body Weights:

Animals were observed twice daily for incidence of abnormal signs. Observations of any unusual conditions were recorded when made. Full physical and neurological assessments were performed weekly. Individual body weights were recorded pretest and weekly during the exposure and/or recovery period.

Analysis of TPP in Corn Oil:

Analysis of the daily dosing solutions of TPP in corn oil was performed using a Tracor 550 Gas Chromatograph.

III. METHODS (Cont.)

B. Post-Mortem:

Dates of Necropsy:	January 6, 1982 (5 male, 5 female) - animal receiving 20 doses. January 8, 1982 (3 male, 3 female) - animals receiving 2 doses.
Sacrifice Method:	Exsanguinated under sodium pentobarbital anesthesia.
Tissues Fixed:	brain (3 sections) sciatic nerve with muscle (both right and left, 3 sections each) spinal cord (four inch section of cervical and lumbar regions)
Fixative:	All tissues - 10% neutral buffered formalin.
Histopathology:	Slides of tissue sections listed below were prepared and examined microscopically from all animals. Sections of all tissues were prepared with H&E stain and luxol fast blue stain.
Sciatic Nerve (right and left):	1 longitudinal section (on each) 2 transverse sections (on each)
Cervical Spinal Cord:	1 longitudinal section 2 transverse sections
Lumbar Spinal Cord:	1 longitudinal section 2 transverse sections

IV. RESULTS AND DISCUSSION

A. Analysis of TPP in Corn Oil Results (Appendix B)

During the first two weeks of the study, the assay for TPP in Corn Oil that were obtained by gas chromatography were approximately 55, 77, 81, and 95% of nominal for Groups II, III, IV, and V, respectively. Due to the low value obtained particularly for Group II, samples in subsequent weeks for all groups were assayed by high pressure liquid chromatography (HPLC). Because the percentage of nominal was slightly higher by HPLC, this method was used for the remainder of the study; these results are presented in Appendix B.

B. Mortality (Animal Termination History - Table 4, page 31)

No test animal mortality occurred.

C. Physical Observations (Physical Examinations - Table 1, page 9)

There were no overt physical signs of toxicity seen in any of the treatment groups considered related to the test material.

D. Neurological Observations (Neurological Examinations - Table 2, page 14)

Neurological examinations during the study revealed sporadic impairment of the extensor postural thrust, placing (tactile), and patellar reflexes. Limb weakness or uncoordination, and knuckling under of paws was observed in males receiving twenty doses in Groups V (20 mg/kg), IV (10 mg/kg), and III (5 mg/kg); and in one female receiving twenty doses in Group II (1.0 mg/kg). Similar observations were noted in one female animal receiving two doses in Group III. Other findings observed were slight and not considered related to the test material.

IV. RESULTS AND DISCUSSION (Cont.)

D. Neurological Observations (cont.)

The findings in the male dogs in Groups V, IV, and III, which received twenty doses, were considered to be related to the dosing of TPP. The findings seen in the two female dogs may be related to treatment, although these signs were not observed at higher dose levels in dogs receiving either two or twenty doses.

E. Body Weights
(Individual Body Weights - Table 3, page 30)

No association between body weights and treatment was observed during the course of the study.

F. Macroscopic Pathology
(Appendix C)

Postmortem findings, observed grossly, either occurred in the treated and control animals or they occurred sporadically; they did not appear to be related to the test material.

G. Microscopic Pathology
(Appendix C)

Microscopic pathology findings revealed mild to moderate generalized vacuolative axonal lesions in the ventral and lateral funicule of the spinal cord at the high dose level (Group V) in both dogs receiving twenty treatments, and may be treatment related. Similar lesions, although minimal and focal in nature, were noted in spinal cord sections at the lower dose levels of 1 and 10 mg/kg TPP. In groups receiving 1 mg/kg TPP only the females exhibited this change following either 2 or 20 doses. These lesions were not seen in any dog receiving only corn oil. No histopathological was seen in the sciatic nerve.

Revised: James B. Terrill

Date: Dec 1, 1982

V. CONCLUSION

There was no treatment related mortality and physical signs were similar in treated and control animals.

Body weight increments for both sexes in treated and control dogs were comparable throughout the treatment and recovery periods.

Evidence of neurological impairment was noted in male dogs receiving twenty doses in Groups III (5 mg/kg), IV (10 mg/kg), and V (20 mg/kg). Similar findings were noted in two female dogs in Groups II and III which received twenty and two treatments, respectively; however, these findings were not noted at higher dose levels in dogs receiving 2 or 20 doses.

Although macroscopic pathology findings were unremarkable, microscopic pathology findings revealed mild to moderate generalized vacuolative axonal lesions in the ventral and lateral funicule of the spinal cord at the high dose level in male and female dogs receiving twenty treatments. Similar lesions, although minimal and focal in nature, were noted in spinal cord sections at the lower dose levels of 1 and 10 mg/kg TPP. In the group receiving 1 mg/kg TPP, only the females exhibited this change following either 2 or 20 doses. These lesions were not seen in any dog receiving only corn oil.

James B. Terrill — Dec 1, 1982

James B. Terrill, Ph.D.
Director Inhalation Toxicology — Date

Geoffrey K. Hogan — 12/2/82

Geoffrey K. Hogan, Ph.D., D.A.B.T.
Vice-President of Toxicology — Date

Bio/dynamics Inc.

Revised: James B. Terrill
Date: Dec 1, 1982

PROJECT NO.: 81-7534A

A FOUR WEEK ORAL TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN THE DOG

ABSTRACT

This study was conducted for the M&T Chemicals to assess the oral toxicity of triphenyl phosphine (TPP) when administered via gastric intubation in a corn oil vehicle to male and female dogs. Four dose levels and two different durations of treatment were used in the study; the specific test regimes were as follows: two dogs (1 male, 1 female) per group received twenty doses over four weeks at 1, 5, 10 and 20 milligrams TPP per kilogram (mg/kg); two doses were administered to two dogs (1 male, 1 female) per group at concentrations of 1, 5 and 20 mg/kg TPP. These shorter duration treatments were followed by a four week recovery period. A concurrent vehicle control group (1 male, 1 female) received twenty doses of corn oil, the volume was comparable to that given the TPP treated animals. Each batch of treated corn oil was prepared fresh daily and the level of TPP in the vehicle was determined by gas chromatographic and/or high pressure liquid chromatographic methods. Body weight measurements, physical observations and neurological examinations were performed pre-test and weekly throughout the study. Complete gross postmortem examinations were performed and selected sections of nervous system tissue were preserved from all animals for histopathological evaluation.

All animals survived the duration of the study.

Signs of neurological impairment were noted in male dogs receiving twenty 5, 10 or 20 mg/kg TPP doses. Similar findings were noted in two female dogs which received 1 or 5 mg/kg TPP doses for twenty or two treatments, respectively; however, these findings were not noted in female dogs receiving either two or twenty treatments at higher dose levels.

Microscopic pathology evaluations (H&E, and luxol fast blue stains) revealed mild to moderate generalized vacuolative axonal lesions in the ventral and lateral funicle of the spinal cord at both sexes of dogs receiving twenty doses of 20 mg/kg TPP. Similar lesions, although miminal and focal in nature, were noted in spinal cord sections in dogs receiving 1 or 10 mg/kg TPP. In the group receiving 1 mg/kg TPP (2 or 20 doses), only females exhibited this change. These lesions were not seen in any dog receiving only corn oil.

Evaluations of body weight, general physical observations (non-neurological) and gross postmortem results were considered unremarkable.

81-7534A

Table 1
A Four Week Oral Toxicity Study
of TPP in the Dog

Physical Examination
Group I

An. No.	Observation	Interval				
		Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
	Males					
1001	Cervical lymph nodes swollen	1+				
	Tonsils swollen	1+				
	Scab on left elbow of forelimb					x
	No observed abnormalities		x	x	x	
	Females					
1501	Popliteal lymph nodes enlarged					1+
	No observed abnormalities	x	x	x	x	

Key: 1+ = slight; x = present.

81-7534A

Table 1 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Physical Examination
Group II

An. No.	Observation	Interval				
		Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
	Males					
2001*	Scab covered and thickening of skin on occipital region of head	x	x	x	x	x
2002	Testicles small and immature	x				
	Alopecia		x	x	x	x
	Popliteal lymph node enlarged			1+	1+	x
	Females					
2501	Both cervical lymph nodes swollen	1+				
	Lymph on top of skull approximately 1.0 cm in diameter				x	
	No observed abnormalities		x	x		x
2502*	Excitable	2+	1+	1+	1+	1+
	Hairloss on medial part of both hind hocks	1+	x	x		

Key: 1+ = slight; 2+ = moderate; x = present; * = two doses only.

Table 1 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Physical Examination
Group III

An. No.	Observation	Interval				
		Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
		Males				
3001*	Both popliteal lymph nodes enlarged	1+		x	1+	1+
	Left popliteal lymph node enlarged		x			
	Tonsils enlarged and red	1+	1+	1+	1+	1+
	Underweight	1+				
3002	Scab covered lesion on middle of muzzle		x			
	Denuded area over lesion			x	x	
	Left mandibular lymph node swollen	1+				
	No observed abnormalities					x
		Females				
3501	No observed abnormalities		x	x	x	x
3502*	Both popliteal lymph nodes enlarged		1+	1+	1+	1+
	Left upper residual canine present	x	x			
	Rash on left shoulder					x

Key: 1+ = slight; x = present; * = two doses only.

Table 1 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Physical Examination
Group IV

An. No.	Observation	Interval				
		Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
		Males				
4001	Testicles small and immature	x				
	No observed abnormalities		x	x	x	x
		Females				
4501	No observed abnormalities	x	x	x	x	x

Key: x = present.

Table 1 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Physical Examination
Group V

An. No.	Observation	Interval				
		Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
		Males				
5001	Popliteal lymph nodes enlarged		1+	1+	1+	1+
	Tonsils red and swollen		1+			
	No observed abnormalities	x				
5002*	Salivation				1+	
	Cervical lymph nodes swollen		1+	1+	1+	1+
	Both popliteal lymph nodes enlarged		1+	1+	1+	1+
	Testicles small and immature	x				
		Females				
5501*	Mouth reddened				1+	1+
	Interdigital swelling				1+	1+
	Both popliteal lymph nodes enlarged		1+	1+	1+	
5502	Timid				1+	
	Salivation				2+	
	Cervical lymph nodes swollen		1+	1+		1+
	Popliteal lymph nodes enlarged			1+	1+	1+
	Alopecia				2+	

Key: 1+ = slight; 2+ = moderate; x = present; * = two doses only.

Table 2
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group I - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #1001					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group I - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #1501					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	-	+
Extensor postural thrust	+	+	+	+	a
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal; - = reflex absent.
[a]Reflex normal; tic in right rear paw.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group II - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #2001*					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
* = two doses only.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group II - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #2002					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	-	-
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal; - = reflex absent.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group II - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #2501					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	c	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	c	+
Extensor postural thrust	+	b	d	b	b
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal.
[b]Right rear paw knuckles under.
[c]Reflex present but slow.
[d]Right rear paw placing uncoordinated.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group II - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #2502*					
Pupillary light	+	+	+	+	e
Consensual light	+	+	+	+	e
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	e
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal; * = two doses only.
[e]Could not evaluate due to excitability.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group III - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #3001*					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	f	+	f	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal; * = two doses only.
[f]Reflex normal; places hind legs widely apart.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group III - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal Number 3002					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	g	+	h
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
[g]Right rear paw knucles over.
[h]Placing right rear paw posterior to left.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group III - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #3501					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group III - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #3502*					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	i	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	j,k	j,l	j,l	j,l
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal; * = two doses only.
[i]Rotating the right hock medially.
[j]Both hind legs placed behind center of gravity.
[k]Right hind leg reluctant to bare weight.
[l]Both hind legs reluctant to bare weight.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group IV - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #4001					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	m	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal.
[m]Hind limbs will not hold weight (slight weakness).

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group IV - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #4501					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	-	-	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal; - = reflex absent.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group V - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #5001					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	o	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	n	+	m	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal.
[m]Hind limbs will not hold weight (slight weakness).
[n]Paw placed near midline - slight knucling under of paws.
[o]Slight uncoordination of hind limb movement, hopping bringing both hind limbs forward.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group V - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #5002*					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	p	p
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal; * = two doses only.
PSlight reaction.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group V - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #5501*					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal; * = two doses only.

Table 2 (cont.)
A Four Week Oral Toxicity Study
of TPP in the Dog

Neurological Examination
Group V - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
Animal #5502					
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal.

Table 3
A Four Week Oral Toxicity Study
of TPP in the Dog

Individual Body Weights (kg)

Group	Animal Number	Sex	Interval				
			Pretest 12/1/81	Week 1 12/14/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/4/82
mg/kg							
I	1001	M	11.4	11.7	11.6	11.7	11.8
Corn Oil	1501	F	9.2	9.3	9.2	9.2	9.2
II	2001*	M	12.1	12.7	12.5	12.7	12.4
1.0	2002	M	10.7	11.0	11.1	11.5	11.6
	2501	F	9.6	9.9	10.2	10.4	10.4
	2502*	F	9.4	9.9	9.4	9.5	9.6
III	3001*	M	9.6	11.2	10.3	10.4	10.5
5.0	3002	M	12.4	12.5	12.6	12.9	13.3
	3501	F	9.9	9.8	10.0	9.8	10.3
	3502*	F	8.4	9.2	8.4	8.6	8.4
IV	4001	M	11.4	11.6	11.7	11.9	12.0
10.0	4501	F	9.2	9.2	9.1	9.2	9.0
V	5001	M	11.6	11.9	12.2	12.1	12.4
20.0	5002*	M	10.5	11.5	10.6	10.9	10.9
	5501*	F	10.5	11.1	10.6	10.9	11.0
	5502	F	8.4	8.6	8.7	9.1	9.3

*Two doses only.

Table 4
A Four Week Oral Toxicity Study
of TPP in the Dog

Animal Termination History

Group	Males				Females			
	An. No.	Days on Test	Date of Death	Type of Death/Week	An. No.	Days on Test	Date of Death	Type of Death/Week
mg/kg								
I Corn Oil	1001	31	1/6/82	T/5	1501	31	1/6/82	T/5
II 1.0	2001*	33	1/8/82	T/5	2501	31	1/6/82	T/5
	2002	31	1/6/82	T/5	2502*	33	1/8/82	T/5
III 5.0	3001*	33	1/8/82	T/5	3501	31	1/6/82	T/5
	3002	31	1/6/82	T/5	3502*	33	1/8/82	T/5
IV 10.0	4001	31	1/6/82	T/5	4501	31	1/6/82	T/5
V 20.0	5001	31	1/6/82	T/5	5501*	33	1/8/82	T/5
	5002*	33	1/8/82	T/5	5502	31	1/6/82	T/5

Key: T = terminal sacrifice; * = two doses only.

Appendix A
A Four Week Oral Toxicity Study
of TPP in the Dog

Methodology and References - General

Parameter	Reference or Description
Physical Examination:	Central Nervous System: examined - behavior movements, reactivity to stimuli, muscle tone. Autonomic Nervous System: examined - pupil size, secretions. Respiration: examined - nostrils, character, rate. Cardiovascular: examined - palpation. Gastrointestinal: examined - abdominal shape, feces consistency and color. Genitourinary: examined - penis, vulva, perineal region. Skin and Fur: examined - color, turgor, integrity. Mucous Membrane: examined - mouth, rectal, vaginal. Eye: examined - eyelids, eyeball, transparency. General Condition.
Neurological Examination:	The following reflexes were observed: righting, patellar, flexor, extensor, visual placing response, corneal and pupillary.
Body Weight	Detecto scale (60-S).

Appendix B

DETERMINATION OF TRIPHENYLPHOSPHINE IN CORN OIL FOR STUDY NO. 81-7534A

MAC Project No.: 81244

Sponsor: M&T Chemicals, Inc.
Rahway, New Jersey

Prepared by: John F. Hamilton Date: June 7, 1982

Issued by: Bio/dynamics, Inc.
Department of Metabolism and
Analytical Chemistry
P.O. Box 43, Mettlers Road
East Millstone, N.J. 08873

SUMMARY

The levels of Triphenylphosphine (TPP) in corn oil were determined over a 4 week period by high performance liquid chromatography (HPLC). Assays were performed initially to confirm data generated by gas chromatography, performed by the Department of Inhalation Toxicology.

Average sample recoveries for week 2 through week 5 were 66.3%, 84.6%, 90.3%, and 93.3% for Groups II through V, respectively.

Low recovery of TPP may have been the result of some interaction with corn oil.

1.0 INTRODUCTION

Dosing solutions were analyzed by High Performance Liquid Chromatography (HPLC) to verify the levels of TPP in corn oil determined by gas chromatography. The levels of TPP in corn oil were determined for dosing solutions prepared during weeks 2, 3, 4 and 5 of study 81-7534A.

2.0 TEST MATERIAL

Technical grade Triphenylphosphine (TPP) (Lot #PNVAH-6KFA) was used as standard material and sample values were uncorrected for standard purity (94.9% purity). Test material was provided by the Department of Inhalation Toxicology.

Dosing solutions were prepared by the Large Animals Department at Bio/dynamics. Samples of the test material were delivered to the Department of Metabolism and Analytical Chemistry for analysis. The dosing solution consisted of test material dissolved in Mazola corn oil. Test material in corn oil was diluted into tetrahydrofuran before injection onto the LC column.

3.0 REAGENTS AND HPLC CONDITIONS

Pump:	Tracor 990 Isochromatographic Pump.
Injector:	Rheodyne 7125 with 20 ul loop
Column:	Partisil 10 ODS 250 x 4.6 mm I.D.
Detector:	Waters Model 440 uv
Wavelength for Detection:	254 nm
Attenuation:	0.5
Recorder:	Tracor Model FB11E
Mobile Phase:	88/12 Methanol/H_2O
Flow Rate:	1.5 ml/minute

Retention time: 5 minute

Methanol: Baker "Resi-Analyzed"

The chromatographic condition may vary to optimize chromatography.

4.0 RESULTS

As shown in Figure 1, TPP concentration as a function of peak area is linear from 50 to 255 ug/ml of TPP with a correlation coefficient of 0.999.

Table I summarizes the levels of TPP found in dosing solutions expressed as % of nominal. The concentration of TPP found was 66.3%, 84.6%, 90.3% and 93.3% of nominal for dose groups II, III, IV and V, respectively. Results for week 4 were not included in the overall mean since recovery values were significantly lower than expected. This was probably due to the 5 day lag between date of sample preparation and date of analysis.

There was some concern that Group II values were consistantly low suggesting some interaction which is less significant at higher levels of TPP. Serial dilutions were made of two samples at nominal TPP concentrations of 1 mg/ml and 20 mg/ml, to theoretical concentrations of 200, 130, 100 and 50 ug/ml, to obtain a 4 point dilution curve for each sample. The ratio of the slope generated for the sample dilution curve versus the actual TPP standard curve is indicative of actual sample concentration. Samples at 100% nominal concentration would generate a curve similar to curve A (Figure II) and have a slope ratio of 1. As seen by Figure II, the serial dilution curve of the 20 mg/ml sample (Gr. V, #649) gives a slope of 1.197. Comparison of this value to the standard curve slope shows $\frac{1.197}{1.368} = 0.875$, or approximately 88% of nominal concentration. Similarly the dilution of the 1 mg/ml sample (Gr. II, #646) shows a value of $\frac{0.860}{1.368} = 0.629$, or approximately 63% of nominal concentration. These values are comparable to average values generated for the dose groups,

81-7534A
81244

and indicate a portion of TPP may be inactive in corn oil. Direct calculations based on average percent nominal values for Groups II and V corresponds to 0.3 to 1.34 mg/ml TPP reacted, respectively. (See Appendix I for calculations.) These values may be time and concentration dependant.

Additional evidence suggesting inactivation of TPP in Group II dosing solution comes from a fortification experiment. As shown in Table I, fortifications of corn oil at 1, 5, 10 and 20 mg/ml with TPP showed 100% recovery at levels of 5, 10 and 20 mg/ml while only 75% was recovered at 1 mg/ml. These analyses were performed 2.5 hours after samples were prepared to minimize time as a factor.

The results indicate that TPP levels in Groups III, IV and V were within 15% of nominal concentration. The concentration of TPP in Group II dosing solutions appeared low at 66.3 $\pm$ 8.2% of nominal. However, if this data is corrected for procedural recovery of the fortified sample the corrected recovery of Group II TPP levels would be 89.6% of nominal.

John F. Hamilton
Supervisor
Department of Metabolism
and Analytical Chemistry

5/20/82
Date

Richard P. McPartland, Ph.D.
Director
Department of Metabolism
and Analytical Chemistry

5-20-82
Date

TABLE I. TPP/CORN OIL

Summary of Data

Week	Date Sample Received	Date of Analysis	Group I (0 mg/ml) Smpl#	Group I (0 mg/ml) % Nom	Group II (1 mg/ml) Smpl#	Group II (1 mg/ml) % Nom	Group III (5 mg/ml) Smpl#	Group III (5 mg/ml) % Nom	Group IV (10 mg/ml) Smpl#	Group IV (10 mg/ml) % Nom	Group V (20 mg/ml) Smpl#	Group V (20 mg/ml) % Nom
2	12/16/81	12/17/81	620	-	621	71	622	82	623	92	624	93
2	12/16/81	12/17/81	635	-	636	73	637	83	638	91	639	93
2	12/18/81	12/18/81	640	-	641	73	642	82	643	87	644	91
2	12/18/81	12/20/81	645	-	646	64	647	85	648	93	649	99
						x = 70.3		x = 83				
3	12/21/81	12/21/81	650	-	651	72	652	86	653	87	654	93
3	12/23/81	12/24/81	655	-	656	52	657	84	658	88	659	95
						51 52						
						x = 62		x = 85				
4*	12/28/81	1/2/82	655A	-	656A	<25%**	657A	50**	658A	73**	659A	79**
	12/30/81	1/2/82	660	-	661	<25%**		55**	663	72**	664	89**
5	1/4/82	1/4/82	665	-	666	59	667	90	668	94	669	89
Fortifications***				-		74**		100**		100**		100**
						x = 66.3 SD = 8.2		x = 84.6 SD = 2.8		x = 90.3 SD = 2.9		x = 93.3 SD = 3.1

*Results inconclusive due to instrument problem/time lapse between sample receipt and analysis.
**Data not used in statistics.
***Samples were weighed, diluted, and anlayzed immediately 2.5 hr elapsed time.

APPENDIX I. CALCULATION OF REACTED FPP

Group II: Avg % Nominal: 66.3% Nominal Value 1 mg/ml

1 mg/ml - (0.663 x 1 mg/ml) = 0.337 mg/ml reacted.

Group V: Avg % Nominal: 93.3% Nominal value 20 mg/ml

20 mg/ml - (0.933 x 20 mg/ml) = 1.34 mg/ml reacted.

FIGURE 1.

MAC Project # 81244
Tox. Study # 81-7534A
Compound TPP/corn oil
Interval wk 2

Signature [signature]
Date Inj. 12/17/81 NBR
Verified by Richard P. McPartland
Date Verified 12-17-81

Cross-Ref. # 81244-1

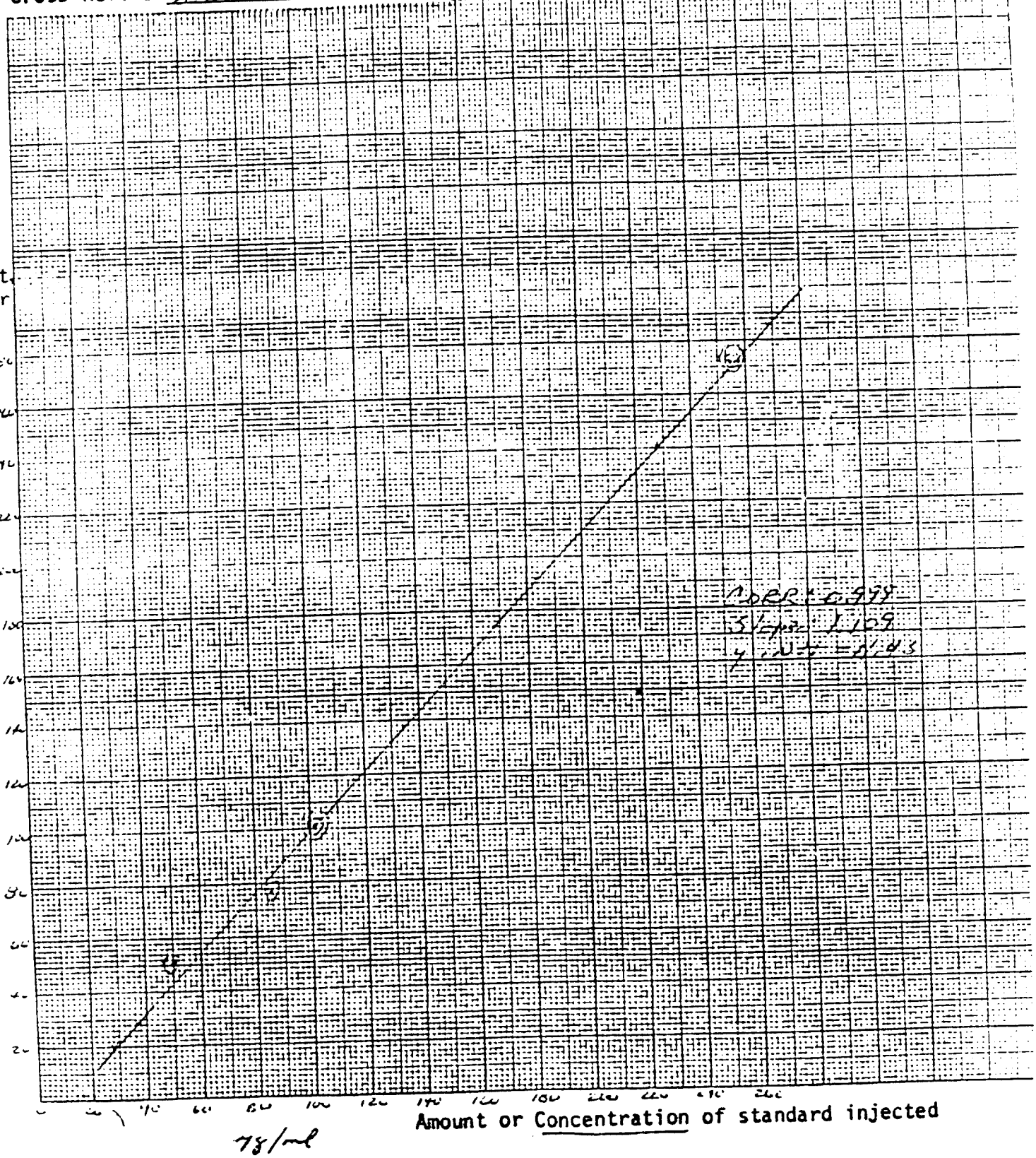


FIGURE II. SAMPLE DILUTIONS

MAC Project # 81244
Tox. Study # 81-2534A
Compound TPP
Interval wk 2 Sample Dilutions
Signature J Hamlet
Date Inj. 12/29/81 NBR
Verified by Richard P. McPartland
Date Verified 12-30-81

Cross-Ref. # 81244-1"

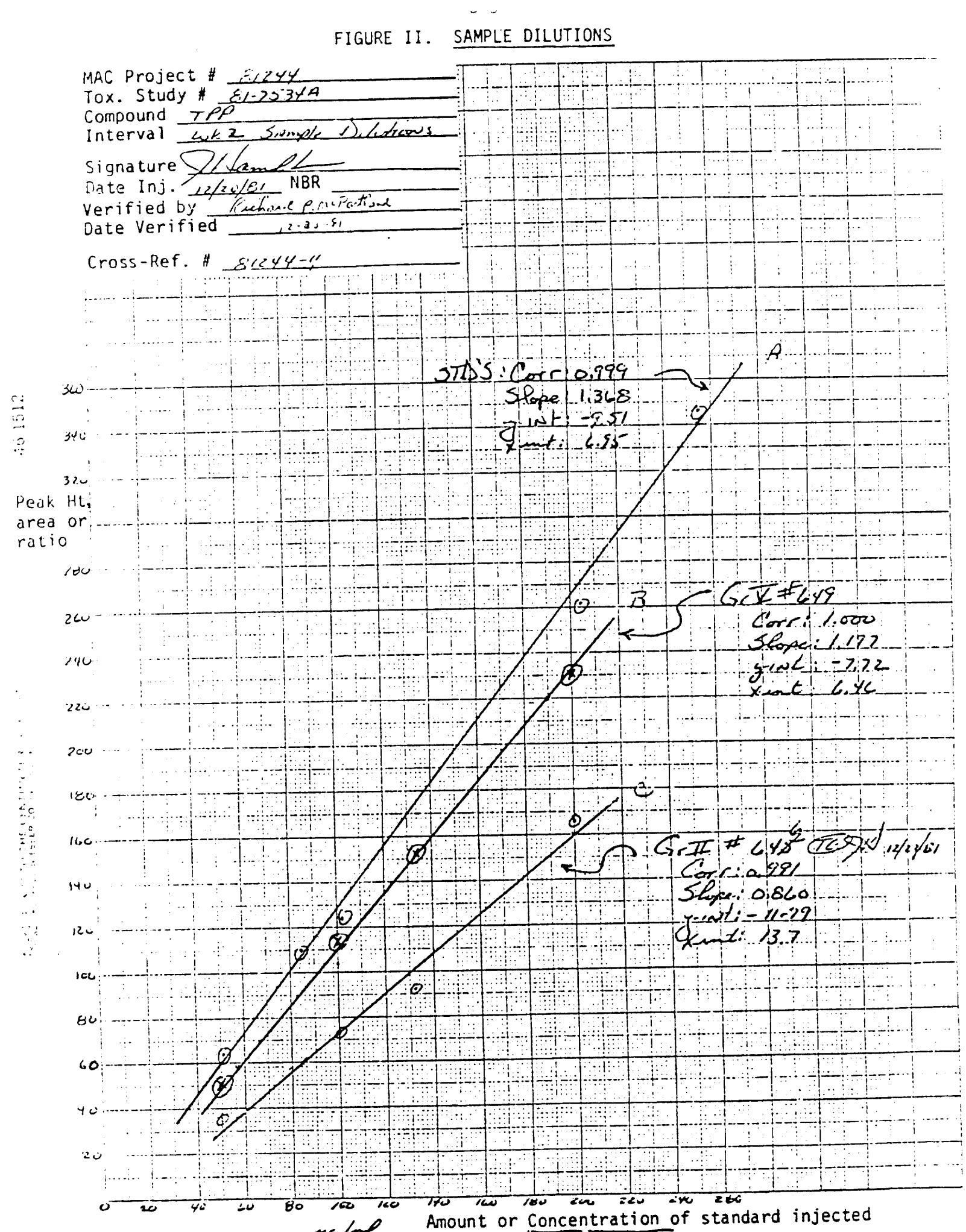
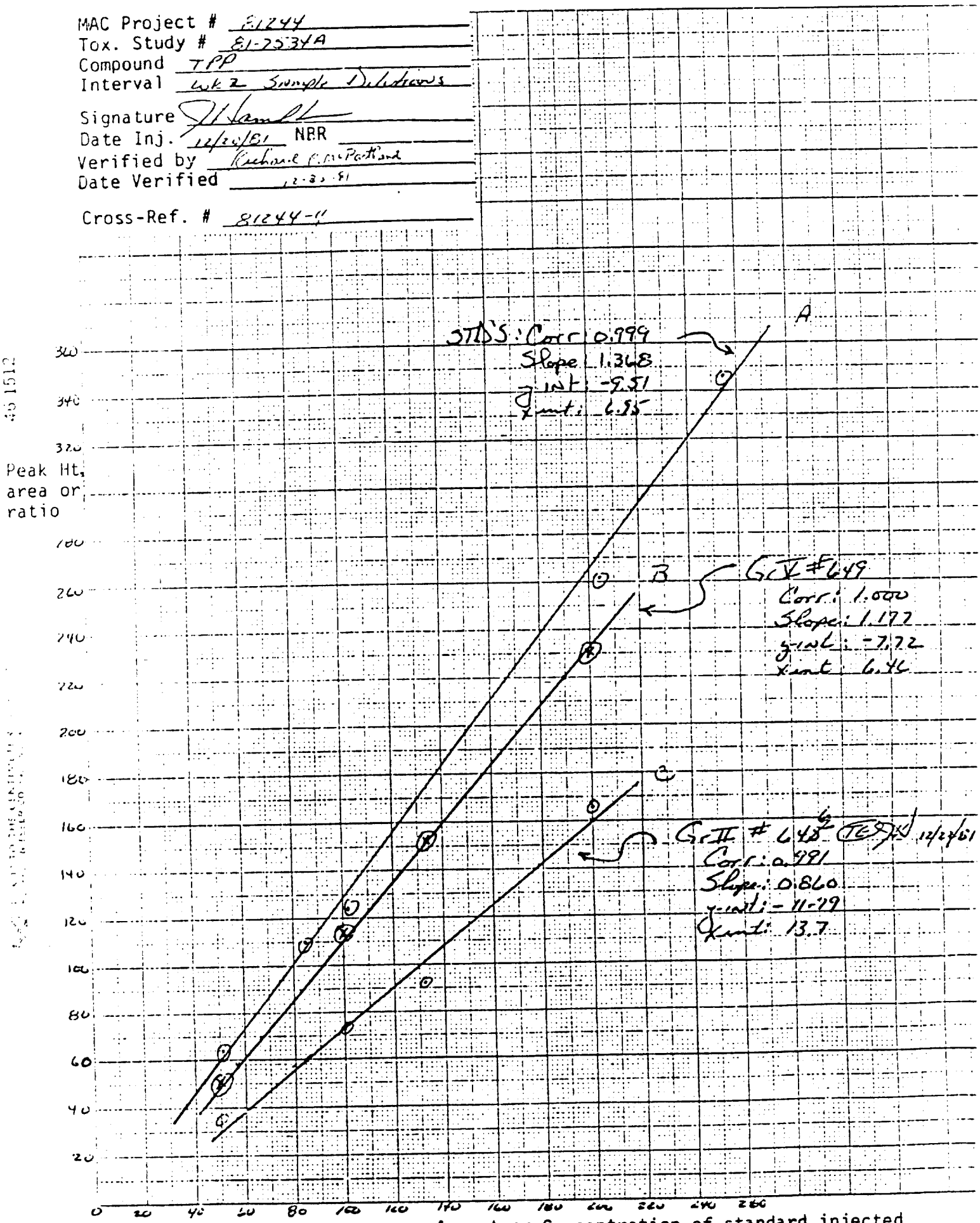


FIGURE II. SAMPLE DILUTIONS

MAC Project # E1244
Tox. Study # E1-2534A
Compound TPP
Interval wk 2 Sample Dilutions
Signature [signature]
Date Inj. 12/26/81 NBR
Verified by Richard C. McPartland
Date Verified 12-30-81

Cross-Ref. # 81244-1'



Appendix C

M-11, 81-7534 A

A Four Week Oral Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

A Four Week Oral Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

TABLE OF CONTENTS

Summary of Gross Postmortem Observations..... pg. 3

Table I - Identification and Disposition
of Individual Animals on Test..... pg. 4

Table II - Gross Postmortem Observations..... pg. 6

Microscopic Evaluation..... pg. 10

Pathology Summary..... pg. 12

Introduction..... pg. 14

Materials and Methods..... pg. 15

Discussion of Microscopic Findings..... pg. 16

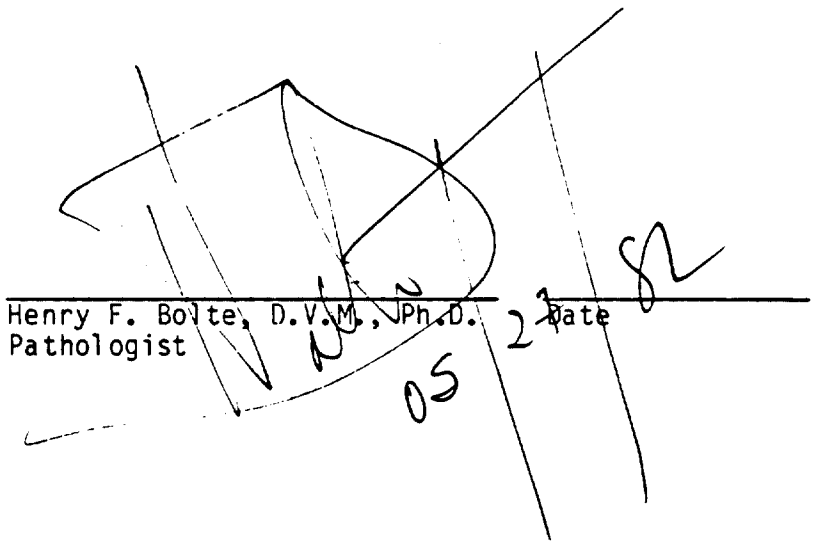
Summary of Microscopic Findings..... pg. 18

Incidence of Microscopic Findings..... pg. 19

A Four Week Oral Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Summary of Gross Postmortem Observations

Postmortem findings, observed grossly, either occurred in the treated and control animals or they occurred sporadically; they did not appear to be related to the test article.



Henry F. Bolte, D.V.M., Ph.D. Date
Pathologist

Table I

A Four Week Oral Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Identification and Disposition of Individual Animals on Test

Table I 81-7534 A

A Four Week Oral Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Identification and Disposition of Individual Animals on Test

Group	Sex	Animal Number	Type of Death	Group	Sex	Animal Number	Type of Death
I	M	1001	Ta	I	F	1501	Ta
II	M	2001	Tb	II	F	2501	Ta
		2002	Ta			2502	Tb
III	M	3001	Tb	III	F	3501	Ta
		3002	Ta			3502	Tb
IV	M	4001	Ta	IV	F	4501	Ta
V	M	5001	Ta	V	F	5501	Tb
		5002	Tb			5502	Ta

K E Y

Ta = Terminal Sacrifice - 20 Day Dose

Tb = Terminal Sacrifice - 2 Day Dose

Table II

A Four Week Oral Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

A Four Week Oral Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

GENERAL KEY TO GROSS POSTMORTEM OBSERVATIONS

N.O.A.	=	No observable abnormalities
R	=	Right
L	=	Left
B	=	Both, Bilateral
X (x)	=	Times
N	=	Normal
cm	=	centimeter
diam.	=	diameter
approx.	=	approximately
G.I.	=	Gastro-intestinal
A.G.	=	Ano-genital
<	=	less than
≤	=	less than or equal to

81-7534 A

A Four Week Oral Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Group	Sex	Animal Number	Organ/ Tissue	Observations
I	M	1001	Elbow (L)	Scab, dark brown.
II	M	2001	Top of Head	3 small lesions (approx. 0.5cm).
		2002	Jejunum	Mid: white, coiled round worm.
III	M	3001	Popliteal Lymph Node	Large.
			Tonsils	Slightly swollen.
		3002	Lungs	L apical lobe: firm, tan, red/brown area (2.0x6.0cm); R apical lobe: tan nodular area (1.0cm); brown areas along edges (2.0x0.5cm); brown/tan area (1.0cm in diam.).
			Jejunum	Upper: round worm (12 cm length) in lumen.
IV	M	4001	-	N.O.A.
V	M	5001	Jejunum	2 worms (5-6cm).
		5002	Popliteal Lymph Node	Large.

81-7534 A

A Four Week Oral Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Group	Sex	Animal Number	Organ/ Tissue	Observations
I	F	1501	Popliteal Lymph Node	Large.
II	F	2501	-	N.O.A.
		2502	-	N.O.A.
III	F	3501	-	N.O.A.
		3502	Fur/Skin	L upper thoracic: red area with slight hair loss (3.0cm).
			Lungs	L diaphragmatic lobe, ventral edge: brown, irregular area (1.5x1.0cm).
			Popliteal Lymph Node	Large.
IV	F	4501	Inguinal Teats	Red.
			Lungs	L apical lobe: tan area (4.0x5.0cm); R apical lobe: tan area (2.0x3.0cm).
V	F	5501	Rear Paw	L: interdigital swelling between 3rd and 4th digits.
		5502	-	N.O.A.

ROBERT F. McCONNELL, D.V.M., P.A.
CONSULTING PATHOLOGY SERVICES
PHONE 201-782-4676

DIPLOMATE
AMERICAN COLLEGE
OF
VETERINARY PATHOLOGISTS

R.D. 7, WELLS ROAD
FLEMINGTON, NEW JERSEY 08822

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534A

A FOUR WEEK ORAL TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN DOGS

PATHOLOGY REPORT

SUBMITTED TO: ALEKSANDAR L. KNEZEVICH, DR. MED. VET.
VICE PRESIDENT, PATHOLOGY
BIO/DYNAMICS, INC.
P. O. BOX 43
METTLERS ROAD
EAST MILLSTONE, NEW JERSEY 08873

Robert F McConnell

ROBERT F. McCONNELL, D.V.M.

APRIL 26, 1982

67/82

B/d M-11, 81-7534A
RFMc 67/82
4/26/82

TABLE OF CONTENTS

	PAGE(S)
PATHOLOGY SUMMARY	12
INTRODUCTION	14
MATERIALS AND METHODS	15
DISCUSSION OF MICROSCOPIC FINDINGS	16
SUMMARY OF MICROSCOPIC FINDINGS TABLE 1	18
INCIDENCE OF MICROSCOPIC FINDINGS IN INDIVIDUAL DOGS BY DOSE GROUP AND SEX TABLE 2	19

RFMc 67/82
4/26/82

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534A

A FOUR WEEK ORAL TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN DOGS

PATHOLOGY REPORT

PATHOLOGY SUMMARY:

Sixteen male and female dogs were divided into 5 groups. Group I served as corn oil vehicle controls. Each of the remaining Groups was treated with triphenyl phosphine (TPP) at levels of 1.0, 5.0, 10.0 and 20.0 mg./kg. for 2 or 20 days.

At the end of the 4-week study the dogs were necropsied and sections of spinal cord and sciatic nerve were submitted for microscopic examination.

TPP related generalized mild to moderate vacuolative lesions were observed in the ventral and lateral funiculi of the cervical and lumbar spinal cord in 1 male and 1 female of the high dose group. No lesion was present in the sciatic nerve of any dog dosed with TPP.

Minimal focal axonal vacuolation was noted in 0/2 ♂, 2/2 ♀ Group II, 0/2 ♂, 0/2 ♀ Group III, 1/1 ♂, 1/1 ♀ Group IV, and 1/2 ♂, 0/2 ♀ Group V dogs. The lesion was present primarily in the cervical cord with only 1 animal having a lesion in the lumbar cord. The significance of this lesion was questionable. It was, however, not present in control dogs.

B/d M-11, 81-7534A
RFMc 67/82
4/26/82

It was concluded under the conditions of this test, that TPP at concentrations of 20 mg./kg./day may cause mild to moderate generalized vacuolative axonal lesions in the ventral and lateral funiculi of the spinal cord in male or female dogs.

Robert F. McConnell

ROBERT F. McCONNELL, D.V.M.
Diplomate, American College
of Veterinary Pathologists

RFMc 67/82
4/26/82

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534A

A FOUR WEEK ORAL TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN DOGS

PATHOLOGY REPORT

INTRODUCTION:

This study was designed to evaluate the effects of triphenyl phosphine (TPP) on selected tissues of the peripheral nervous system (spinal cord and sciatic nerve) of the dog following two or twenty oral doses of the experimental agent.

Sixteen male and female beagle dogs were divided into 5 groups. Group I (1 ♂ - 1 ♀) served as corn oil vehicle controls. Group II (2 ♂ - 2 ♀) received 1.0 mg./kg. of TPP + corn oil vehicle, Group III (2 ♂ - 2 ♀) received 5.0 mg./kg. of TPP + vehicle, Group IV (1 ♂ - 1 ♀) received 10 mg./kg. of TPP + vehicle, and Group V (2 ♂ - 2 ♀) received 20 mg./kg. of TPP + vehicle.

At the end of the 4-week study the dogs were anesthetized with sodium pentobarbital and exsanguinated. Tissues were collected for microscopic examination. The results of the histopathological evaluation form the basis of this pathology report.

RFMc 67/82
4/26/82

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534A

A FOUR WEEK ORAL TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN DOGS

PATHOLOGY REPORT

MATERIALS AND METHODS:

At necropsy the following tissues or their representative sample were taken and preserved in 10% neutral buffered formalin: brain, sciatic nerve (right and left), and spinal cord from both cervical and lumbar regions.

Slides of the tissue sections listed below were prepared routinely from all animals. The tissue sections were stained with H & E and luxol fast blue.

Sciatic nerve, right and left	1 longitudinal section on each
	2 transverse sections on each
Cervical spinal cord	1 longitudinal section
	2 transverse sections
Lumbar Spinal cord	1 longitudinal section
	2 transverse sections

RFMc 67/82
4/26/82

Revised By: [signature] 5/17/82
Pathologist Date

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534A

A FOUR WEEK ORAL TOXICITY STUDY OF
TRIPHENYL PHOSPHINE IN DOGS

PATHOLOGY REPORT

DISCUSSION OF MICROSCOPIC FINDINGS:

The incidence and severity of microscopic findings are listed according to individual dog, sex, and dose group in TABLE 2. These data are summarized to aid in comparison in TABLE 1.

In this study the vacuolative lesion was limited to the meylinated axonal tracts in the spinal cord; the sciatic nerve was uninvolved. In this test the lesion occurred as a mild to moderate generalized change in 1/2 ♂ and 1/2 ♀ of the 20 mg./kg. group. It was present in both the cervical and lumbar sections of the spinal cord. No lesion was present in the sciatic nerve sections.

Focal and usually solitary nerve fiber swelling was observed in 2/2 ♀ Group II, 1/1 ♂ and 1/1 ♀ Group IV, and 1/2 ♂ Group V dogs. The significance of the minimal solitary lesions is questionable.

The generalized lesions observed at the high dose level only were best seen in the longitudinal spinal cord sections. They were characterized by multiple dilated or vacuolated axon sheaths with accumulations of scattered clumps of eosinophilic debris and occasional dark round bits of material suggestive of nuclear chromatin. One dog at the high dose level had no peripheral nervous tissue lesion (5501).

scattered clumps of eosinophilic debris and occasional dark round bits of material suggestive of nuclear chromatin. One dog at the high dose level had no peripheral nervous tissue lesion (5501).

The minimal focal lesions observed as previously mentioned may not have been compound associated. The incidence rate of the finding among the various dose groups suggested that it may have been a spontaneous occurrence. It was notable that no animals were affected in Group III while there were 2 in Group II, 2 in Group IV, and 1 in Group V. The change was observed primarily in the cervical cord but infrequently in the lumbar cord.

RFMc 67/82
4/26/82

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534A

A FOUR WEEK ORAL TOXICITY STUDY OF
TRIPHENYL PHOSPHINE IN DOGS

SUMMARY OF MICROSCOPIC FINDINGS

TABLE 1

	DOSE GROUP									
	I		II		III		IV		V	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
NUMBER/DOGS/GROUP	1	1	2	2	2	2	1	1	2	2
TISSUE/RESPONSE										
CERVICAL SPINAL CORD:										
Vacuolation of lateral and ventral funiculi;										
(a) Focal, minimal	0	0	0	2	0	0	1	1	1	0
(b) Diffuse, mild/moderate	0	0	0	0	0	0	0	0	1	1
LUMBAR SPINAL CORD:										
Vacuolation of lateral and ventral funiculi;										
(a) Focal, minimal	0	0	0	0	0	0	1	0	0	0
(b) Diffuse, mild/moderate	0	0	0	0	0	0	0	0	1	1

TISSUES WITHIN NORMAL HISTOLOGICAL LIMITS: Right and Left Sciatic Nerves.

RFMc 67/82
4/26/82

BIO/DYNAMICS, INC.
A FOUR WEEK ORAL TOXICITY STUDY OF
TRIPHENYL PHOSPHINE IN DOGS

INCIDENCE OF MICROSCOPIC FINDINGS

PROJECT 81-7534A - ROUTE - ORAL

TABLE 2

GROUP	I, CORN OIL		II, 1.0 mg./kg. TPP				III, 5.0 mg./kg. TPP				IV, 10 mg./kg. TPP		V, 20 mg./kg. TPP			
SEX	♂	♀	♂		♀		♂		♀		♂	♀	♂		♀	
ANIMAL NUMBER	1001	1501	2001	2002	2501	2502	3001	3002	3501	3502	4001	4501	5001	5002	5501	5502
TISSUE/RESPONSE (Days on Test)	20	20	2	20	20	2	2	20	20	2	20	20	20	2	2	20
CERVICAL SPINAL CORD:	N	N	N	N			N	N	N	N					N	
Vacuolation, lateral and ventral funiculus	-	-	-	-	(1)	(1)	-	-	-	-	(1)	(1)	3	(1)	-	2
LUMBAR SPINAL CORD:	N	N	N	N	N	N	N	N	N	N		N		N	N	
Vacuolation, lateral and ventral funiculus	-	-	-	-	-	-	-	-	-	-	(1)	-	3	-	-	2
RIGHT SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
LEFT SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

LEGEND FOR TABLE:

N = tissue within normal histological limits
1 = minimal
2 = mild
3 = moderate
( ) = focal or localized change
- = indicated change not present

Appendix D
A Four Week Oral Toxicity Study
of TPP in the Dog

PERSONNEL

DEPARTMENT/TITLE	NAME	DEGREE
Study Director, Inhalation	James B. Terrill	Ph.D.
Supervisor, Inhalation	Doug Reigle	B.S.
Supervisor, Large Animals	Dan Pisacreta	B.S.
Study Monitor	Jeff Krietzman	B.S.
Pathologist	Henry F. Bolte	D.V.M., Ph.D.
	Robert F. McConnell	D.V.M.
	Asa Mays	D.V.M.
Quality Assurance		
Director	Joseph B. Townsend	
Supervisor	Craig Lamb	B.A.

QUALITY ASSURANCE STATEMENT

****

STUDY NO.: 81-7534A TEST SUBSTANCE/ARTICLE: Triphenyl Phosphine

STUDY SPONSOR: M & T Chemicals

Listed below are dates that this study was inspected by the Quality Assurance Unit and the dates findings were reported to the Study Director and Management

DATE(S) OF INSPECTION	REPORTED TO STUDY DIRECTOR	REPORTED TO MANAGEMENT
12/14/81	12/29/81	12/29/81 1/5/82
12/21/81	12/22/81	1/6/82
3/18-22/82	3/26/82	1/5/82
5/11/82	6/11/82	6/11/82 6/11/82
6/7-8/82	6/11/82	6/11/82 6/11/82

Craig Lamb 6/11/82

CRAIG LAMB
MANAGER, QUALITY ASSURANCE

DATE

sheaths of nerves extending from the sciatic nerve up to the cervical spinal cord. The long central nervous system tracts (e.g., gracile, corticospinal, spinocerebellar) had undergone considerable degeneration. This was most apparent in dog Number 5001 followed by 5002 and 4001 (see Figures 1 and 2). Changes in the sciatic nerve consisted of focal myelin vacuolation (Figures 3 and 4). Vacuolation was difficult to interpret since the dogs were not perfusion fixed and considerable fixation artifact was present resulting in a diffuse, subtle bubbling and granularity to the myelin sheaths in spinal cords and peripheral nerves from all of the dogs.

In the male dogs from the lower dosed groups, only minimal myelin fragmentation was seen in the sciatic nerve of Number 3002. Animal Number 3001 had minimal myelin bubbling (vacuolation). No significant changes were seen in Numbers 2002 and 1001. In Number 2001, the only change was minimal to slight hemorrhage or perivascular hemorrhage and edema, changes which could have been agonal.

Females

Changes in the females were even less consistent. Number 5502, in Group V, had degenerative changes that were observed in longitudinal sections of the sciatic nerve, ascending into the gracile tract of the lumbar spinal cord. The cervical spinal cord appeared unaffected. Number 5501, also in Group V, appeared relatively unaffected with only slight myelin bubbling and vacuolation in the sciatic nerves. Similarly,

the Group IV female dog, Number 4501, had only minimal to moderate myelin fragmentation and bubbling in the sciatic nerves (see Figures 5-8). In the mid-dose group, Number 3502 had only a small focus of minimal myelin bubbling and one focus of minimal hemorrhage in the lumbar spinal cord. No significant changes were seen in Number 3501 and Number 1501. Minimal to slight changes of questionable significance were seen in Numbers 2501 and 2502. The minimal myelin fragmentation and bubbling observed could be the result of fixation artifact since these changes were not seen consistently in any one spinal cord tract.

CONCLUSION AND SUMMARY

In conclusion, microscopic examination of the spinal cord and sciatic nerve of dogs treated with varying dosages of TPP for 20 days revealed changes indicative of a central-peripheral distal axonal neuropathy such as the "dying-back" neuropathy caused by various chemical neurotoxins (see: Experimental and Clinical Neurotoxicology, Spencer, P.S. and Schaumburg, H.H. eds., Williams and Wilkins, Baltimore, 1980). Characteristic changes were axonal degeneration with axons fragmented and staining darker than normal and bubbling and fragmentation of the myelin sheaths with moderate to marked vacuolation and focal deposition of myelin debris. Phagocytes, the gitter cells, moved in to remove the myelin and lipid debris. Occasional perivascular hemorrhage and edema accompanied the neural changes. In the high dose animals, the

extensive multifocal changes in the peripheral nervous system were accompanied by considerable degeneration of the long central nervous system tracts (e.g., the gracile, corticospinal, and spinocerebellar).

Although were was considerable fixation artifact in these canine nervous tissues, the severity of treatment-related lesions in the nervous system might be summarized as follows:

MALES

Animal Number	5002	5001	4001	3002	3001	2002	2001	1001
Average Severity	2+	3+	2+	0	±1	0	±1	0

FEMALES

Animal Number	5502	5501	4501	3502	3501	2502	2501	1501
Average Severity	2+	1+	1+	±1	0	0	0	0

Thus, there seemed to be a clear effect in the nervous systems of dogs treated with 20 or 10 mg/kg TPP. In the 5 mg/kg dose group, 1/2 females had questionable effects while 1/2 male dogs had questionable lesions. Similarly, in the 1 mg/kg dose group, 1/2 males had questionable effects while 2/2 females were negative. Because of the small numbers of animals in Groups III and II and the presence of considerable fixation artifact in the nervous tissues, it is difficult to draw any conclusions about these treatment groups. However, the trend is

suggestive of no significant lesions in dogs treated with both 5 and 1 mg/kg for the 20 day period under the conditions of this experiment.

BEVERLY Y. COCKRELL, D.V.M., Ph.D.
Pathologist

BYC/wk

HISTOPATHOLOGY INCIDENCE TABLES

GROUP	DOSE (MG/KG)
I	0
II	1
III	5
IV	10
V	20

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Male dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V									
ANIMAL	10001		20001	20002		30001	30002		40001		50001	50002								
SCIATIC NERVE, LEFT - 27	X		X	X		X	X													
Axonal Degeneration									1		3	1								
Myelin Bubbling (Vacuolation)									2		2	1								
Myelin Fragmentation									2											
SCIATIC NERVE, LEFT - 27 LFB	X		X	X		X														
Axonal Degeneration									2		3	2								
Myelin Bubbling (Vacuolation)									2		3	2								
Myelin Fragmentation							1		2		3									
SCIATIC NERVE, LEFT - 27a	X		X	X		X	X													
Axonal Degeneration									1		2	2								
Myelin Bubbling (Vacuolation)									2		2	2								
SCIATIC NERVE, LEFT - 27a LFB	X		X	X		X	X													
Axonal Degeneration									2		2	2								
Myelin Bubbling (Vacuolation)									2		2	2								
SCIATIC NERVE, RIGHT - 27b	X		X	X		X	X													
Axonal Degeneration									1		3	2								
Myelin Bubbling (Vacuolation)									1		2	1								
SCIATIC NERVE, RIGHT - 27b LFB	X		X	X			X													
Axonal Degeneration									2		3	2								
Myelin Bubbling (Vacuolation)						1			2		3	2								
Myelin Debris												1								
Myelin Fragmentation											3									

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Male dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V									
ANIMAL	1 0 0 1		2 0 0 1	2 0 0 2		3 0 0 1	3 0 0 2		4 0 0 1		5 0 0 1	5 0 0 2								
SCIATIC NERVE, RIGHT - 27c	X		X	X		X	X													
Axonal Degeneration									1		2	1								
Myelin Bubbling (Vacuolation)									1		2	1								
SCIATIC NERVE, RIGHT - 27c LFB	X		X	X		X	X													
Axonal Degeneration									2		2	2								
Myelin Bubbling (Vacuolation)									2		2	2								
SPINAL CORD, CERIVCAL, CROSS -																				
3a LFB	X		X	X		X	X													
Axonal Degeneration									1		3	2								
Myelin Bubbling (Vacuolation)									2		4	2								
SPINAL CORD, CERVICAL, CROSS -																				
3a	X		X	X		X	X													
Axonal Degeneration									1		3	2								
Myelin Bubbling (Vacuolation)									2		4	2								
Perivascular Hemorrhage and																				
Edema																				
SPINAL CORD, CERVICAL,																				
LONGITUDINAL - 3	X			X			X													
Axonal Degeneration									3		3	3								
Gitter Cells												1								
Myelin Bubbling (Vacuolation)						1			3		3	3								
Myelin Debris												1								
Myelin Fragmentation									3		2	1								

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Male dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V	
ANIMAL	1001		2001	2002		3001	3002		4001		5001	5002
SPINAL CORD, CERVICAL,												
LONGITUDINAL - 3 (CONTINUED)												
Perivascular Hemorrhage and												
Edema			1						2		2	
SPINAL CORD, CERVICAL,												
LONGITUDINAL - 3 LFB	X		X	X			X					
Axonal Degeneration									3		3	3
Gitter Cells											3	2
Myelin Bubbling (Vacuolation)						1			3			3
Myelin Debris									2		3	2
Myelin Fragmentation									3			1
SPINAL CORD, CERVICAL,												
LONGITUDINAL - 3CUT1	X		X	X		X	X					
Axonal Degeneration									3		3	3
Gitter Cells											2	1
Myelin Bubbling (Vacuolation)									3		3	3
Myelin Debris									1		2	2
Myelin Fragmentation									2		3	1
SPINAL CORD, CERVICAL,												
LONGITUDINAL - 3CUT2	X		X	X		X	X					
Axonal Degeneration									3		3	3
Gitter Cells											3	2
Myelin Bubbling (Vacuolation)									3		4	3
Myelin Debris									1		3	2

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Male dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V										
ANIMAL	1001		2001	2002		3001	3002		4001		5001	5002									
SPINAL CORD, CERVICAL,																					
LONGITUDINAL - 3CUT2																					
(CONTINUED)																					
Myelin Fragmentation									2		3	2									
SPINAL CORD, CERVICAL,																					
LONGITUDINAL - 3CUT3	X		X	X		X	X														
Axonal Degeneration									3		3	2									
Gitter Cells									1		3	1									
Myelin Bubbling (Vacuolation)									3		4	2									
Myelin Debris									2		3	1									
Myelin Fragmentation									2		3	1									
Perivascular Hemorrhage and																					
Edema									1												
SPINAL CORD, CERVICAL,																					
LONGITUDINAL - 3CUT4	X		X	X		X	X														
Axonal Degeneration									3		3	2									
Gitter Cells									1		3	1									
Myelin Bubbling (Vacuolation)									3		4	2									
Myelin Debris									1		3	1									
Myelin Fragmentation									2		3	1									
Perivascular Hemorrhage and																					
Edema									1												

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Male dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V										
ANIMAL	10001		20001	20002		30001	30002		40001		50001	50002									
SPINAL CORD, LUMBAR, CROSS -																					
3c	X			X		X	X														
Axonal Degeneration									2		3	3									
Hemorrhage			2																		
Myelin Bubbling (Vacuolation)									3		4	3									
Myelin Debris																					
Myelin Fragmentation																					
Perivascular Hemorrhage and Edema																					
SPINAL CORD, LUMBAR, CROSS -																					
3c LFB	X			X		X	X														
Axonal Degeneration									2		3	2									
Gitter Cells											2										
Hemorrhage			2																		
Myelin Bubbling (Vacuolation)									3		4	2									
Myelin Debris											2										
Myelin Fragmentation																					
SPINAL CORD, LUMBAR,																					
LONGITUDINAL - 3BCUT3	X		X	X		X	X														
Axonal Degeneration									3		4	3									
Gitter Cells											3	1									
Myelin Bubbling (Vacuolation)									3		5	3									
Myelin Debris									1		3	1									
Myelin Fragmentation									2		3	2									

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Male dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V									
ANIMAL	1 0 0 1		2 0 0 1	2 0 0 2		3 0 0 1	3 0 0 2		4 0 0 1		5 0 0 1	5 0 0 2								
SPINAL CORD, LUMBAR,																				
LONGITUDINAL - 3BCUT3																				
(CONTINUED)																				
Perivascular Hemorrhage and																				
Edema											2	1								
SPINAL CORD, LUMBAR,																				
LONGITUDINAL - 3b	X			X		X	X													
Axonal Degeneration									2		3	2								
Gitter Cells											2	2								
Hemorrhage																				
Myelin Bubbling (Vacuolation)									2		3	2								
Myelin Debris									1		2									
Myelin Fragmentation									2		2	1								
Perivascular Hemorrhage and																				
Edema			1						1		3	2								
SPINAL CORD, LUMBAR,																				
LONGITUDINAL - 3b LFB	X		X	X		X	X													
Axonal Degeneration									2		3	2								
Gitter Cells											3	2								
Myelin Bubbling (Vacuolation)									2		4	2								
Myelin Debris											3	1								
Myelin Fragmentation									2		2	1								
Perivascular Hemorrhage and																				
Edema											3	2								

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Male dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V	
ANIMAL	10001		20001	20002		30001	30002		40001		50001	50002
SPINAL CORD, LUMBAR,												
LONGITUDINAL - 3bCUT1	X		X	X		X	X					
Axonal Degeneration									3		3	3
Gitter Cells											2	2
Myelin Bubbling (Vacuolation)									3		4	3
Myelin Debris									1		2	2
Myelin Fragmentation									3		2	2
Perivascular Hemorrhage and Edema									1		2	1
SPINAL CORD, LUMBAR,												
LONGITUDINAL - 3bCUT2	X		X	X		X	X					
Axonal Degeneration									2		4	3
Gitter Cells											3	2
Myelin Bubbling (Vacuolation)									3		4	3
Myelin Debris											3	2
Myelin Fragmentation									2		3	2
Perivascular Hemorrhage and Edema											3	1
SPINAL CORD, LUMBAR,												
LONGITUDINAL - 3bCUT4	X		X	X		X	X					
Axonal Degeneration									3		4	3
Gitter Cells											3	2
Myelin Bubbling (Vacuolation)									3		4	3
Myelin Debris									1		3	2
Myelin Fragmentation									3		3	2

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Male dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V									
ANIMAL	10001		20001	20002		30001	30002		40001		50001	50002								
SPINAL CORD, LUMBAR,																				
LONGITUDINAL - 3bCUT4																				
(CONTINUED)																				
Perivascular Hemorrhage and																				
Edema											3									

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

GROUP	DOSE (MG/KG)
I	0
II	1
III	5
IV	10
V	20

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Female dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V									
ANIMAL	1501		2501	2502		3501	3502		4501		5501	5502								
SCIATIC NERVE, LEFT - 27	X		X	X		X	X		X		X									
Axonal Degeneration																				
Myelin Bubbling (Vacuolation)												2								
Myelin Fragmentation																				
SCIATIC NERVE, LEFT - 27 LFB	X		X	X		X														
Axonal Degeneration																				
Myelin Bubbling (Vacuolation)							1		3		2	2								
Myelin Fragmentation									1											
SCIATIC NERVE, LEFT - 27a	X		X	X		X	X		X		X	X								
Axonal Degeneration																				
Myelin Bubbling (Vacuolation)																				
SCIATIC NERVE, LEFT - 27a LFB	X		X	X		X	X		X		X	X								
Axonal Degeneration																				
Myelin Bubbling (Vacuolation)																				
SCIATIC NERVE, RIGHT - 27b	X		X	X		X	X		X		X									
Axonal Degeneration																				
Myelin Bubbling (Vacuolation)												2								
SCIATIC NERVE, RIGHT - 27b LFB	X		X	X		X	X													
Axonal Degeneration																				
Myelin Bubbling (Vacuolation)									2		2	2								
Myelin Debris																				
Myelin Fragmentation											2									

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Female dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V									
ANIMAL	1501		2501	2502		3501	3502		4501		5501	5502								
SCIATIC NERVE, RIGHT - 27c	X		X	X		X	X		X		X	X								
Axonal Degeneration																				
Myelin Bubbling (Vacuolation)																				
SCIATIC NERVE, RIGHT - 27c LFB	X		X	X		X	X		X		X	X								
Axonal Degeneration																				
Myelin Bubbling (Vacuolation)																				
SPINAL CORD, CERIVCAL, CROSS -																				
3a LFB	X		X	X		X	X		X		X	X								
Axonal Degeneration																				
Myelin Bubbling (Vacuolation)																				
SPINAL CORD, CERVICAL, CROSS -																				
3a	X			X		X	X		X		X	X								
Axonal Degeneration																				
Myelin Bubbling (Vacuolation)																				
Perivascular Hemorrhage and																				
Edema			2																	
SPINAL CORD, CERVICAL,																				
LONGITUDINAL - 3	X		X			X	X		X		X	X								
Axonal Degeneration																				
Gitter Cells																				
Myelin Bubbling (Vacuolation)				1																
Myelin Debris																				
Myelin Fragmentation																				

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Female dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V										
ANIMAL	1501		2501	2502		3501	3502		4501		5501	5502									
SPINAL CORD, CERVICAL,																					
LONGITUDINAL - 3 (CONTINUED)																					
Perivascular Hemorrhage and																					
Edema																					
SPINAL CORD, CERVICAL,																					
LONGITUDINAL - 3 LFB	X		X			X	X		X		X	X									
Axonal Degeneration																					
Gitter Cells																					
Myelin Bubbling (Vacuolation)				1																	
Myelin Debris																					
Myelin Fragmentation																					
SPINAL CORD, CERVICAL,																					
LONGITUDINAL - 3CUT1	X		X	X		X	X		X		X	X									
Axonal Degeneration																					
Gitter Cells																					
Myelin Bubbling (Vacuolation)																					
Myelin Debris																					
Myelin Fragmentation																					
SPINAL CORD, CERVICAL,																					
LONGITUDINAL - 3CUT2	X		X	X		X	X		X		X	X									
Axonal Degeneration																					
Gitter Cells																					
Myelin Bubbling (Vacuolation)																					
Myelin Debris																					

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Female dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V									
ANIMAL	1501		2501	2502		3501	3502		4501		5501	5502								
SPINAL CORD, CERVICAL,																				
LONGITUDINAL - 3CUT2																				
(CONTINUED)																				
Myelin Fragmentation																				
SPINAL CORD, CERVICAL,																				
LONGITUDINAL - 3CUT3	X		X	X		X	X		X		X	X								
Axonal Degeneration																				
Gitter Cells																				
Myelin Bubbling (Vacuolation)																				
Myelin Debris																				
Myelin Fragmentation																				
Perivascular Hemorrhage and																				
Edema																				
SPINAL CORD, CERVICAL,																				
LONGITUDINAL - 3CUT4	X		X	X		X	X		X		X	X								
Axonal Degeneration																				
Gitter Cells																				
Myelin Bubbling (Vacuolation)																				
Myelin Debris																				
Myelin Fragmentation																				
Perivascular Hemorrhage and																				
Edema																				

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Female dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V									
ANIMAL	15011		25012	25022		35013	35023		45014		55015	55025								
SPINAL CORD, LUMBAR, CROSS -																				
3c	X		X	X		X	X				X									
Axonal Degeneration																				
Hemorrhage																				
Myelin Bubbling (Vacuolation)												3								
Myelin Debris												2								
Myelin Fragmentation												3								
Perivascular Hemorrhage and																				
Edema									1											
SPINAL CORD, LUMBAR, CROSS -																				
3c LFB	X		X	X		X	X		X		X									
Axonal Degeneration																				
Gitter Cells																				
Hemorrhage																				
Myelin Bubbling (Vacuolation)												3								
Myelin Debris												2								
Myelin Fragmentation												3								
SPINAL CORD, LUMBAR,																				
LONGITUDINAL - 3BCUT3	X		X	X		X	X		X		X									
Axonal Degeneration												3								
Gitter Cells												3								
Myelin Bubbling (Vacuolation)												4								
Myelin Debris												4								
Myelin Fragmentation												4								

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Female dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V									
ANIMAL	1501		2501	2502		3501	3502		4501		5501	5502								
SPINAL CORD, LUMBAR,																				
LONGITUDINAL - 3BCUT3																				
(CONTINUED)																				
Perivascular Hemorrhage and																				
Edema												1								
SPINAL CORD, LUMBAR,																				
LONGITUDINAL - 3b	X			X		X			X		X									
Axonal Degeneration												3								
Gitter Cells												1								
Hemorrhage							1													
Myelin Bubbling (Vacuolation)												3								
Myelin Debris																				
Myelin Fragmentation			1									3								
Perivascular Hemorrhage and																				
Edema												1								
SPINAL CORD, LUMBAR,																				
LONGITUDINAL - 3b LFB	X		X	X		X	X		X		X									
Axonal Degeneration												3								
Gitter Cells												1								
Myelin Bubbling (Vacuolation)												3								
Myelin Debris																				
Myelin Fragmentation												3								
Perivascular Hemorrhage and																				
Edema												1								

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Female dogs

	GROUP I		GROUP II			GROUP III			GROUP IV		GROUP V	
ANIMAL	1501		2501	2502		3501	3502		4501		5501	5502
SPINAL CORD, LUMBAR,												
LONGITUDINAL - 3bCUT1	X		X	X		X	X		X		X	
Axonal Degeneration												3
Gitter Cells												2
Myelin Bubbling (Vacuolation)												3
Myelin Debris												2
Myelin Fragmentation												3
Perivascular Hemorrhage and Edema												1
SPINAL CORD, LUMBAR,												
LONGITUDINAL - 3bCUT2	X		X	X		X	X		X		X	
Axonal Degeneration												3
Gitter Cells												3
Myelin Bubbling (Vacuolation)												4
Myelin Debris												3
Myelin Fragmentation												3
Perivascular Hemorrhage and Edema												
SPINAL CORD, LUMBAR,												
LONGITUDINAL - 3bCUT4	X		X	X		X	X		X		X	
Axonal Degeneration												3
Gitter Cells												3
Myelin Bubbling (Vacuolation)												4
Myelin Debris												4
Myelin Fragmentation												4

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

HISTOPATHOLOGY INCIDENCE TABLE

81-7534A
20 Day Sacrifice
Female dogs

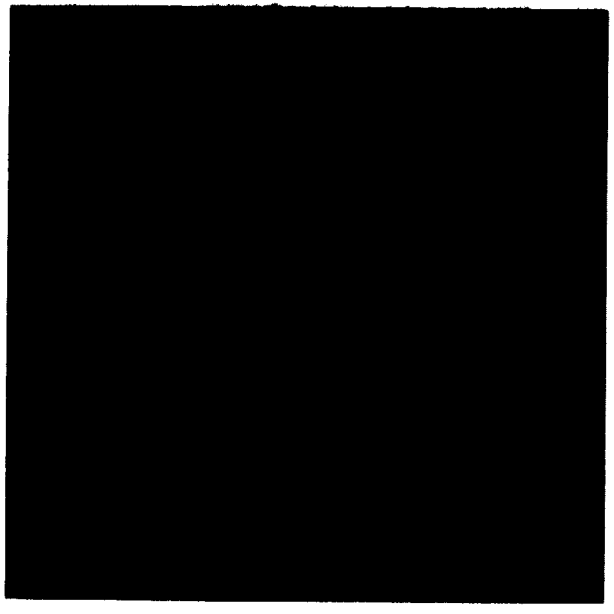
	GROUP I		GROUP II			GROUP III			GROUP IV			GROUP V								
ANIMAL	1501		2501	2502		3501	3502		4501			5501	5502							
SPINAL CORD, LUMBAR,																				
LONGITUDINAL - 3bCUT4																				
(CONTINUED)																				
Perivascular Hemorrhage and																				
Edema													1							

Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minima, 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
u=moribund sac./death

APPENDIX

BEST COPY AVAILABLE

1



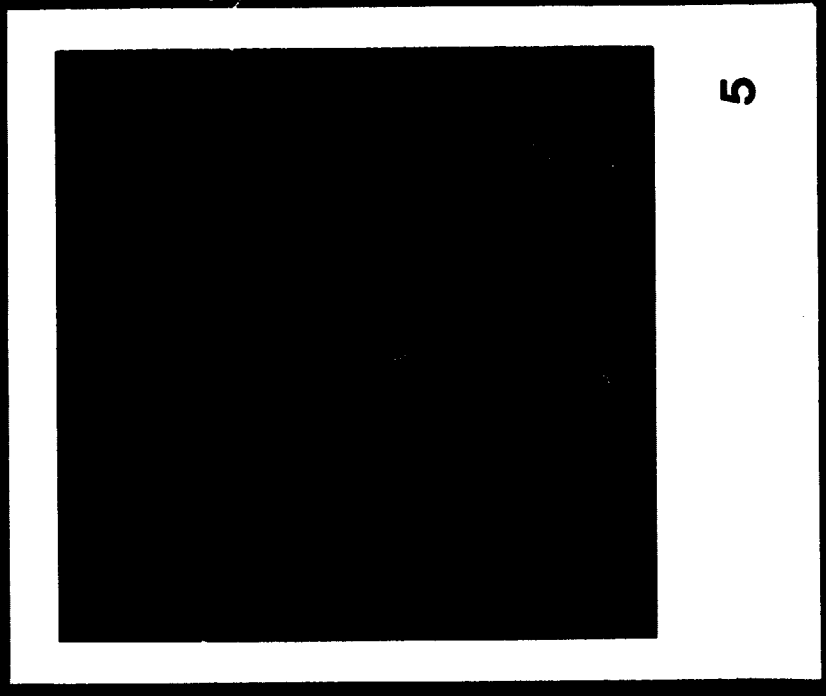
2

BEST COPY AVAILABLE

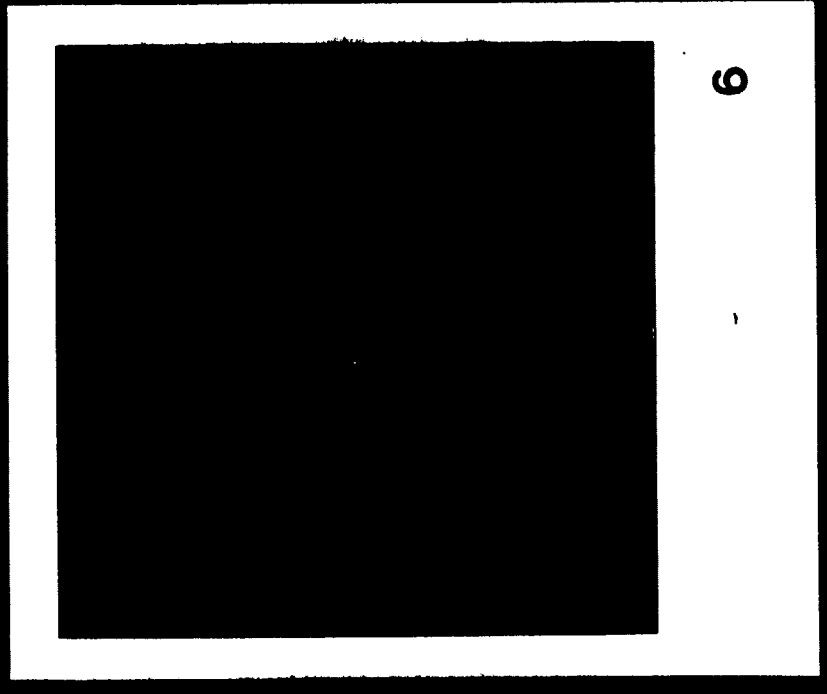
3

4

BEST COPY AVAILABLE

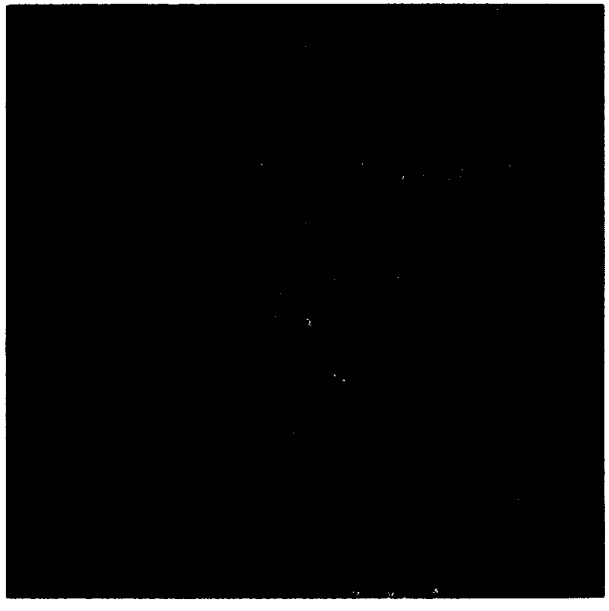


5

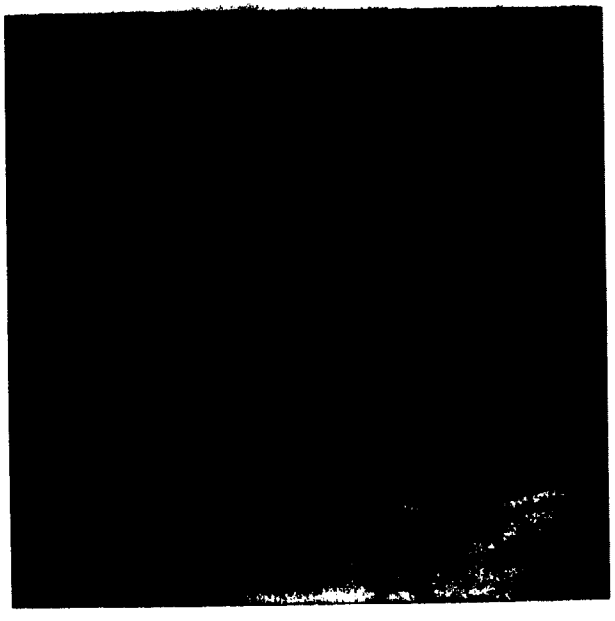


6

BEST COPY AVAILABLE



7



8

M-555

REPORT ON SECTIONS OF SPINAL CORD FROM STUDIES NUMBERED 81-7534A AND 81-7534B.

From: Professor J. B. Cavanagh, MD, FRCP, FRCPath.
Institute of Neurology, Queen Square House, WC1N 3BG

Sections consisted of transverse (T.S.) and longitudinal (L.S.) sections of spinal cord, the latter apparently being cut in a dorsoventral (sagittal or parasagittal) direction, so that they often showed dorsal columns along one side and ventral columns along the other. Occasionally obliquity of section allowed some of the lateral columns to be seen in L.S. The tissue was reasonably well fixed by immersion and was stained either with haematoxylin and eosin or with luxol fast blue. The former was the more useful; the latter is not a suitable stain for such a study as the blue stained normal myelin obscures the few degenerating fibres and the associated glial changes. It would have been more informative to have had one stained for glial cells using cresyl fast violet, and another for axons (Glees & Marsland for instance). Most of the changes reported were seen in H & E stained sections and have often been marked with an ink dot to aid localization.

Oral Study 81-7534A

1001 M control 20 days.

A few degenerating axons seen in 3 (L.S.) and in 3a and 3c with a glial focus.

1501 F control 20 days.

No abnormality seen.

1.0 mg/kg 2 day

2001 M A very occasional degenerating axon in 3b (L.S.) but none in 3, 3a or 3c.

2502 F One degenerating fibre in 3, and 3a; nil abnormal in 3b and 3c.

Ditto 20 day

2002 M No abnormality seen.

2501 F About 2 degenerating axons in 3 (L.S.) and 5 or 6 in 3a (T.S.), and one in 3b, but nil abnormal in 3c.

5.0 mg/kg 2 day

3001 M One degenerating fibre seen in 3; no abnormality in 3a, b, or c.

3502 F One degenerating fibre in 3b (L.S.), but none in 3, 3a, or 3c.

Ditto 20 day

3002 M No abnormality seen.

3501 F One degenerating fibre seen in 3c, 3, 3a and 3b no abnormality.

10 mg/kg 20 day

4001 M In all sections, moderately numerous recently degenerating axons with associated glial increase and mild macrophage infiltration. The appearance of mild chromatolysis in two motor neurons. The degenerating axons were largely confined to the lateral, dorsolateral and ventral tracts, and were about equal in 3a(L.S.) and 3b (L.S.).

10 mg/kg 20 day (Cont'd.)

The dorsal columns showed very few degenerating axons. No changes in spinal roots, and no haemorrhages or inflammatory infiltration.

4501 F One degenerating axon seen in 3, but no abnormality found in 3a, 3b or 3c.

20 mg/kg 2 day

5002 M No abnormality seen.

5501 F One degenerating axon seen only in 3b (L.S.): no abnormality in remainder.

20 mg/kg 20 day

5001 M Numerous degenerating axons of recent origin in all sections, with associated increase in glial cells and mild macrophage infiltration. The degenerating axons occur peripherally in the lateral and ventral tracts and to a minor degree in the white matter adjacent to the grey matter. The capillaries of the grey matter are congested and an occasional small haemorrhage is present, probably agonal in origin. Virtually no degenerating fibres in dorsal columns.

5502 F Numerous degenerating axons of recent origin in lateral and ventral columns in all sections, with associated glial increase and mild macrophage infiltration. The dorsal columns show very few

degenerating axons. No inflammatory infiltration and no degeneration seen in spinal roots. The capillaries in the grey matter show congestion and an occasional, probably agonal, haemorrhage.

Inhalation study 81-7534B

Control 20 days

1001 M No abnormality seen.

1501 F One degenerating axon in 3a (T.S.), but none in 3, 3b, or 3c.

0.5 mg/m^3 2 day

2002 M One fragmenting axon in 3 (L.S.) but nil abnormal in 3a, 3b, or 3c.

2501 F Three degenerating axons seen in 3 (L.S.), but nil abnormal in 3a, 3b or 3c.

Ditto 20 day

2001 M One degenerating axon seen in 3, but none in 3a, 3b, or 3c. Small capillary haemorrhages in grey matter, probably agonal.

2502 F An occasional degenerating axon seen in 3 (L.S.) and two each with a knot of glial cells in 3a (T.S.) but no changes seen in 3b or 3c.

3 mg/m^3 2 day

3002 M A very occasional degenerating axon seen in 3, 3a, 3b, but nil abnormal in 3c.

3 mg/m^3 2 day (Cont'd.)

3501 F A very occasional degenerating axon seen in all four sections about 2 or 3 per section.

Ditto 20 day

3001 M A few degenerating axons seen in 3 (L.S.), but no abnormality in 3a, 3b or 3c.

3502 F No abnormality found.

10 mg/m^3 20 day

4001 M One degenerating axon seen in 3 (L.S.) and 3a (T.S.) and two in 3b (L.S.), but none seen in 3c (T.S.)

4501 F No abnormality seen.

30 mg/m^3 2 day

5001 M Two degenerating fibres seen each in 3 (L.S.) and 3a (T.S.) but none seen in 3b, or 3c.

5501 F No abnormality seen.

30 mg/kg 20 day

5002 M Massive recent axon degeneration seen in all four sections with glial increase and mild macrophage infiltration. The axonal degeneration is largely confined to lateral and to ventral tracts but may encroach on the regions next to the grey matter. The dorsal columns show very few degenerating axons.

30 mg/kg 20 day (Cont'd.)

In 3b (L.S.) there is mild glial activation in the grey matter and small capillary haemorrhages that may be agonal. No inflammatory infiltration and spinal roots appear normal.

5502 F Massive axonal degeneration of recent origin in lateral and ventral columns encroaching on those fibres near the grey matter. There is associated glial activation and increase and mild macrophage infiltration. Dorsal columns show very few degenerating fibres. No inflammatory infiltration and no changes in spinal roots. Grey matter shows small probably agonal capillary haemorrhages.

Comments on these observations

Two kinds of change are apparent, both in the oral and in the inhalation groups, the kinds of change being different only in quantity, not in quality.

The first change is the occurrence of a few recent degenerating axons in the lateral and ventral columns, and to a lesser degree in the dorsal columns in animals in all groups including control animals. These are isolated axons undergoing fragmentation with vacuolation and degeneration of the associated myelin with the presence of an occasional myelophage sometimes lying within a vacuole. These myelophages have small nuclei and a finely foamy cytoplasm: they are probably of microglial or macrophage origin. Other cells of macrophage type are present along these isolated lines of vacuoles, and there is a mild increase in associated astroglia.

These isolated degenerating axons are seen in two of four control animals, in six of eight animals in the lowest group of the two studies, in six of eight animals in the next highest. None of these animals were seriously affected though they might show from one to six or so degenerating axons; the rest, a small percent, showed no such changes in these samplers.

The second change seen was essentially the same as the first but now large numbers of axons showed recent degeneration and along each degenerating axon the same cellular features were evident, but now in much greater quantity. The distribution of the degenerating fibres is important. It was always greatest in the periphery of the lateral and ventral tracts, perhaps sometimes greatest in the dorsolateral regions, sometimes in the lateral regions. There was never much difference between upper and lower segments of cord and thus no real suggestion of systematized distal degeneration of axons. Also there was often evidence of degeneration in the white matter close to the grey matter, which is unusual in highly systematized degeneration since these axons are often quite short intersegmental fibres. Most striking of all was the relative sparing of the dorsal columns. In some sections two or three degenerating axons were seen, in others none, even when severe damage occurred in the other tracts. This was true of both upper and lower spinal cord. Moreover, there were no changes seen in any nerve root, nor presumably in the peripheral nerves in any animal.

In the grey matter an occasional cell with a chromatolytic appearance was seen, and occasional probably agonal capillary haemorrhages were present, probably due to handling of a rather congested cord at necropsy. No real significance can be placed on these findings. No evidence suggesting infection or inflammation could be found.

It should be noted that the inhalation animals 5502 (B) and 5001 (B) were probably the worst affected, these closely followed by 5001 (A) and 5502 (A) from the oral group. 4001 (A) in the 10 mg/kg group was the least affected but degenerating fibres were many more than in the remainder. It should also be noted that 5501 (B), 5002 (A) and 5501 (A) appeared to be quite normal, and only the animals in the groups that had the longest exposure at that dose showed these effects (i.e. 20 days). In this long-term group this appeared to be a dose-related event but only one of two animals in the 10 mg/kg group was in fact affected, so that consistency was not complete. 4501 (A) indeed, seemed to be quite normal.

Are these changes a toxic effect?

Firstly, there is no doubt that these changes are not related to the systematized distal degeneration seen with neurotoxic organophosphorus compounds.

Secondly, they are quite unlike the changes associated with triphenylphosphate intoxication so far as we can see from the only existing study of the effects of this substance. Lillie and Smith's (1932) description is far from satisfactory but it is clear nonetheless that long tract degeneration was not a major feature of the condition. They emphasized the nerve

cell destruction in various parts of the nervous system. I do not know whether triphenylphosphine would be converted to or be contaminated by triphenylphosphate, but the changes found here would not lead me to believe that this was occurring.

On the other hand, the fact that there were minor changes of the same kind with the same distribution occurring in a high proportion of the animals from all groups regardless of the dosing schedule and including half the controls, should suggest the conclusion that perhaps the potentially toxic chemical might not be playing a primary role in the production of these changes.

I would like to know more about these animals and the way they have been kept, fed and handled. I would like to know particularly about their diet and whether they have been 'paunch fed' on entrails of cattle. Recently (Palmer and Medd, 1981) I have seen occurring spontaneously in 'paunch fed' beagles degeneration of axons with exactly the same features and pattern as found here. This change may be associated also with chromatolysis of nerve cells in certain brain stem nuclei, such as the dentate nuclei of the cerebellum, and of other centres. The nature of this condition is quite unknown but seems to be associated with this type of diet. I think it is unlikely to be a coincidence that your animals are showing this pattern of change, which is so remarkably similar. In your series, however, you have killed animals off before they could have developed the full change, and exposure to the triphenylphosphine seems

in some way apparently to have precipitated the condition in a proportion of animals. I do not, however, think that the chemical was primarily responsible for this pattern of neurological damage.

(J. B. Cavanagh)
MD FRCP FRCPath.

Lillie, R.D. & Smith, M.I. (1932)
Histopathology of some neurotoxic phenol esters.
Nat. Inst. Hlth. Bull. No. 160.

Palmer, A.C. & Medd, R.H. (1981)
Hound ataxia. Vet. Rec. 109, 43.

GROUP 7534A - ORAL

Animal	Sex	Amount of degeneration	Treatment	Time of exposure (Days)
1001	M	A few seen	Control	20
1501	F	Normal	"	20
2001	M	Very occasional	1.0 mg/kg	2
2502	F	About 2 seen	"	2
2002	M	Normal	"	20
2501	F	About six or so	"	20
3001	M	One only seen	5.0 mg/kg	2
3502	F	One only seen	"	2
3002	M	Normal	"	20
3501	F	One only seen	"	20
4001	M	Moderately numerous	10.0 mg/kg	20
4501	F	Normal	"	20
5002	M	Normal	20.0 mg/kg	2
5501	F	Normal	"	2
5001	M	Numerous seen	"	20
5502	F	Numerous seen	"	20

Summary of findings in oral dosing group

GROUP 7534 B - INHALATION

Animal number	Sex	Amount of degeneration	Treatment	Time of Exposure (Days)
1001	M	Normal	Control	20
1501	F	One only seen	"	20
2002	M	Normal	0.5 mg/m^3	2
2501	F	Three only seen	"	2
2001	M	One only seen	"	20
2502	F	Several seen	"	20
3002	M	Occasional seen	3.0 mg/m^3	2
3501	F	Occasional seen	"	2
3001	M	A few seen	"	20
3502	F	Normal	"	20
4001	M	About three seen	10.0 mg/m^3	20
4501	F	Normal	"	20
5001	M	About four seen	30.0 mg/m^3	2
5501	F	Normal	"	2
5002	M	Massive degeneration	"	20
5502	F	Massive degeneration	"	20

Summary of findings in inhalation dosing group

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

HOECHST CELANESE
BIO/DYNAMICS STUDY NUMBER 81-7534A

RE-EVALUATION OF A 20-DAY STUDY IN DOGS WITH TRIPHENYL PHOSPHINE (TPP)

PATHOLOGY REPORT

Submitted to:

Hoechst Celanese
Summit, NJ 07901

August 4, 1988

TABLE OF CONTENTS

Page

PATHOLOGY SUMMARY 1

HISTOPATHOLOGY INCIDENCE TABLES

Males I-1
Females I-9

APPENDIX: Photographic Illustrations

PATHOLOGY SUMMARY

HOECHST CELANESE
BIO/DYNAMICS STUDY NUMBER 81-7534A

RE-EVALUATION OF A 20-DAY STUDY IN DOGS WITH TRIPHENYL PHOSPHINE (TPP)

PATHOLOGY SUMMARY

Hematoxylin and eosin and luxol fast blue stained microscopic slides of spinal cord and sciatic nerve from 16 dogs were submitted for evaluation of central and peripheral nervous system lesions. Following is the experimental design:

Groups	I	II	III	IV	V
Dose (mg/kg)	0	1	5	10	20
Males (Nos.)	1001	2001 2002	3001 3002	4001	5001 5002
Female (Nos.)	1501	2501 2502	3501 3502	4501	5501 5502

Each hematoxylin and eosin and luxol fast blue stained slide was examined microscopically. The findings from this review appear in the attached Histopathology Incidence Tables. Summary Incidence Tables were not prepared because of the relatively few number of animals in each treatment group.

RESULTS

Males

Significant changes were seen in the Group IV and V male dogs. These changes consisted of fragmentation and bubbling of the myelin

ROBERT F. McCONNELL, D.V.M., P.A.
CONSULTING PATHOLOGY SERVICES
PHONE 201-782-4676

DIPLOMATE
AMERICAN COLLEGE
OF
VETERINARY PATHOLOGISTS

R.D. 7, WELLS ROAD
FLEMINGTON, NEW JERSEY 08822

BIO/DYNAMICS, INC.
PROJECT M-11

81-7534 A
A FOUR WEEK ORAL TOXICITY STUDY
OF TRIPHENYL PHOSPHINE
IN DOGS

81-7534 B
A FOUR WEEK INHALATION TOXICITY STUDY
OF TRIPHENYL PHOSPHINE
IN DOGS

HISTOPATHOLOGICAL EVALUATION
OF ADDITIONAL LONGITUDINAL SECTIONS
OF CERVICAL AND LUMBAR SPINAL CORD

A SUPPLEMENT TO PATHOLOGY REPORTS
RFMc 67/82

SUBMITTED TO:

ALEKSANDAR L. KNEZEVICH, DR. MED. VET.
VICE PRESIDENT, PATHOLOGY
BIO/DYNAMICS, INC.
P. O. BOX 43
METTLERS ROAD
EAST MILLSTONE, NEW JERSEY 08873

ROBERT F. McCONNELL, D.V.M.
9/7/1983

67/82

TABLE OF CONTENTS

	PAGE(S)
INTRODUCTION/MATERIALS AND METHODS	1 - 3
RESULTS/DISCUSSION	4 - 7
PATHOLOGY SUMMARY	8 - 10
INCIDENCE OF MICROSCOPIC FINDINGS:	
81-7534 A (Oral)	11 - 13
81-7534 B (Inhalation)	14 - 16
INDEX OF PHOTOMICROGRAPHS	17

BIO/DYNAMICS, INC.
PROJECT M-11

81-7534 A
A FOUR WEEK ORAL TOXICITY STUDY
OF TRIPHENYL PHOSPHINE IN DOGS

81-7534 B
A FOUR WEEK INHALATION TOXICITY STUDY
OF TRIPHENYL PHOSPHINE IN DOGS

HISTOPATHOLOGICAL EVALUATION OF ADDITIONAL LONGITUDINAL SECTIONS OF CERVICAL AND LUMBAR SPINAL CORD

A SUPPLEMENT TO PATHOLOGY
REPORTS RFMc 67/82

INTRODUCTION:

On April 26, 1982 two separate histopathology reports were submitted to Bio/dynamics which contained the data from studies M-11, 81-7534A and 81-7534B. The "A" report contained data from the oral toxicity study of triphenyl phosphine in dogs and the "B" report contained data on the inhalation toxicity of triphenyl phosphine in dogs.

In both studies one cross and one longitudinal section of cervical and lumbar spinal cord and sciatic nerve were evaluated microscopically for effects of triphenyl phosphine toxicity.

As a consequence of the microscopic evaluation, a clear compound effect was established at the high dose 20 day exposure for both the inhalation and oral studies (30 mg./m^3 inhalation and 20 mg./kg. oral). In both the lumbar and cervical spinal cord sections from dogs at these levels numerous axons were undergoing degenerative changes.

In several other dosage groups of both the oral and inhalation tests, however, single or scattered individual degenerating axons were also noted. The axonal lesion was not observed in control dogs. The sporadic axonal change was present in dogs of lower dosage groups but was not present at higher levels and in some cases occurred in 2-day exposure dogs but not in 20-day exposure dogs at the same dosage level. There was no clear dose effect and the data did not establish a "no effect level" for which the study had been designed.

Due to the sporadic occurrence of the low level axonal degeneration at the lower dosage levels, it was difficult to determine whether the lesions were treatment associated or whether they were an on-going natural degenerative process.

The slides were submitted to Professor J. B. Cavanagh, M.D., of the Institute of Neurology in London for a second opinion. His evaluation confirmed the findings in the treated animals. In addition, Dr. Cavanagh observed what he interpreted to be axonal lesions in 1 oral vehicle control male and in 1 control inhalation female. He concluded, however, that the lesions were not primarily due to triphenyl phosphine treatment. (See Cavanagh report.)

It was decided that additional spinal cord sections should be evaluated. Four longitudinal step sections of cervical and lumbar spinal cord were prepared from the original paraffin blocked specimens. The longitudinal orientation of the cord sections plus the additional 4 sections of each cord segment provided a greater number of axons for examination.

The histopathological evaluation of these additional sections forms the basis of this supplemental pathology report.

MATERIALS AND METHODS:

The paraffin blocked specimens of cervical and lumbar spinal cord from animals in 81-7534 A and 81-7534 B were resectioned at wide intervals to provide an increased surface area for axonal examination. Four new sections from each block of longitudinal cervical and lumbar cord were prepared and stained with hematoxylin and eosin.

Each slide was identified by the project number, dog number, location [cervical (3), lumbar (3b)] and cut number (1 thru 4).

In the evaluation of the cord section a single vacuole without evidence of axonal degeneration or a single clump of glial cells without evidence of axonal degeneration were not included in the findings.

These alterations were not characteristic of the spinal cord lesions induced by triphenyl phosphine in high dosage level dogs.

The microscopic findings for dogs from 81-7534 A and 81-7534 B can be found on pages 11 thru 16 of this report.

BIO/DYNAMICS, INC.
PROJECT M-11

81-7534 A
A FOUR WEEK ORAL TOXICITY STUDY
OF TRIPHENYL PHOSPHINE IN DOGS

81-7534 B
A FOUR WEEK INHALATION TOXICITY STUDY
OF TRIPHENYL PHOSPHINE IN DOGS

HISTOPATHOLOGICAL EVALUATION OF ADDITIONAL
LONGITUDINAL SECTIONS
OF CERVICAL AND LUMBAR SPINAL CORD

A SUPPLEMENT TO PATHOLOGY
REPORTS RFMc 67/82

RESULTS:

The evaluation of 4 cervical and 4 lumbar spinal cord sections from each dog on each study allowed for a much greater surface area to be studied. Since the axonal lesions tended to run in a longitudinal direction, often the course of the damaged axon could be traced for a long distance in the specimen. When multiple damaged axons were present, counting each one affected was difficult because they were often not parallel within the plane of sectioning and coursed in and out of the thinly cut specimen.

This evaluation revealed damaged axons in 2 control dogs of Study 81-7534 A (oral). The male dog had one damaged axon in (3b) cut 2 and the female had 2 damaged axons in (3b) cut 2. The male and female control dogs from Study 81-7534 B (inhalation) had no axonal lesion.

As can be seen in the microscopic findings of the oral study, pages 11 thru 13, dogs of the 2 and 20 day 1.0 and 5.0 mg./kg. levels had no lesion or only sporadic axons involved. At the 20 mg./kg. 2 day level both dogs were similar to controls.

A dose effect appeared to have been present only in the 10 and 20 mg./kg. dogs dosed orally for 20 days. In the 10 mg./kg. dogs numerous axons were involved but at the 20 mg./kg. level the damaged axons were too numerous to count.

Similar results were noted in the 81-7534 B (inhalation) study. Although both controls were free of the axonal lesions, controls in the "A" study established that a low level of axonal lesions are to be expected. If this is the case, then a "no effect" level was noted for the 0.5 and 3.0 mg./m^3 dogs at 2 and 20 days; it was present for 10 mg./m^3 dogs at 20 days; and was present in 30 mg./m^3 dogs after 2 days of inhalation exposure.

Based on this new data, the only toxicological effect occurred in dogs at the high dose level (30 mg./m^3) after 20 days of inhalation exposure and at 10 and 20 mg./kg. after 20 days of oral dosing.

DISCUSSION:

Axons have but a single way morphologically to express injury. They undergo wallerian degeneration which is a well documented pathological phenomena(1). In the human, it has been estimated that up to 25% of all adult nerve cells die by the eighth or ninth decade of life. Subsequently the associated axons also die and undergo the process of wallerian degeneration.(2) It should not be unexpected then to see a normal background incidence of wallerian degeneration in spinal cord specimens from every individual if an extensive search is conducted. One would expect that certain individuals would be more affected than others due to

1. Robbins, S.L. "Pathologic Basis of Disease" W.B. Saunders Co., Phila. 1974, p. 1529.
2. Gardner, E. "Fundamentals of Neurology" W.B. Saunders Co., Phila. 3rd Ed. 1958, p. 85.

biological variability. Such appears to have been the case in both of these studies. The low level incidence noted in the initial pathology reports was based on a subjective grading and a small sampling. The data could not be evaluated accurately and a "no effect level" was not achieved.

By increasing the sample size and by reviewing specimens in their optimum plane of orientation necessary to expose the axons, control animals were also shown to have the axonal lesions. The sporadic low level lesions noted in treated dogs were then put into the proper prospective when compared to control animals. They were then judged to have been unassociated with treatment.

The following are conclusions based on this supplemental histopathology study of triphenyl phosphine treated dogs:

1. Sporadic wallerian degeneration of axons is an expected finding in spinal cord sections of "normal" animals.
2. Low level axonal degeneration in the spinal cord of treated dogs from the oral and inhalation studies was considered to have been similar to axonal lesions noted in 2 control dogs. At this incidence level it was judged to have been unrelated to treatment.
3. For the oral toxicity study of triphenyl phosphine a "no effect level" was shown to be present at 1.0 and 5.0 mg./kg. for 2 and 20 days of oral treatment and at 20.0 mg./kg. for 2 days of oral treatment. Compound related axonal degeneration was noted at 10.0 and 20.0 mg./kg. after 20 days of continuous oral treatment.

4. For the inhalation toxicity study of triphenyl phosphine, a "no effect level" was shown to be present at 0.5 and 3.0 mg./m^3 for 2 and 20 days, at 10.0 mg./m^3 for 20 days, and at 30.0 mg./m^3 for 2 days of inhalation exposure.

   Inhalation exposure to triphenyl phosphine at levels of 30.0 mg./m^3 for 20 days resulted in widespread spinal cord axonal degeneration.

BIO/DYNAMICS, INC.
PROJECT M-11

81-7534 A
A FOUR WEEK ORAL TOXICITY STUDY
OF TRIPHENYL PHOSPHINE IN DOGS

81-7534 B
A FOUR WEEK INHALATION TOXICITY STUDY
OF TRIPHENYL PHOSPHINE IN DOGS

HISTOPATHOLOGICAL EVALUATION OF ADDITIONAL
LONGITUDINAL SECTIONS
OF CERVICAL AND LUMBAR SPINAL CORD

A SUPPLEMENT TO PATHOLOGY
REPORTS RFMc 67/82

PATHOLOGY SUMMARY:

Two studies of the toxicity of triphenyl phosphine were conducted in dogs. One was by the oral route, the other by inhalation exposure. The oral study was conducted at levels of 1.0, 5.0, and 20 mg./kg. for 2 and 20 days and at 10 mg./kg. for 20 days only. The inhalation study was conducted at levels of 0.5, 3.0, and 30.0 mg./m^3 for 2 and 20 days and at 10 mg./m^3 for 20 days only. One male and one female were utilized in each dosage group and for each duration period. Two controls were present in each study.

In a prior pathology report, compound associated axonal degeneration was noted in cervical and lumbar spinal cord sections at the two highest dosage levels in each study (20 mg./kg. oral and 30 mg./m^3 inhalation) after 20 days of treatment. A "no effect level", however, was not established for any of the remaining test groups due to the sporadic low level incidence of axonal degeneration which was noted in several dogs in various dosage groups. Controls were not affected.

A second opinion from a neuropathologist confirmed the spinal cord lesions. In addition, his report indicated that axonal degeneration was present in 2 control animals.

It was decided to examine additional longitudinal sections of lumbar and cervical cord from the original paraffin blocked specimens. Four additional step-sectioned longitudinal H and E stained sections of cervical and lumbar spinal cord from each dog were examined microscopically.

Two control dogs were found to have axonal lesions. These findings indicated that a normal background low incidence of axonal degeneration is present in spinal cord sections of untreated dogs. This additional information allowed for the proper interpretation of low level axonal lesions in dogs of the lower dosage groups of both studies.

As a result of this new data, the following conclusions concerning the two studies were made:

1. Sporadic wallerian degeneration of axons is an expected finding in spinal cord sections of "normal" animals.
2. Low level axonal degeneration in the spinal cord of treated dogs from the oral and inhalation studies were considered to have been similar to axonal lesions noted in 2 control dogs. They were judged to have been unrelated to treatment.
3. For the oral toxicity study of triphenyl phosphine a "no effect level" was shown to be present at 1.0 and 5.0 mg./kg. for 2 and 20 days of oral treatment and at 20.0 mg./kg. for 2 days of oral treatment. A compound related axonal degeneration was noted at 10.0 and 20.0 mg./kg. after 20 days of continuous oral treatment.

4. For the inhalation toxicity study of triphenyl phosphine, a "no effect level" was shown to be present at 0.5 and 3.0 mg./m^3 after 2 and 20 days of inhalation exposure, at 10.0 mg./m^3 after 20 days of inhalation exposure, and at 30.0 mg./m^3 after 2 days of inhalation exposure. When dogs were exposed to 30.0 mg./m^3 for 20 days by the inhalation route widespread compound related spinal cord axonal degeneration was induced.

BIO/DYNAMICS, INC.
A FOUR WEEK ORAL TOXICITY STUDY
OF TRIPHENYL PHOSPHINE
IN DOGS

I, CONTROL CORN OIL

♂	1001	(3)	Cut 1	WNL	♀	1501	(3)	Cut 1	WNL
	20 days on test	(3)	Cut 2	WNL		20 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	WNL			(3)	Cut 3	WNL
		(3)	Cut 4	WNL			(3)	Cut 4	WNL
		(3b)	Cut 1	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 2	One fiber affected but an equivocal finding			(3b)	Cut 2	Two fibers affected.
							(3b)	Cut 3	WNL
		(3b)	Cut 3	WNL					
		(3b)	Cut 4	WNL					

II, 1.0 mg./kg. TPP

♂	2001	(3)	Cut 1	WNL	♀	2501	(3)	Cut 1	WNL
	2 days on test	(3)	Cut 2	WNL		20 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	One fiber affected			(3)	Cut 3	WNL
							(3)	Cut 4	WNL
		(3)	Cut 4	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 1	WNL			(3b)	Cut 2	One fiber affected.
		(3b)	Cut 2	WNL			(3b)	Cut 3	WNL
		(3b)	Cut 3	WNL			(3b)	Cut 4	One fiber affected.
		(3b)	Cut 4	WNL					
♂	2002	(3)	Cut 1	WNL	♀	2502	(3)	Cut 1	One fiber affected.
	20 days on test	(3)	Cut 2	WNL		2 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	WNL			(3)	Cut 3	WNL
		(3)	Cut 4	WNL			(3)	Cut 4	WNL
		(3b)	Cut 1	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 2	WNL			(3b)	Cut 2	WNL
		(3b)	Cut 3	WNL			(3b)	Cut 3	WNL
		(3b)	Cut 4	WNL			(3b)	Cut 4	WNL

WNL = within normal limits

BIO/DYNAMICS, INC.
A FOUR WEEK ORAL TOXICITY STUDY
OF TRIPHENYL PHOSPHINE
IN DOGS

III, 5.0 mg./kg. TPP

♂	3001	(3)	Cut 1	WNL	♀	3502	(3)	Cut 1	WNL
	2 days on test	(3)	Cut 2	WNL		2 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	WNL			(3)	Cut 3	WNL
		(3)	Cut 4	WNL			(3)	Cut 4	WNL
		(3b)	Cut 1	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 2	WNL			(3b)	Cut 2	WNL
		(3b)	Cut 3	WNL			(3b)	Cut 3	WNL
		(3b)	Cut 4	WNL			(3b)	Cut 4	WNL
♂	3002	(3)	Cut 1	WNL	♀	3501	(3)	Cut 1	WNL
	20 days on test	(3)	Cut 2	WNL		20 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	WNL			(3)	Cut 3	WNL
		(3)	Cut 4	WNL			(3)	Cut 4	WNL
		(3b)	Cut 1	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 2	WNL			(3b)	Cut 2	WNL
		(3b)	Cut 3	WNL			(3b)	Cut 3	WNL
							(3b)	Cut 4	WNL

IV, 10 mg./kg. TPP

♂	4001	*(3)	Cut 1	4 fibers affected	♀	4501	(3)	Cut 1	~ 2 fibers affected
	20 days on test	(3)	Cut 2	4 fibers affected		20 days on test	(3)	Cut 2	1 fiber affected.
		(3)	Cut 3	~ 5 fibers affected			(3)	Cut 3	WNL
		(3)	Cut 4	~ 5 fibers affected			(3)	Cut 4	~ 4 fibers affected
		(3b)	Cut 1	~ 6 fibers affected			(3b)	Cut 1	WNL) Cut close to) mid line, may
		(3b)	Cut 2	~ 6 fibers affected			(3b)	Cut 2	WNL) not be in area) where most
		(3b)	Cut 3	~ 2 fibers affected			(3b)	Cut 3	WNL) fibers are) affected.
		(3b)	Cut 4	~ 3 fibers affected			(3b)	Cut 4	WNL)

*All changes are nearing the final clean-up stage - are less prominent.

WNL = within normal limits

BIO/DYNAMICS, INC.
A FOUR WEEK ORAL TOXICITY STUDY
OF TRIPHENYL PHOSPHINE
IN DOGS

V, 20 mg./kg. TPP

♂	5002	(3)	Cut 1	WNL		♀	5501	(3)	Cut 1	WNL	
	2 days on test	(3)	Cut 2	WNL			2 days on test	(3)	Cut 2	2 fibers affected	
		(3)	Cut 3	One fiber affected				(3)	Cut 3	WNL	
								(3)	Cut 4	WNL	
		(3)	Cut 4	WNL				(3b)	Cut 1	WNL	
		(3b)	Cut 1	WNL				(3b)	Cut 2	WNL	
		(3b)	Cut 2	One fiber affected				(3b)	Cut 3	WNL	
		(3b)	Cut 3	WNL				(3b)	Cut 4	WNL	
		(3b)	Cut 4	WNL							
♂	5001	(3)	Cut 1	TNTC	Lesions only in ventral columns	♀	5502	(3)	Cut 1	TNTC	Both dorsal and ventral tracts
	20 days on test	(3)	Cut 2	TNTC			20 days on test	(3)	Cut 2	TNTC	
		(3)	Cut 3	TNTC				(3)	Cut 3	TNTC	
		(3)	Cut 4	WNL				(3)	Cut 4	TNTC	
		(3b)	Cut 1	TNTC				(3b)	Cut 1	TNTC	
		(3b)	Cut 2	TNTC				(3b)	Cut 2	TNTC	
		(3b)	Cut 3	TNTC				(3b)	Cut 3	TNTC	
		(3b)	Cut 4	TNTC				(3b)	Cut 4	TNTC	

WNL = within normal limits
TNTC = too numerous to count

BIO/DYNAMICS, INC.
A FOUR WEEK INHALATION TOXICITY STUDY
OF TRIPHENYL PHOSPHINE
IN DOGS

LONGITUDINAL SECTIONS OF CERVICAL & LUMBAR SPINAL CORD

I, XYLENE VEHICLE CONTROL

♂	1001	(3)	Cut 1	WNL	♀	1501	(3)	Cut 1	WNL
	20 days on test	(3)	Cut 2	WNL		20 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	WNL			(3)	Cut 3	WNL
		(3)	Cut 4	WNL			(3)	Cut 4	WNL
		(3b)	Cut 1	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 2	WNL			(3b)	Cut 2	WNL
		(3b)	Cut 3	WNL			(3b)	Cut 3	WNL
		(3b)	Cut 4	WNL			(3b)	Cut 4	WNL

II, 0.5 mg./m^3 TPP

♂	2001	(3)	Cut 1	2 foci appear to be same axon	♀	2502	(3)	Cut 1	WNL
	20 days on test	(3)	Cut 2	WNL		20 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	WNL			(3)	Cut 3	WNL
		(3)	Cut 4	WNL			(3)	Cut 4	WNL
		(3b)	Cut 1	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 2	WNL			(3b)	Cut 2	WNL
		(3b)	Cut 3	WNL			(3b)	Cut 3	WNL
		(3b)	Cut 4	WNL			(3b)	Cut 4	WNL
♂	2002	(3)	Cut 1	WNL	♀	2501	(3)	Cut 1	WNL
	2 days on test	(3)	Cut 2	WNL		2 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	WNL			(3)	Cut 3	WNL
		(3)	Cut 4	WNL			(3)	Cut 4	WNL
		(3b)	Cut 1	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 2	WNL			(3b)	Cut 2	One fiber affected
		(3b)	Cut 3	WNL			(3b)	Cut 3	One fiber affected
		(3b)	Cut 4	WNL			(3b)	Cut 4	One fiber affected

WNL = within normal limits

BIO/DYNAMICS, INC.
A FOUR WEEK INHALATION TOXICITY STUDY
OF TRIPHENYL PHOSPHINE
IN DOGS

III, 3.0 mg./m^3 TPP

♂	3002	(3)	Cut 1	WNL	♀	3501	(3)	Cut 1	One fiber affected
	2 days on test	(3)	Cut 2	One fiber affected		2 days on test	(3)	Cut 2	One fiber affected
		(3)	Cut 3	WNL			(3)	Cut 3	Four fibers affected
		(3)	Cut 4	One fiber affected			(3)	Cut 4	One fiber affected
		(3b)	Cut 1	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 2	WNL			(3b)	Cut 2	Two fibers affected
		(3b)	Cut 3	WNL			(3b)	Cut 3	One fiber affected
		(3b)	Cut 4	WNL			(3b)	Cut 4	One fiber affected
♂	3001	(3)	Cut 1	WNL	♀	3502	(3)	Cut 1	WNL
	20 days on test	(3)	Cut 2	One fiber affected		20 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	WNL *			(3)	Cut 3	WNL
		(3)	Cut 4	WNL			(3)	Cut 4	WNL
		(3b)	Cut 1	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 2	WNL			(3b)	Cut 2	WNL
		(3b)	Cut 3	WNL			(3b)	Cut 3	WNL
		(3b)	Cut 4	WNL			(3b)	Cut 4	WNL

*Considerable artifactual shrinkage and vacuolation - analysis was difficult.

IV, 10.0 mg./m^3 TPP

♂	4001	(3)	Cut 1	WNL	♀	4501	(3)	Cut 1	WNL
	20 days on test	(3)	Cut 2	One fiber affected		20 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	WNL			(3)	Cut 3	WNL
		(3)	Cut 4	One fiber affected			(3)	Cut 4	WNL
		(3b)	Cut 1	WNL			(3b)	Cut 1	WNL
		(3b)	Cut 2	WNL			(3b)	Cut 2	WNL
		(3b)	Cut 3	WNL			(3b)	Cut 3	WNL
		(3b)	Cut 4	WNL			(3b)	Cut 4	WNL

WNL = within normal limits

BIO/DYNAMICS, INC.
A FOUR WEEK INHALATION TOXICITY STUDY
OF TRIPHENYL PHOSPHINE
IN DOGS

V, 30 mg./m^3 TPP

♂	5001	(3)	Cut 1	One fiber affected	♀	5501	(3)	Cut 1	WNL
	2 days on test	(3)	Cut 2	Two fibers affected		2 days on test	(3)	Cut 2	WNL
		(3)	Cut 3	WNL			(3)	Cut 3	WNL
		(3)	Cut 4	3 fibers affected			(3)	Cut 4	WNL
		(3b)	Cut 1	One fiber affected			(3b)	Cut 1	WNL
		(3b)	Cut 2	Two fibers affected			(3b)	Cut 2	WNL
		(3b)	Cut 3	WNL			(3b)	Cut 3	WNL
		(3b)	Cut 4	WNL			(3b)	Cut 4	WNL
♂	5002	(3)	Cut 1	Too numerous to count	♀	5502	(3)	Cut 1	TNTC
	20 days on test	(3)	Cut 2	TNTC		20 days on test	(3)	Cut 2	TNTC
		(3)	Cut 3	TNTC			(3)	Cut 3	TNTC
		(3)	Cut 4	TNTC			(3)	Cut 4	TNTC
		(3b)	Cut 1	TNTC			(3b)	Cut 1	TNTC
		(3b)	Cut 2	TNTC			(3b)	Cut 2	TNTC
		(3b)	Cut 3	TNTC			(3b)	Cut 3	TNTC
		(3b)	Cut 4	TNTC			(3b)	Cut 4	TNTC

WNL = within normal limits
TNTC = too numerous to count

81-7534A
81-7534B

PHOTOMICROGRAPHS OF REPRESENTATIVE SPINAL CORD
AXONAL LESIONS

1. 81-7534A ♀ 1501 xylene control 20 days (3b) cut 2
   Single axon degeneration. Note the vacuolated areas near the center of the field. 100x

2. 81-7534A ♀ 1501 xylene control 20 days (3b) cut 2
   Same as above. 250x

3. 81-7534B ♂ 2001 0.5 mg./m.^{3} TPP 20 days (3) cut 1
   Single axon degeneration. 100x

4. 81-7534B ♂ 2001 0.5 mg./m.^{3} TPP 20 days (3) cut 1
   Same axon as shown above. 250x

5. 81-7534B ♂ 5001 20 mg./kg. oral TPP 20 days (3) cut 1
   Multiple affected axons. Too numerous to count in each microscopic field. 100x

6. 81-7534B ♂ 5001 20 mg./kg. oral TPP 20 days (3) cut 1
   Same field as above. 250x

7. 81-7534B ♂ 5002 30 mg./m.^{3} inhalation TPP 20 days (3b) cut 4
   Multiple affected axons. Too numerous to count in each microscopic field. 100x

8. 81-7534B ♂ 5002 30 mg./m.^{3} inhalation TPP 20 days (3b) cut 4
   Same field as above. 250x

81-7534A
81-7534B

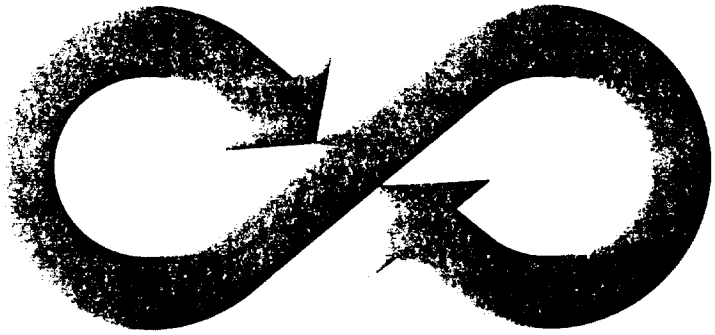
Quality Assurance Statement

A report entitled "Histopathological Evaluation of Additional Longitudinal Sections of Cervical and Lumbar Spinal Cord, A Supplement to Pathology Reports" was inspected by the Quality Assurance Unit of Bio/dynamics, Inc. on 9/1/83 and 9/2/83. All findings were reported to the Study Director on 9/13/83 and to Management on 9/13/83.

Craig M. Lamb, B.A.
Manager of Quality Assurance
and Science Information

9/13/83
Date

Contains No CBI



Bio/dynamics Inc.

Toxicological Resources Unit

PROJECT NO. 5082-78

AN ACUTE ORAL TOXICITY STUDY IN HENS

COMPOUND: Triphenylphosphine (M&T)

Submitted to: M & T Chemicals, Inc.
Rahway, New Jersey

Date: June 22, 1978

I. GENERAL

An acute oral toxicity study was conducted in hens for M & T Chemicals, Inc., using Triphenylphosphine (M & T). The test material, a white powder, was received on January 31, 1978 and was labeled: 9-10-77; 2-3; $\emptyset_3P$ FCVAH-13K; Retain: R. Hontu.

II. MATERIALS AND METHODS

White Leghorn hens (Kerr Hatcheries; Frenchtown, New Jersey), weighing 1.35 to 1.60 kg, were used in this study. The animals were housed individually with food and water provided ad libitum.

Following a preliminary range-finding study, the test material was prepared as a 25% w/v solution in corn oil (gentle heating required for full dissolution) and administered to ten hens at a single dose level of 5.0 g/kg (20.0 ml/kg). The test solution was administered by oral intubation using an eight-inch length of bevelled tygon tubing.

Observations for mortality and overt signs of effect were performed at one and four hours following dosing and daily thereafter for fourteen days. Observations for signs of neurological impairment were performed pretest (Day 0), and on Days 1, 3, 7 and 14. Body weights were recorded initially and terminally.

At the end of the fourteen-day study period all hens were sacrificed using an overdose of sodium pentobarbital. A gross necropsy was performed on each hen.

III. RESULTS

Individual body weights and necropsy observations are presented in Table I. Individual results of each neurological evaluation, along with a

III. RESULTS (cont.)

description of the scoring scale, are presented in Table II.

No animals died during this study. The oral LD_{50} for Triphenylphosphine (M & T) in hens is therefore greater than 5.0 g/kg.

Five hens exhibited doubtful (or minor) signs of neurological impairment on Day 1; none of the hens exhibited these signs on Day 3. One hen displayed doubtful (or minor) signs on Day 7, while another hen received a doubtful (or minor) score on Day 14.

The following in-life signs of effect were noted during this study: liquid stool (24 hours - 5 of 10 animals, 48 hours - 5/10) fecal staining of the abdomen (24 hours - 5/10, 48 hours - 5/10), and lethargy (24 hours - 1/10, 48 hours - 4/10, 72 hours - 3/10). Lethargy was noted sporadically throughout the study. Ataxia was noted in three animals on Day 5, two on Day 6, one on Days 7 and 8, and two on Day 9. One animal lost weight over the fourteen-day study period.

At the terminal sacrifice discoloration and/or mottling of the liver was noted in seven of the ten hens. No other abnormalities were noted in any animal.

Under the conditions of this study Triphenylphosphine (M&T) was not found to produce permanent locomotor impairment.

Pamela R. Heenehan, B.S.
Staff Supervisor

William E. Rinehart, Sc.D.
Director, Division of Biology
and Safety Evaluation

William G. Braun, M.S.
Manager

TABLE I

AN ACUTE ORAL TOXICITY STUDY IN HENS

COMPOUND: Triphenylphosphine (M&T)

Individual Body Weights and Necropsy Observations

Dose Level g/kg	Animal Number	Body Weights - kg Initial	Terminal	Necropsy Observations
5.0	88	1.50	1.75	Liver: brown
	89	1.40	1.55	Liver: left lobe brown
	90	1.35	1.40	NOA
	91	1.45	1.50	Liver: tan
	92	1.40	1.60	Liver: yellow
	93	1.60	1.65	Liver: mottled
	94	1.40	1.35	Liver: mottled, red and brown
	95	1.40	1.45	NOA
	96	1.40	1.55	Liver: brown
	97	1.45	1.50	NOA

NOA - No Observable Abnormalities

TABLE II

AN ACUTE ORAL TOXICITY STUDY IN HENS

COMPOUND: Triphenylphosphine (M&T)

Individual Neurological Scores

Dose Level g/kg	Animal Number	Day 0 (Pretest)	1	3	7	14
5.0	88	0	1	0	0	0
	89	0	0	0	1	0
	90	0	1	0	0	0
	91	0	1	0	0	0
	92	0	0	0	0	0
	93	0	1	0	0	0
	94	0	0	0	0	1
	95	0	1	0	0	0
	96	0	0	0	0	0
	97	0	0	0	0	0

Scoring Scale:

Symptom free..... 0
Doubtful or minor symptoms (includes leg weakness)..... 1
Positive paralytic symptoms (lack of leg coordination, loss of balance, tendency to fall backwards)..... 2
Advanced paralytic symptoms (inability to walk and hyperextension, ataxia, complete prostration, morbidity)..... 3
Death..... 4

Bio/dynamics Inc.

T-11

Acute Toxicity of TPPO to Dogs

Preliminary Report

METHODS:

TPPO, as supplied by M & T Chemicals, was administered to Beagle dogs, per os, in corn oil. Four (4) dogs, 2 male and 2 female, were given a single dose of 500mg/kg and six (6) dogs, 3 male and 3 female, were given a single dose of 300mg/kg. Concentrations in corn oil were 100mg/ml for both dose levels. Animals were dosed on November 30, 1978 from 9:00 - 10:20 AM for the 500mg/kg animals and from 10:30 AM - 12:00 Noon for the 300mg/kg animals.

RESULTS:

Within about 15 minutes of dosing, classic cholinergic signs of toxicity were observed. Included were tremors, emesis, frothing, diarrhea, prostration and convulsions which proceeded to death in all animals from both groups. Numbers and approximate time to death were as follows:

500mg/kg			300mg/kg		
Number	Sex	Time to Death	Number	Sex	Time to Death
4364	M	11:00AM 11/30/78	4356	M	Found dead 8:30AM 12/1/78
4370	M	Found dead 8:30AM 12/1/78	4361	M	12:00PM 11/30/78
4381	F	11:30AM 11/30/78	4367	M	12:00PM 12/1/78*
4385	F	12:00PM 11/30/78	4377	F	1:00PM 11/30/78
			4378	F	3:30PM 11/30/78
			4391	F	1:30PM 11/30/78

* Euthanized in extreme moribund condition.

Mettlers Road • East Millstone, New Jersey 08873 • (201) 873-2550

RESULTS (Cont.):

Gross necropsies were performed on all animals and the following tissues were preserved for possible histopathological evaluation:

brain
spinal cord (lumbar, cervical)
sciatic nerve (R/L)
lung

Findings on autopsy included:

hemorrhaging of lungs (all animals except 4385)
stomach distention (4377, 4378, 4364, 4361)
firm (contracted) heart (4385, 4364, 4378)

DISCUSSION:

TPPO, administered to beagle dogs per os, at dose levels of 500 or 300 mg/kg produced a rapid onset of the classic signs of cholinergic toxicity. All animals succumbed to these dose levels. The oral LD_{50} to dogs is, therefore, less than 300mg/kg. Additional work is contemplated at lower levels to more precisely define the acute toxic level in dogs.

Daniel J. Pisacreta, Supervisor
Large Animal Section

William E Rinehart 12/11/78

William E. Rinehart, Sc.D.
Vice President - Science

Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO. 79-2379

A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS WITH TRIPHENYLPHOSPHINE OXIDE

Submitted to: M&T Chemicals, Inc.
Rahway, New Jersey 07065

Date: June 29, 1979

79-2379

TABLE OF CONTENTS

I. INTRODUCTION..... 1

II. MATERIALS AND METHODS..... 1

A. Test Material..... 1
B. Vehicle..... 1
C. Test Animals..... 1
D. Selection and Group Assignments..... 2
E. Animal Identification..... 2
F. Experimental Outline..... 3
G. Husbandry..... 3
H. Test Material Administration..... 3
I. Observations..... 4
J. Body Weight..... 4
K. Laboratory Studies..... 4
L. Post-Mortem..... 4

III. RESULTS AND DISCUSSION..... 5

A. Mortality..... 5
B. Observations..... 5
C. Neurological Examinations..... 6
D. Body Weights..... 6
E. Cholinesterase..... 6
F. Gross Pathology..... 6

IV. SUMMARY..... 7

TABLES

I. Individual Body Weights and Mortality Data..... 9
II. Individual Neurological Evaluations..... 10
III. Individual Plasma and Brain Cholinesterase Values..... 16

APPENDICES

A. Methodology and References..... A-1
B. Gross Pathology Summary..... B-1

79-2379

I. INTRODUCTION

The purpose of this study, conducted for M&T Chemicals, Inc., was to assess the reversibility of any neurotoxic or other adverse effects following daily administration of triphenylphosphine oxide (TPPO) for 21 days. Following the twenty-one day dosing regimen, the animals were observed for an additional four weeks to evaluate the possible reversibility of effects. All animals were sacrificed on Day 51 of the study. This study was performed at Bio/dynamics, Inc., East Millstone, New Jersey.

II. MATERIALS AND METHODS

A. Test Material:	Triphenylphosphine oxide
Concentration:	50 mg/kg/day
Supplier:	M&T Chemicals, Inc. Rahway, New Jersey
Description:	Off-white powder
Date Received:	26 September 1978
Label Information:	T.P.P.O. VYVCD - IR #2
Storage:	Room Temperature
B. Vehicle:	Mazola® Corn Oil
C. Test Animals:	Dogs
Strain:	Beagle
Number of Animals:	12 (6/sex)
Purchased:	22 (11/sex)
Placed on Test:	12 (6/sex)
Supplier:	Marshall Research Animals, Inc. North Rose, New York

79-2379

Date Received:	20 February 1979
Age at Receipt:	5-7 months
Weight at Initiation of Treatment (kilograms):	

	Mean	Range
Males	7.4	6.6 - 8.4
Females	7.3	6.8 - 8.0

Immunization: Against distemper, hepatitis, bordetalla, leptospirosis, and oral papillomas by supplier. Treatment for rabies performed at Bio/dynamics, Inc.

Parasites (intestines): Anthelmintics (piperazine, thiabendazole) were routinely administered by supplier as required.

Physical Examination: (Meth. and Ref., Appendix A)

Pretest examinations were conducted by Bio/dynamics' Staff Veterinarian (H.E. Blatt, V.M.D.)

D. Selection and Group Assignment: Animals were randomly selected for dose groups using the table of random numbers (8,000 numbers) by M.G. Kendal and B.B. Smith starting at Third Thousand Row 19 column 1-4 and ending at Fourth Thousand Row 9 column 17-20. These numbers were used in pairs to correspond to the last two numbers in the Bio/dynamics ear tag number. The starting point was arbitrarily selected.

E. Animal Identification: Each dog was assigned a unique Bio/dynamics identification number which appeared on the animal's cage. In addition, each animal bore an ear tag tattoo with a USDA number.

F. Experimental Outline

Group	Treatment	Dose mg/kg/day	No. of Animals M	No. of Animals F	Duration Treatment	Duration Recovery	Lab Studies	Sacrifice/ Necropsy -	Interval
I	Corn Oil	0	3	3	21 days	28 days	All	All	51 days
II	Triphenyl-phosphine oxide (TPPO)	50	3	3	21 days	28 days	All	All	51 days

[a]Plasma cholinesterase was evaluated on the day prior to treatment and three hours following each animal's last treatment; brain (medulla) cholinesterase was evaluated immediately after death.

G. Husbandry:

Acclimation Period: 20 February - 12 March 1978

Housing: Individually in elevated metal grid cages.

Food: 400 grams of standard laboratory diet (Wayne Bite Size®)

Water: ad libitum. By automated water system (Elizabethtown Water Company)

Environmental Conditions: 12 hour light/dark cycle. Temperature monitored twice daily.

H. Test Material Administration:

Route: Oral Intubation

Method: Appropriate mixtures of test substance in corn oil were administered. Individual dose volumes were adjusted by most recent weekly body weight.

Dose Volume: 1 ml/kg

Frequency: 21-Day Repeated Treatment. Daily (7 days per week) (13 March - 2 April 1979)

I. Observations:

For Mortality and Gross Signs of Toxicologic or Pharmacologic Effects:	Daily
Neurologic:	Days 0, 1, 4, 7, 10, 14, 21, 28, 35, 42 and 49. (performed 3 hours after dosing)

J. Body Weight: (Meth. and Ref., Appendix A)

Pretest and weekly thereafter.

K. Laboratory Studies: (Meth. and Ref., Appendix A)

Blood was obtained from unanesthetized dogs via jugular venipuncture.

Number of Animals and Time Intervals: See Experimental Outline.

Parameter Evaluation

Clinical Chemistry:

cholinesterase
brain
plasma

L. Post-Mortem:

Necropsy:	2 May 1979
Number of Animals:	All
Sacrifice Method:	Exsanguination under sodium pentobarbital anesthesia.
Tissues Preserved:	All animals.

brain (2 transverse sections)
cervical cord (1 longitudinal, 2 transverse)
lumbar cord (1 longitudinal, 2 transverse)
sciatic nerve (both; 1 longtudinal, 2 transverse)

Preservatives:	10% neutral buffered formalin: all tissues.
Tissues Examined Histopathologically:	None. Tissues retained at Bio/dynamics for possible future examination.

III. RSULTS AND DISCUSSION

A. Mortality (Table I)

No deaths occurred in the vehicle control group or in any animal treated with TPPO. All animals were sacrificed on Day 51 of the study.

B. Observations

All animals in the vehicle control and TPPO-treated groups exhibited loose stools and feed emesis sporadically throughout the study.

Five of the six dogs receiving TPPO (two males and three females) exhibited violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming" movements, excessive salivation, vocalization and dyspnea following the first dose. Signs appeared approximately one to two hours after dosing and subsided within four hours after dosing. Three dogs, all females, exhibited slight intermittent head tremors following the second dose. Except for two episodes of convulsions in a single animal (described below), no further physical signs indicative of neurologic effects of the test material were seen for the remainder of the dosing period or during the post-dose observation period.

On two occasions (Days 4 and 17) one animal, male No. 4436, choked immediately following dosing and became rigid and exhibited convulsions. The animal was immediately lifted with the head held downward and a small amount of the dosing mixture was expelled. Signs seen subsequently included convulsions, tremors, dyspnea, excessive salivation and inability to stand, followed by rapid recovery. The dog appeared normal within thirty minutes of dosing on Day 4 and within fifteen minutes of dosing on Day 17. The signs observed in this animal appear to be associated with partial aspiration of the dosing mixture; however, it is possible that they represent a direct effect of the test material.

C. Neurological Examinations (Table II)

There were no signs of any neurological impairment noted in any of the dogs in either the control group or the TPPO-treated group during any of the neurological examinations.

D. Body Weights (Table I)

Weight gains of males receiving TPPO were comparable to or greater than those of control males. Two females receiving TPPO exhibited slight weight losses during the first and/or second weeks of study. However, weight gains of TPPO-treated females during the recovery period and overall weight gains for this group were considered comparable to gains of control females.

E. Cholinesterase Values (Table III)

There was no evidence of cholinesterase inhibition in plasma or brain samples from animals receiving 50 mg/kg of TPPO for twenty-one consecutive treatments.

F. Gross Pathology (Appendix B)

Gross postmortem examinations revealed no findings related to TPPO administration.

IV. SUMMARY

This study was designed to evaluate the reversibility of any neurotoxic or other adverse effects following daily administration of triphenylphosphine oxide (TPPO) to dogs (3 males and 3 females) for twenty-one days followed by a four week recovery period. A vehicle control group (3 males, 3 females) received corn oil, following the same dosing regimen as the TPPO group. Observations for mortality and gross signs of toxicologic or pharmacologic effects were made daily and observations for neurological effects were made on Days 0, 1, 4, 7, 10, 14, 21, 28, 35, 42 and 49 of the study. Plasma cholinesterase was measured pretest and three hours after the last dose; brain cholinesterase was measured terminally.

All dogs survived throughout the study. Five of the six animals which received TPPO exhibited violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming" movements, excessive salivation, vocalization and dyspnea following the first dose. Three dogs exhibited slight head tremors following the second dose. Except for two episodes in a single animal of prostration, convulsions, tremors, dyspnea and excessive salivation, which occurred immediately after dosing and may have been associated with aspiration of the test material, no subsequent signs indicative of neurologic effects were noted. No impairment of reflexes was evident during neurologic examinations and no inhibition of plasma or brain cholinesterase was apparent. Body weight gains of control and TPPO-treated dogs over the seven weeks of study were comparable. Gross postmortem examinations revealed no evidence of an effect of TPPO.

79-2379

In conclusion, acute neurotoxic effects occurred in five of six dogs receiving TPPO at a dose of 50 mg/kg. No delayed neurotoxicity was seen in dogs observed for four weeks following twenty-one daily doses at 50 mg/kg.

Carol S. Auletta, B.A.
Study Director

William E. Rinehart, Sc.D.
Vice-President, Science and
Market Development

Prepared with the technical assistance of:
Jeffrey P. Lefrancois, B.S., Manager, Large Animal Section
Daniel J. Pisacreta, B.S., Supervisor
John P. Kovach, B.Sc., AAS, M.T. (A.S.C.P.) Supervisor, Clinical Pathology
Daniel T. La Rosa, Jr., B.A., Report Preparation

TABLE I
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Individual Body Weights and Mortality Data

Group	Day: An.No.	0[a]	7	14	21[b]	28	35	42	49	Weight Gain (kg) Days 0-21	21-49	0-49	Death Type[c]	Day
		Body Weights (kg)												
							Males							
I	4438	6.9	7.0	7.2	7.4	7.6	8.2	8.6	8.4	0.5	1.0	1.5	T	51
Corn Oil	4439	6.6	6.9	6.6	6.9	7.0	7.2	7.6	7.7	0.3	0.8	1.1	T	51
(0 mg/kg)	4440	7.0	7.1	7.4	7.6	8.1	8.6	8.6	9.2	0.6	1.6	2.2	T	51
II	4436	8.4	8.6	8.8	9.2	9.7	10.2	10.2	10.4	0.8	1.2	2.0	T	51
TPPO	4437	7.6	7.7	8.4	8.6	9.2	9.5	9.5	10.2	1.0	1.6	2.6	T	51
(50 mg/kg)	4441	7.9	8.0	8.8	8.9	9.4	10.1	10.6	10.9	1.0	2.0	3.0	T	51
							Females							
I	4448	7.2	7.4	7.7	7.9	8.0	8.5	8.4	8.6	0.7	0.7	1.4	T	51
Corn Oil	4449	6.8	6.8	6.8	7.3	7.5	8.0	8.1	8.3	0.5	1.0	1.5	T	51
(0 mg/kg)	4451	7.7	8.1	8.3	8.6	8.9	9.4	9.4	9.8	0.9	1.2	2.1	T	51
II	4447	8.0	7.8	7.6	7.7	8.2	8.4	8.5	9.0	-0.3	1.3	1.0	T	51
TPPO	4450	6.8	6.5	7.0	7.2	7.4	8.0	8.0	8.4	0.4	1.2	1.6	T	51
(50 mg/kg)	4452	7.0	7.3	7.5	8.1	8.3	8.8	9.0	9.1	1.1	1.0	2.1	T	51

[a]Day 0 represents the day prior to dosing.
[b]Day 21 = Last day of dosing.
[c]T = Terminal Sacrifice.

Bio/dynamics Inc.

79-2379

TABLE II
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Individual Neurological Evaluations

SPECIES: Dog ANIMAL NO.: 4438 SEX: M GROUP: I DOSE: 0 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

SPECIES: Dog ANIMAL NO.: 4439 SEX: M GROUP: I DOSE: 0 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

79-2379

TABLE II
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Individual Neurological Evaluations

SPECIES: Dog ANIMAL NO.: 4440 SEX: M GROUP: I DOSE: 0 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

SPECIES: Dog ANIMAL NO.: 4436 SEX: M GROUP: II DOSE: 50 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

79-2379

TABLE II
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Individual Neurological Evaluations

SPECIES: Dog ANIMAL NO.: 4437 SEX: M GROUP: II DOSE: 50 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

SPECIES: Dog ANIMAL NO.: 4441 SEX: M GROUP: II DOSE: 50 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

79-2379

TABLE II
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Individual Neurological Evaluations

SPECIES: Dog ANIMAL NO.: 4448 SEX: F GROUP: I DOSE: 0 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

SPECIES: Dog ANIMAL NO.: 4449 SEX: F GROUP: I DOSE: 0 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

79-2379

TABLE II
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Individual Neurological Evaluations

SPECIES: Dog ANIMAL NO.: 4451 SEX: F GROUP: I DOSE: 0 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

SPECIES: Dog ANIMAL NO.: 4447 SEX: F GROUP: II DOSE: 50 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

79-2379

TABLE II
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Individual Neurological Evaluations

SPECIES: Dog ANIMAL NO.: 4450 SEX: F GROUP: II DOSE: 50 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

SPECIES: Dog ANIMAL NO.: 4452 SEX: F GROUP: II DOSE: 50 mg/kg

ORGAN SYSTEM	OBSERVATION	Day 0	1	4	7	10	14	21	28	35	42	49
NEUROLOGIC EXAMINATION	RIGHTING REFLEX	0	0	0	0	0	0	0	0	0	0	0
	FLEXOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	EXTENSOR REFLEX	0	0	0	0	0	0	0	0	0	0	0
	VISUAL PLACING	0	0	0	0	0	0	0	0	0	0	0
	LOCOMOTION	0	0	0	0	0	0	0	0	0	0	0
	STANDING	0	0	0	0	0	0	0	0	0	0	0

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

79-2379

TABLE III
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Individual Plasma and Brain Cholinesterase Values

Group	An.No.	Plasma (M/min)[a]		Brain (M/min)[a]
		Pretest	3 Hours Following Final Treatment	Terminal
			Males	
I	4438	2.3	2.2	1.3
Corn Oil	4439	3.0	2.8	1.5
(0 mg/kg)	4440	3.5	2.8	1.5
II	4436	2.4	2.7	1.3
TPPO	4437	3.2	3.0	1.2
(50 mg/kg)	4441	3.4	2.9	1.2
			Females	
I	4448	3.2	3.2	1.3
Corn Oil	4449	3.0	2.6	1.3
(0 mg/kg)	4451	3.1	2.9	1.5
II	4447	3.3	3.1	1.5
TPPO	4450	3.1	3.1	1.1
(50 mg/kg)	4452	3.2	3.8	1.2

[a]Activity expressed as micro moles of substrate hydrolyzed per minute per ml of plasma or per gram of brain tissue.

79-2379

Appendix A
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Methodology and References

General

Parameter	Reference or Description
Physical Examination	Examined pretest for each animal: Central Nervous System - behavior movements, reactivity to stimuli and muscle tone. Autonomic Nervous System - pupil size and secretions. Respiratory - nostrils, character and rate. Cardiovascular - palpation. Gastrointestinal - abdominal shape and feces consistency and color. Genitourinary - penis/vulva and perineal region. Skin and Fur - color, turgor and integrity. Mucous Membranes - conjunctiva, mouth, rectal and vaginal. Eye - eyelids, eyeball and transparency. Other - general condition.
Body Weight	Detecto Scale (60-S)

79-2379

Appendix A
A SEVEN WEEK NEUROTOXICITY STUDY
IN DOGS

Methodology and References

General

Parameter	Specimen	Reporting Units	Reference or Description
Cholinesterase	Plasma	µm/ml/min	Modification of Nabb. D.P. and F. Whitefield Determination of Cholinesterase by an automated pH-stat. Method. Arch. Enviromental Health Vol. 15, August 1967, pp. 147-154.
	Brain	µm/g/min	

79-2379

Appendix B
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Gross Pathology Summary

A few gross lesions were noted in the lungs and spleen of both control and treated groups. They are incidental findings and are not related to the test material.

Teofilo M. Saulog, D.V.M.
Pathologist

79-2379

Appendix B (cont.)
A SEVEN WEEK NEUROTOXICITY STUDY IN DOGS

Gross Pathology Summary

Group	An.No	Tissue/Organ	Observation
			Males
I Corn Oil (0 mg/kg)	4438	Lungs	Left apical lobe, firm, palpable nodules, 0.5 cm. diameter; color indistinguishable from surrounding tissue.
	4439	Lungs	All lobes, all surfaces, tan raised areas approximately 0.2-0.6cm. diameter.
	4440	Lungs	All lobes, all surfces dark and scattered, nodules approximately 0.4 cm. diameter.
II TPPO (50 mg/kg)	4436		N.O.A.
	4437	Lung	Left diaphragmatic slightly hemorrhagic.
	4441	Lungs	All lobes, all surfaces, tan nodules 0.2-0.4 cm. diameter.
			Females
I Corn Oil (0 mg/kg)	4448		N.O.A.
	4449	Lungs	Firm, tan, palpable nodules, 0.4-0.7 cm. diameter on dorsal surface of all lobes.
	4451	Spleen	Scar approximately 0.8 cm. diameter white on dorsal surface.
II TPPO (50 mg/kg)	4447	Spleen	Scar approximately 2.0 cm. diameter on dorsal surface.
		Lung	Right diaphragmatic lobe nodule 0.9 cm. diameter, clear.
	4450		N.O.A.
	4452	Lung	Left diaphragmatic lobe, tan, palpable 0.5 cm. diameter nodule on dorsal surface.

N.O.A. = No Observable Abnormalities.

APPENDIX C

79-2379

A Seven Week Neurotoxicity Study in Dogs
With Triphenylphosphine Oxide
Pathology Report

Submitted: November 14, 1979

79-2379

A Seven Week Neurotoxicity Study in Dogs With Triphenylphosphine Oxide Pathology Report

TABLE OF CONTENTS

Introduction and Purpose

Experimental Design

Materials and Methods

Results

Summary

Microscopic Findings
Table I

79-2379

A Seven Week Neurotoxicity Study in Dogs With Triphenylphosphine Oxide Pathology Report

INTRODUCTION AND PURPOSE

The purpose of this study is to assess the reversibility of any neurologic or other adverse effects following daily oral administration of Triphenylphosphine Oxide (TPPO) for 21 days in Beagle dogs.

EXPERIMENTAL DESIGN

Group	Treatment	Dose mg/kg/day	No. of Animals	Duration of Treatment	Terminal Sacrifice and Gross Post-mortem Examination	Microscopic Examination
I	Corn Oil	-	3M 3F	21 days	Day 51 - all tissues examined	Specified Tissues[1]
II	TPPO	50	3M 3F	21 days	Day 51 - all tissues examined	Specified Tissues

[1] The specified tissues are: 1 longitudinal and 2 transverse sections of (a) each sciatic nerve, (b) lumber spinal cord and (c) cervical spinal cord; and a transverse section of the cerebrum and cerebellum with brain stem. Two stains will be utilized... hematoxylin and eosin, and luxol fast blue.

MATERIALS AND METHODS

A. Compound: Triphenylphosphine Oxide (TPPO)

B. Animals: Beagle Dogs

C. Drug Administration: Daily by Intubation.

D. Gross Postmortem Procedures: Animals were killed by exsanguination under sodium pentibarbitol anesthesia. A complete postmortem examination was done on each animal, and any gross abnormality was preserved in 10% neutral buffered formalin, in addition to the specified tissues.

E. Microscopic Methods: Sections of brain, cervical spinal cord, lumbar spinal cord and right and left sciatic nerves of each animal, were preserved in 10% neutral buffered formalin. After fixation, these tissues were parafin embedded, sectioned at 6 microns, and stained with hematoxylin and eosin, the number of each piece of tissue as per experimental design - specified tissues. In addition, for every H&E completed, a corresponding section was cut at 10 microns and stained with luxol fast blue.

79-2379

A Seven Week Neurotoxicity Study in Dogs With Triphenylphosphine Oxide Pathology Report

RESULTS

A Summary of gross lesions were presented in Appendix B of the previous report of project #79-2379, dated June 29, 1979. Gross postmortem examination did not reveal any nervous system lesions.

Microscopic findings are reported in Table I, the focal meningitis characterized by fibrosis, hemorrhage, and congestion exhibited by animal #4447 (79-6202) is not consistent with a drug related toxic lesion, but rather, it is more compatible with a physical trauma or parasite migration, although the precise etiology could not be determined.

Bio/dynamics Inc.

PROJECT NO. 79-2379

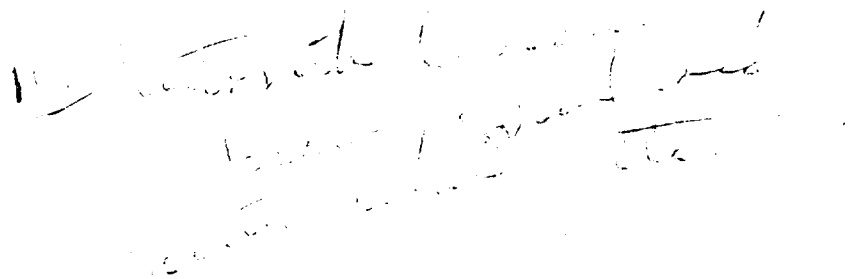
A SEVEN WEEK NEUROTOXICITY STUDY

IN DOGS WITH TRIPHENYLPHOSPHINE OXIDE

ABSTRACT

This study was designed to evaluate the reversibility of any neurotoxic or other adverse effects following daily administration of triphenylphosphine oxide (TPPO) to dogs (3 males and 3 females) for twenty-one days followed by a four week recovery period. A vehicle control group (3 males, 3 females) received corn oil, following the same dosing regimen as the TPPO group. Observations for mortality and gross signs of toxicologic or pharmacologic effects were made daily and observations for neurological effects were made on Days 0, 1, 4, 7, 10, 14, 21, 28, 35, 42 and 49 of the study. Plasma cholinesterase was measured pretest and three hours after the last dose; brain cholinesterase was measured terminally.

All dogs survived throughout the study. Five of the six animals which received TPPO exhibited violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming" movements, excessive salivation, vocalization and dyspnea following the first dose. Three dogs exhibited slight head tremors following the second dose. Except for two episodes in a single animal of prostration, convulsions, tremors, dyspnea and excessive salivation, which occurred immediately after dosing and may have been associated with aspiration of the test material, no subsequent signs indicative of neurologic effects were noted. No impairment of reflexes was evident during neurologic examinations and no inhibition of plasma or brain cholinesterase was apparent. Body weight gains of control and TPPO-treated dogs over the seven weeks of study were comparable. Gross postmortem examinations revealed no evidence of an effect of TPPO.



79-2379

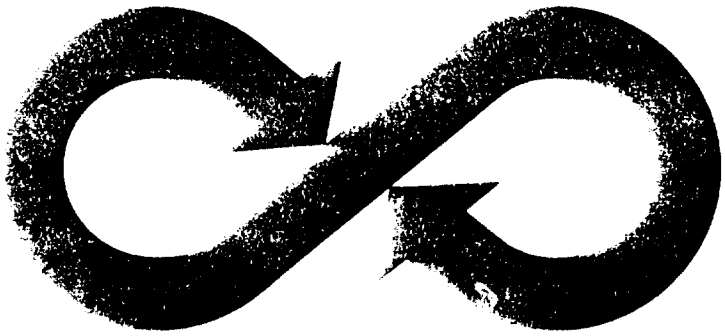
A Seven Week Neurotoxicity Study in Dogs With Triphenylphosphine Oxide Pathology Report

Microscopic Findings

Animal Number	443838	443939	444040	444848	444949	445051	443636	443737	444141	444747	445052
Sex	M			F			M			F	
Pathology Number 79-	619393	619494	619595	619696	619797	619898	619999	620700	620101	620202	620303	620404
Group	I						II					
RIGHT SCIATIC NERVE												
-transverse sections (2)	N	N	N	N	N	N	N	N	N	N	N	N
-longitudinal section (1)	N	N	N	N	N	N	N	N	N	N	N	N
LEFT SCIATIC NERVE												
-transverse sections (2)	N	N	N	N	N	N	N	N	N	N	N	N
-longitudinal section (1)	N	N	N	N	N	N	N	N	N	N	N	N
LUMBAR SPINAL CORD												
-transverse sections (2)	N	N	N	N	N	N	N	N	N	N	N	N
-longitudinal section (1)	N	N	N	N	N	N	N	N	N	N	N	N
CERVICAL SPINAL CORD												
-transverse sections (2)	N	N	N	N	N	N	N	N	N	N	N	N
-longitudinal section (1)	N	N	N	N	N	N	N	N	N	N	N	N
CEREBRUM												
-meningitis, chronic, focal, with congestion and hemorrhage	N	N	N	N	N	N	N	N	N		N	N
										2		
CEREBELLUM AND BRAIN STEM	N	N	N	N	N	N	N	N	N	N	N	N

KEY:

N = Not remarkable
1 = Minimal degree or amount of indicated change.
2 = Mild degree or amount of indicated change.
3 = Moderate degree or amount of indicated change.
4 = Marked degree or amount of indicated change.



Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO.: 6218-80

M-416

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine (TPP)

Submitted to: M&T Chemicals, Inc.
Rahway, New Jersey

Date: June 30, 1980

I. INTRODUCTION

This study was conducted for M&T Chemicals Inc. in order to evaluate the acute oral toxicity of Triphenylphosphine. The study was performed at Bio/dynamics, Inc., Mettlers Road, East Millstone, New Jersey, 08873, using procedures based on the methods described in 40 CFR Part 163.81-1. (ENVIRONMENTAL PROTECTION AGENCY PESTICIDE PROGRAMS. Proposed Guidelines for Registering Pesticides in the U.S.; Hazard Evaluation: Humans and Domestic Animals. Acute Oral Toxicity Study.)

II. MATERIALS AND METHODS

A. Dates of Study

LD_{50} Determination

Animal Receipt:	April 8, 1980
Initiation (Dosing):	April 23, 1980
Termination:	May 7, 1980

B. Study Personnel

Study Director:	George R. Lankas, Ph.D.
Laboratory Supervisor:	Donna L. Blaszcak, B.S.
Technician in Charge:	Gary Trimmer, B.S.
Study Monitor (Report Preparation):	Carol Puma, B.S.

C. Test Animals

Albino Rats

Strain:	Sprague-Dawley CD^R
Reason for Selection:	Standard laboratory animal
Supplier:	Charles River Breeding Laboratories, Inc. Wilmington, Massachusetts 01887
	LD_{50} Determination: Sixty (five/sex/dose level)

II. MATERIALS AND METHODS (cont.)

Age:	Young adults
Weight: (prefasted)	Males : 267 to 329 grams Females: 220 to 288 grams
Equilibration Period:	15 days
Observations:	All animals were checked for viability twice daily during the equilibration period. Prior to assignment to study all animals received a physical examination.
Husbandry:	
Housing:	Group-housed (six/cage) during equilibration. Individually-housed during study.
Cages:	Suspended, stainless steel with wire mesh bottoms.
Environmental Conditions:	Temperature: monitored twice daily Temperature Range: 64°F to 72°F Humidity: monitored daily Light cycle: 12 hours light, 12 hours dark
Food:	Purina Laboratory Rodent Diet ad libitum.
Water:	Automatic watering system, ad libitum. Municipal water supply (Elizabethtown Water Co.)
Identification:	Each animal was identified with a monel ear tag bearing a unique number prior to testing.
Selection:	Animals considered unsuitable for study because of poor health or outlying body weights were excluded from selection. Animals for study were selected from those remaining; each animal from a group-housed unit was placed in a different test group.

D. Test Material:

Label Information:	Triphenylphosphine 500 grams for 6218-80
Lot. No.:	PNVAH-6KF
Description:	Cream, white colored granular powder
Date of Receipt:	April 11, 1980

II. MATERIALS AND METHODS (cont.)

Received from: M&T Chemicals Inc.

Storage: Ambient temperature

Analysis: Detailed analysis of triphenylphosphine performed by M&T Chemicals Inc., is presented in Appendix A.

Preparation of Dosing Material Mixtures:

LD_{50}:

Concentration: 0.2 g/ml (20%)

Vehicle: Peanut oil

Procedure: Prior to mixing, the test material was ground with a mortor and mestle until extremely fine. 30 grams of the test material were placed in an appropriate container and vehicle was added to achieve a total volume of 150 ml. The mixture was then stirred to achieve a homogeneous mixture.

Temperature of dosing mixture: room temperature

E. Test Design:

LD 5 0			
No. of Animals		Dose	Dose Volume*
M	F	(mg/kg)	(ml/kg)
5	5	Vehicle Control	7.12
5	5	350	1.75
5	5	497	2.49
5	5	706	3.53
5	5	1002	5.01
5	5	1423	7.12

*Concentration 0.2 g/ml

Vehicle Control:

Ten animals (five/sex) were dosed with 7.12 ml/kg of peanut oil.

II. MATERIALS AND METHODS (cont.)

F. Administration of Test Material:

Animals were fasted overnight (for approximately 17 hours) prior to dosing. The test and control material was administered by oral intubation, using a ball-tipped intubation needle. Doses were calculated using pre-fasted body weights.

G. Experimental Evaluation (In-Life):

Viability Check: Twice Daily

Observations of Pharmacologic and Toxicologic Signs:

Approximately 1, 2, and 4 hours after dosing and daily thereafter for fourteen days.

Body Weights:

Pre-fast (weights used for calculation of doses)
Post-fast (just prior to dosing)
Day 7 and Day 14
Terminal: Any animals which did not survive for 14 days were weighed at the time of death or at the time they were found dead.

H. Post Mortem:

Gross post mortem examinations were performed on all animals which died during the study. All animals surviving at termination of the observation period (Day 14) were killed by carbon dioxide inhalation and examined grossly. All abnormalities were recorded but no tissues were saved.

I. Raw Data Storage:

All raw data and the original study protocol will be retained permanently on file in the Bio/dynamics Archives.

J. References-LD_{50} Calculations:

Miller, Lloyd C. and M.L. Tainter., Estimation of the ED_{50} and Its Error by Means of Logarithmic-Probit Graph Paper, Proc. Soc. Exp. Bio. Med 57: 261-264 (1944).

III. RESULTS

Individual body weights are presented in Table 1. A summary of in-life observations is presented in Table II. Necropsy observations are presented in Table III.

A. Mortality

Dose levels, mortality data and LD_{50} calculations for the LD_{50} study were as follows:

Dose Level mg/kg	Mortality Male	Female	Total
Vehicle Control	0/5	0/5	0/10
350	0/5	0/5	0/10
497	0/5	0/5	0/10
706	0/5	1/5[a]	1/10[a]
1002	2/5	3/5	5/10
1423	3/5	4/5	7/10
LD_{50}:	1200mg/kg	980mg/kg	1150 mg/kg
95% Confidence Limits (mg/kg):	828 to 1572	805 to 1155	991 to 1309

[a]Accidental death, test material found in thoracic cavity.

LD_{50} calculations and dose response curves and slopes are presented in Appendix B.

B. Body Weights (Table I)

Those animals dying prior to termination of the study exhibited body weight losses. The animals surviving the fourteen-day post-dose observation period exhibited body weight gains. However, a few animals in the 2 highest dose groups exhibited weight losses at Day 7.

Bio/dynamics Inc.

79-2379

A Seven Week Neurotoxicity Study in Dogs With Triphenylphosphine Oxide Pathology Report

SUMMARY

Six beagle dogs were given 50mg/kg/day of Triphenylphosphine Oxide (TPPO) by oral intubation for 21 days and allowed to recover for an additional 28 days at which time terminal sacrifice and complete gross postmortem evaluations were done. Both sciatic nerves, portions of the cervical and lumbar spinal cord, cerebrum and cerebellum with brain stem were examined microscopically (hematoxylin and eosin and Luxol fast blue stains) with the result that no drug induced morphologic alterations could be detected.

Fred A. Hines, D.V.M.
Pathologist
11/5/79

TABLE I

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
Vehicle Control	2860 M	268	244				321	53	347	79
	2901 M	270	239				322	52	361	91
	2880 M	279	255				329	50	363	84
	2875 M	271	245				322	51	354	83
	2845 M	296	263				346	50	377	81
	2924 F	244	222				263	19	286	42
	2952 F	244	223				265	21	269	25
	2916 F	260	244				287	27	294	34
	2931 F	240	218				252	12	260	20
	2919 F	255	241				280	25	289	34

[a]Change from prefasted weight (grams).

TABLE I (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level mg/kg	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
350	2867 M	283	257				339	56	373	90
	2886 M	293	271				325	32	367	74
	2893 M	286	255				335	49	370	84
	2838 M	290	270				337	47	373	83
	2842 M	323	290				381	58	426	103
	2966 F	250	228				265	15	278	28
	2929 F	248	227				276	28	280	32
	2920 F	235	215				253	18	247	12
	2951 F	251	230				276	25	288	37
	2943 F	253	230				277	24	289	36

[a]Change from prefasted weight (grams).

TABLE I (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level mg/kg	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
497	2883 M	304	280				350	46	408	104
	2900 M	306	275				350	44	387	81
	2834 M	288	265				325	37	364	76
	2862 M	276	250				308	32	352	76
	2878 M	292	265				329	37	367	75
	2946 F	256	232				280	24	290	34
	2941 F	254	236				272	18	286	32
	2912 F	250	233				274	24	280	30
	2965 F	248	235				262	14	272	24
	2928 F	220	201				245	25	249	29

[a]Change from prefasted weight (grams).

TABLE I (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level mg/kg	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
706	2879 M	317	287				345	28	338	21
	2892 M	286	254				300	14	355	69
	2876 M	295	266				321	26	366	71
	2852 M	279	247				323	44	389	110
	2859 M	292	255				315	23	349	57
	2909 F	287	258	238	2	-49				
	2940 F	256	235				266	10	301	45
	2942 F	263	244				269	6	278	15
	2903 F	262	240				273	11	277	15
	2947 F	288	260				319	31	342	54

[a]Change from prefasted weight (grams).

TABLE I (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level mg/kg	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
1002	2897 M	268	243	231	2	-37				
	2841 M	287	263				277	-10	330	43
	2856 M	280	253				302	22	356	76
	2868 M	267	242				276	9	286	19
	2884 M	285	260	245	3	-40				
	2917 F	250	226	213	2	-37				
	2914 F	262	241	224	1	-38				
	2934 F	233	211				229	-4	254	21
	2922 F	236	216				256	20	266	30
	2959 F	234	217	205	3	-29				

[a]Change from prefasted weight (grams).

TABLE I (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level mg/kg	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
1423	2836 M	329	301				332	3	400	71
	2866 M	286	255				280	-6	336	50
	2839 M	308	280	258	3	-50				
	2887 M	296	270	240	4	-56				
	2858 M	281	257	237	2	-44				
	2963 F	243	225				248	5	267	24
	2930 F	250	230	213	3	-37				
	2907 F	243	226	219	3	-24				
	2902 F	244	225	211	2	-33				
	2936 F	248	225	203	3	-45				

[a]Change from prefasted weight (grams).

TABLE II

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose	Interval: Day / Hrs.	1				2	3	4	5-7	8-14	Time last Observed
			1	2	4	24						
			M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F	
Fecal Staining	Vehicle		1/0	0	0/1	0	0	0	0	0	0	4 Hours
Soft Stool	Control		2/0	2/1	1/1	0	0	0	0	0	0	4 Hours

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose (mg/kg)	Interval: Day 1 Hrs. 1 M/F	2 M/F	4 M/F	24 M/F	2 M/F	3 M/F	4 M/F	5-7 M/F	8-14 M/F	Time last Observed
Fecal Staining	350	0	0	1/0	0	0	0	0	0	0	4 Hours
Soft Stool		0	0	1/0	0	0	0	0	0	0	4 Hours
Alopecia		0	0	0	0	0/1	0/1	0/1	0/1	0/1	Day 14

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose (mg/kg)	Interval: Day 1 Hrs. 1 M/F	2 M/F	4 M/F	24 M/F	2 M/F	3 M/F	4 M/F	5-7 M/F	8-14 M/F	Time last Observed
Urinary Staining	497	0	0	0	0/1	0	0	0	0	0	24 Hours
Fecal Staining		0	0/1	0/1	0	0	0	0	0	0	4 Hours
Soft Stool		0	0/1	0/1	0	0	0	0	0	0	4 Hours
Piloerection		0	0	0	0	0/1	0/1	0	0	0	Day 3
Abdominal Griping		0	0	0	0	0/1	0	0	0	0	Day 2
Motor Activity Decrease		0	0	2/0	0	0	0	0	0	0	4 Hours
Alopecia (Ano-Genital Area)		0	0	0	0/1	0	0	0	0	0	24 Hours

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Interval: Day Hrs. / Dose (mg/kg)	1 — 1 M/F	1 — 2 M/F	1 — 4 M/F	1 — 24 M/F	2 M/F	3 M/F	4 M/F	5-7 M/F	8-14 M/F	Time last Observed
Dead	706	0	0	0	0	0/1	0/1	0/1	0/1	0/1	
Fine Tremors		0	0	0	0	0/1	0	0	0	0	Day 2
Red Ocular Discharge		0	0	0	0/1	0	0	0	0	0	24 Hours
Urinary Staining		0	0	0	0	0/1	0	0	0	0	Day 2
Fecal Staining		0	1/0	0	0	0	0	0	0	0	2 Hours
General Poor Condition		0	0	0	0	0	0/1	0	0	0	Day 3
Soft Stool		0	1/0	0	0	0	0	0	0	0	2 Hours
Motor Activity Decrease		0	0	0	1/1	0	0	0	0	0	24 Hours
Hypothermia		0	0	0	0/1	0	0	0	0	0	24 Hours
Irregular Breathing		0	0	0	0/1	0	0	0	0	0	24 Hours
Alopecia (Ano-Genital Area)		0	0	0	0/1	0	0	0	0	0	24 Hours
Unthrifty		0	0	0	0	0/1	0	0	0	0	Day 2

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex or of those surviving exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose (mg/kg)	Interval: Day / Hrs.	1				2	3	4	5-7	8-14	Time last Observed
			1	2	4	24						
			M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F	
Dead	1002		0	0	0	0/1	1/2	2/3	2/3	2/3	2/3	
Fine Tremors			0	0	0	0	2/0	1/0	0	0	0	Day 3
Red Nasal Discharge (slight, dry)			0	0	0	0	0	0	0/1	0	0	Day 4
Respiratory Rate Decrease			0	0	1/0	0/1	0	0	0	0	0	24 Hours
Red Ocular Discharge			0	0	0	0	0/1	0	0	0	0	Day 2
Fecal Staining			0	0/1	0/1	0	0	0	0	1/0	0	Day 7
Soft Stool			0	0/1	0/1	0	0	0	0	0	0	4 Hours
Piloerection			0	0	0	0	0/1	0/1	0	0	0	Day 3
Abdominal Griping			0	0	0	0	1/1	0	0	0	0	Day 2
Motor Activity Increase			0	0	0	0	0	0	0	0/1	0/1	Day 12
Motor Activity Decrease			0	0	2/1	0	0	0	0	0	0	4 Hours
Prostration			0	0	0	1/1	0	0	0	0	0	24 Hours
Hypothermia			0	0	0	1/1	0	0	0	0	0	24 Hours
Food Consumption Decrease			0	0	0	0	0	0	0	0/1	0	Day 5

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex or of those surviving exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose (mg/kg)	Interval: Day Hrs. 1 1 M/F	1 2 M/F	1 4 M/F	1 24 M/F	2 M/F	3 M/F	4 M/F	5-7 M/F	8-14 M/F	Time last observed
Dead	1423	0	0	0	0	1/1	2/4	3/4	3/4	3/4	
Ataxia		0	0	0	0	1/0	0	0	0	0	Day 2
Convulsions		0	0	0	0	0/1	0	0	0	0	Day 2
Coarse Tremors		0	0	0	0	1/1	0	0	0	0	Day 2
Red Nasal Discharge		0	0	0	0	0	1/0	1/1	0	0	Day 4
Respiratory Rate Decrease		1/0	1/2	0	1/1	0/1	0	0	0	0	Day 2
Irregular Breathing		0	0	0/1	0	0	0	0	0	0	4 Hours
Wet Rales		0/1	0	0	0	0	0	0	0	0	1 Hours
Clear Oral Discharge		1/0	0/1	0	0	0	0	0	0	0	2 Hour
Red Ocular Discharge		0	0	0	1/0	0	0	0	0	0	24 Hours
Urinary Staining		0	0	0	0/1	0	0	0	0	0	24 Hours
Fecal Staining		0	1/1	0/1	0	0	0	0/1	0	0	Day 4
Soft Stool		0	1/1	0/1	0	0	0	0	0	0	24 Hours
Piloerection		0	0	0/2	1/0	1/2	0	0	0	0	Day 2

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex or of those surviving exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose (mg/kg)	Interval: Day Hrs.	1				2	3	4	5-7	8-14	Time last Observed
			1	2	4	24						
			M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F	
Abdominal Griping	1423		0	0	0	1/0	1/2	0	0	0	0	Day 2
Motor Activity Decrease	(cont.)		0	0	2/2	0	2/0	1/1	1/0	1/0	0	Day 6
Hypothermia			0	0	0	1/1	0	0	0	0	0	24 Hours
Aggressive			0	0	0	0	0	0	0	1/0	0	Day 5
Alopecia (Ano-Genital Area)			0	0	0	1/2	0/2	0	0	0	0	Day 2
Prostration			0	0	0	1/1	1/1	1/0	0	0	0	Day 3
Unthrifty			0	0	0	0	1/0	0	0	0	0	Day 2

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex or of those surviving exhibiting sign one or more times during interval.

TABLE III

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
Vehicle Control	2860 M	14	T	Lungs: mottled dark red 75%; spleen: roughened; kidneys: mottled pale 10%; adrenals: dark red foci.
	2901 M	14	T	Lungs: mottled dark red 75%; spleen: roughened; kidneys: mottled pale; adrenals: dark red foci.
	2880 M	14	T	Lungs: mottled dark red 100%; spleen: roughened; adrenals: brown red foci.
	2875 M	14	T	Lungs: mottled dark red 40%, dark red foci throughout; adrenals: dark red foci.
	2845 M	14	T	Lungs: pale dark red foci 40%; kidneys: mottled dark red; adrenals: dark red.
	2924 F	14	T	Lungs: mottled dark red 60%; adrenals: dark red foci.
	2952 F	14	T	Lungs: mottled dark red 50%; spleen: roughened; adrenals: dark red foci.
	2916 F	14	T	Lungs: pale, dark red areas 50%; spleen: roughened.
	2931 F	14	T	Lungs: dark red patches 40%; spleen: slightly roughened; kidneys: mottled pale; adrenals: dark red foci.
	2919 F	14	T	Lungs: mottled dark red 90%, dark red foci 60%; spleen: roughened; adrenals: dark red foci.

[a]T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
350	2867 M	14	T	Lungs: mottled dark red 40%; spleen: slightly roughened; kidneys: mottled pale; adrenals: mottled red.
	2886 M	14	T	Lungs: mottled pale, dark red foci 100%; spleen: slightly roughened; adrenals: dark red foci.
	2893 M	14	T	Lungs: pale; spleen: roughened; kidneys: mottled pale 50%; adrenals: brown red foci.
	2838 M	14	T	Lungs: pale, dark red foci throughout; spleen: roughened; kidneys: mottled pale; adrenals: dark red foci.
	2842 M	14	T	Lungs: mottled pale with dark red foci 100%; spleen: slightly roughened; adrenals: dark red.
	2966 F	14	T	Lungs: pale, dark red foci throughout; spleen: slightly roughened; kidneys: mottled pale; adrenals: dark red foci.
	2929 F	14	T	Alopecia: stomach, left groin area; lungs: dark red foci 100%; spleen: dark, slightly roughened.
	2920 F	14	T	Lungs: mottled dark red 40%; spleen: roughened; adrenals: dark red foci.
	2951 F	14	T	Lungs: mottled pale 20%; spleen: slightly roughened; adrenals: dark red foci.
	2943 F	14	T	Lungs: dark red areas; stomach: vascularized; spleen: slightly roughened; kidneys: mottled tan 25%.

[a]T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
497	2883 M	14	T	Lungs: pale with dark red dots; spleen: roughened; adrenals: dark red foci.
	2900 M	14	T	Lungs: mottled pale 80%; spleen: slightly roughened; adrenals: red foci.
	2834 M	14	T	Lungs: pale dark red foci 80%; spleen: slightly roughened; kidneys: mottled pale; adrenals: red.
	2862 M	14	T	Lungs: dark red areas; spleen: roughened; kidneys: mottled pale 25%; left adrenal: dark red foci.
	2878 M	14	T	Lungs: pale dark red foci 30%; stomach: vascularized; kidneys: mottled pale; adrenals: dark red foci.
	2946 F	14	T	Lungs: mottled pale 30%; stomach: slightly vascularized; spleen: slightly roughened; adrenals: red foci; mesentery lymph nodes: dark red.
	2941 F	14	T	Lungs: mottled pale 40%; spleen: slightly roughened; adrenals: red foci.
	2912 F	14	T	Lungs: dark red foci throughout, mottled dark red 40%; spleen: roughened; kidneys: mottled pale; adrenals: dark red.
	2965 F	14	T	Spleen: roughened.
	2928 F	14	T	Lungs: pale mottled dark red 30%; liver: mottled pale 20%; spleen: slightly roughened; adrenals: dark red foci.

[a]T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
706	2879 M	14	T	Lungs: mottled pale with dark red foci 100%; stomach: pronounced vascularization; spleen: slightly roughened; adrenals: dark red foci; mesenteric lymph nodes: red.
	2892 M	14	T	Lungs: mottled pale 20%, dark red foci 100%; spleen: slightly roughened; adrenals: red; mesenteric lymph nodes: dark red.
	2876 M	14	T	Lungs: dark red foci 100%, mottled pale 40%; stomach: pronounced vascularization; spleen: slightly roughened.
	2852 M	14	T	Lungs: pale, dark red foci throughout; spleen: slightly roughened; kidneys: mottled pale; adrenals: dark red; mesentery lymph nodes: dark red.
	2859 M	14	T	Lungs: pale, with dark red foci; stomach: empty; spleen: roughened.
	2909 F	2	F	Red, dry ocular discharge; yellow dry oral discharge; brain: pronounced vascularization; hole in eosphagus and trachea; thoracic cavity: contained food and test material.
	2940 F	14	T	Spleen: dark, roughened; adrenals: dark red foci.
	2942 F	14	T	Lungs: mottled dark red 20%, mottled brown red 80%; stomach: vascularized; spleen: slightly roughened; adrenals: mottled red.
	2903 F	14	T	Lungs: mottled dark red 30%; liver: mottled pale 20%; adrenals: dark red foci.
	2947 F	14	T	Lungs: mottled pale, dark red areas; spleen: slightly roughened; adrenals: brown-red foci; uterus: blood vessels injected.

[a]F=Found dead (Day given is day found dead).
T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
1002	2897 M	2	F	Pale, brown, wet urinary staining; brain: pronounced vascularization; lungs: mottled dark red 20%, bright red 80%; stomach: pronounced vascularization, red foci 30%, distended 3 times normal; intestines: pronounced vascularization, viscous orange fluid; adrenals: red; testes: pronounced vascularization; urinary bladder: contains thin green fluid.
	2841 M	14	T	Lungs: mottled dark red 20%, mottled pale 10%, dark red foci 100%; spleen: slightly roughened; adrenals: pale red.
	2856 M	14	T	Lungs: mottled pale 70%; spleen: slightly roughened; kidneys: mottled pale; adrenals: pale red.
	2868 M	14	T	Thoracic cavity: contains test material surrounding heart, small white particles.
	2884 M	3	F	Red dry nasal discarge; brain: pronounced vascularization; lungs: mottled dark red 50%, brown red 20%; liver: mottled pale 100%; stomach: pyloric region - contains black material mixed with other contents of stomach; intestines: contain viscous black fluid with yellow suspension; spleen: diminished, slightly roughened; kidneys: pale; adrenals: pale red; urinary bladder: contains red-yellow fluid.

[a]F=Found dead (Day given is day found dead).
T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
1002 cont.	2917 F	2	F	Alopecia of the ano-genital region; brain: pronounced vascuarization; lungs: mottled extreme dark red 80%, caseous, pale red patches approx. 1x1cm; liver: clear edges; stomach: distended; intestines: contain viscous yellow fluid; spleen: diminished; adrenals: pale red.
	2914 F	1	F	Dry, dark red oral discharge; alopecia: ano-genital area; dry urinary staining; brain: pronounced vascularization; lungs: mottled dark red 40%; liver: mottled pale 30%; stomach: pronounced vascularization, contains viscous brown fluid; intestines: pronounced vascularization, contain viscous brown fluid; spleen: slightly roughened; adrenals: pale red.
	2934 F	14	T	Lungs: red foci 100%; stomach: pronounced vascularization; spleen: slightly roughened; adrenals: red foci.
	2922 F	14	T	Lungs: mottled pale 10%; stomach: pronounced vascularization of cardiac and pyloric region; spleen: slightly roughened; adrenals: mottled pale red; lymph nodes (left side): red.
	2959 F	3	F	Dry red nasal discharge; yellow brown urinary staining; brain: pronounced vascularization; lungs: mottled bright red 80%, mottled dark red 20%; liver: mottled pale 80%; stomach: pyloric region - black areas on inside wall, contains yellow substance mixed with green substance; intestines: contain red-yellow fluid, yellow suspension; spleen: slightly roughened; kidneys: pale; adrenals: pale red; urinary bladder: empty.

[a]F=Found dead (Day given is day found dead).
T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
1423	2836 M	14	T	Lungs: mottled pale 70%; adrenals: pale red.
	2866 M	14	T	Lungs: mottled pale 80%, dark red foci 100%; stomach: slightly vascularized; spleen: roughened; right kidney: mottled pale; adrenals: pale red.
	2839 M	3	F	Red dry oral and nasal discharge; brown dry urinary and fecal staining; lungs: mottled dark red 20%, mottled brown red 80%, dark red foci 100%; liver: mottled pale throughout, dark red foci 100%; stomach: pyloric region - inside wall has black areas; intestines: contain brown yellow fluid; spleen: slightly roughened; kidneys: pale; adrenals: pale red; testes: undescended.
	2887 M	4	F	Brown oral discharge; red dry nasal discharge; red-yellow urinary staining; brain: pronounced vascularization; lungs: mottled dark red 75%, mottled brown red 25%; liver: mottled pale 100%; stomach: pyloric region - inside wall, small black patches; intestines: distended with gas, contain yellow-brown fluid with yellow suspension; spleen: pale; kidneys: pale; adrenals: pale red; urinary bladder: contains red-yellow fluid.
	2858 M	2	F	Clear wet nasal discharge; dry, yellow urinary staining; brain: pronounced vascularization; lungs: mottled dark red 90%; liver: mottled pale; stomach: pronounced vascularization, pyloric region - dark black foci, distended with viscous brown substance; spleen: diminished; adrenals: mottled pale.

[a]F=Found dead (Day given is day found dead).
T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
1423 cont.	2963 F	14	T	Lungs: mottled pale 20%, dark red foci 80%; spleen: roughened; adrenals: mottled pale red.
	2930 F	3	F	Dry red oral and nasal discharge; brown yellow urinary and fecal staining; brain: pronounced vascularization; lungs: mottled dark red 75%; liver: pale 80%; stomach: contains green-yellow material; intestines: contain red-yellow fluid, yellow suspension; kidneys: pale; adrenals: pale red.
	2907 F	3	F	Brown dry oral discharge; red dry nasal discharge; brown wet urinary staining; lungs: mottled dark red 20%, brown red 80%; dark red foci 100%; liver: mottled pale 80%; stomach: black foci inside wall; intestines: viscous yellow fluid with yellow suspension; spleen: dark red patch at tip; adrenals: dark red foci; urinary bladder: empty.
	2902 F	2	F	Dry red nasal discharge; wet yellow urinary staining; lungs: mottled dark red 80%; liver: mottled pale 30%; stomach: distended with gas and pale green substance; intestines: slightly pronounced vascularization, contain viscous white creamy substance; adrenals: enlarged, pale red; uterus: greyish.
	2936 F	3	F	Red dry nasal discharge; brown yellow urinary staining; lungs: mottled dark red 90%; liver: pale 80%, yellow patch on lobe; stomach: cardiac region - distended with air, pyloric region - black areas on inside wall; intestines: contain red-yellow fluid, viscous; kidneys: pale; adrenals: pale red.

[a]F=Found dead (Day given is day found dead).
T=Terminal (Killed on Day 14).

APPENDIX A

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

ANALYSIS OF TRIPHENYLPHOSPHINE

The analysis of the triphenylphosphine for Lot PNVAH-6KF (flaked) is as follows:

Triphenylphosphine	=	99.4%
Triphenylphosphine Oxide	=	0.6%
Loss on Drying (LOD)	=	0.1%
Light Transmission*	=	98% 650 nm
APHA Color*	=	50
Ash	=	25 ppm
Chlorides	=	9.9 ppm
Magnesium	=	1.4 ppm
Iron	=	1.4 ppm
Sodium	=	1.6 ppm

*measured for 25% acetone solution

APPENDIX B

PROJECT NO.: 6218-80

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine (TPP)

LD_{50} CALCULATIONS, DOSE RESPONSE CURVES AND SLOPE

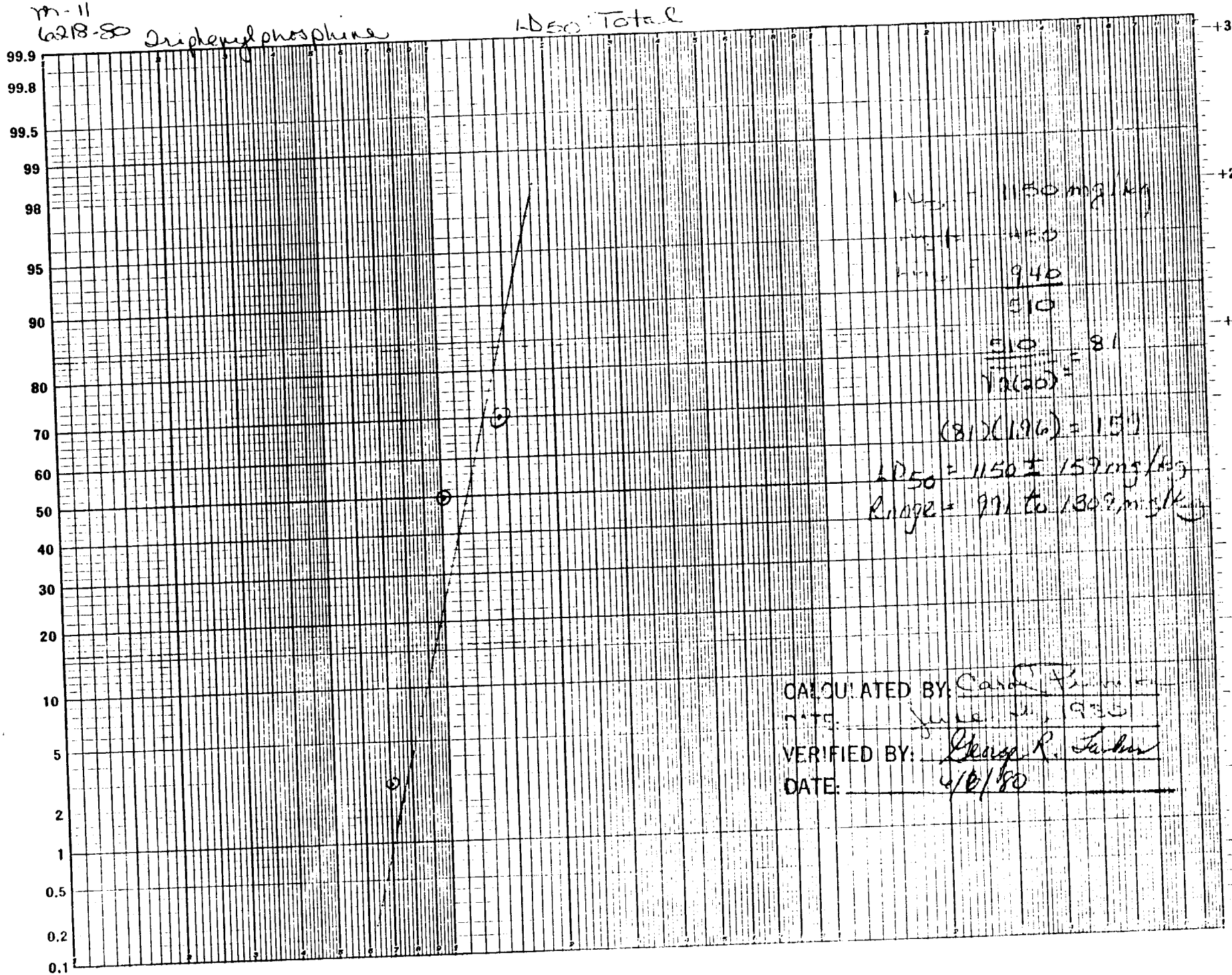
NO. 32.376. LOGARITHMIC NORMAL.

GRAPH PAPER ®

PRINTED IN U.S.A.

m-11
6218-80 Triphenylphosphine

LD50: Total



LD50 = 1150 mg/kg

[illegible] = 450

[illegible] = 940

510

$\frac{510}{\sqrt{2(20)}} = 81$

(81)(1.96) = 159

LD50 = 1150 ± 159 mg/kg

Range = 991 to 1309 mg/kg

CALCULATED BY: [illegible]

DATE: June [illegible], 1980

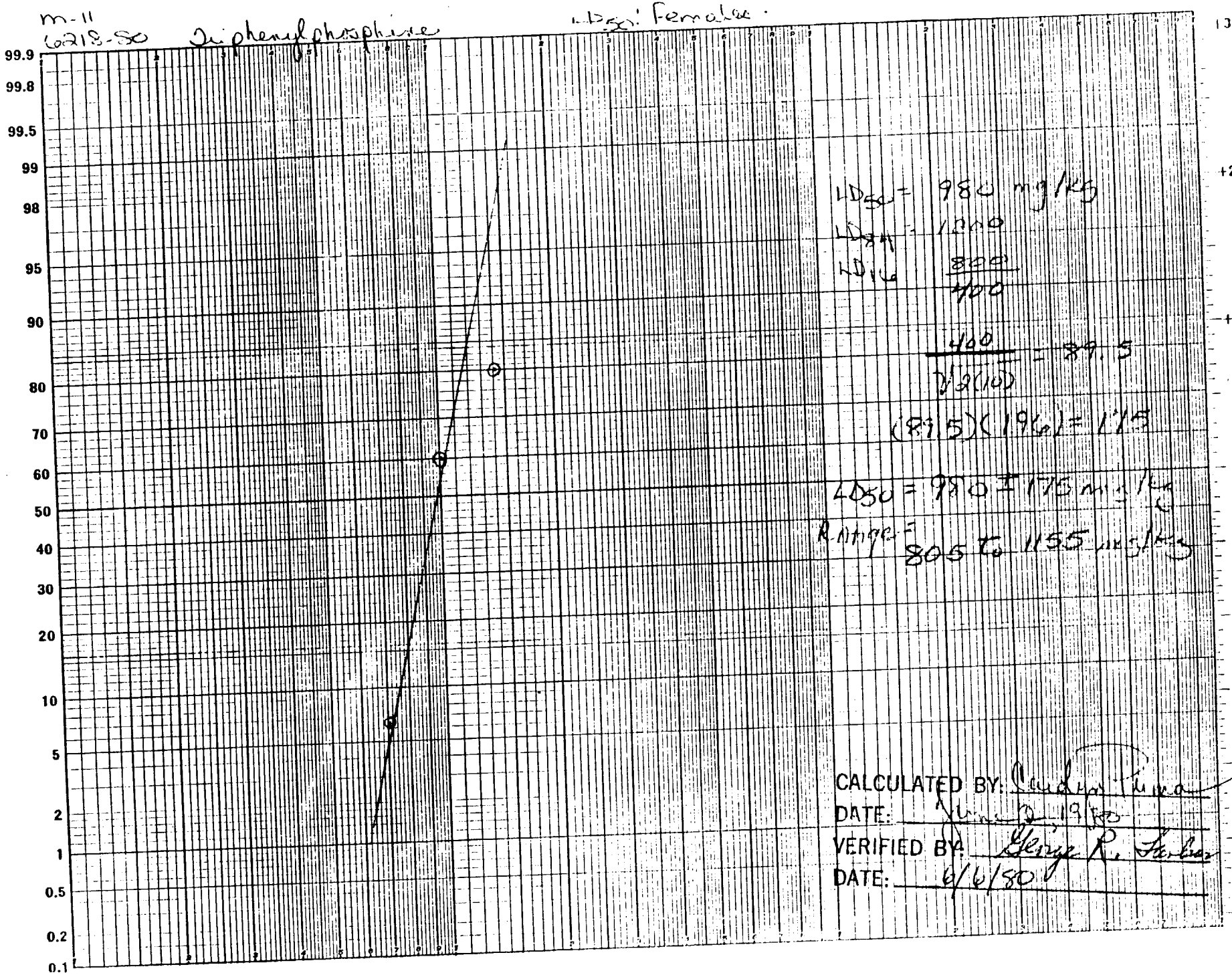
VERIFIED BY: George R. [illegible]

DATE: 6/6/80

Bio dynamics Inc.

NO. 32,376. LOGARITHMIC NORMAL. CODEX GRAPH PAPER PRINTED IN U.S.A.

m-11
6218-80 Triphenylphosphine LD50: Females



LD_{50} = 980 mg/kg
LD_{84} = 1200
LD_{16} 800
400

$\frac{400}{\sqrt{2(10)}} = 89.5$

(89.5)(196) = 175

LD_{50} = 980 ± 175 mg/kg
Range = 805 to 1155 mg/kg

CALCULATED BY: Carolyn Pima
DATE: June 2, 1980
VERIFIED BY: George R. Farber
DATE: 6/6/80

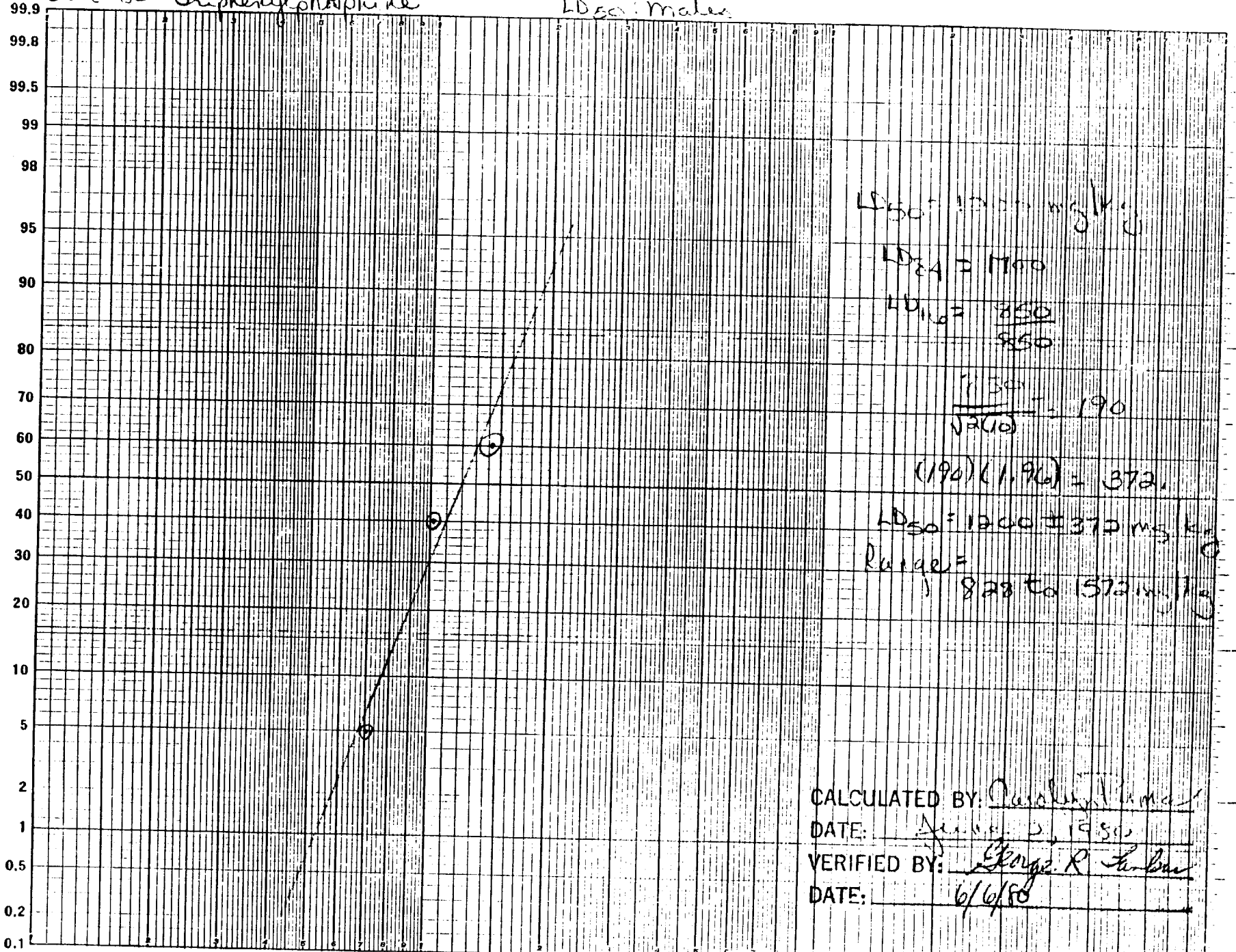
Bio dynamics Inc.

GRAPH PAPER ®
IN STOCK DIRECT FROM COLE-PARMER
PRINTED IN U.S.A.

M-11

6218-50 Triphenylphosphine

LD50: Males



Bio dynamics Inc.

Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO.: 81-7534B

A FOUR WEEK INHALATION TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN THE DOG

Submitted to: M&T Chemicals
Rahway, N.J. 07065

Attention: Mr. Arthur Sheldon

Date: May 31, 1982

Revised Date: December 17, 1982

III. RESULTS (cont.)

C. In-Life Observations (Table II)

Fecal staining and soft stool were exhibited by several animals at all dose levels usually within the first four hours after dosing due to the cathartic effect of the vehicle. At the higher dose levels (706, 1002, and 1423 mg/kg) ataxia, fine or coarse tremors, red ocular discharge, irregular breathing and/or respiratory rate decrease, motor activity decrease and hypothermia were exhibited by several animals. Other signs noted sporadically are outlined in Table II.

IV. DISCUSSION

The results presented here can be contrasted with an earlier acute study (Bio/dynamics Project No. 2575-75) in which the LD_{50} for triphenylphosphine was approximately 30-fold greater than in the present study. This difference can be attributed to the fact that in the earlier study the test material was administered orally as an aqueous, particulate suspension in 1% Methocel. The relatively larger volume used coupled with the insolubility of the test substance in an aqueous medium would likely render a substantial fraction unavailable for absorption, thereby increasing the LD50.

George R. Lankas 6/30/80

George R. Lankas, Ph.D. Date
Study Director

William E. Rinehart 6/30/80

William E. Rinehart, Sc.D. Date
Vice-President

TABLE OF CONTENTS

I. INTRODUCTION 1

II. MATERIALS
- A. Test Material 1
- B. Test Animals 1
- C. Selection 2
- D. Animal Identification 3

III. METHODS
- A. In-Life 3
  - Experimental Design 3
  - Husbandry 3
  - Test Material Administration 4
  - Chamber Operation 4
  - Atmospheric Sampling 5
  - Animal Observations and Body Weights 5
- B. Post Mortem 6

IV. RESULTS AND DISCUSSION
- A. Chamber Concentrations 7
- B. Mortality 7
- C. Physical Observations 7
- D. Neurological Examinations 7
- E. Body Weights 8
- F. Macroscopic Pathology 8
- G. Microscopic Pathology 9

V. CONCLUSION 10

TABLES

1. Daily Mean and Integrated Mean Analytical Concentrations 11
2. Daily Nominal Concentrations 12
3. Summary of Particle Size Distribution 13
4. Physical Examinations 14
5. Neurological Examinations 19
6. Individual Body Weights 35
7. Animal Termination History 36

APPENDICES

- A. Methodology and References A-1
- B. Analytical Methodology B-1
- C. Pathology Report C-1
- D. Personnel D-1

I. INTRODUCTION

This study, conducted for M&T Chemicals, was designed to evaluate effects produced by inhalation exposure to Triphenyl Phosphine (TPP) when administered to the breathing atmosphere of beagle dogs for 6 hours per day, 5 days per week for up to four weeks. Target concentrations of 0.5, 3.0, 10.0, and 30.0 milligrams per meter cubed (mg/m^3) were selected for the exposures based upon previous studies in which the nervous system was affected (Bio/dynamics Project Numbers 79-7349, 79-7350, and 79-7380). Two dogs in all exposure groups received 20 six hour exposures over four weeks. In addition, two dogs from the 0.5, 3.0, and 30.0 mg/m^3 groups received two exposures only and were not exposed during the remainder of the four week period (recovery). A control group, consisting of two dogs, was treated in the same manner, except they received twenty exposures to xylene which was the solvent vehicle. At termination, necropsy and a histopathological assessment of the spinal cord and sciatic nerve was performed. This study was conducted at Bio/dynamics, Inc., Mettlers Road, East Millstone, New Jersey 08873. However, histopathology was performed by Robert F. McConnell, D.V.M., P.A.; Consulting Pathology Services; R.D.7, Wells Road, Flemington, N.J. 08852. All raw data and specimens will be stored in the archives Bio/dynamics, Inc.

II. MATERIALS

A. Test Material: Triphenyl Phosphine

Supplier:	M&T Chemicals Rahway, N.J. 07065
Description:	Cream colored white granular powder
Lot Number:	PNVAH-6KFA
Active Ingredient:	94.9%

II. MATERIALS (Cont.)

A. Test Material (cont.)

Amount Received:	Gross weight - 3.325 kg Estimated net weight - 2.5 kg
Date Received:	December 2, 1981
Expiration Date:	December, 1982
Storage Information:	60 - 85°F
Vehicle Control Material:	Xylene
Supplier:	VWR Scientific Inc. P.O. Box 999 South Plainfield, N.J. 07080
Description:	Clear liquid
Lot Number:	108826
Label Information	CS 9490-5
Date Received:	November 20, 1981 - 24 containers

B. Test Animals: Dog

Strain:	Beagle		
Number of Animals:	16 (8 males, 8 females)		
Supplier:	Marshall Research Animals, Inc. North Rose, New York 14516		
Date Received:	September 29, 1981		
Age at Receipt:	4-5 months		
Age at Initiation of Exposure:	6-7 months		
		Mean	Range
Weight at Initiation of Exposure (kilograms):	Males:	11.4	9.6 - 13.2
	Females:	9.2	8.2 - 10.4
Immunization:	Against Distemper, hepatitis, and leptospirosis by supplier. Treatment for rabies performed at Bio/dynamics, Inc. upon receipt of animals.		

II. MATERIALS (Cont.)

B. Test Animals (cont.)

Parasites: Fecal examination conducted during pretest on all animals at Bio/dynamics, Inc. during the equilibration period. All results were negative.

C. Selection: More animals were purchased and equilibrated than were required for the study. Animals considered unsuitable for the study on the basis of pretest physical examinations were eliminated prior to initiation of exposure to the test substance (Methodology and References, Appendix A). Animals were sorted into groups on a random basis from those animals considered suitable for study purposes.

D. Animal Identification: Each dog was assigned a unique Bio/dynamics, Inc. identification number which appeared on an ear tag and on the animal's cage. In addition, each animal bore an ear tattoo with a USDA number.

III. METHODS

A. In-Life

EXPERIMENTAL DESIGN:

Group	Concentration	Exposure Time	Number of Exposures		Number of Animals[1]	
			Two[1,2]	Twenty[1,3]	Necropsy	Histopathology
I	Xylene Control[4]	6 Hr/Day	0	2	2	2
II	0.5 mg/m^3	6 Hr/Day	2	2	4	4
III	3.0 mg/m^3	6 Hr/Day	2	2	4	4
IV	10.0 mg/m^3	6 Hr/Day	0	2	2	2
V	30.0 mg/m^3	6 Hr/Day	2	2	4	4
		Totals:	6	10	16	16

1 - Evenly divided by sex.
2 - Exposed for two days, sacrificed 30 days after last exposure.
3 - Due to intervening holidays (Christmas and New Years), animals were exposed over a five week period 3-5 days/week, and sacrificed one day after the last exposure.
4 - The level of xylene was 150 ppm or less in all chambers - different strength solutions of TPP in xylene were prepared so that the level of xylene was approximately the same in all chambers.

III. METHODS (Cont.)

A. In-Life (cont.)

Husbandry:

Equilibration Period:	Minimum 4 weeks
Housing:	Individually in elevated metal grid cages during exposure and non-exposure periods.
Food:	Standard laboratory diet (Wayne Bite-Size®), approximately 400 g/day during non-exposure hours only.
Water:	City tap water (Elizabethtown Water Company) with automatic watering system ad libitum during non-exposure hours only.
Lights:	12 hours on, 12 hours off (animals exposed during lights "on" period).

Test Material Administration:

A solution of TPP and xylene was placed in a 500 milliliter volumetric flask that was connected to an FMI fluid metering pump (Model G-20). The test solution was fed directly into an air atomizing nozzle (Spraying Systems Company; 1/4 J nozzle) and dry air at a back pressure of 20 pounds per square inch (psi) was passed through the atomizer to generate the aerosol. The resultant aerosol was drawn into the horizontal intake portal of a 10 cubic meter (m^3) glass and stainless steel exposure chamber. Chamber concentrations were controlled by adjusting the rate of flow of the solution to the spray atomizer. The solutions used for each exposure group were as follows:

Group I - control; administration of solvent only (xylene)
Group II - 0.5 mg/m^3, 0.25% (weight/volume) solution
Group III - 3.0 mg/m^3, 1.50% (weight/volume) solution
Group IV - 10.0 mg/m^3, 5.00% (weight/volume) solution
Group V - 30.0 mg/m^3, 15.00% (weight/volume) solution

III. METHODS (Cont.)

Test Material Administration (cont.)

Chamber Operation:

The stainless steel and glass exposure chambers in which the animals were exposed had a total volume of 10 cubic meters. These chambers were operated dynamically at an air flow of 3000 (Group I - IV) or 2900 (Group V) liters per minutes. These flow rates provided one complete air change every 3.3 or 3.4 minutes with equilibrium times (T_{99}) every 15.3 or 15.9 minutes, respectively.

Atmospheric Sampling:

At least three samples per day were drawn from each exposure chamber through a glass fiber filter. Samples were pulled at various flow rates and times, the collected TPP desorbed in a solvent and the resultant solution analyzed by gas chromotography. A full detailed description of the sampling procedure and methodology is presented in Appendix B.

Determination of particle size distribution was done for each chamber, including the control, once each week using a Royco particle monitor (Model 227).

Animal Observations and Body Weights:

Animals were observed twice daily for incidence of abnormal signs. Observations of any unusual conditions were recorded when made. Full physical and neurological assessments were performed weekly. Individual body weights were recorded pretest and weekly during the exposure and/or recovery period.

III. METHODS (Cont.)

B. Post-Mortem:

Dates of Necropsy:	January 7, 1982 (5 male, 5 female) - animal receiving 20 exposures. January 8, 1982 (3 male, 3 female) - animals receiving 2 exposures.
Sacrifice Method:	Exsanguinated under sodium pentobarbital anesthesia.
Tissues Fixed:	brain (3 sections) sciatic nerve with muscle (both right and left, 3 sections each) spinal cord (four inch section of cervical and lumbar regions)
Fixative:	All tissues - 10% neutral buffered formalin.
Histopathology:	Slides of tissue sections listed below were prepared and examined microscopically from all animals. Sections of all tissues were prepared with H&E stain and luxol fast blue stain.
Sciatic Nerve (right and left):	1 longitudinal section (on each) 2 transverse sections (on each)
Cervical Spinal Cord:	1 longitudinal section 2 transverse sections
Lumbar Spinal Cord:	1 longitudinal section 2 transverse sections

incl [illegible] - 192 Tissues

IV. RESULTS AND DISCUSSION

A. Chamber Concentrations

(Daily and Integrated Mean Concentration - Table 1, page 11; Nominal Concentrations - Table 2, page 12; Particle Size Distribution - Table 3, page 13; Analytical Chemistry Methodology - Appendix B)

The cumulative integrated mean concentrations for dogs receiving 20 exposures were 0.5, 3.2, 9.7, and 28 mg/m^3 for Groups II, III, IV and V, respectively; while dogs receiving two exposures only received integrated mean concentrations of 0.3, 3.6, and 24 mg/m^3 for Groups II, III, and V. The average nominal TPP concentrations for 20 exposures was 0.78, 3.5, 15, and 51 mg/m^3 for Groups II, III, IV, and V, respectively. The particle size assays indicated that a respirable aerosol (count median diameter 0.5 microns or less) was present in the 0.5 and 3.2 mg/m^3 chambers. The concentration of aerosol at 9.9 and 28 mg/m^3 was above the upper range of the instrument. Because both the same generation conditions were used throughout and the nominal to analytical ratio is comparable over all test chambers, the particle size is believed to be about the same respirable size in all test chambers.

B. Mortality

(Animal Termination History - Table 7, page 36)

No test animal mortality occurred.

C. Physical Observations

(Physical Examinations - Table 4, page 14)

There were no overt physical signs of toxicity seen in any of the treatment groups. Although excitability was noted at least in one dog in every group, in most cases this was noted prior to initiation of treatment and continued partially or throughout the study.

D. Neurological Observations

(Neurological Examinations - Table 5, page 19)

Both the male and female dogs receiving 20 exposures in Group V (30.0 mg/m^3) showed indications of neurological impairment. This was noted in the

IV. RESULTS AND DISCUSSION (Cont.)

D. Neurological Observations (cont.)

extensor postural thrust reflex; both dogs had a tendency to knuckle over, and have unsteadiness and weakness in the hind limbs. The locomotion reflex in the male dog also appeared unsteady and to be knuckling over. The male dog receiving two exposures in Group V (30.0 mg/m^3) also exhibited some extensor postural thrust reflex impairment as uncoordination in rear legs, and rotating the body in one direction. The female receiving two exposures in this group did not exhibit any neurological impairment. The male dog in Group IV (10 mg/m^3) exhibited erratic placement of limbs on Weeks 1 and 4 and one male dog in Group III (3.0 mg/m^3) which received two exposures exhibited some limb placement incoordination throughout the study. Other transient findings were seen in some dogs which occurred either pretest or in single incidences.

The findings in both male dogs and the one female dog receiving 20 exposures in Group V were considered to be related to the exposure of TPP. While similar findings in the male dog each in Group IV and III, they were not considered related to treatment since the dog in Group IV (20 exposure) did not show persistent or severe signs, and the Group III dog only received two exposures. No other signs were considered related to the test material.

E. Body Weights
(Individual Body Weights - Table 6, page 35)

No association between body weights and treatment was observed during the course of the study.

F. Macroscopic Pathology
(Appendix C)

Postmortem findings, observed grossly, either occurred in the treated and control animals or they occurred sporadically; they did not appear to be related to the test material.

IV. RESULTS AND DISCUSSION (Cont.)

G. Microscopic Pathology
(Appendix C)

Microscopic pathology revealed moderate generalized vacuolative degeneration of the ventral and lateral funicule of both the cervical and lumbar spinal cord at the high level (Group V) in the dogs receiving 20 exposures and this was considered treatment related. Similar lesions, although minimal and focal in nature, were noted in spinal cord sections of dogs exposed to 0.5 or 3.2 mg/m^3 TPP. In the dogs receiving 0.5 mg/m^3 TPP the lesion was noted in one male following 20 exposures and in one female following only two exposures. These lesions were not seen in any dogs exposed to the vehicle alone. No histopathological effect was seen in the sciatic nerve.

Bio dynamics Inc.

Revised: James B. Terrill

Date: Dec. 1, 1982

V. CONCLUSION

There was no treatment related mortality and physical signs were similar in treated and control animals.

Body weight increments for both sexes in treated and control dogs were comparable throughout the treatment and recovery periods.

Evidence of neurological impairment was noted at the high exposure level (30.0 mg/m^3) in both male dogs and in the one female dog which received twenty exposures.

Although macroscopic pathology findings were unremarkable, microscopic pathology findings revealed modulate generalized vacuolative degeneration of the ventral and lateral funicule of the cervical and lumbar spinal cord on dogs receiving 20 exposures at the high level. Similar lesions, although minimal and focal in nature, were noted in spinal cord sections of dogs exposed to 0.5 or 3.2 mg/m^3 TPP. In the dogs receiving 0.5 mg/m^3 TPP the lesion was noted in one male following 20 exposures and in one female following only two exposures.

James B. Terrill — Dec 1, 1982

James B. Terrill, Ph.D. — Date
Director Inhalation Toxicology

Geoffrey K. Hogan — 12/2/82

Geoffrey K. Hogan, Ph.D., D.A.B.T. — Date
Vice-President of Toxicology

Table 1
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Daily Mean and Integrated Mean Analytical Concentrations

Date	Test Day	Exposure Number	Group II - 0.5 mg/m^3		Group III - 3.0 mg/m^3		Group IV - 10 mg/m^3		Group V - 30 mg/m^3	
			Daily Mean Concentration (mg/m^3)	Integrated Mean Concentration (mg/m^3)	Daily Mean Concentration (mg/m^3)	Integrated Mean Concentration (mg/m^3)	Daily Mean Concentration (mg/m^3)	Integrated Mean Concentration (mg/m^3)	Daily Mean Concentration (mg/m^3)	Integrated Mean Concentration (mg/m^3)
12/8/81	1	1	0.3	0.3	4.1	4.1	4.5*	4.5	27	27
12/9/81	2	2	0.3	0.3	3.1	3.6	8.7	6.6	20	24
12/10/81	3	3	0.6*	0.4	2.9*	3.4	10*	7.7	34*	27
12/11/81	4	4	0.6	0.5	2.8	3.2	9.0	8.1	28	27
12/14/81	7	5	0.8	0.5	3.4	3.3	12	8.8	29	28
12/15/81	8	6	0.6	0.5	3.8	3.4	9.3	8.9	36	29
12/16/81	9	7	0.6	0.5	3.2	3.3	12	9.4	29	29
12/17/81	10	8	0.6	0.6	3.5	3.4	9.6	9.4	29	29
12/18/81	11	9	0.5	0.5	2.8	3.3	9.3	9.4	29	29
12/21/81	14	10	0.5	0.5	3.5	3.3	12	9.6	28	29
12/22/81	15	11	0.5	0.5	2.6	3.2	9.2	9.6	34	29
12/23/81	16	12	0.4	0.5	4.3	3.3	11	9.7	26	29
12/24/81	17	13	0.5	0.5	2.8	3.3	8.4	9.6	14	28
12/28/81	21	14	0.5	0.5	2.8	3.3	10	9.6	26*	28
12/29/81	22	15	0.5	0.5	3.3	3.3	9.3	9.6	36	28
12/30/81	23	16	0.5	0.5	3.3	3.3	9.4	9.6	26	28
12/31/81	24	17	0.6	0.5	3.3	3.3	12	9.7	33	28
1/4/82	28	18	0.5	0.5	2.8	3.2	9.5	9.7	24	28
1/5/82	29	19	0.4	0.5	3.3	3.2	8.2	9.7	20	28
1/6/82	30	20	0.4	0.5	2.9	3.2	11	9.7	33	28

*Based on four samples.

Table 2
A Four Week Inhalation Toxicity Study
of TPP in the Dog

81-7534B

Daily Nominal Concentrations

Date	Test Day	Exposure Number	Group II - 0.5 mg/m^3 Total Solution	Group II - 0.5 mg/m^3 Total* TPP	Group III - 3.0 mg/m^3 Total Solution	Group III - 3.0 mg/m^3 Total* TPP	Group IV - 10 mg/m^3 Total Solution	Group IV - 10 mg/m^3 Total* TPP	Group V - 30 mg/m^3 Total Solution	Group V - 30 mg/m^3 Total* TPP
12/8/81	1	1	206	0.52	349	5.2	235	12	223	33
12/9/81	2	2	289	0.72	306	4.6	284	14	296	44
12/10/81	3	3	381	0.95	254	3.8	309	15	287	43
12/11/81	4	4	392	0.98	241	3.6	301	15	263	39
12/14/81	7	5	343	0.86	259	3.9	312	16	393	59
12/15/81	8	6	326	0.82	210	3.2	240	12	329	49
12/16/81	9	7	332	0.83	184	2.8	247	12	259	39
12/17/81	10	8	301	0.75	205	3.1	308	15	301	45
12/18/81	11	9	310	0.78	172	2.6	315	16	322	48
12/21/81	14	10	304	0.76	180	2.7	301	15	290	44
12/22/81	15	11	291	0.73	141	2.1	265	13	312	47
12/23/81	16	12	259	0.65	247	3.7	291	15	313	47
12/24/81	17	13	317	0.79	153	2.3	258	13	519	78
12/28/81	21	14	283	0.71	186	2.8	274	14	273	41
12/29/81	22	15	312	0.78	195	2.9	293	15	346	52
12/30/81	23	16	350	0.88	257	3.9	345	17	277	42
12/31/81	24	17	287	0.71	203	3.0	294	15	259	39
1/4/82	28	18	290	0.73	307	4.6	349	17	354	53
1/5/82	29	19	323	0.81	296	4.4	438	22	519	79
1/6/82	30	20	364	0.91	346	5.2	476	24	659	99
		Mean:	313	0.78	235	3.5	307	15	340	51

*Total TPP Nominal Delivery = Total Solution Nominal Delivery X $\frac{\%\ \text{Solution}}{100}$

Table 3
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Summary of Particle Size Distribution Using
The Royco Particle Monitor Model 227

Date of Sample	Stage Constant (um) 0.5	0.7	1.4	3.0	5.0		Count Median Diameter (um)	Geometric Standard Deviation
			Group I - Xylene (control)					
12/10/81	1243	510	100	9	4		0.58	2.1
12/18/81	1372	307	29	1	0		0.46	1.8
12/21/81	2836	980	164	17	9		0.55	2.0
12/30/81	3535	921	286	55	19		0.41	2.4
						Mean:	0.50	2.1
			Group II - 0.5 mg/m^3					
12/10/81	123932	16780	2416	14	6		0.42	1.9
12/18/81	217714	43206	7948	5	4		0.40	2.0
12/21/81	261568	54905	7817	9	2		0.41	1.9
12/30/81	978497	71039	20124	145	130		0.27	2.1
						Mean:	0.38	2.0
			Group III - 3.0 mg/m^3					
12/10/81[a]	657959	50622	60601	74	3		-	-
12/18/81	564140	63718	9715	48	3		0.33	1.9
12/21/81	595112	83560	15715	42	6		0.33	2.1
12/30/81	420806	92030	67953	52958	164		-	-
						Mean:	0.35	2.1
			Group IV - 10 mg/m^3					
12/10/81[a]	675458	11066	65767	7857	14		-	-
12/18/81[a]	718708	32429	64394	7303	46		-	-
12/21/81[a]	690630	28717	80326	7656	17		-	-
12/30/81[a]	280788	73587	64986	8000	4684		-	-
						Mean:	-	-
			Group V - 30 mg/m^3					
12/10/81[a]	637265	47187	10922	11158	11		-	-
12/18/81[a]	437667	87634	40807	68233	1463		-	-
12/21/81	720623	90486	25388	19535	892		0.24	2.7
12/30/81[b]	333204	83449	26537	621	304		0.35	2.6
						Mean:	0.30	2.7

[a]Particle size analysis invalid due to equipment malfunction or technician error.
[b]Suspected invalid determinations.

Table 4
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Physical Examination
Group I

An. No.	Observation	Interval				
		Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
		Males				
1001	Salivation					1+
	Throat inflamed					1+
	No observed abnormalities	x	x	x	x	
		Females				
1501	Excitable	1+	1+	1+	2+	1+
	Tonsils red and swollen	1+	2+	1+	1+	1+
	Popliteal lymph nodes swollen		1+	1+	1+	1+

Key: 1+ = slight; 2+ = moderate; x = present.

Table 4 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Physical Examination
Group II

An. No.	Observation	Interval				
		Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Males					
2001	Excitable	2+	2+	2+		2+
	Both residual canines present			x	x	
	Right residual canine present	x				
2002*	Popliteal lymph nodes swollem	1+	1+	1+	1+	1+
	Testicles small and immature	x				
	Females					
2501*	No observed abnormalities	x	x	x	x	x
2502	Left popliteal enlarged	1+		1+	1+	
	No observed abnormalities		x			x

Key: 1+ = slight; 2+ = moderate; x = present; * = two exposures only.

Table 4 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Physical Examination
Group III

An. No.	Observation	Interval				
		Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Males					
3001	Excitable	2+	2+	3+	2+	3+
	Popliteal lymph nodes swollen			1+	1+	1+
	Scab covered lesion below left eye	1+				
	Dry scaly denuded area below left eye		1+	1+		
3002*	Excitable		2+			
	Right pupillary response slow	1+				
	Left popliteal enlarged				1+	
	No observed abnormalities			x		x
	Females					
3501*	Excitable	1+	2+			3+
	Cervical lymph nodes enlarged			1+	1+	
	Both residual canines present	x	x	x		x
	Popliteal lymph nodes enlarged			1+		
3502	Excitable		3+	3+	1+	1+
	Mouth reddened			1+	1+	
	Vulva swollen					1+
	No observed abnormalities	x				

Key: 1+ = slight; 2+ = moderate; 3+ = marked;
x = present; * = two exposures only.

Table 4 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Physical Examination
Group IV

An. No.	Observation	Interval				
		Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
		Males				
4001	Popliteal lymph nodes swollen	1+	1+	1+	1+	1+
		Females				
4501	Excitable		2+	2+	1+	1+
	Both residual canines present	x				
	Malaclusion	x				
	Hair thinning	1+	1+	1+	1+	1+

Key: 1+ = slight; 2+ = moderate; x = present.

Table 4 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Physical Examination
Group V

An. No.	Observation	Interval				
		Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
		Males				
5001*	No observed abnormalities	x	x	x	x	x
5002	Testicles small and immature	x				
	No observed abnormalities		x	x	x	x
		Females				
5501*	Excitable	3+	3+	2+	2+	3+
	Both residual canines present	x				
	Popliteal lymph nodes enlarged			1+	1+	
5502	Popliteal lymph nodes swollen			1+	1+	1+
	No observed abnormalities	x	x			

Key: 1+ = slight; 2+ = moderate; 3+ = marked;
x = present; * = two exposures only.

81-7534B

Table 5
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group I - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 1001				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	a	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	a	+	+	+	+
Placing-tactile	a	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
[a]Response positive but sluggish due to passive behavior.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group I - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 1501				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group II - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 2001				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	b	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
[b]Response positive but slow or sluggish.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group II - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 2002*				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
* = two exposures only.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group II - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 2501*				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
* = two exposures only.

81-7534B

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group II - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 2502				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	b	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
[b]Response positive but slow or sluggish.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group III - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 3001				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	d	+	+
Extensor (crossed)	+	+	d	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	c	c	+	+	e
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
[c]Response positive, but animal places feet very widely apart.
[d]Could not evaluate due to excitability.
[e]Does not place feet evenly on floor.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group III - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 3002*				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	-	-	g
Extensor postural thrust	+	f	f	+	h
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
- = reflex absent
* = two exposures only.
[f]Response positive but uncoordinated.
[g]Response weak.
[h]Place left paw posteriorly and uncoordinated.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group III - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 3501*				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
* = two exposures only.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group III - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 3502				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	b	+	+	+	+
Placing-tactile	b	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
[b]Response positive but slow or sluggish.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group IV - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 4001				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	i	+	+	j
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
[i]Response positive, but places feet irratically.
[j]Places feet or legs close to midline of body.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group IV - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 4501				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group V - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 5001*				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	-	-	+	+	+
Extensor postural thrust	+	+	k	+	l
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	b	+	+	+	+

Key: + = reflex normal; - = Reflex absent.
* = two exposures only.
[b]Response positive but slow or sluggish.
[k]Some uncoordination in rear legs.
[l]Rotates body to the right.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group V - Male

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 5002				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	j,p
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	m	o	q
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	n	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
[j]Places feet or legs close to midline of body.
[m]Tendeny to knuckle over (both paws).
[n]Unable to do tonic neck reactions due to weakness in all limbs.
[o]Unsteady reaction, weakness and unsteadiness in hindlimbs.
[p]Both rear legs are toeing over.
[q]Locomotion appears unsteady.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group V - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 5501*				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	+	+
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	+	+	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	+	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
* = two exposures only.

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Neurological Examination
Group V - Female

Observation	Interval				
	Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
	Animal Number 5502				
Pupillary light	+	+	+	+	+
Consensual light	+	+	+	+	+
Palpebral, blink	+	+	+	+	+
Palpebral, corneal	+	+	+	+	+
Gag	+	+	+	+	+
Optic righting	+	+	+	+	+
Startle (oculo-acoustic)	+	+	+	+	+
Startle (aural-acoustic)	+	+	+	+	+
Flexor (withdrawl)	+	+	+	+	+
Extensor (crossed)	+	+	+	+	+
Patellar	+	+	+	-	g
Locomotion	+	+	+	+	+
Extensor Tone	+	+	+	+	+
Placing-visual	+	+	+	+	+
Placing-tactile	+	+	+	+	+
Extensor postural thrust	+	+	m	r	+
Righting reactions	+	+	+	+	+
Tonic neck reactions	+	+	+	n	+
Hopping reflex	+	+	+	+	+

Key: + = reflex normal
- = reflex absent
[g]Response weak.
[m]Tendency to knuckle over (both paws).
[n]Unable to do tonic neck reactions due to weakness in all limbs.
[r]Response positive but unsteady; unsteadiness of gait due to slight hindlimb weakness.

Table 6
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Individual Body Weights (kg)

Group mg/m^3	Animal Number	Sex	Interval Pretest 12/1/81	Week 1 12/15/81	Week 2 12/21/81	Week 3 12/28/81	Week 4 1/5/82
I	1001	M	11.5	11.6	11.5	11.6	11.6
Xylene Control	1501	F	9.4	9.5	9.6	9.8	9.7
II	2001	M	11.4	11.6	11.7	11.7	11.9
0.5	2002*	M	11.1	11.0	11.3	11.5	11.6
	2501*	F	9.4	9.4	9.4	9.5	9.3
	2502	F	8.4	9.1	8.9	9.0	9.1
III	3001	M	10.4	10.6	10.3	10.7	11.0
3.0	3002*	M	12.0	11.6	11.7	11.7	11.9
	3501*	F	8.2	8.4	8.3	8.6	8.6
	3502	F	10.4	10.2	10.0	10.2	10.7
IV	4001	M	12.0	12.5	12.4	12.4	12.3
10.0	4501	F	9.4	9.6	9.5	9.6	10.0
V	5001*	M	13.2	13.4	13.7	13.6	13.5
30.0	5002	M	9.6	10.4	10.2	10.4	10.6
	5501*	F	8.7	8.6	8.5	8.6	8.7
	5502	F	9.5	9.6	9.6	9.4	9.6

*Two exposures only.

Table 7
A Four Week Inhalation Toxicity Study
of TPP in the Dog

Animal Termination History

Group mg/m^3	Males				Females			
	An. No.	Days on Test	Date of Death	Type of Death/Week	An. No.	Days on Test	Date of Death	Type of Death/Week
I Xylene Control	1001	31	1/7/82	T/5	1501	31	1/7/82	T/5
II 0.5	2001	31	1/7/82	T/5	2501*	32	1/8/82	T/5
	2002*	32	1/8/82	T/5	2502	31	1/7/82	T/5
III 3.0	3001	31	1/7/82	T/5	3501*	32	1/8/82	T/5
	3002*	32	1/8/82	T/5	3502	31	1/7/82	T/5
IV 10.0	4001	31	1/7/82	T/5	4501	31	1/7/82	T/5
V 30.0	5001*	32	1/8/82	T/5	5501*	32	1/8/82	T/5
	5002	31	1/7/82	T/5	5502	31	1/7/82	T/5

Key: T = terminal sacrifice; * = two exposures only.

Appendix A
A Four Week Inhalation Toxicity Study of TPP in the Dog

Methodology and References - General

Parameter	Reference or Description
Physical Examination:	Central Nervous System: examined - behavior movements, reactivity to stimuli, muscle tone. Autonomic Nervous System: examined - pupil size, secretions. Respiration: examined - nostrils, character, rate. Cardiovascular: examined - palpation. Gastrointestinal: examined - abdominal shape, feces consistency and color. Genitourinary: examined - penis, vulva, perineal region. Skin and Fur: examined - color, turgor, integrity. Mucous Membrane: examined - mouth, rectal, vaginal. Eye: examined - eyelids, eyeball, transparency. General Condition.
Neurological Examination:	The following reflexes were observed: righting, patellar, flexor, extensor, visual placing response, corneal and pupillary.
Body Weight	Detecto scale (60-S).

Appendix B

AN ANALYTICAL METHOD FOR THE DETERMINATION OF (TPP) TRIPHENYL PHOSPHINE IN INHALATION CHAMBER ATMOSPHERES

DEVELOPED BY: James C. Eschbach Date: 7/1/82 J C Eschbach

ISSUED BY: Bio/dynamics Inc.
Inhalation Department
Box 43
Mettlers Road
East Millstone
N.J. 08873

1.0 Scope:

Inhalation chamber atmospheres containing triphenyl phosphine were monitored during animal exposures. This method describes the procedures for collection, desorption and determination of TPP concentrations by gas chromatography. The method is sensitive to 10 ng of TPP.

2.0 Principle:

T.P.P. is collected from the chamber atmospheres by filtration. Chamber air is drawn through glass fiber filters at a calibrated flow rate for a specific time depending on the concentration.

The filters are removed from their holders, placed in scintillation vials and desorbed with acetonitrile. Aliquots of the acetonitrile solutions are then injected into a gas chromatograph.

The gas chromatograph used for analysis is equipped with a flame ionization detection system.

3.0 Equipment:

3.1	Gas Chromatograph	Tracor 550 or equivalent equipped with a Flame Ionization detection system.
3.2	Strip Chart Recorder	Linear 10" or equivalent
3.3	20 ml Scintillation Vials	Wheaton or equivalent, glass
3.4	10 ul Syringe	Hamilton 701N or equivalent
3.5	Chromatographic Column	6'x1/8" glass
3.6	Glass Fiber Filter Paper	3.7 cm Whatman or equivalent

3.0 Equipment (cont.)

3.7 Filter Holders	Millipore or equivalent
3.8 TSI Mass Flow Sensor	
3.9 Oil-less Vacuum Pump	Thomas, Leeson or Welch
3.10 Calibrated critical orifice	Millipore or equivalent

4.0 Reagents:

4.1 Acetonitrile	Baker Resi-analized or equivalent
4.2 Gas Chromatographic Column Packing	3% OV-17 on Chromosorb W HP 80/100 mesh packed in a glass coil 6'x4mm O.D.x2 mm I.D.

4.3 Triphenyl Phosphine (TPP) M & T Chemical Co., Rahway, N.J. 94.9% pure.

5.0 Procedure:

5.1 Sample Collection:

Place glass fiber filters in holders with attached critical orifice and connect them to a vacuum pump calibrated to draw approximately 10 liters per minute for one minute at the high dose and adjust this sampling time to collect equivalent amounts at all dose levels.

5.1.1 Typical Sample Collections:

Dose Level mg/m^3	Flow (lpm)	Collection (min)	Theoretical Amt. Collected (ug)
0.5	9.8	15	73.5
3.0	9.6	2	57.6
10.0	9.6	1	96.0
30.0	9.6	1	288.0

5.2 Sample Desorption:

Remove the glass fiber filters from the holders and place them in individual scintillation vials. Add 4 ml of acetonitrile to Groups II, III, IV and 8 ml to Group V to desorb the collected TPP. Allow the filters to desorb, with occasional swirling, for 2 minutes. Save these samples for gas chromatographic analysis.

5.3 Gas Chromatographic Analysis:

Aliquots of the acetonitrile solution containing TPP are injected into a gas chromatograph equipped with a flame ionization detector.

5.3 Gas Chromatographic Analysis (cont.)

Table I lists the operating conditions for the use of a Tracor Model 550 gas chromatograph.

Gas chromatographic separations are made using a 3% OV-17 Chromosorb W HP 80/100 mesh column.

5.3.1 Preparation of Standard Solutions:
TPP in Acetonitrile (Store stock solutions in a refrigerator)

a. Prepare a standard stock solution by weighing out 10 mg of TPP and transfer to a 100 ml volumetric flask, dilute to volume with acetonitrile. This solution contains 0.1 mg/ml (Stock Solution A). Stock Solution A should be prepared fresh every month or upon depletion.

b. Five standard solutions for the gas chromatographic analysis of chamber atmospheres were prepared according to the following dilution scheme.

5.3.1.1 Dilutions of Stock Solution A can be made for calibration of the gas chromatograph. A set of suggested dilutions follows:

ml Stock Solution A	Final Dilution Volume (ml)	Final Concentration Nanograms/Microliter of TPP
0.5	10	5
1.0	10	10
2.0	10	20
3.0	10	30
6.0	10	60

This range can be adjusted depending on the concentrations being assayed.

5.3.2 Calibration of the Flame Ionization Detector:

a. Inject 2 microliter aliquots of the standard solutions containing TPP from section 5.3.1.1 at 5, 10, 20, 30 and 60 nanograms/microliter into the gas chromatograph. The gas chromatograph should be operated at the approximate conditions given in Table I. Typical chromatograms of standards are presented in Figure 1.

b. Determine the peak height or area for each standard injected.

c. Construct a standard curve by plotting detector response (Peak height or area) versus concentration (ng) of standard injected. A typical standard curve is shown in Figure 2.

5.0 Procedure (cont.)

5.3.3 Chamber Concentration Determination:

a. Aliquots of samples collected in Section 5.2 (2 ul amounts) are injected into gas chromatograph and the peak height or area for TPP determined.

b. Directly compare the peak heights or areas of the unknown samples injected with the appropriate standard curve and extrapolate the concentration of TPP in the injected aliquot.

c. Determine the chamber concentration in terms of mg/m^3 using the following equation:

$$\left[\frac{\text{ng found}}{\text{ul injected}} \times \frac{10^3 \text{ ul}}{\text{ml}} \times \text{ml final volume} \times \frac{\text{ug}/10^3\text{ng}}{\text{Total Air (L)}}\right] \times \frac{\text{mg}}{10^3 \text{ ug}} \times \frac{10^3 \text{L}}{\text{m}^3} = \text{mg/m}^3$$

6.0 Recovery Studies:

6.1 TPP was placed on Gelman filters and weighed (1.52, 2.17 and 2.98 mg) and room air was drawn through the filters at 10 lpm for 10 minutes.

6.2 The filters were then removed from their holders and were placed in individual scintillation vials for desorption.

6.3 The filters were desorbed using 4 ml of acetonitrile or more if necessary for gas chromatographic analysis.

6.4 Aliquots of the acetonitrile solutions were injected into a gas chromatograph for determination of the residual TPP.

6.5 Results of the analysis demonstrate that an average recovery for TPP of 98.7% was obtained. Table II illustrate these results.

TABLE I GAS CHROMATOGRAPHIC CONDITIONS FOR THE ANALYSIS OF TPP IN INHALATION CHAMBER ATMOSPHERES

1.	Instrument	Tracor 550 gas chromatograph or equivalent
2.	Detector	Flame Ionization
3.	Column	6'x1/4" O.D.x2 mm I.D. coiled glass
4.	Packing	3% OV-17 on Chromosorb W HP 80/100 mesh
5.	Recorder	Omni scripe 11" strip chart or equivalent, 1 cm/minute chart speed
6.	Temperatures	
	Detector	250°C
	Column	220°C
	Inlet	250°C
	Outlet	250°C
7.	Gas Flows	
	Hydrogen	35 ml/minute
	Helium (Carrier)	45 ml/minute
	Air	250 ml/minute
8.	Attenuation	10x8

TABLE II RECOVERY OF TPP FROM FILTERS USED TO COLLECT CHAMBER AIR SAMPLES

Air Flow: 10 liters/minute for 10 minutes.

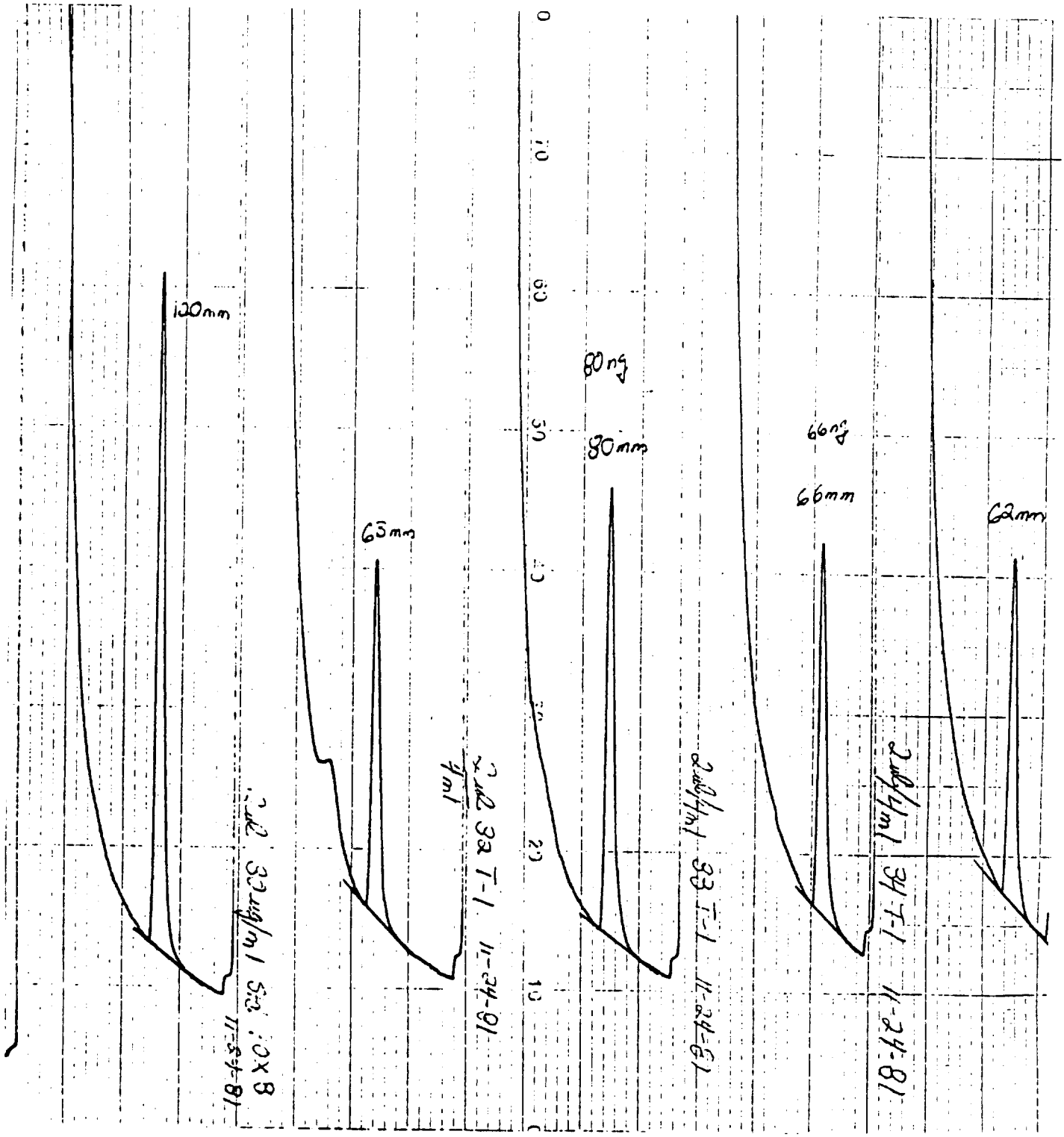
Amount of TPP on Filter mg	Final Dilution (mls)	Theoretical Concert. (ug/ml)	Actual Conc. (ug/ml)	% Recovery
1.52	20	76	78	103
2.17	80	27	28	104
2.98	80	37	33	89

Mean: 98.7

AN ANALYTICAL METHOD FOR THE DETERMINATION OF (TPP)

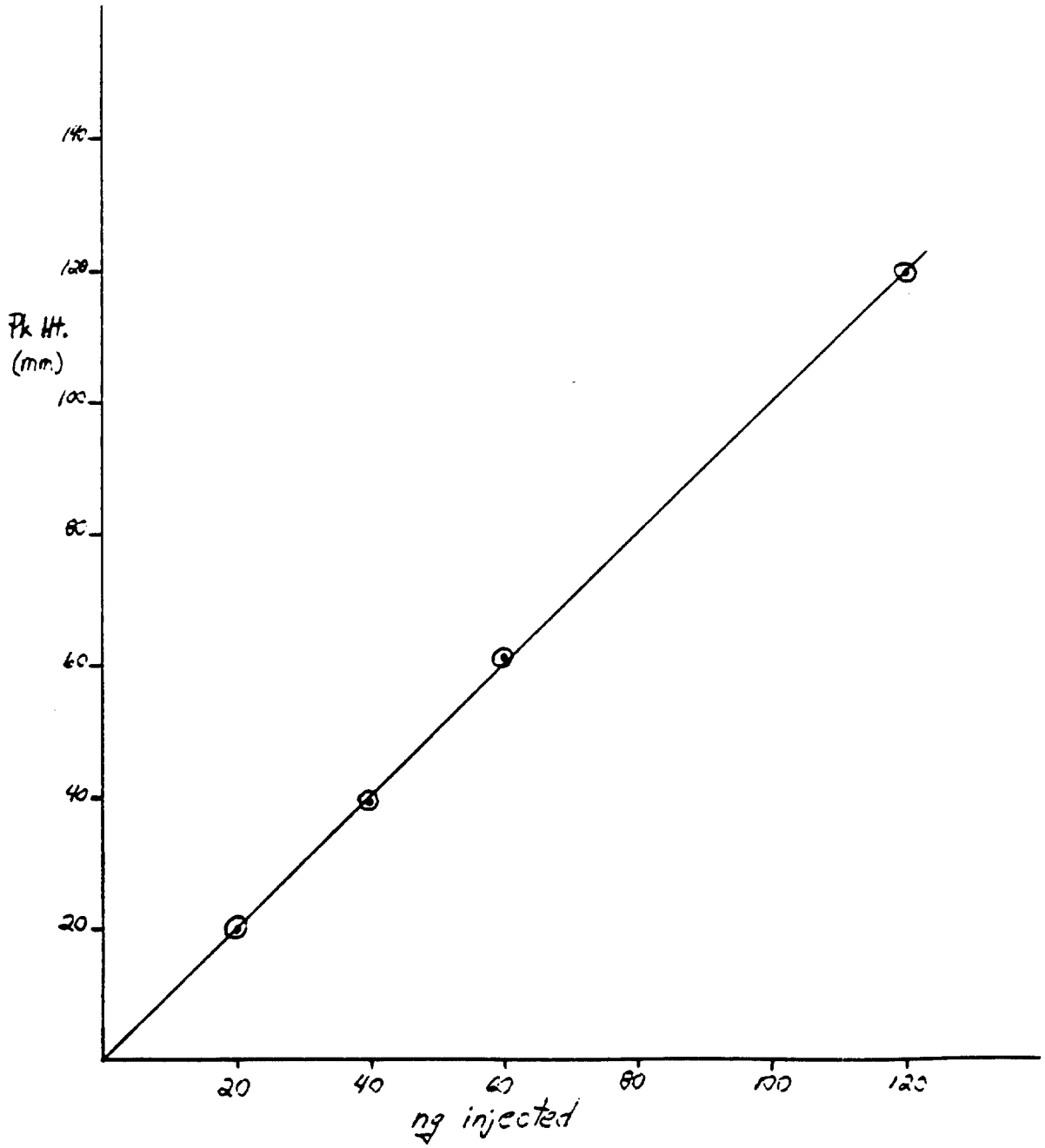
TRIPHENYL PHOSPHINE IN INHALATION CHAMBER ATMOSPHERES

FIGURE I - Typical Chromatograms for TPP Standards and Chamber Air Samples.



AN ANALYTICAL METHOD FOR THE DETERMINATION OF (TPP)
TRIPHENYL PHOSPHINE IN INHALATION CHAMBER ATMOSPHERES

FIGURE 2 - A Typical Standard Curve for TPP Determined by Gas Chromatograph.



Appendix C

M-11, 81-7534 B

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

TABLE OF CONTENTS

Summary of Gross Postmortem Observations..... pg. 3

Table I - Identification and Disposition
of Individual Animals on Test..... pg. 4

Table II - Gross Postmortem Observations..... pg. 6

Microscopic Evaluation..... pg. 10

Pathology Summary..... pg. 12

Introduction..... pg. 14

Materials and Methods..... pg. 15

Discussion of Microscopic Findings..... pg. 16

Summary of Microscopic Findings..... pg. 18

Incidence of Microscopic Findings..... pg. 19

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Summary of Gross Postmortem Observations

Postmortem findings, observed grossly, either occurred in the treated and control animals or they occurred sporadically; they did not appear to be related to the test article.

Henry F. Bolte, D.V.M., Ph.D. Date
Pathologist

Table I

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Identification and Disposition of Individual Animals on Test

Table I

81-7534 B

A Four Week Inhalatin Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Identification and Disposition of Individual Animals on Test

Group	Sex	Animal Number	Type of Death	Group	Sex	Animal Number	Type of Death
I	M	1001	Ta	I	F	1501	Ta
II	M	2001	Ta	II	F	2501	Tb
		2002	Tb			2502	Ta
III	M	3001	Ta	III	F	3501	Tb
		3002	Tb			3502	Ta
IV	M	4001	Ta	IV	F	4501	Ta
V	M	5001	Tb	V	F	5501	Tb
		5002	Ta			5502	Ta

K E Y

Ta = Terminal Sacrifice - 20 Day Dose

Tb = Terminal Sacrifice - 2 Day Dose

Table II

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

GENERAL KEY TO GROSS POSTMORTEM OBSERVATIONS

N.O.A.	=	No observable abnormalities
R	=	Right
L	=	Left
B	=	Both, Bilateral
X (x)	=	Times
N	=	Normal
cm	=	centimeter
diam.	=	diameter
approx.	=	approximately
G.I.	=	Gastro-intestinal
A.G.	=	Ano-genital
<	=	less than
≤	=	less than or equal to

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Group	Sex	Animal Number	Organ/ Tissue	Observations
I	M	1001	Duodenum	Slightly red.
			Colon	Slightly red.
II	M	2001	Lungs	L apical lobe: irregular gray area (3.5cm) centrally located; R cardiac lobe: tan/brown area (1.0cm).
		2002	Popliteal Lymph Node	Large.
III	M	3001	Popliteal Lymph Node	Large.
			Lungs	L apical lobe, dorsal surface: aggregation of small tan nodules (area of 1.5cm); R apical lobe: few scattered tan nodules near edge (1.0cm).
			Area of Pyloric Sphincter	Multiple red foci.
			Duodenum	Red.
		3002	Lungs	All lobes: pinpoint red foci scattered throughout; L diaphragmatic lobe: red/brown, irregular area (3.0cm) along edge.
			Mediastinal Lymph Nodes	Dark red.
			Ileo-Colic Junction	Slightly red.
IV	M	4001	Popliteal Lymph Node	Large.
			Area of Pyloric Sphincter	Multiple red foci.
V	M	5001	Lungs	L apical lobe: brown/tan area (3.5x3.0 cm), slightly raised, extends through parenchyma.
		5002	Duodenum	Lower portion: round worm (8.0cm); areas of scattered redness.

81-7534 B

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Group	Sex	Animal Number	Organ/ Tissue	Observations
I	F	1501	Popliteal Lymph Node	Large.
			Tonsils	Red and swollen.
II	F	2501	-	N.O.A.
		2502	Lungs	L diaphragmatic lobe: brown area (0.5cm); L apical lobe: aggregation of small tan nodules (1.0cm).
			Mesenteric Lymph Nodes	Center: dark red.
			Duodenum	Slightly red.
III	F	3501	Ileo-Colic Junction	Red.
		3502	Vulva	Swollen.
IV	F	4501	Ascending Colon	Slightly red longitudinal streaks.
			Fur/Skin	Hindlegs: slight alopecia.
V	F	5501	-	N.O.A.
		5502	Popliteal Lymph Node	Large.

ROBERT F. McCONNELL, D.V.M., P.A.
CONSULTING PATHOLOGY SERVICES
PHONE 201-782-4676

DIPLOMATE
AMERICAN COLLEGE
OF
VETERINARY PATHOLOGISTS

R.D. 7, WELLS ROAD
FLEMINGTON, NEW JERSEY 08822

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534B

A FOUR WEEK INHALATION TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN DOGS

PATHOLOGY REPORT

SUBMITTED TO: ALEKSANDAR L. KNEZEVICH, DR. MED. VET.
VICE PRESIDENT, PATHOLOGY
BIO/DYNAMICS, INC.
P. O. BOX 43
METTLERS ROAD
EAST MILLSTONE, NEW JERSEY 08873

Robert F. McConnell

ROBERT F. McCONNELL, D.V.M.

APRIL 26, 1982

B/d M-11, 81-7534B
RFMc 67/82
4/26/82

TABLE OF CONTENTS

	PAGE(S)
PATHOLOGY SUMMARY	12
INTRODUCTION	14
MATERIALS AND METHODS	15
DISCUSSION OF MICROSCOPIC FINDINGS	16
SUMMARY OF MICROSCOPIC FINDINGS TABLE 1	18
INCIDENCE OF MICROSCOPIC FINDINGS IN INDIVIDUAL DOGS BY DOSE GROUP AND SEX TABLE 2	19

RFMc 67/82
4/26/82

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534B

A FOUR WEEK INHALATION TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN DOGS

PATHOLOGY REPORT

PATHOLOGY SUMMARY:

Sixteen male and female beagle dogs were divided into 5 groups. Group I dogs served as xylene vehicle controls and were exposed at inhalation levels of 600 ppm or lower. Groups II, III, IV, and V were exposed to TPP in xylene vehicle at levels of 0.5, 3.0, 10.0, and 30.0 mg./m^3. At the end of the test the dogs were killed, necropsied, and peripheral nervous tissue collected for microscopic examination.

Sections of the cervical and lumbar spinal cord and right and left sections of the sciatic nerve were evaluated.

One male and 1 female at the high dose level had moderate generalized vacuolative degenerative changes in axons of the ventral and lateral funiculi of both the cervical and lumbar portions of the spinal cord.

Minimal focal vacuolative lesions, usually of single axons, occurred in the cervical spinal cord of 1/2 ♂, 0/2 ♀, Group II; 1/2 ♂, 1/2 ♀ Group III; 0/1 ♂, 0/1 ♀, Group IV; and 1/2 ♂, 0/2 ♀, Group V dogs. The significance of these minimal focal lesions was questionable.

The sciatic nerves of all treated and control dogs were within normal histological limits.

RFMc 67/82
4/26/82

It was concluded, under the conditions of this test, that TPP at inhalation levels of 30 mg./m^3 may cause moderate generalized vacuolative degeneration of axons in the ventral and lateral funiculi of the cervical and lumbar spinal cord but does not cause an effect in the sciatic nerve.

ROBERT F. McCONNELL, D.V.M.
Diplomate, American College
of Veterinary Pathologists

RFMc 67/82
4/26/82

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534B
A FOUR WEEK INHALATION TOXICITY STUDY OF
TRIPHENYL PHOSPHINE IN DOGS

PATHOLOGY REPORT

INTRODUCTION:

This study was designed to evaluate the effects of triphenyl phosphine (TPP) in selected tissues of the peripheral nervous system (spinal cord and sciatic nerve) of the dog when administered by inhalation at levels of 0.5, 3.0, 10.0, and 30.0 mg./m^3 for 2 or 20 days.

Sixteen male and female beagle dogs were divided into 5 groups. Group I (1 ♂ - 1 ♀) served as an inhalation vehicle control and was dosed with xylene at 600 ppm or less. Dogs in Group II (2 ♂ - 2 ♀) 0.5; Group III (2 ♂ - 2 ♀) 3.0; Group IV (1 ♂ - 1 ♀) 10.0; and Group V (2 ♂ - 2 ♀) 30.0 mg./m^3 were dosed similarly to controls with TPP dissolved in xylene.

At the end of the 4-week study the dogs were anesthetized with sodium pentobarbital and exsanguinated. Tissues were collected for microscopic examination. The results of the histopathological evaluation form the basis of this pathology report.

RFMc 67/82
4/26/82

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534B

A FOUR WEEK INHALATION TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN DOGS

PATHOLOGY REPORT

MATERIALS AND METHODS:

At necropsy the following tissues or their representative sample were taken and preserved in 10% neutral buffered formalin: brain, sciatic nerve (right and left), and spinal cord from both cervical and lumbar regions.

Slides of the tissue sections listed below were prepared routinely from all animals. The tissue sections were stained with H & E and luxol fast blue.

Sciatic nerve, right and left	1 longitudinal section on each
	2 transverse sections on each
Cervical spinal cord	1 longitudinal section
	2 transverse sections
Lumbar Spinal cord	1 longitudinal section
	2 transverse sections

Revised By: [signature] 12/17/82
Pathologist Date

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534B

A FOUR WEEK INHALATION TOXICITY STUDY OF
TRIPHENYL PHOSPHINE IN DOGS

PATHOLOGY REPORT

DISCUSSION OF MICROSCOPIC FINDINGS:

The incidence and severity of microscopic findings are listed according to individual dog, sex, and dose group in TABLE 2. These data are summarized for ease of comparison in TABLE 1.

In this study a vacuolative degenerative axonal lesion was confined to the spinal cord and the sciatic nerve was spared. The lesion in this test occurred as a moderate generalized vacuolative axonal degeneration in the ventral and lateral funiculi of the cervical and lumbar spinal cord. No lesion was present in the sciatic nerve. The affected dogs included ♂5002 and ♀5502 of the 30.0 mg./m^3 high dose group.

In addition, minimal focal vacuolation was observed in occasional axonal nerve fibers in the cervical spinal cord of the following dogs: 0/1 ♂, 0/1♀ control; 1/2 ♂, 0/2 ♀, 0.5; 1/2 ♂, 1/2 ♀, 3.0; 0/1 ♂, 0/1 ♀, 10.0; and 1/2 ♂, 0/2 ♀, 30.0 mg./m^3. Similar minimal focal lesions were present in the lumbar cord of 1/2 0.5 mg./m^3 dogs. In one dog (3501) of the 3.0 mg./m^3 group the lesion was also observed in both the cervical and lumbar cord sections. The significance of these focal lesions was questionable.

B/d M-11, 81-7534B
RFMc 67/82
4/26/82

The generalized lesions observed in dogs at the high dose level were best seen in the longitudinal sections of the cord. They were easily recognized with both the H & E and luxol fast blue stains. The lesion was characterized by multiple dilated or vacuolated axon sheaths with accumulations of scattered eosinophilic debris and occasional dark rounded bits of what appeared to have been nuclear chromatin. Dog 5501 ♀ at the high dose level had no peripheral nervous system lesion.

The minimal focal spinal cord lesions may have been spontaneous in character as they had no dose dependent relationship. They were, however, not present in control dogs.

RFMc 67/82
4/26/82

Revised By: _____
Pathologist Date

BIO/DYNAMICS, INC.
PROJECT M-11, 81-7534B

A FOUR WEEK INHALATION TOXICITY STUDY OF
TRIPHENYL PHOSPHINE IN DOGS

SUMMARY OF MICROSCOPIC FINDINGS

TABLE 1

	DOSE GROUP									
	I		II		III		IV		V	
NUMBER DOGS/GROUP	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	1	1	2	2	2	2	1	1	2	2
TISSUE/RESPONSE										
CERVICAL SPINAL CORD:										
Vacuolation of lateral and ventral funiculi;										
(a) Focal, minimal	0	0	1	0	1	1	0	0	1	0
(b) Diffuse, moderate	0	0	0	0	0	1	0	0	1	1
LUMBAR SPINAL CORD:										
Vacuolation of lateral and ventral funiculi;										
(a) Focal, minimal	0	0	0	1	0	1	0	0	0	0
(b) Diffuse, moderate	0	0	0	0	0	0	0	0	1	1

TISSUES WITHIN NORMAL HISTOLOGICAL LIMITS: Right and Left Sciatic Nerves.

RFMc ..,82
4/26/82

Revised By: [signature] 11/26/82
Pathologist Date

BIO/DYNAMICS, INC.
A FOUR WEEK INHALATION TOXICITY STUDY OF
TRIPHENYL PHOSPHINE IN DOGS

INCIDENCE OF MICROSCOPIC FINDINGS

PROJECT 81-7534B - ROUTE - INHALATION

TABLE 2

GROUP	I, XYLENE VEHICLE CONTROL		II, 0.5 mg./m³ TPP				III, 3.0 mg./m³ TPP				IV, 10.0 mg./m³ TPP		V, 30.0 mg./m³ TPP			
SEX	♂	♀	♂		♀		♂		♀		♂	♀	♂		♀	
ANIMAL NUMBER	1001	1501	2001	2002	2501	2502	3001	3002	3501	3502	4001	4501	5001	5002	5501	5502
TISSUE/RESPONSE (Days on Test)	20	20	20	2	2	20	20	2	2	20	20	20	2	20	2	20
CERVICAL SPINAL CORD:	N	N		N	N	N	N			N	N	N			N	
Vacuolation, lateral and ventral funiculus	-	-	(1)	-	-	-	-	(1)	(1)	-	-	-	(1)	3	-	3
LUMBAR SPINAL CORD:	N	N	N	N		N	N	N		N	N	N	N		N	
Vacuolation, lateral and ventral funiculus	-	-	-	-	(1)	-	-	-	(1)	-	-	-	-	3	-	3
RIGHT SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
LEFT SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

LEGEND FOR TABLE:

N = tissue within normal histological limits
1 = minimal
2 = mild
3 = moderate
( ) = focal or localized change
- = indicated change not present

Appendix D
A Four Week Inhalation Toxicity Study
of TPP in the Dog

PERSONNEL

DEPARTMENT/TITLE	NAME	DEGREE
Study Director, Inhalation	James B. Terrill	Ph.D.
Supervisor, Inhalation	Doug Reigle	B.S.
Supervisor, Large Animals	Dan Pisacreta	B.S.
Study Monitor	Jeff Krietzman	B.S.
Pathologist	Henry F. Bolte	D.V.M., Ph.D.
	Robert F. McConnell	D.V.M.
	Asa Mays	D.V.M.
Quality Assurance		
Director	Joseph B. Townsend	
Supervisor	Craig Lamb	B.A.

QUALITY ASSURANCE STATEMENT

****

STUDY NO.: 81-7534B TEST SUBSTANCE/ARTICLE Triphenyl Phosphine

STUDY SPONSOR: M & T Chemicals

Listed below are dates that this study was inspected by the Quality Assurance Unit and the dates findings were reported to the Study Director and Management

DATE(S) OF INSPECTION	REPORTED TO STUDY DIRECTOR	REPORTED TO MANAGEMENT
12/14/81	12/29/81	12/29/81 1/5/82
12/17/81	1/7/82	1/8/82 1/14/82
12/21/81	12/22/81	1/6/82
5/11/82	6/11/82	6/11/82 6/11/82
5/28-6/4/82	6/11/82	6/11/82 6/11/82

Craig Lamb 6/11/82

CRAIG LAMB
MANAGER, QUALITY ASSURANCE

DATE

Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO. 79-2445

A SEVEN WEEK NEUROTOXICITY STUDY WITH TRIPHENYLPHOSPHINE IN DOGS

Final Report

Submitted to: M & T Chemicals, Inc.
Rahway, New Jersey 07065

Date: August 22, 1980

Revised: James B. Lovell

Date: Dec 15, 1982

Bio/dynamics Inc.

PROJECT NO.: 81-7534B

A FOUR WEEK INHALATION TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN THE DOG

ABSTRACT

This study was conducted for M&T Chemicals to assess the inhalation toxicity of triphenyl phosphine (TPP) when administered as a respirable aerosol in a xylene vehicle to male and female beagle dogs. Four atmospheric concentration levels and two different durations of treatment were used in the study; the specific test regimes were as follows: two dogs (1 male, 1 female) per group received twenty 6-hour exposures over four weeks at exposure levels of 0.5, 3.2, 9.7 and 28 milligrams TPP per meter cubed (mg/m^3); two 6-hour exposures were administered to two dogs (1 male, 1 female) per group at concentrations of 0.3, 3.6 and 24 mg/m^3 TPP. These shorter duration treatments were followed by a four week recovery period. A concurrent vehicle control group (1 male and 1 female dog) received twenty 6-hour exposures to 150 parts per million (v/v in air) of xylene, a level of solvent which was comparable to that in each test exposure chamber. Chambers were monitored analytically for TPP levels at least 3 times/chamber/day. Particle size distribution were determined periodically throughout the study. Body weight measurements, physical observations and neurological examinations were performed pre-test and weekly throughout the study. Complete gross postmortem examinations were performed and selected sections of nervous system tissue were preserved from all test animals for histopathological evaluation.

All animals survived the duration of the study.

Signs of neurological impairment were noted during the study in male dogs receiving 2 exposures at 24 mg/m^3 TPP or 20 exposures at 28 mg/m^3 TPP and in the female dogs receiving 20 exposures at 28 mg/m^3 TPP. Histopathological examinations (H&E, and luxol fast blue stains) revealed moderate generalized vacuolative degeneration of the ventral and lateral funicle of the cervical and lumbar spinal cord of the dogs which received twenty exposures to 28 mg/m^3 TPP. Similar lesions, although minimal and focal in nature, were noted in spinal cord sections of dogs receiving 0.5 or 3.2 mg/m^3 TPP. In the lowest treatment group animals, this lesion was observed in one male following 20 exposures and in one female following only two exposures. These lesions were not seen in any vehicle control dog.

Evaluations of body weight, general physical observations (non-neurological) and gross postmortem results were considered unremarkable.

P.O. Box 43 • East Millstone, New Jersey 08873 • (201) 873-2550 • Telex: 844597 • Cable: BIO/DYN

Bio/dynamics Inc.

PROJECT NO. 79-2445

A SEVEN WEEK NEUROTOXICITY STUDY WITH TRIPHENYLPHOSPHINE IN DOGS

ABSTRACT

A total of 12 Beagle dogs were randomly distributed into two groups of 3 animals/sex/group. The test material, triphenylphosphine (TPP), was administered to Group II animals by gastric intubation at a dose level of 25 mg/kg/day for 21 consecutive days. The Group I (vehicle control) animals received the equivalent amount of corn oil alone. Following the 3 week dosing period, all animals were observed for an additional 4 weeks prior to sacrifice.

The administration of TPP did not significantly affect body weight or food consumption. However, 3 out of the 6 animals in Group II exhibited signs of neurological impairment. The signs were first evident approximately 14 days following initiation of TPP treatment and were characterized principally by ataxia and hindlimb weakness.

There was a slight improvement noted in the affected animals following cessation of compound administration. Thereafter, no further improvement was noted. No such effects were observed in the vehicle control group.

Complete gross postmortem examinations were performed on all animals after 7 weeks. No compound-related effects were observed.

Histopathologic evaluation of selected tissues revealed myelin degeneration in the spinal cord of all Group II animals. Similar lesions were also noted to a lesser extent in the cerebrum. Peripheral nerves were not affected. Those animals in Group II which showed clinical signs of neurological damage had the most severe lesions histopathologically. No abnormalities were observed in the Group I (vehicle control) animals.

P.O. Box 43 • Mettlers Road • East Millstone, New Jersey 08873 • (201) 873-2550 • Telex: 844597 • Cable: BIO/DYN

TABLE OF CONTENTS

I. INTRODUCTION..... 1

II. MATERIALS AND METHODS

A. Test Substance..... 1
B. Vehicle..... 2
C. Test Animals..... 2
D. Selection and Group Assignment..... 2
E. Animal Identification..... 3
F. Experimental Outline..... 3
G. Husbandry..... 3
H. Test Substance Administration..... 4
I. Observations
Physical..... 5
Neurological..... 5
J. Body Weight..... 5
K. Food Consumption..... 6
L. Postmortem..... 6

III. RESULTS AND DISCUSSION

A. Mortality..... 7
B. Physical Observations..... 7
C. Neurological Examinations..... 7-9
D. Body Weight..... 9
E. Food Consumption..... 9
F. Pathology..... 10

IV. APPENDICES

A. Methodology and References..... A-1
B. Animal Termination History..... B-1
C. Mean and Individual Body Weight Values..... C-1
D. Individual Neurological Examination Values..... D-1
E. Summary of Neurological Examination Values..... E-1
F. Determination of Triphenylphosphine in Corn Oil Dosing Solutions..... F-1
G. Pathology..... G-1
H. Personnel..... H-1
I. Quality Assurance Statement..... I-1

I. INTRODUCTION

This study, conducted for M & T Chemicals, Inc., was designed to determine if triphenylphosphine (TPP) induced any neurotoxic or other adverse effects when administered to Beagle dogs. A previous study (Bio/dynamics Project No. 78-2317) showed that TPP produced a delayed neuropathy in Beagle dogs when administered by gastric intubation as a single dose of 300 mg/kg followed by daily doses of 50 mg/kg/day for 13 days. Therefore, the current study was conducted by administering TPP by this same route of administration at a daily dose level of 25 mg/kg/day for a total dosing period of 3 weeks to determine whether this lower dose would be without effect. Since delayed neuropathy typically becomes observable 10 days to 2 weeks following compound administration, a 4 week post-dose observation period was included to monitor any delayed effects and the potential for their reversibility. This study was conducted at Bio/dynamics, Inc., Mettlers Road, East Millstone, New Jersey 08873. All data are stored in the archives at Bio/dynamics, Inc.

II. MATERIALS AND METHODS

A. Test Substance: Triphenylphosphine (TPP)

Concentration:	99.7% Active Ingredient
Supplier:	M & T Chemicals, Inc. Rahway, N.J. 07065
Description:	Off-white powder
Date Received:	26 September 1978
Lot No.:	WT VAH-352K
Density:	1.194g/ml (from Bio/dynamics Project No.: 78-2317)
Sampling:	An archival sample of TPP was removed following termination of the study and is stored in the Archives at Bio/dynamics, Inc.
Storage:	Room temperature

B. Vehicle: Mazola® Corn Oil

Date Received: 3 March 1980

Label Information: Expiration Date: 12 December 1980

Storage: Room temperature

C. Test Animals: Dogs

Strain: Beagle

Number of Animals: 12 animals were placed on test, 6 male and 6 female.

Supplier: Marshall Research Animals, North Rose, New York 14516

Date Received: Animal No. 4435 (male) 2/20/79; all other animals 4/17/79.

Age at Receipt: 6-8 Months

Age at Initiation of Treatment: 17-19 Months

Weight at Initiation of Treatment (kilograms):

	Mean	Range
Males:	10.8	7.7 - 14.1
Females:	9.8	8.0 - 12.3

Immunization: Against distemper, hepatitis and leptospirosis by supplier. Treatment for rabies performed at Bio/dynamics, Inc.

Parasites (intestinal): Fecal examination conducted on all dogs at Bio/dynamics, Inc. during the equilibration period. Anthelmintics (piperazine and dichlorvos) were routinely administered by supplier as required.

D. Selection and Group Assignment: Animals for this study were selected from among the Bio/dynamics, Inc. in-house colony in such a way as to most nearly equalize body weight means for each group and sex. Animals were ranked by body weight and distributed into 2 blocks of 3 animals per sex so that body weight means for each group were comparable. Each block was given a two-digit number and using a random numbers table was designated as either Group I (vehicle control) male and female or Group II (TPP) male and female.

E. Animal Identification: Each dog was assigned a unique Bio/dynamics, Inc. identification number which appeared on an ear tag and on the animal's cage. In addition each animal bore an ear tattoo with a USDA number. Periodically, identification and tattoo numbers were compared to the cage numbers to insure correct animal identification.

F. Experimental Outline:

Group	Treatment	Dose (mg/kg/day)	Number of Animals M	Number of Animals F	Duration Treatment	Duration Recovery	Histopathology
I	Corn Oil (Vehicle Control)[a]	0	3	3	21 Days	28 Days	All Survivors
II	Triphenyl-phosphine (TPP)	25	3	3	21 Days	28 Days	All Survivors

[a]Control animals received the same volume of corn oil (1 ml/kg/day) as the TPP-treated animals.

G. Husbandry:

Acclimation Period: 13 February 1980 to 23 March 1980

Housing: Individually in elevated metal grid cages.

Food: 400 grams of standard laboratory diet (Wayne Bite Size®).

Water: ad libitum. By automated water system (Elizabethtown Water Company).

Environmental Conditions: 12 hour light/dark cycle.

Temperature monitored twice daily.

H. Test Substance Administration:

Route: Oral intubation; individual doses were adjusted using the most recent body weight data.

Method of Dosing Solution Preparation: Initially, the following procedure was used to prepare the daily dosing solutions of TPP in corn oil (final concentration = 25 mg/ml):

1. Using a 250 ml graduated cylinder, 117.5 ml of corn oil were measured out. About 1/4 of this amount was poured into an electric blender.

2. 3.0 grams of TPP were placed into the blender. The remaining corn oil was used to rinse out the container containing the TPP, and the rinse added to the blender.

3. The above mixture was blended for 5 minutes at low speed and poured into an appropriately labelled stock containers.

Prior to initiating the study, samples prepared using the above method were analyzed for TPP as described in Appendix F. The results indicated the samples contained 95% of the nominal concentration. Therefore, the study was initiated using the above preparation procedure. However, during the first 3 days of the study, concentrations from the sampling of the dosing solutions ranged from 60% - 85% of nominal. These values were considered unacceptable and since no readily apparent explanation could be found, the method of preparation was changed as follows:

1. Into a clean and completely dry 150 ml volumetric flask were placed 3.75 grams of TPP.

2. The container holding the TPP was rinsed with corn oil and the rinse added to the flask. Corn oil was added to the flask until the final volume of 150 ml was achieved.

Method of Dosing Solution Preparation (cont.):

3. This mixture was shaken by hand for 2-3 minutes and then placed into a sonicator for 15 minutes.

4. The mixture was then placed in a water bath at 40°C for 5 minutes.

5. Steps 3 and 4 were repeated until all TPP was dissolved.

This method was employed for the 18 consecutive dosing solution preparations subsequent to the first 3 days of the study. Samples from solutions prepared in this manner yielded recoveries ranging from 94%-110%. Therefore, Group II (TPP treated) animals were slightly underdosed only for the first 3 days of the study. This is not considered to have seriously affected the results obtained.

Frequency: Daily (7 days per week)

Duration: 21 days

Dates of Treatment: 24 March 1980 through 14 April 1980

Sampling: Samples of control corn oil and the TPP dosing solution were analyzed in duplicate for TPP concentraton on Day 0 (first day of dosing) and on Days 1, 2, 3, 4, 7, and 14. Analytical methodology and results of analysis are presented in Appendix F.

I. Observations:

For Mortality and Gross Signs of Toxicologic or Pharmacologic Effects: Twice daily

Neurologic Examination: (Meth. and Ref., Appendix A)

4 days pretest, just prior to dosing, 3 hours after first dosing, Days 4, 7, 10, 14, 21, 28, 35, 42 and 49.

J. Body Weight: (Meth. and Ref., Appendix A)

Pretest, weekly during treatment and terminally (after fasting).

K. Food Consumption: (Meth. and Ref., Appendix A)

Pretest and weekly thereafter. Estimated 4 times weekly beginning one week pretest.

L. Postmortem:

Necropsy: 13 May 1980

Number of Animals: 12 total

Sacrifice Method: Exsanguination under sodium pentobarbital anesthesia.

Tissues Preserved:

brain (2 sections)
nerve (left and right sciatic)
spinal cord (cervical and lumbar in the intact vertebral column)
gross lesions (including a section of normal-appearing portion of same tissue)

Preservatives: 10% neutral buffered formalin: all tissues.

Stain: Hematoxylin and Eosin
Luxol-fast Blue (Meth. and Ref., Appendix A)

Tissues Examined Histopathologically: Sections of each of the following tissues were prepared histologically; one set of sections was stained with hematoxylin and eosin and the other set with luxol-fast blue.

sciatic nerve	1 longitudinal, 2 transverse
cervical spinal cord	1 longitudinal, 2 transverse
lumbar spinal cord	1 longitudinal, 2 transverse
brain	2 transverse

III. RESULTS AND DISCUSSION

A. Mortality (Appendix B):

No mortality was observed during the course of this study.

B. Physical Observations:

Animal No. 4515 (Group I female) was noted to have sores on the right, rear paw similar to those commonly observed in Beagle dogs housed in metal grid cages. In addition, this animal also showed transient excessive lacrimation of the left eye. Animal No. 4550 (Group II female) also had cage sores of the left front paw as well as alopecia and dry coat. Animal No. 4544 (Group I female) had hyperplasia of the lymphoid follicle of the third eyelid in the left eye. This is also a very common finding in Beagle dogs. Animal Nos. 4435 and 4537 (both Group II males) exhibited excessive salivation pretest and throughout the study.

Animals were observed in regard to fecal output and feed emesis daily during the equilibration and study intervals. Both Group I and Group II animals exhibited soft, loose stools throughout the dosing period. This is considered a normal finding in view of the cathartic effect of the corn oil administered. The other observations noted above are considered to be unremarkable and unrelated to compound administration. Those physical observations related to neurological function are discussed below.

C. Neurological Examinations (Appendix D and E):

Results of the neurological examinations on Group I (vehicle control) animals indicated consistently normal responses pretest, during the period of vehicle administration, and during the recovery period. Occasional sluggish responses were noted in several observed reflexes but these were transient and not considered significant. The only consistent finding among the Group I animals was a slight to moderate impairment of the flexor response in Animal No. 4544 (female). This is not unusual since, as mentioned above, long-term

C. Neurological Examinations (Appendix D and E) (cont.):

housing in metal grid cages can damage the interdigital area of the paws resulting in a toughening and insensitivity of the affected area.

Among the Group II (TPP-treated) animals, 3 out of 6 animals (2 males and 1 female) exhibited normal neurological responses throughout the course of this study. Again, as noted in the controls, occasional sluggish responses were noted in some reflexes but these were considered to be unrelated to the administration of TPP.

Among the remaining 3 out of 6 TPP-treated animals (1 male and 2 females), distinct alterations in neurological function related to TPP administration were noted as follows: Animal No. 4517 (female) first showed signs of neurological impairment 14 days following initiation of treatment. This was evident by the animal's inability to stand and by clearly impaired extensor postural thrust and extensor reflexes in the hindlimbs. The animal was alert and active despite this deficit. At Day 21 the animal's condition worsened. The animal was unable to stand despite repeated attempts. The righting reflex was clearly impaired as well as both hind- and forelimb function. One week following cessation of dosing, the animal's condition improved slightly. The dog was able to stand normally but continued to fall when movement was attempted. Hindlimb reflexes were present but clearly impaired. During the remainder of the recovery period the animal's condition remained unchanged with no further improvement noted. This animal was the most severely affected by TPP treatment with moderate to severe impairment of affected reflexes.

Animal No. 4537 (Group II male) showed signs of moderate impairment on Day 21. The animal had difficulty in standing and was slightly ataxic. Both fore- and hindlimb reflexes as well as the righting reflex were slightly impaired. One week later definite improvement was noted. Slight ataxia was

C. Neurological Examinations (Appendix D and E) (cont.):

still present but the animal was able to walk without falling. Slight hindlimb deficit was still evident. The animal's condition did not change significantly during the remainder of the recovery period. Animal No. 4550 (Group II female) showed slight tremors in the hindlimbs just prior to dosing (Day 0). The tremors persisted during the course of the study and appeared to become more severe during the period of compound administration. On Day 21 of the study the animal was slightly ataxic with some evidence of a hindlimb deficit. One week later the animal's condition was essentially normal with the exception of the persistence of the slight hindlimb tremors. Thereafter, the animal's condition remained unchanged.

In summary of the neurological evaluations, 3 out of 6 animals in Group II showed one or more signs of impairment during the study compared to no significant effects in the Group I animals. The nature of the neurological effects was such as to cause ataxia, hindlimb weakness, and incoordination of forelimb movements. Although improvement was observed following cessation of TPP administration, effects were generally still present following the 4 week recovery period. Therefore, the neurological effects caused by TPP may be irreversible.

D. Body Weight (Appendix C):

Mean body weights for both sexes in Groups I and II decreased slightly between Weeks -1 and 0. Thereafter, slight increases in mean body weight were observed for males and females in Groups I and II.

E. Food Consumption:

Food consumption values during the study were not significantly different from those noted pretest for both groups. Exceptions were Animal No. 4519 (Group I male) whose food consumption decreased midway into the study and Animal No. 4546 (Group II female) who stopped eating upon initiating TPP

E. Food Consumption (cont.)

administration. Both dogs were switched to one can of Wayne® dog food per day. Following this change in diet, food consumption was normal. Both dogs were gradually weaned off the canned food and returned to the dry ration without further problems.

F. Pathology (Appendix G):

Gross postmortem examination revealed that there was no pattern of lesions which would suggest a compound-related effect.

Microscopic evaluation of tissues from the nervous system revealed minimal to severe myelin degeneration in the central nervous system of all TPP-treated animals. No such lesions were found in the vehicle control group. The lesions were found principally in the cervical and lumbar spinal cord and to a lesser extent in the cerebrum. No lesions were noted in the sciatic nerves or in the spinal nerves from the cervical and lumbar regions.

The animals which showed clinical signs of neurological damage (Animal Nos. 4517, 4537 and 4550) had the most severe lesions microscopically. However, the remainder of the TPP-treated animals had no clinical signs yet showed microscopic evidence of damage to the central nervous system.

One interesting finding in the present study is that in contrast to the "classical" lesions found in delayed neuropathy, no lesions were present in the peripheral nervous tissue while the brain and spinal cord were clearly affected. Usually, the peripheral nerves are first affected and the lesions progress in a cephalic direction. In the earlier study with higher dose levels (Project No. 78-2317) both central and peripheral nerves were damaged by TPP.

F. Pathology (cont.):

Therefore, the results of the present study suggest that the central nervous system may be more sensitive to the effects of low level TPP administration as compared to the peripheral nervous system.

George R. Lankas

George R. Lankas, Ph.D.
Study Director

William E. Rinehart

William E. Rinehart, Sc.D.
Vice-President, Science

Appendix A
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Methodology and References - General

Parameter	Reference or Description
Method of Test Substance Administration	Appropriate amounts of test substance were mixed with the vehicle daily. Doses were adjusted by most recent body weights. Animals were dosed daily via esophageal intubation.
Neurological Examination	The following reflexes and responses were observed: locomotion, standing, pupillary reflex, corneal reflex, visual placing, extensor postural thrust, extensor, flexor, tonic neck, walking reactions (wheelbarrowing), startle reflex, and righting reflex. The method of neurological examination was obtained from: Textbook of Veterinary Internal Medicine, Vol. 1, ed. by Stephen J. Ettinger, Philadelphia; W.B. Saunders, pp. 293-298, 1975.
Body Weight	Detecto bench scale (60-S).
Food Consumption	A visual estimate of amount of food consumed per day was made for each dog four times per week.
Stain - Luxol-fast Blue	Armed Forces Institute. Manual for Histologic Technics (Modified). 2nd Edition. New York: McGraw-Hill Book Co., Inc., 1949.

Appendix B
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Animal Termination History

Group mg/kg/day	Males An. No.	Date of Death	Type of Death[a]	Days on Test[b]	Females An. No.	Date of Death	Type of Death[a]	Days on Test[b]
I	4519	5/13/80	T	50	4515	5/13/80	T	50
0	4520	5/13/80	T	50	4543	5/13/80	T	50
	4521	5/13/80	T	50	4544	5/13/80	T	50
II	4435	5/13/80	T	50	4517	5/13/80	T	50
25	4537	5/13/80	T	50	4546	5/13/80	T	50
	4538	5/13/80	T	50	4550	5/13/80	T	50

[a]T = Terminal Sacrifice
[b]Includes both dosing and recovery periods.

Appendix C
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Mean and Individual Body Weights (kilograms)[a]

Group mg/kg/day	An. No.	Week: -3	-1	(Day) 0	1	2	3	4	5	6	7
					Males						
I	4519	9.5	9.9	9.7	9.7	9.1	9.6	9.8	9.8	10.5	10.5
0	4520	8.5	8.5	8.5	7.7	8.1	8.2	8.3	8.4	8.4	8.5
	4521	14.7	14.9	14.9	14.1	14.3	14.6	14.5	14.6	14.7	15.0
	Mean	10.9	11.1	11.0	10.5	10.5	10.8	10.9	10.9	11.2	11.3
	S.E.	0.7	1.9	0.7	1.9	1.9	1.9	1.9	1.9	1.9	1.9
	N	3	3	3	3	3	3	3	3	3	3
II	4435	10.4	10.5	10.0	10.0	9.9	10.0	10.0	10.0	9.9	10.0
25	4537	10.0	10.7	9.6	10.1	10.1	9.9	10.1	10.5	10.2	10.4
	4538	12.2	12.7	12.0	12.1	12.1	12.1	12.6	12.9	13.2	13.2
	Mean	10.9	11.3	10.5	10.7	10.7	10.7	10.9	11.1	11.1	11.2
	S.E.	0.7	0.7	0.7	0.7	0.7	0.7	0.9	0.9	1.1	1.0
	N	3	3	3	3	3	3	3	3	3	3
					Females						
I	4515	11.6	12.4	11.8	12.4	12.2	11.9	12.1	12.6	12.4	12.6
0	4543	9.9	10.1	9.5	9.5	9.4	9.4	9.2	9.2	9.3	9.3
	4544	7.9	8.3	8.3	8.1	8.1	8.4	8.4	8.5	9.0	9.2
	Mean	9.8	10.3	9.9	10.0	9.9	9.9	9.9	10.1	10.2	10.4
	S.E.	1.1	1.5	1.0	1.3	1.2	1.0	1.1	1.3	1.1	1.1
	N	3	3	3	3	3	3	3	3	3	3
II	4517	12.8	13.3	12.3	12.3	12.4	12.5	12.5	12.4	12.4	12.6
25	4546	9.3	9.4	9.1	8.5	9.0	9.1	9.5	9.6	9.7	10.0
	4550	8.1	8.2	8.0	7.8	8.1	8.3	8.5	8.6	8.6	8.2
	Mean	10.1	10.3	9.8	9.5	9.8	10.0	10.2	10.2	10.2	10.3
	S.E.	1.4	1.5	1.3	1.4	1.3	1.3	1.2	1.1	1.1	1.3
	N	3	3	3	3	3	3	3	3	3	3

[a]Body weights for Week -2 were not recorded due to technician error.

Appendix D
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group I (0 mg/kg/day)
Animal No. 4519M

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	0	0	0	0	0
Standing	0	0	0	0	0	0	0	0	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Flexor Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Reflex	0	0	0	1	0	0	0	0	0	0	0	0
Tonic Neck Reflex	0	0	0	1	0	0	0	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	0	0	0	0	0	0	0	0	0
Startle Reflex	0	0	0	0	1	1	1	0	1	0	0	0
Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group I (0 mg/kg/day)
Animal No. 4520M

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	0	0	0	0	0
Standing	0	0	0	0	0	0	0	0	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Flexor Reflex	0	0	0	1	0	0	0	0	0	0	0	0
Extensor Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Tonic Neck Reflex	0	0	0	1	0	0	1	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	0	0	0	0	0	0	0	0	0
Startle Reflex	1	0	0	0	0	0	0	0	0	0	0	0
Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group I (0 mg/kg/day)
Animal No. 4521M

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	0	0	0	0	0
Standing	0	0	0	0	0	0	0	0	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	1	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Flexor Reflex	1	0	0	0	0	0	0	0	0	0	0	0
Extensor Reflex	1	0	0	0	0	0	0	0	0	0	0	0
Tonic Neck Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	0	0	0	0	0	0	0	0	0
Startle Reflex	1	0	0	0	0	0	0	0	0	0	0	0
Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group I (0 mg/kg/day)
Animal No. 4515F

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	0	0	0	0	0
Standing	0	0	0	0	0	0	0	0	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Flexor Reflex	1	0	0	0	0	0	0	0	0	0	0	0
Extensor Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Tonic Neck Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	0	0	0	0	0	0	0	0	0
Startle Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group I (0 mg/kg/day)
Animal No. 4543F

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	0	0	0	0	0
Standing	0	0	0	0	0	0	0	0	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	1	0	0	0	0	0	0	0	0
Flexor Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Reflex	1	0	0	1	1	0	0	0	0	0	1	0
Tonic Neck Reflex	0	0	0	1	0	0	0	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	0	0	0	0	0	0	0	0	0
Startle Reflex	1	0	1	1	1	0	0	0	0	0	0	0
Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group I (0 mg/kg/day)
Animal No. 4544F

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	0	0	0	0	0
Standing	0	0	0	0	0	0	0	0	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	1	0	0	0	0	0	0	0	0
Flexor Reflex	1	0	0	1	3	2	2	1	1	1	1	1
Extensor Reflex	0	1	0	0	0	0	0	0	0	0	0	0
Tonic Neck Reflex	0	0	0	1	0	0	0	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	0	0	0	0	0	0	0	0	0
Startle Reflex	1	0	0	0	1	0	0	0	1	0	0	0
Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group II (25 mg/kg/day)
Animal No. 4435M

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	0	0	0	0	0
Standing	0	0	0	0	0	0	0	0	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Flexor Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Reflex	0	1	1	1	0	0	0	0	0	0	0	0
Tonic Neck Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	0	0	0	0	0	0	0	0	0
Startle Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group II (25 mg/kg/day)
Animal No. 4537M

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	0	1	0	0	1
Standing	0	0	0	0	0	0	0	1	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	1	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	0	0	0	0	1	0	1	2	1
Flexor Reflex	0	0	0	2	2	2	0	2	1	0	0	0
Extensor Reflex	0	0	0	2	1	1	0	1	1	1	0	0
Tonic Neck Reflex	0	0	0	1	0	0	0	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	1	0	0	0	0	0	0	2	0	1	0	1
Startle Reflex	0	0	0	0	0	0	0	0	0	0	0	1
Righting Reflex	0	0	0	0	0	0	0	1	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group II (25 mg/kg/day)
Animal No. 4538M

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	0	0	0	0	0
Standing	0	0	0	0	0	0	0	0	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	0	0	1	0	1	0	0	0	1
Flexor Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Reflex	0	1	0	1	0	0	0	0	0	0	0	0
Tonic Neck Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	0	0	0	0	0	0	0	0	0
Startle Reflex	0	0	0	0	0	0	1	0	0	0	0	0
Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group II (25 mg/kd/day)
Animal No. 4517F

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	3	1	1	1	2
Standing	0	0	0	0	0	0	2	2	2	1	2	1
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	1	0	0	2	2	1	2	1	1
Flexor Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Reflex	0	1	0	1	0	0	2	3	2	2	2	2
Tonic Neck Reflex	0	0	0	0	0	0	0	1	1	1	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	0	0	0	0	2	1	1	2	1
Startle Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Righting Reflex	0	0	0	0	0	0	0	2	2	1	2	1

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group II (25 mg/kg/day)
Animal No. 4546F

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	0	0	0	0	0	0
Standing	0	0	0	0	0	0	0	0	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Flexor Reflex	1	1	2	3	2	0	0	1	0	0	0	0
Extensor Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Tonic Neck Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	0	0	0	0	0	0	0	0	0
Startle Reflex	0	0	1	1	0	0	0	0	0	0	0	0
Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix D (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Individual Neurological Examination Values
Group II (25 mg/kg/day)
Animal No. 4550F

Observation	Day: -4	0[a]	3 hrs.[b]	4	7	10	14	21	28	35	42	49
Locomotion	0	0	0	0	0	0	1	0	0	0	0	0
Standing	0	0	0	0	0	0	0	0	0	0	0	0
Pupillary Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Corneal Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Visual Placing Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Extensor Postural Thrust Reflex	0	0	0	0	0	0	0	0	0	0	0	0
Flexor Reflex	0	1	1	2	2	3	3	2	2	1	1	1
Extensor Reflex	1	1	1	1	1	0	1	0	0	0	0	0
Tonic Neck Reflex	0	0	1	1	1	0	0	0	0	0	0	0
Walking Reactions (Wheelbarrowing)	0	0	0	1	1	0	0	0	0	0	0	0
Startle Reflex	0	0	0	1	0	0	0	0	0	0	0	0
Righting Reflex	0	0	0	0	0	0	0	0	0	0	0	0

[a]Evaluation performed just prior to first dosing.
[b]Evaluation performed 3 hours after first dosing.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Pretest (4 Days Prior to Dosing)

Neurological Score:	Group I (0 mg/kg/day) 0	1	2	3	Group II (25 mg/kg/day) 0	1	2	3
Observation[a]								
Locomotion	3/3				3/3			
Standing	3/3				3/3			
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	2/3	1/0			2/3	1/0		
Extensor Postural Thrust Reflex	3/3				3/3			
Flexor Reflex	2/1	1/2			3/2	0/1		
Extensor Reflex	2/2	1/1			3/2	0/1		
Tonic Neck Reflex	3/3				3/3			
Walking Reactions (Wheelbarrowing)	3/3				2/3	1/0		
Startle Reflex	1/1	2/2			3/3			
Righting Reflex	3/3				3/3			

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Pretest (Just Prior to Dosing)

Neurological Score:	Group I (0 mg/kg/day) 0	1	2	3	Group II (25 mg/kg/day) 0	1	2	3
Observation[a]								
Locomotion	3/3				3/3			
Standing	3/3				3/3			
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/3				3/3			
Flexor Reflex	3/3				3/2			
Extensor Reflex	3/2	0/1			1/1	2/2		
Tonic Neck Reflex	3/3				3/3			
Walking Reactions (Wheelbarrowing)	3/3				3/3			
Startle Reflex	3/3				3/3			
Righting Reflex	3/3				3/3			

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Day 0 (3 Hours After First Dose)

Neurological Score:	Group I (0 mg/kg/day) 0	1	2	3	Group II (25 mg/kg/day) 0	1	2	3
Observation[a]								
Locomotion	3/3				3/3			
Standing	3/3				3/3			
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/3				3/3			
Flexor Reflex	3/3				3/1	0/1	0/1	
Extensor Reflex	3/3				2/2	1/1		
Tonic Neck Reflex	3/3				3/2	0/1		
Walking Reactions (Wheelbarrowing)	3/3				3/3			
Startle Reflex	3/2	0/1			3/2	0/1		
Righting Reflex	3/3				3/3			

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Day 4

Neurological Score:	Group I (0 mg/kg/day) 0	1	2	3	Group II (25 mg/kg/day) 0	1	2	3
Observation[a]								
Locomotion	3/3				3/3			
Standing	3/3				3/3			
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/2	0/1			3/2	0/1		
Flexor Reflex	1/3	2/0			2/1	0/0	1/1	0/1
Extensor Reflex	3/3	1/1			0/1	2/2	1/0	
Tonic Neck Reflex	1/2	2/1			2/2	1/1		
Walking Reactions (Wheelbarrowing)	3/3				3/2	0/1		
Startle Reflex	3/2	0/1			3/1	0/2		
Righting Reflex	3/3				3/3			

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Day 7

Neurological Score:	Group I (0 mg/kg/day) 0	1	2	3	Group II (25 mg/kg/day) 0	1	2	3
Observation[a]								
Locomotion	3/3				3/3			
Standing	3/3				3/3			
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/3				3/3			
Flexor Reflex	3/2	0/0	0/0	0/1	2/1	0/0	1/2	
Extensor Reflex	3/2	0/1			2/2	1/1		
Tonic Neck Reflex	3/3				3/2	0/1		
Walking Reactions (Wheelbarrowing)	3/3				3/2	0/1		
Startle Reflex	2/1	1/2			3/3			
Righting Reflex	3/3				3/3			

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Day 10

Neurological Score:	Group I (0 mg/kg/day) 0	1	2	3	Group II (25 mg/kg/day) 0	1	2	3
Observation[a]								
Locomotion	3/3				3/3			
Standing	3/3				3/3			
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/3				2/3	1/0		
Flexor Reflex	3/2	0/0	0/1		2/2	0/0	1/0	0/1
Extensor Reflex	3/3				2/3	1/0		
Tonic Neck Reflex	3/3				3/3			
Walking Reactions (Wheelbarrowing)	3/3				3/3			
Startle Reflex	2/3	1/0			3/3			
Righting Reflex	3/3				3/3			

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Day 14

Neurological Score:	Group I (0 mg/kg/day) 0	1	2	3	Group II (25 mg/kg/day) 0	1	2	3
Observation[a]								
Locomotion	3/3				3/2	0/1		
Standing	3/3				3/2	0/0	0/1	
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/3				3/2	0/0	0/1	
Flexor Reflex	3/2	0/0	0/1		3/2	0/0	0/0	0/1
Extensor Reflex	3/3				3/1	0/1	0/1	
Tonic Neck Reflex	2/3	1/0			3/3			
Walking Reactions (Wheelbarrowing)	3/3				3/3			
Startle Reflex	2/3	1/0			2/3	1/0		
Righting Reflex	3/3				3/3			

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Day 21

Neurological Score:	Group I (0 mg/kg/day)				Group II (25 mg/kg/day)			
	0	1	2	3	0	1	2	3
Observation[a]								
Locomotion	3/3				3/2	0/0	0/0	0/1
Standing	3/3				2/2	1/0	0/1	
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/3				1/2	2/0	0/1	
Flexor Reflex	3/2	0/1			2/1	0/1	1/1	
Extensor Reflex	3/3				2/2	1/0	0/0	0/1
Tonic Neck Reflex	3/3				3/2	0/1		
Walking Reactions (Wheelbarrowing)	3/3				2/2	0/0	1/1	
Startle Reflex	3/3				3/3			
Righting Reflex	3/3				2/2	1/0	0/1	

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Day 28

Neurological Score:	Group I (0 mg/kg/day) 0	1	2	3	Group II (25 mg/kg/day) 0	1	2	3
Observation[a]								
Locomotion	3/3				2/2	1/1		
Standing	3/3				3/2	0/0	0/1	
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/3				3/2	0/1		
Flexor Reflex	3/2	0/1			2/2	1/0	0/1	
Extensor Reflex	3/3				2/2	1/0	0/1	
Tonic Neck Reflex	3/3				3/2	0/1		
Walking Reactions (Wheelbarrowing)	3/3				3/2	0/1		
Startle Reflex	2/2	1/1			3/3			
Righting Reflex	3/3				3/2	0/0	0/1	

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Day 35

Neurological Score:	Group I (0 mg/kg/day)				Group II (25 mg/kg/day)			
	0	1	2	3	0	1	2	3
Observation[a]								
Locomotion	3/3				3/2	0/1		
Standing	3/3				3/2	0/1		
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/3				2/2	1/0	0/1	
Flexor Reflex	3/2	0/1			3/2	0/1		
Extensor Reflex	3/3				2/2	1/0	0/1	
Tonic Neck Reflex	3/3				3/2	0/1		
Walking Reactions (Wheelbarrowing)	3/3				2/2	1/1		
Startle Reflex	3/3				3/3			
Righting Reflex	3/3				3/2	0/1		

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Day 42

Neurological Score:	Group I (0 mg/kg/day) 0	1	2	3	Group II (25 mg/kg/day) 0	1	2	3
Observation[a]								
Locomotion	3/3				3/2	0/1		
Standing	3/3				3/2	0/0	0/1	
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/3				2/2	0/1	1/0	
Flexor Reflex	3/2	0/1			3/2	0/1		
Extensor Reflex	3/2	0/1			3/2	0/0	0/1	
Tonic Neck Reflex	3/3				3/3			
Walking Reactions (Wheelbarrowing)	3/3				3/2	0/0	0/1	
Startle Reflex	3/3				3/3			
Righting Reflex	3/3				3/2	0/0	0/1	

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix E (cont.)
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

Summary of Neurological Examination Values
Interval: Day 49

Neurological Score:	Group I (0 mg/kg/day) 0	1	2	3	Group II (25 mg/kg/day) 0	1	2	3
Observation[a]								
Locomotion	3/3				2/2	1/0	0/1	
Standing	3/3				3/2	0/1		
Pupillary Reflex	3/3				3/3			
Corneal Reflex	3/3				3/3			
Visual Placing Reflex	3/3				3/3			
Extensor Postural Thrust Reflex	3/3				1/2	2/1		
Flexor Reflex	3/2	0/1			3/2	0/1		
Extensor Reflex	3/3				3/2	0/0	0/1	
Tonic Neck Reflex	3/3				3/			
Walking Reactions (Wheelbarrowing)	3/3				2/2	1/1		
Startle Reflex	3/3				2/3	1/0		
Righting Reflex	3/3				3/2	0/1		

[a]Number represents number of males/females exhibiting indicated response at above interval.

Code:

0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Appendix F
Determination of Triphenylphosphine (TPP) in Corn Oil Dosing Solutions for Study No. 79-2445

Report No.: 80015

Sponsor: M & T Chemicals, Inc.
Rahway, New Jersey 07065

Prepared by: Blanche Del Casale Date: June 30, 1980

Issued by: Bio/dynamics, Inc.
Department of Metabolism and Analytical Chemistry

Summary

The levels of triphenylphosphine (TPP) in corn oil dosing solutions were determined at various intervals during a seven week oral neurotoxicity study in dogs. Single samples were analyzed on days 0, 1, 2, 3 and 4. Thereafter samples were assayed once per week for 2 weeks into the study. Pretest stability data indicated that corn oil solutions would have to be made fresh daily just prior to administration to minimize degradation of TPP.

Joseph O'Grodnick 8-20-80
Joseph O'Grodnick, Ph.D.
Director
Department of Metabolism and Analytical Chemistry

Appendix F (cont.)

1.0 INTRODUCTION

A seven week oral neurotoxicity study of triphenylphosphine in dogs was performed by the Department of Toxicology of Bio/dynamics, Inc. Dose solution concentration was 25,000 ug/ml. Samples of the corn oil dosing solutions were sent to the Department of Metabolism and Analytical Chemistry for assay at the time of preparation. The purpose of this study was to confirm the stability of the test compound in the corn oil solutions and to verify the concentration of the prepared dosing solutions.

2.0 EXPERIMENTAL

2.1 Materials

Triphenylphosphine, Analytical	Supplied by the sponsor
General Laboratory Glassware	
Liquid Chromatograph with u Bondapak column and a Co Pell ODS pre-column	Tracor 990 with 960 U.V. Detector Waters Associates Whatman

2.2 Determination of Triphenylphosphine in Corn Oil Dosing Solution

2.2.1. Sample Preparation

Appropriate dilutions of the corn oil solutions were made with tetrahydrofuran for injection into the liquid chromatograph.

2.2.2. Liquid Chromatographic Analysis

A standard curve was generated by injecting known (varying) amounts of triphenylphosphine. Standards were plotted as the peak area of TPP vs. ng of standard injected. Sample concentrations were determined by comparing peak areas of samples with the standard curve.

A typical standard curve and examples of standard and sample injections are shown in Figures 1-3, respectively. Liquid Chromatographic conditions are given in Table I.

Appendix F (cont.)

2.2.3. Calculations

$$\frac{(\text{total ug found as read from std. curve}) \times \text{final volume in ml} \times 100}{\text{ug/ml added}} = \% \text{ recovery}$$

3.0 RESULTS AND CONCLUSIONS

Assays for triphenylphosphine at the time of preparation ranged from 60% recovery to 110% (average 85%). Complete results of weekly analyses are given in Table II.

Assays for pre-test seven day stability ranged from 90 to 98% of nominal concentration on day one, 32-52% of nominal on day four and 70% of nominal on day seven. Complete results of seven day stability are found in Table III.

These data verify that the animals on this study were administered dosing solutions which closely conform to the intended dosages. The unusually low recoveries for Days 0, 1 and 2 were due to problems encountered in the Dose solution mixing procedure. The problem was corrected subsequent to Day 2.

Appendix F (cont.)

TABLE I. Liquid Chromatographic Conditions For The Analysis of TPP in Oral Corn Oil Dosing Solutions

Instrument:	Tracor 960 liquid Chromatograph or equivalent
Detector:	Tracor Fixed Wavelength 254 nm
Column:	Waters u Bondapak 30 cm x 3.9 mm i.d.
Pre column:	Co Pell ODS
Mobile Phase:[a]	88:12 Methanol: Water
Flow Rate[a]:	2.0 ml/minute
AUFS[a]	32
Chart Speed:	0.30 inches/minute

[a]These parameters may be changed to achieve optimum sensitivity.

Appendix F (cont.)

TABLE II. LEVELS OF TRIPHENYLPHOSPHINE IN CORN OIL DOSING SOLUTIONS FOR STUDY NO. 79-2445

Day	Group	Date Sampled	Date Analyzed	TPP, ug/ml Added	TPP, ug/ml Found	% Nominal
Day 0	I-1	3/24/80	3/24/80	0	<6250	-
	I-2	3/24/80	3/24/80	0	<6250	-
	I-3	3/24/80	3/24/80	0	<6250	-
	II-1	3/24/80	3/24/80	25,000	18,000	72
	II-2	3/24/80	3/24/80	25,000	18,250	73
	II-3	3/24/80	3/24/80	25,000	15,000	60
	II-4	3/24/80	3/24/80	25,000	15,000	60
	II-5	3/24/80	3/24/80	25,000	17,500	70
Day 1	II-1	3/25/80	3/25/80	25,000	21,250	85
	II-2	3/25/80	3/25/80	25,000	20,750	83
Day 2	I-1	3/26/80	3/26/80	0	<6250	-
	I-2	3/26/80	3/26/80	0	<6250	-
	II-1	3/26/80	3/26/80	25,000	15,250	61
	II-2	3/26/80	3/26/80	25,000	17,000	68
Day 3	I-1	3/27/80	3/27/80	0	<6250	-
	I-2	3/27/80	3/27/80	0	<6250	-
	II-1	3/27/80	3/27/80	25,000	26,250	105
	II-2	3/27/80	3/27/80	25,000	25,000	100
Day 4	I-1	3/28/80	3/28/80	0	<6250	-
	I-2	3/28/80	3/28/80	0	<6250	-
	II-1	3/28/80	3/28/80	25,000	26,750	107
	II-2	3/28/80	3/28/80	25,000	27,500	110
Day 7	I-1	3/31/80	3/31/80	0	<6250	-
	I-2	3/31/80	3/31/80	0	<6250	-
	II-1	3/31/80	3/31/80	25,000	26,250	105
	II-2	3/31/80	3/31/80	25,000	23,750	95
Day 14	I-1	4/7/80	4/7/80	0	<6250	-
	I-2	4/7/80	4/7/80	0	<6250	-
	II-1	4/7/80	4/7/80	25,000	23,750	95
	II-2	4/7/80	4/7/80	25,000	23,250	93

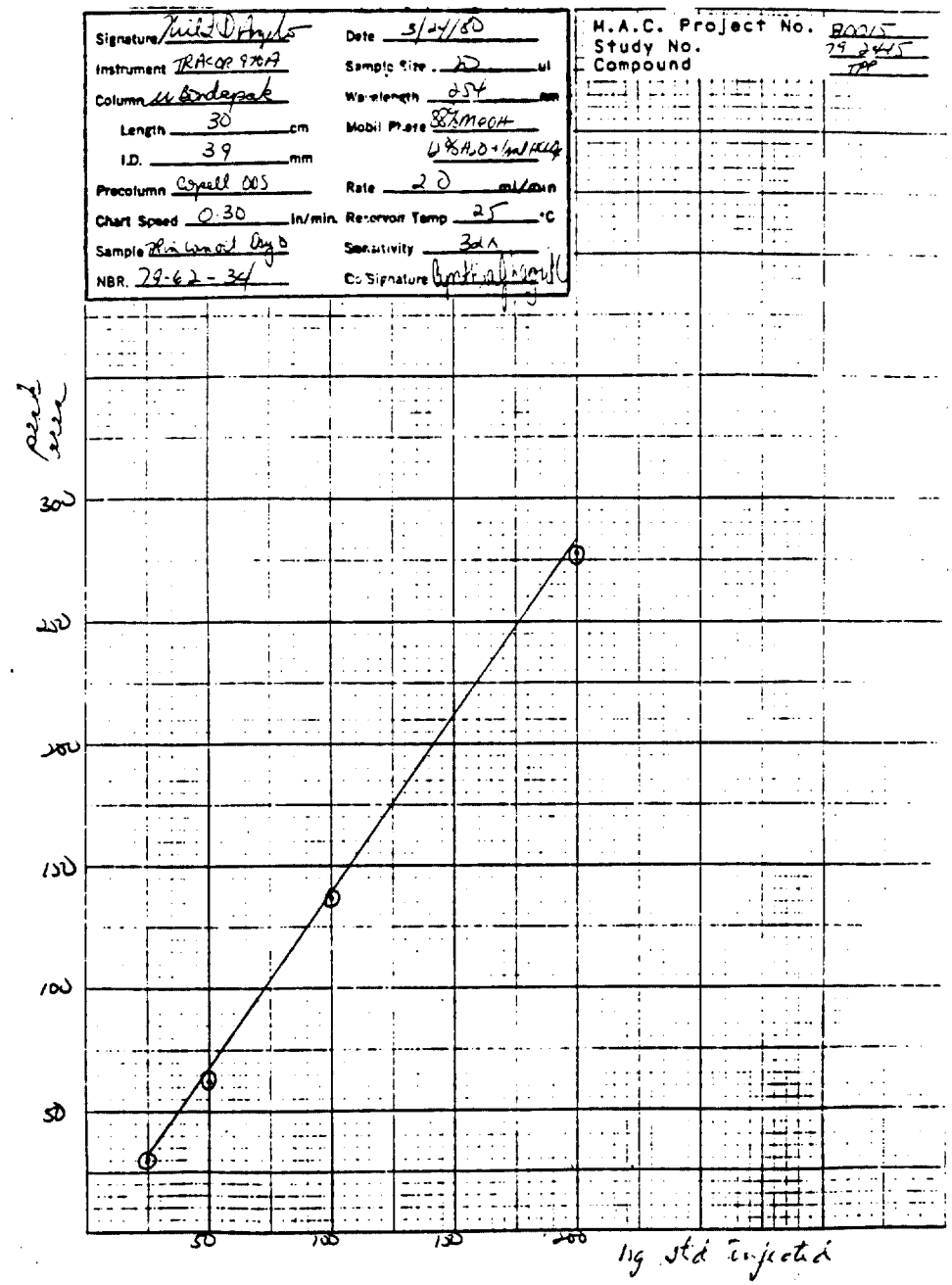
Appendix F (cont.)

TABLE III. PRE-TEST 14 DAY STABILITY DATA FOR TPP STUDY NO. 79-2445

Interval Day	Date Prepared	Dose Group	TPP Content, ug/ml Added	Found	Recovery, % Nominal
1	3/10/80	I	0	<6250	-
		II-1	25,000	24,500	98
		II-2	25,000	22,500	90
		II-3	25,000	24,500	98
		II-4	25,000	23,750	95
4	3/10/80	I	0	<6250	-
		II-1	25,000	13,000	52
		II-2	25,000	12,500	50
		II-3	25,000	8,000	32
		II-4	25,000	10,750	43
7	3/10/80	I-1	0	<6250	-
		I-2	0	<6250	-
		II-1	25,000	17,500	70
		II-2	25,000	17,500	70
		II-3	25,000	17,500	70
		II-4	25,000	17,500	70

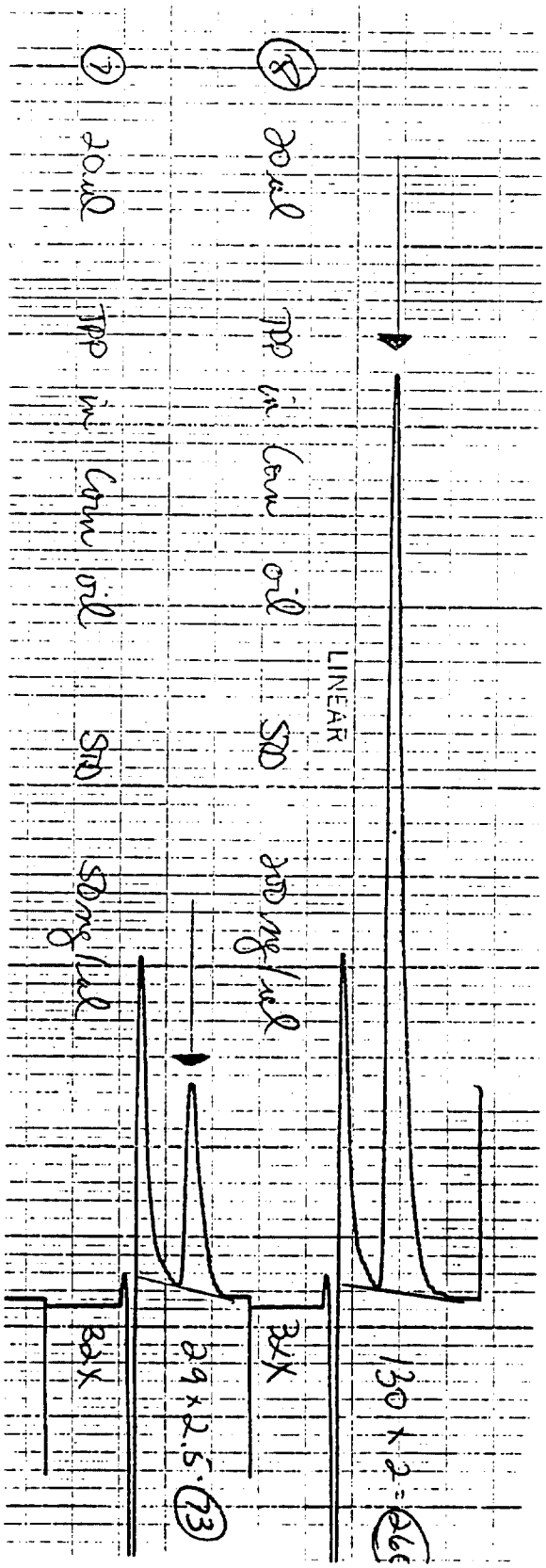
Appendix F (cont.)

FIGURE 1. TYPICAL STANDARD CURVE FOR TPP



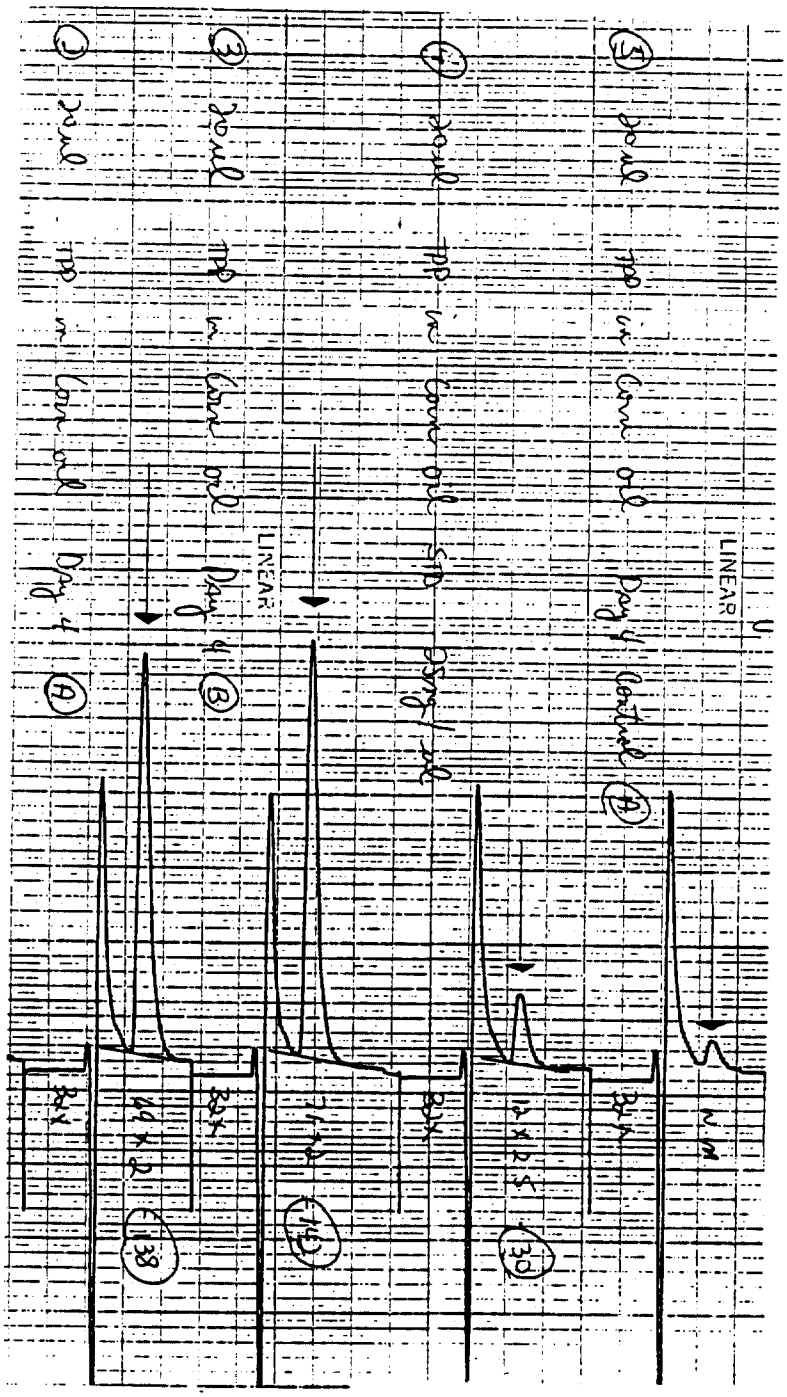
Appendix F (cont.)

FIGURE 2. TYPICAL CHROMATOGRAM OF TPP STANDARD



Appendix F (cont.)

FIGURE 3. TYPICAL CHROMATOGRAM OF TPP IN CORN OIL DOSING SOLUTIONS



Appendix G

A Seven Week Neurotoxicity Study in Dogs with Triphenylphosphine Pathology Report

Appendix G (cont.)
A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

Table of Contents

INTRODUCTION

MATERIALS AND METHODS
- Test Substance
- Test Animals
- Test Substance Administration
- Experimental Design
- Gross Postmortem Methods
- Microscopic Methods

RESULTS

SUMMARY

TABLE I
- Gross Postmortem Observations

TABLE II
- Microscopic Findings

TABLE III
- Summary of Microscopic Findings

Appendix G (cont.)
A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

INTRODUCTION

This study, conducted for the M and T Chemical Company, was designed to assess the reversibility of any neurotoxic or other adverse effects on Beagle dogs (3/sex/group) of Triphenylphosphine when administered daily for 21 days at a dose level of 25 mg/kg/day. The treatment period was followed by a 28-day recovery period.

MATERIALS AND METHODS

Test Substance: Triphenylphosphine

Test Animals: Beagle dogs

Test Substance Administration: Oral, via intubation

Experimental Design:

Group	Dose mg/kg/day	Number of Animals					
		Initial		Necropsy		Histopathology	
		M	F	M	F	M	F
I	0 (Vehicle Control)	3	3	All		All	
II	25	3	3	All		All	

Gross Postmortem Methods:

At termination, dogs were sacrificed by exsanguination under sodium pentobarbital anesthesia. Observations were made for grossly detectable tissue or organ abnormalities. The entire brain, one section each of the cervical and lumbar spinal cords (in the intact vertebral column) and both sciatic nerves were taken from each animal and preserved in 10% neutral buffered formalin. In addition, any tissues or organs exhibiting any gross abnormalities were taken and preserved.

Appendix G (cont.)
A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

MATERIALS AND METHODS (cont.)

Microscopic Methods:

The brain, cervical and lumbar sections of the spinal cord and both sciatic nerves were trimmed, processed in the duoautotechnicon, embedded in paraffin and sectioned at 6u. Two representative sections of each were then placed on glass slides, one being stained with hematoxylin and eosin and the other with luxol fast blue counterstained with cresyl violet.

RESULTS

Tables I, II and III present gross, microscopic and summary of microscopic findings, respectively.

No pattern of gross lesions was found which would suggest a test substance related effect.

Microscopically, a minimal to severe, patchy to segmental myelin degeneration was observed in the central nervous tissue of treated animals only. This myelin degeneration was most severe in the peripheral ventral and lateral spinal tracts of the cervical spinal cord; a sparing effect of the dorsal spinal tracts is an interesting, but unexplained, observation. The lesion was also present in the lumbar spinal cord and, to a lesser extent, in the white tracts of the cerebrum. The sciatic nerve and the spinal nerves examined from the cervical and lumbar portions of the spinal cord were unaffected. Whether this lesion represents a primary myelin defect, or is secondary to axonal degeneration can not be determined without appropriate silver stains that specifically stain axons. Apparently, there is no reversibility of this myelin degeneration given a 28-day post-treatment recovery period.

Appendix G (cont.)
A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

SUMMARY

Twelve beagle dogs were distributed equally by sex into two experimental groups. Group I animals served as controls and received only the corn oil vehicle. Group II animals were administered, via intubation, 25mg/kg/day of triphenylphosphine over a 21-day period. The treatment period was followed by a 28-day recovery period at the end of which the animals were sacrificed and given a gross postmortem examination. Selected tissues were saved and examined microscopically.

No pattern of gross lesions was found which would suggest a test substance related effect.

Microscopic examination of selected tissues revealed a minimal to severe myelin degeneration in the cervical and lumbar portions of the spinal cord and, to a lesser extent, in the spinal tracts of the cerebrum. No peripheral nerve involvement was found. Furthermore, there was no apparent reversibility of this lesion during the 28-day recovery period.

Fred A. Hines / M L Knezevich

Fred A. Hines, D.V.M.
Pathologist

Appendix G (cont.)

Table I

A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Appendix G (cont.)

Table I
(cont.)

79-2445

A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

GENERAL KEY TO GROSS POSTMORTEM OBSERVATIONS

N.O.A.	=	No observabe abnormalities
R	=	Right
L	=	Left
X	=	Times
cm	=	centimeters
diam.	=	diameter
approx.	=	approximately

Appendix G (cont.)

Table I
(cont.)

79-2445

A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Group	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
I	M	4519	80-2834	Lungs	L posterior lobe: two embedded red nodules (approx. 1.0x0.6cm) firm and solid.
		4520	80-2835	Lungs	L median lobe: embedded, red, firm nodule (0.7cm in diam.).
		4521	80-2836	Lungs	R anterior lobe: tan, firm distention (2.0cm in diam.) in parenchyma with red center(approx. 0.8cm in diam.).
	F	4515	80-2837	-	N.O.A.
		4543	80-2838	-	N.O.A.
		4544	80-2839	Eye (L)	Mass: (1.0cm in diam.) protruding from lower lid, pink and solid.

Appendix G (cont.)

Table I
(cont.)

79-2445

A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Group	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
II	M	4435	80-2840	-	N.O.A.
		4537	80-2841	-	N.O.A.
		4538	80-2842	Spleen	Raised tan and gray areas along edge of head and tail.
	F	4517	80-2843	Spleen	Raised black and tan areas around edge of head.
				Lungs	All lobes: varying degrees of tan mottling; R posterior lobe: embedded red nodules (less than or equal to 0.6cm in diam.); mid lobes: areas of constriction with tan mottling.
		4546	80-2844	-	N.O.A.
		4550	80-2845	-	N.O.A.

Appendix G (cont.)

Table II

A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

Microscopic Findings
Terminal Sacrifice

Appendix G (cont.)

Table II
(cont.)

79-2445

A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

GENERAL KEY TO HISTOPATHOLOGY TABLES

N	=	Not remarkable, within normal histologic limits
1	=	Minimal degree of indicated change
2	=	Mild degree of indicated change
3	=	Moderate degree of indicated change
4	=	Marked degree of indicated change

Appendix G (cont.)
Table II
(cont.)

79-2445

A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

Microscopic Findings
Terminal Sacrifice
Hematoxylin and Eosin-Stained Sections

Animal Number	4519	4520	4521	4515	4543	4544	4435	4537	4538	4517	4546	4550
Sex	M	M	M	F	F	F	M	M	M	F	F	F
Pathology Number 80-	2834	2835	2836	2837	2838	2839	2840	2841	2842	2843	2844	2845
Group		I			I			II			II	
BRAIN												
-1st transverse section	N	N	N	N	N	N			N			
patchy degeneration of myelin characterized by a status spongiosum and swollen macrophages containing fibrilar or globular eosinophilic or amphophilic material.							1	1		2	1	1
-2nd transverse section	N	N	N	N	N	N	N		N		N	
patchy degeneration of myelin characterized by a status spongiosum and swollen macrophages containing fibrilar or globular eosinophilic or amphophilic material.								1		3		1
RIGHT SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N	N	N
LEFT SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N	N	N
CERVICAL SPINAL CORD	N	N	N	N	N	N						
patchy degeneration of myelin characterized by a status spongiosum and swollen macrophages containing fibrilar or globular eosinophilic or amphophilic material.							2	3	2	3	3	3
LUMBAR SPINAL CORD	N	N	N	N	N	N						
patchy degeneration of myelin characterized by a status spongiosum and swollen macrophages containing fibrilar or globular eosinophilic or amphophilic material.							3	1	2	2	2	3

Appendix G (cont.)
Table II
(cont.)

79-2445

A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

Microscopic Findings
Terminal Sacrifice
Luxol Fast Blue-Stained Sections

Animal Number	4519	4520	4521	4515	4543	4544	4435	4537	4538	4517	4546	4550
Sex	M	M	M	F	F	F	M	M	M	F	F	F
Pathology Number 80-	2834	2835	2836	2837	2838	2839	2840	2841	2842	2843	2844	2845
Group		I			I			II			II	
BRAIN												
-1st transverse section patchy degeneration of myelin characterized by a status spongiosum and swollen macrophages containing fibrilar or globular eosinophilic or amphophilic material.	N	N	N	N	N	N	N	N	N	2	N	N
-2nd transverse section patchy degeneration of myelin characterized by a status spongiosum and swollen macrophages containing fibrilar or globular eosinophilic or amphophilic material.	N	N	N	N	N	N	N	1	N	2	N	N
RIGHT SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N	N	N
LEFT SCIATIC NERVE	N	N	N	N	N	N	N	N	N	N	N	N
CERVICAL SPINAL CORD patchy degeneration of myelin characterized by a status spongiosum and swollen macrophages containing fibrilar or globular eosinophilic or amphophilic material.	N	N	N	N	N	N	3	4	2	4	3	3
LUMBAR SPINAL CORD patchy degeneration of myelin characterized by a status spongiosum and swollen macrophages containing fibrilar or globular eosinophilic or amphophilic material.	N	N	N	N	N	N	2	3	1	2	3	4

Appendix G (cont.)

Table III

A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

Summary of Microscopic Findings
Terminal Sacrifice

Appendix G (cont.)

Table III
(cont.)

79-2445

A Seven Week Neurotoxicity Study
in Dogs with Triphenylphosphine
Pathology Report

Summary of Microscopic Findings
Terminal Sacrifice

Organ/Tissue -Observation		H&E-Stained Sections				L.F.B.-Stained Sections			
	Group	I		II		I		II	
	Sex	M	F	M	F	M	F	M	F
	Number of Animals	3	3	3	3	3	3	3	3
BRAIN (1st transverse section)									
-not remarkable		3	3	1	0	3	3	3	2
-D.M.*		0	0	2	3	0	0	0	1
BRAIN (2nd transverse section)									
-not remarkable		3	3	2	1	3	3	2	2
-D.M.*		0	0	1	2	0	0	1	1
SCIATIC NERVE (RIGHT)									
-not remarkable		3	3	3	3	3	3	3	3
SCIATIC NERVE (LEFT)									
-not remarkable		3	3	3	3	3	3	3	3
CERVICAL SPINAL CORD									
-not remarkable		3	3	0	0	3	3	0	0
-D.M.*		0	0	3	3	0	0	3	3
LUMBAR SPINAL CORD									
-not remarkable		3	3	0	0	3	3	0	0
-D.M.*		0	0	3	3	0	0	3	3

* D.M. = patchy degeneration of myelin characterized by a status spongiosum and swollen macrophages containing fibrilar or globular eosinophilic, amphophilic or L.F.B.$^+$ material.

Appendix H
A Seven Week Neurotoxicity Study
with Triphenylphosphine in Dogs

PERSONNEL

DEPARTMENT/TITLE	NAME	DEGREE
STUDY DIRECTOR	George R. Lankas	Ph.D.
LARGE ANIMAL SECTION		
MANAGER	Jeffrey P. Lefrancois	B.S.
SUPERVISOR	Daniel J. Pisacreta	B.S.
TECHNICIAN-IN-CHARGE	Patricia R. Presti	B.S.
METABOLISM AND ANALYTICAL CHEMISTRY		
MANAGER	Joseph O'Grodnick	Ph.D.
SUPERVISOR	Ben Gulizia	B.S.
VETERINARIAN	Fred A. Hines	D.V.M.
PATHOLOGY		
DIRECTOR	Aleksandar L. Knezevich	D.M.V.
PATHOLOGIST	Fred A. Hines	D.V.M.
SUPERVISOR, HISTOTECHNOLOGY	Joanna V. Rovee	H.T. (ASCP)
QUALITY ASSURANCE		
SUPERVISOR	Craig Lamb	B.S.

Appendix I
BIO/DYNAMICS INC.
METTLERS ROAD
EAST MILLSTONE, N.J. 08873

QUALITY ASSURANCE STATEMENT

* * * * * * * * * * * *

Study # 79-2445 Test Article/Substance Triphenylphosphine (TPP)

Sponsor M and T Chemical Company

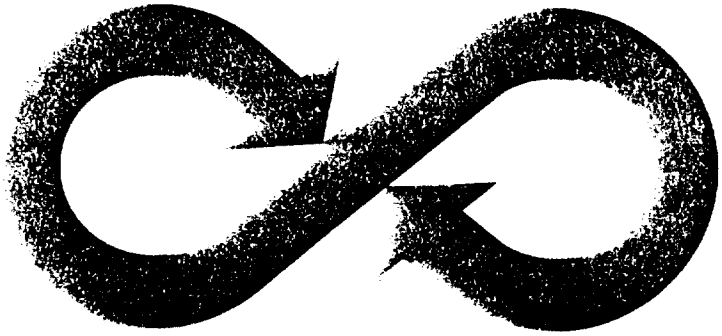
Listed below are dates that this study was inspected by the Quality Assurance Unit and the dates findings were reported to the Study Director and to Management.

Date(s) of Inspection	Date(s) findings reported to Study Director	Date(s) findings reported to Management
4/11/80	4/14/80	4/27/80 5/5/80
4/21/80	4/21/80	4/21/80 4/23/80
4/30/80	4/30/80	4/30/80
5/13/80	5/15/80	5/15/80
7/21/80	8/13/80	8/13/80 8/18/80
8/11/80	8/15/80	8/20/80 8/21/80

Unless noted to the contrary herein, it is my opinion that the raw data upon which this report is based is free from error that would affect the integrity of the study and that the final report accurately summarizes these data.

8-22-80
date

Craig Lamb
Supervisor, Quality Assurance



Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO. 79-7349

A FOUR WEEK INHALATION TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN DOGS

Submitted to: M & T CHEMICALS
Rahway, New Jersey 07065

Attention: Arthur Sheldon

Date: December 31, 1980

BEST COPY AVAILABLE

Bio/dynamics Inc.

PROJECT NO. 79-7349

A FOUR WEEK INHALATION TOXICITY STUDY OF TRIPHENYL PHOSPHINE IN DOGS

ABSTRACT

Groups of two male and two female Beagle dogs were exposed to concentrations of 18.0 or 94.8 mg/m^3 of triphenyl phosphine (TPP) for 6 hours/day, 5 days/week for four weeks; a similarly constituted group were exposed for the same period to xylene vapor and served as controls. After treatment, all dogs were observed for a four week recovery period before necropsy.

Visual evidence of locomotor dysfunctions were seen at the high level from the second week of treatment. This was neither progressive during the exposure period nor recuperable in the recovery period.

Elevations of serum alkaline phosphatase activities and cholesterol concentrations were seen at both levels with some evidence of normalization during the recovery period.

Patchy to segmental degeneration of the myelin in the white tracts of the brain and spinal cord were seen at both levels.

A group of one male and one female Beagle dogs were exposed for 6 hours/day for two days to concentrations of 216 and 162 mg/m^3. These dogs did not show any visual evidence of locomotor impairment but histopathological evidence of myelin degeneration was found.

P.O. Box 43 • Mettlers Road • East Millstone, New Jersey 08873 • (201) 873-2550 • Telex: 844597 • Cable: BIO/DYN

TABLE OF CONTENTS

I. INTRODUCTION 1

II. MATERIALS 1
A. Test Material 1
B. Test Animals 2
C. Selection 2
D. Animal Identification 2

III. METHODS 3
A. In-Life 3
Experimental Design 3
Husbandry 3
Test Material Administration 4
Chamber Operation 4
Atmospheric Sampling 4
Animal Observations and Body Weights 5
Ophthalmoscopic Examination 5
Laboratory Studies 5
B. Post Mortem 7

IV. RESULTS
A. Chamber Concentrations 9
B. Physical Observations 9
C. Body Weights 9
D. Hematology 10
E. Clinical Chemistry 10
F. Organ Weights 10
G. Macroscopic Pathology 10
H. Microscopic Pathology 10
I. Addendum 11

V. DISCUSSION 12

TABLES

1. Cumulative Exposure Records 14
2. Particle Size Distribution 16
3. Summary of Physical Observations 17
4. Individual and Mean Body Weights 20
5. Individual and Mean Hematology Values 22
6. Individual and Mean Clinical Chemistry Values 30
7. Individual and Mean Terminal Body Weights, Organ Weights, and Organ/Body Weight Ratios 38

APPENDICES

A. Methodology and References A-1
B. Analytical Chemistry Results B-1
C. Pathology Report C-1
D. Animal Termination History D-1
E. Addendum Results E-1

I. INTRODUCTION

This study, conducted for M & T Chemicals, was designed to assess the toxic effects, paying particular attention to neurological responses, of Triphenyl Phosphine (TPP) when administered by inhalation to dogs for 6 hours per day, 5 days a week for four weeks. Target concentrations of 10.0 and 100.0 milligrams per cubic meter (mg/m^3) were selected for the exposures. Hematology and clinical chemistry parameters were determined on all animals twice prior to exposure, at termination of exposure, and at the end of the four week recovery period. Gross necropsy examinations were performed on all animals following the post-exposure period. In the light of certain findings in the main study, a supplementary pair of dogs were exposed for 6 hours per day for two days to a target concentration of 200 mg/m^3 as study 79-7380 and is included as an Addendum (p-11) and Appendix E. All testing was conducted at Bio/dynamics Inc., P.O. Box 43, Mettlers Road, East Millstone, New Jersey, 08873. All raw data are stored at Bio/dynamics Inc.

II. MATERIALS

A. Test Material	Triphenyl Phosphine
Supplier	M & T Chemicals Rahway, New Jersey 07065
First Shipment	
Description	Fine, white powder
Label Information	M & T Chemicals Co.; Triphenylphosphine; WTVAH-352K 4354-78; 1000g
Lot Number	WTVAH-352K
Date Received	January 16, 1980
Amount Received	1 glass container - 1000 grams
Second Shipment	
Description	Thin, white flakes
Label Information	M & T Chemicals Co.; Triphenylphosphine; PNVAH-6KF; #3
Lot Number	PNVAH-6KF
Date Received	February 29, 1980
Amount Received	800 grams
Dates of Exposure	January 29, 1980 - February 22, 1980

II. MATERIALS (Cont.)

B. Test Animals: Dogs

Strain: Beagle

Supplier: Marshall Research Animals, Inc.
North Rose, New York

Number of Animals: 12 (6 males, 6 females) selected from the Bio/dynamics, Inc. in-house animal colony.

Age at Receipt: 5-6 months

Age at Initiation of Exposures: 7-13 months (includes one dog from 12/13/79 shipment).

Weight at Initiation of Exposures (kilograms):

	Mean	Range
Males:	12.8	11.0-15.1
Females:	10.2	8.4-11.4

Immunization: Against distemper, hepatitis, and leptospirosis by supplier. Treatment for rabies performed at Bio/dynamics, Inc. upon receipt of animals.

Parasites: Fecal examination conducted during pretest on all dogs at Bio/dynamics, Inc. during the equilibration period. All results were negative.

C. Selection: More animals were purchased and equilibrated than were required for the study. Animals considered unsuitable for the study on basis of pretest laboratory studies and physical or ophthalmoscopic examinations were eliminated prior to initiation of exposure to the test substance (Meth. and Ref., Appendix A). Animals were sorted into groups on a random basis from those animals considered suitable for study purposes.

D. Animal Identification: Each dog was assigned a unique Bio/dynamics, Inc. identification number which appeared on an ear tag and on the animal's cage. In addition, each animal bore an ear tattoo with a USDA number.

III. METHODS

A. In-Life

Experimental Design

Group	Target Concentration	Exposure Time	Number of Animals[1] Exposed	Pretests 1 & 2 Weeks 4 & 8	Necropsy	Histopathology
I	None (Vehicle Control)	6 Hr/Day 5 Day/Wk 4 Weeks	4	4	4	4
II	10 mg/m^3	6 Hr/Day 5 Day/Wk 4 Weeks	4	4	4	4
III	100 mg/m^3	6 Hr/Day 5 Day/Wk 4 Weeks	4	4	4	4
		TOTALS:	12	12	12	12

[1]Equally divided male and female.

Husbandry:

Equilibration Period: Minimum 4 weeks

Housing: Individually in elevated metal grid cages. Doubly housed during exposure.

Food: Standard laboratory diet (Wayne Bite Size®) 400 grams/dog/day during non-exposure hours only.

Water: Tap-city water (Elizabethtown Water Company) with automatic watering system - ad libitum during nonexposure hours only.

Lights: 12 hours on, 12 hours off (animals exposed during lights "on" period).

III. METHODS (Cont.)

A. In-Life (cont.)

Test Material Administration

A solution of Triphenyl Phosphine and xylene was placed in a flask connected to an FMI fluid metering pump (Model G-20). The test material was fed directly into an air atomizing nozzle (Spraying System Company; 1/4 J nozzle with a #IA spray set up) and dry air at a flow rate of 21.0 liters per minute was passed through the atomizer to generate the aerosol. The resultant aerosol was drawn into the horizontal portal of a 760 liter glass and stainless steel exposure chamber. Chamber concentrations were controlled by adjusting the rate of flow of the solution to the spray atomizer. The solutions used for each exposure group were as follows:

Group I - control; administration of solvent only (xylene)
Group II - 10 mg/m^3, 1.5% (weight/volume) solution on Test Days 2 & 3; 6% (weight/volume) solution on Test Day 4-19; and a 1% (weight/volume) solution on Test Day 22-26.
Group III - 100 mg/m^3, 15% (weight/volume) solution during the first three weeks of exposure, and 12% (weight/volume) solution during the fourth week.

Chamber Operation

The stainless steel and glass exposure chambers in which the animals were exposed had a total volume of one cubic meter with an effective exposure volume of 760 liters. These chambers were operated dynamically at an air flow rate of 219 liters per minute. This provided one complete air change every 4.6 minutes with a equilibrium time (T_{99}) every 21.2 minutes.

Atmospheric Sampling

At least three samples were drawn from each exposure chamber each day using a glass fiber filter. The samples were pulled at 10 liters per minute for 30 and 5 minutes for Groups II and III, respectively. These samples were analyzed by high pressure liquid chromatography using the method provided by the sponsor (Appendix B).

III. METHODS (Cont.)

A. In-Life (cont.)

Atmospheric Sampling (cont.)

Determination of particle size distribution was done for each chamber three times during the first week and once each week thereafter using a Batelle cascade impactor (Model DCI-6). The mass distribution was determined from the material collected on the slides.

Animal Observations and Body Weights

Animals were observed twice daily for incidence of abnormal signs. Observations of any unusual condition were recorded when made. A full recorded physical assessment was performed weekly. Individual body weights were recorded for three weeks prior to exposure and weekly during the exposure and recovery period.

Ophthalmoscopic Examination:	(Methodology and References, Appendix A)
Pretest:	January 22, 1980
Number of Animals:	All including extras
Week 4 exposure termination:	February 22, 1980
Number of Animals:	All survivors
Week 4 post-exposure:	March 20, 1980
Number of Animals:	All survivors
Laboratory Studies	
Pretest 1:	January 9, 1980
Number of Animals:	All including extras

III. METHODS (Cont.)

A. In-Life (cont.)

Laboratory Studies (cont.)

Pretest 2:	January 23, 1980
Number of Animals:	All including extras
Week 4 exposure termination:	February 22, 1980
Number of Animals:	All survivors
Week 4 post-exposure:	March 19, 1980
Number of Animals:	All survivors
Blood Collection:	Venipuncture of the jugular vein

Parameter Evaluated:

Hematology[2]:

hemoglobin
hematocrit
erythrocyte count
leukocyte count (total and differential)
platelet count
reticulocyte count (only counted if signs of anemia was present)

Clinical Chemistry[2]:

blood urea nitrogen
serum glutamic pyruvic transaminase
serum alkaline phosphatase
glucose
albumin
total protein
globulin (calculated)
A/G ratio (calculated)
serum lactic dehydrogenase
bilirubin (direct and total)
sodium
potassium
calcium
total cholesterol

---

[2]Animals fasted overnight prior to blood sampling via jugular vein.

III. METHODS (Cont.)

B. Post Mortem

Post-Exposure Necropsies[3]:

Date of Necropsy:	March 24, 1980 (5 male, 3 female) March 25, 1980 (1 male, 3 female)
Sacrifice Method:	Exsanguinated under sodium pentobarbital anesthesia

Organs Weighed:

adrenals	kidneys
brain	liver
gonads (ovaries or testicles paired)	pituitary
heart	thyroid (with parathyroid)

Tissues Fixed

abdominal aorta	mammary gland (right inguinal)
adrenals (2)	pancreas
bone and bone marrow (sternum)	pituitary
brain (2 sections)	prostate
esophagus	major salivary glands
eyes (2) with optic nerve	sciatic nerve with muscle (both right and left, 3 sections each)
gall bladder	skin (inguinal, taken with mammary gland)
gonads	spinal cord (cervical and lumbar, 3 sections each)
ovaries	spleen
testes with epididymides	stomach (2 sections)
heart (with coronary vessels)	thymus
intestine	trachea
colon	thyroid/parathyroid
duodenum	urinary bladder[5]
ileum	uterus (corpus and cervix uteri)
kidneys	gross lesions (to include a border of normal tissue)
liver (2 sections)	tissue masses or suspect tumors and regional lymph nodes
lungs[4] (2 sections, at least 2 lobes and mainstem bronchi)	
lymph nodes (peribronchial and mesenteric)	

---

[3]Animals were fasted overnight prior to terminal sacrifice.
[4]Lungs were infused via the trachea with an amount of formalin approximately equal to 75% of the lung capacity.
[5]Urinary bladders were opened and examined prior to formalin fixation.

III. METHODS (Cont.)

B. Post Mortem (cont.):

Fixative:	All tissues - 10% neutral buffered formalin
Tissues Examined Histopathologically:	
sciatic nerve (right and left)	1 longitudinal section (on each) 2 transverse sections (on each)
cervical spinal cord	1 longitudinal section 2 transverse sections
lumbar spinal cord	1 longitudinal section 2 transverse sections
brain	2 transverse sections
Stain:	Sections of all tissues were prepared with H & E stain and a duplicate set, with the exception of the brain, was prepared with Luxol fast blue stain (counterstained with cresyl violet).

IV. RESULTS

A. Chamber Concentrations

(Individual, Daily Mean, and Integrated Mean Analytical Concentrations - Table 1, page 14; Particle Size Distribution - Table 2, page 16; Analytical Chemistry Results - Appendix B)

Due to the presence of dogs in the chamber and their tendency to obstruct or interfere with the sampling probe, considerable variations in chamber concentration were found during and between exposures. The cumulative integrated mean concentrations were 18.1 and 94.8 mg/m^3 for the low and high levels, respectively. Particle size distribution analysis indicated the test material had a mean aerodynamic mass median diameter of 2.02 and 2.11 micrometer for Groups II and III, respectively, with mean geometric standard deviation of 2.62 and 2.37.

B. Physical Observations

(Summary of Physical Observations - Table 3, page 17)

Signs of hind limb incoordination were seen in dogs exposed to the high level during the second week of treatment but not at the low level. These responses were confirmed to be gait abnormalities and neurological examination excluded any effects on cranial or segmental reflexes. The incoordination was not progressive and during the recovery period did not show any signs of recovery.

C. Body Weights

(Individual and Mean Body Weights - Table 4, page 20)

During the exposure period all dogs lost weight; this was most marked in males and after cessation of exposure the return towards the pre-test values was absent in all except a low level dog (#4593). In females, weight gains during the recovery period were seen in all dogs. In neither sex was there any association with treatment or exposure level.

IV. RESULTS (Cont.)

D. Hematology
(Individual and Mean Hematology Values - Table 5, page 22)

The erythrocyte and leukocytic characteristics of treated and control dogs were within the limits of normal expectation and were undisturbed by exposure to TPP.

E. Clinical Chemistry
(Individual and Mean Clinical Chemistry Values - Table 6, page 30)

Marked variations between dogs and sampling intervals existed for most measured characteristics, but the elevations of alkaline phosphatase and cholesterol at both levels in both sexes at the end of the exposures appears treatment related. However, there was some evidence of normalization during the recovery period. All other measured characteristics were undisturbed by treatment.

F. Organ Weights
(Individual and Mean Terminal Body Weights and Organs Weight Ratios - Table 7, page 38)

Apart from a higher than normal absolute liver weight in a male (#4568) and relative liver weights in both males at the high level, there were no effects on the absolute or relative organ weights.

G. Macroscopic Pathology
(Appendix C)

The incidence or type of lesions were unrelated to treatment.

H. Microscopic Pathology
(Appendix C)

Patchy to segmental degeneration of the myelin in the white tracts of the brain and spinal cord were seen at both levels with no apparent abnormality in the peripheral nerves.

I. ADDENDUM

In the light of the neurological effects observed in vivo of dogs at the high level, a pair of dogs were exposed for two days to levels approximating to the peak excursions seen at the high level on exposure days 16 (205 mg/m^3) and 19 (228 mg/m^3) to indicate whether isolated high level excursions were the causal factor. The animals were exposed and treated identically to the main study except as follows:

Exposure concentration:	(a) Target 200 mg/m^3 (b) Actual 216 (Day 1) and 162 (Day 2) mg/m^3.
Exposure period:	Two consecutive days (5/1/80 - 5/2/80)
Observation period:	28 days (5/1/80 - 5/28/80)
Hematology and Clinical Chemistry:	Pretest (4/25/80) and Post-Exposure (5/27/80)

RESULTS

Detailed results are included in Appendix E. Neither of the dogs showed any reaction to treatment or neurological impairment during the exposure on observation periods.

Increased alkaline phosphatase levels were seen in both treated dogs following exposure and appear to be treatment-related. All other clinical chemistry parameters were unaffected.

Body weights, hematology parameters, and necropsy findings were within the limits of normal expectation.

Microscopic examination of selected neutral tissues revealed a minimal to mild toxic degeneration of myelin in both animals. Whether the lesion represents a progressive degeneration of the myelin or the healing stage of a reversible test material related effect could not be determined from this experiment.

V. DISCUSSION

The two studies indicate that both cumulative exposure to 18 and 95 mg/m^3 with excursions to 41 and 228 mg/m^3 respectively on isolated occasions and two exposures to 216 and 162 mg/m^3 elicited micropathological changes in the central nervous system.

The presence of microscopic lesions at the low level (without functional impairment) cannot be exclusively ascribed either to the cumulative exposure levels or the transient high level on exposure Day 6; distinction between these two possibilities is impossible based on the data presented.

Roger Ben-Dyke, B.Sc., M.I. Biol.
Study Director, Inhalation Toxicology

1-14-81
Date

Geoffrey K. Hogan, Ph.D.
Vice President of Toxicology

1/16/81
Date

Participating Senior Staff Personnel:

Cathy A. Hoffman, B.S., Supervisor, Inhalation
Dan Pisacreta, B.S., Supervisor, Large Animals
James A. Anagnost, B.S., Technician-in-Charge, Inhalation
Patty Presti, B.S., Technician-in-Charge, Large Animals
Jeff I. Krietzman, B.S., Study Monitor, Inhalation

Table 1
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Individual, Daily Mean, and Integrated
Mean Analytical Concentrations
Group II - 10 mg/m^3

Date	Test Day	Exposure Number	Individual Daily Concentrations 1	2	3	4	5	Daily Mean Concentration	Integrated Mean Concentration
1-29-80	2	1	<0.08	<0.08	<0.08	<0.08		-	-
1-30-80	3	2	5.2	3.9	19.0			9.4	4.7
1-31-80	4	3	24.0	22.5	132.0[b]			23.3	10.9
2-1-80	5	4	23.2	2.7	10.2			12.0	11.2
2-2-80	6	5	15.5	13.8	38.0			22.4	13.4
2-4-80	8	6	58.0	52.2	40.2	13.3		40.9	18.0
2-5-80	9	7	12.5	41.3	40.0			31.3	19.9
2-6-80	10	8	26.5	23.7	26.5			25.6	20.6
2-7-80	11	9	15.0	9.0	8.7			10.9	19.5
2-8-80	12	10	20.2	25.0	3.0			16.1	19.2
2-11-80	15	11	23.8	29.5	22.5			25.3	19.7
2-12-80	16	12	35.5	32.0	26.2			31.2	20.7
2-13-80	17	13	14.3	30.0	14.5			19.6	20.6
2-14-80	18	14	26.5	26.0	21.7			24.7	20.9
2-15-80	19	15	16.5	10.8	18.0			15.1	20.5
2-18-80	22	16	22.3	22.3	19.0			21.2	20.6
2-19-80	23	17	6.5	10.2	7.3			8.0	19.8
2-20-80	24	18	8.7	7.5	9.0			8.4	19.2
2-21-80	25	19	14.0	9.8	9.5			11.1	18.8
2-22-80	26	20	7.0	5.3	2.8			5.0	18.1

[a]No measurable amount of test material was collected on fiber filter.
[b]Value not used in mean calculations.

Table 1 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual, Daily Mean, and Integrated Mean Analytical Concentrations
Group III - 100 mg/m^3

Date	Test Day	Exposure Number	Individual Daily Concentrations										Daily Mean Concentration	Integrated Mean Concentration
			1	2	3	4	5	6	7	8	9	10		
1-29-80	2	1	38.5	38.0	51.0								42.5	42.3
1-30-80	3	2	<0.5[a]	0.5[a]	<0.5[a]								-	21.3
1-31-80	4	3	26.6	26.2	79.0								43.9	28.8
2-1-80	5	4	0.6	9.8	37.0								15.8	25.6
2-2-80	6	5	31.0	37.2	245.0								104.4	41.3
2-4-80	8	6	126.0	33.0	17.0	35.4							52.9	43.3
2-5-80	9	7	25.0	91.5	93.0	72.0							70.4	47.1
2-6-80	10	8	62.0	142.0	186.0								130.0	57.5
2-7-80	11	9	81.0	42.0	48.0								57.0	57.4
2-8-80	12	10	160.8	133.2	68.0								120.7	63.8
2-11-80	15	11	<0.5[b]	0.6[b]	136.8	123.0	147.0	130.8					134.4	70.2
2-12-80	16	12	121.2	72.0	64.0								85.7	71.5
2-13-80	17	13	96.0	5.7[b]	18.9[b]	141.0	126.0						121.0	75.3
2-14-80	18	14	5.6[b]	18.9[b]	1.9[b]	5.5[b]	75.0						75.0	75.3
2-15-80	19	15	207.0	94.0	86.0	162.0							137.3	79.4
2-18-80	22	16	212.5	210.0	238.0	180.0	177.5	201.0	186.0	265.2	177.5		205.3	87.3
2-19-80	23	17	78.0	63.0	75.0	73.0	61.0	59.0	64.0	55.0			66.0	86.0
2-20-80	24	18	60.0	58.0	63.0	42.0	165.0	114.0	177.0	96.0	94.8		96.6	86.6
2-21-80	25	19	105.0	852.0	162.0	105.0	183.0	138.0	147.0	129.0			227.6	94.0
2-22-80	26	20	105.0	108.0	97.2	69.0	22.5	70.0	39.0	57.0	416.0		109.3	94.8

[a]Sample not used in daily mean concentration.
[b]Due to pump failure samples are considered invalid and not used in mean calculations.

Table 2
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Particle Size Distribution
Using the Batelle Cascade Impactor[a]

Group mg/m^3	Date of Sample	Aerodynamic Mass Median Diameter (um)	Geometric Standard Deviation	% of Particles 10 um or less in Diameter
II	1-30-80	1.74	2.33	98.10
10	1-31-80	1.61	2.33	98.44
	2-1-80	2.35	2.34	95.59
	2-4-80	1.46	3.17	95.26
	2-11-80	2.40	2.10	97.27
	2-18-80	2.79	3.21	86.35
	2-20-80	1.80	2.88	94.78
	Mean:	2.02	2.62	95.11
III	1-31-80	1.85	2.27	98.02
100	2-1-80	2.04	2.20	97.83
	2-1-80	2.00	2.93	93.30
	2-4-80	2.49	2.29	95.33
	2-15-80	2.16	2.16	97.64
	Mean:	2.11	2.37	96.42

[a]Probit Analysis: Finney, D.J. Statistical Method in Biological Assay. 2nd Edition. London: Charles Griffin and Co., 1964. pp-468-490. Weighted Regression Analysis: Ames,, Iowa: Iowa State University Press, 1967. p.438.

Table 3
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Summary of Physical Observations
Group I - Control

Observations	Pretest	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Total
No observed abnormalities	4	2	3	1	2	4	3	2	2	23
Mucoid nasal discharge					1					1
Excessive salivation				2			1	1		4
Red vaginal discharge				1						1
Dilated pupils		2	1							3
Hair loss				1	1		1	1		4
Dry coat								1	2	3

Table 3 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Summary of Physical Observations
Group II - 10 mg/m^3

Observations	Pretest	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Total
No observed abnormalities	4	4	1	2	2	3	3	3	4	26
Excessive salivation			1	1			1	1		4
Excitable			1		1	1				3
Red sore			1							1
Rash				1	1					2

Table 3 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Summary of Physical Observations
Group III - 100 mg/m^3

Observations	Pretest	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Total
No observed abnormalities	4	4	1	3	4					16
Unable to stand or walk						1	1			2
Pale mucous membranes						1				1
Uncoordination			4*	4*	4*	4	4	4	4	16
Tremors (jaw)							1			1
Excessive salivation			1	1			2	2	1	7
Shakes all over when walking						1		1	1	3
Hyper-extension of rear legs						1		1	1	3
Opens and closes mouth						2	2			4
Inside of ears red			1							1
Discoloration of fur			1							1
Lack of tongue mobility						1				1
Constantly moving						1	1			2

*Observed and annotated by note to file from Study Director.

Table 4
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Body Weights - kilograms

Group mg/m^3	An.No.	Pretest 1	Pretest 2	Pretest 3	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
					Males							
I	4587	12.7	12.7	12.8	11.6	12.0	11.6	11.9	11.7	11.5	11.1	10.9
0	4585	12.4	13.0	12.9	12.7	12.3	11.8	11.8	11.5	11.6	11.2	10.9
	Mean	12.6	12.9	12.9	12.2	12.2	11.7	11.9	11.6	11.6	11.2	10.9
II	4592	11.5	11.6	11.8	10.3	10.8	10.4	11.0	10.5	10.9	10.7	10.8
10	4593	14.8	15.0	15.1	14.2	13.9	13.5	13.8	14.0	14.5	14.2	14.4
	Mean	13.2	13.3	13.5	12.3	12.4	12.0	12.4	12.3	12.7	12.5	12.6
III	4573	11.0	10.6	11.0	9.8	9.4	8.8	8.8	8.5	8.7	8.7	8.6
100	4568	12.8	12.6	13.2	12.1	11.3	10.9	11.2	10.6	10.6	10.4	10.4
	Mean	11.9	11.6	12.1	11.0	10.4	9.9	10.0	9.6	9.7	9.6	9.5

Table 4 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Body Weights - kilograms

Group mg/m^3	An.No.	Pretest 1	Pretest 2	Pretest 3	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
					Females							
I	4624	9.3	9.5	9.4	8.6	9.0	8.8	8.6	9.6	9.6	9.4	9.1
0	4626	9.8	9.6	9.6	9.1	9.4	8.8	9.1	9.3	9.8	9.7	9.5
	Mean	9.6	9.6	9.5	8.9	9.2	8.8	8.9	9.5	9.7	9.6	9.3
II	4621	11.2	11.4	11.4	10.4	10.4	10.0	9.7	10.1	10.2	10.2	9.8
10	4623	10.8	11.1	10.9	10.4	10.3	10.0	9.7	10.0	10.0	10.2	9.9
	Mean	11.0	11.3	11.2	10.4	10.4	10.0	9.7	10.1	10.1	10.2	9.9
III	4750	a	8.2	8.4	7.9	7.5	7.2	7.0	7.1	7.7	7.3	7.2
100	4622	9.2	9.6	9.4	9.0	8.5	8.5	8.3	8.5	8.5	8.5	8.6
	Mean	9.2	8.9	8.9	8.5	8.0	7.9	7.7	7.8	8.1	7.9	7.9

[a]Animal no. 4750 was taken from extra colony on 1/21/80 to replace animal no. 4626. Pretest 1 body weight for this animal was not taken.

Table 5
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Hematology Values
Pretest 1 - Males

Group mg/m^3	An.No.	HGB g%	HCT %	RBC $10^6/mm^3$	PLAT $10^5/mm^3$	WBC $10^3/mm^3$	Differential - % WBC					
							Neutrophils					
							Mon	Lym	Seg	Ban	Eos	Bas
I	4587	18.2	52	7.54	3.4	9.4	2	31	63	1	3	0
0	4585	17.7	50	7.08	2.7	10.2	2	29	67	1	1	0
	Mean	18.0	51	7.31	3.1	9.8		30	65			
II	4593	14.7	41	6.34	2.5	10.1	0	19	78	0	3	0
10	4592	18.6	52	7.48	2.5	14.3	3	20	74	0	3	0
	Mean	16.7	47	6.91	2.5	12.2		20	76			
III	4573	19.2	54	7.73	3.3	8.6	1	28	70	0	1	0
100	4568	17.4	48	7.44	2.5	10.7	0	23	70	1	6	0
	Mean	18.3	51	7.59	2.9	9.7		26	70			

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Hematology Values
Pretest 1 - Females

Group mg/m^3	An.No.	HGB g%	HCT %	RBC 10^6/mm^3	PLAT 10^5/mm^3	WBC 10^3/mm^3	Differential - % WBC					
							Neutrophils					
							Mon	Lym	Seg	Ban	Eos	Bas
I	4624	19.5	53	7.69	2.4	17.6	0	22	66	0	12	0
0	4626	19.4	53	7.50	4.3	11.1	0	27	63	0	10	0
	Mean	19.5	53	7.60	3.4	14.4		25	65			
II	4621	19.0	49	7.37	3.6	9.6	0	33	59	0	8	0
10	4623	18.8	51	7.51	4.1	14.0	0	37	57	1	5	0
	Mean	18.9	50	7.44	3.9	11.8		35	58			
III	4750	16.2	47	5.95	4.6	8.5	0	39	55	1	5	0
100	4622	20.5	59	8.19	3.1	8.0	0	26	65	0	9	0
	Mean	18.4	53	7.07	3.9	8.3		33	60			

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Hematology Values
Pretest 2 - Males

Group	An.No.	HGB	HCT	RBC	PLAT	WBC	Differential - % WBC					
							Neutrophils					
mg/m^3		g%	%	$10^6/mm^3$	$10^5/mm^3$	$10^3/mm^3$	Mon	Lym	Seg	Ban	Eos	Bas
I	4587	18.8	49	6.79	2.5	9.3	0	31	64	0	5	0
0	4585	19.6	50	6.85	3.3	9.2	0	72	22	0	6	0
	Mean	19.2	50	6.82	2.9	9.3		52	43			
II	4593	16.8	45	6.41	4.3	12.9	0	34	59	0	7	0
10	4592	20.4	51	7.28	2.1	13.0	0	15	83	0	2	0
	Mean	18.6	48	6.85	3.2	13.0		25	71			
III	4573	20.0	52	7.27	2.9	8.3	0	34	61	0	5	0
100	4568	18.3	48	7.05	2.8	9.8	1	34	57	0	8	0
	Mean	19.2	50	7.16	2.9	9.1		34	59			

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Hematology Values
Pretest 2 - Females

Group mg/m^3	An.No.	HGB g%	HCT %	RBC 10^6/mm^3	PLAT 10^5/mm^3	WBC 10^3/mm^3	Differential - % WBC					
							Neutrophils					
							Mon	Lym	Seg	Ban	Eos	Bas
I	4624	21.2	53	7.33	2.8	18.0	0	30	67	0	3	0
0	4626	19.8	52	7.05	4.3	10.7	0	26	66	0	8	0
	Mean	20.5	53	7.19	3.6	14.4		28	67			
II	4621	20.9	51	7.27	1.8	17.5	0	32	62	0	5	1
10	4623	20.3	54	7.26	3.3	12.8	0	28	65	0	4	0
	Mean	20.6	53	7.27	2.6	15.2		30	64			
III	4750	14.7	39	5.13	3.3	10.0	0	28	71	0	1	0
100	4622	21.7	56	7.66	3.9	8.9	0	35	53	0	12	0
	Mean	18.2	48	6.40	3.6	9.5		32	62			

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Hematology Values
Exposure Termination - Males

Group mg/m^3	An.No.	HGB g%	HCT %	RBC $10^6/mm^3$	PLAT $10^5/mm^3$	WBC $10^3/mm^3$	Differential - % WBC					
							Neutrophils					
							Mon	Lym	Seg	Ban	Eos	Bas
I	4587	17.1	48	6.45	3.13	9.9	1	25	71	0	3	0
0	4585	17.1	46	6.24	2.73	11.2	3	21	72	0	4	0
	Mean	17.1	47	6.35	2.93	10.6		23	72			
II	4592	17.6	49	6.60	2.28	12.4	5	21	69	0	5	0
10	4593	15.6	42	5.96	2.44	11.1	0	14	79	0	7	0
	Mean	16.6	46	6.28	2.36	11.8		18	74			
III	4568	15.7	43	6.30	4.10	12.4	3	18	73	0	6	0
100	4573	18.1	50	6.83	4.49	6.1	3	25	71	0	1	0
	Mean	16.9	47	6.57	4.30	9.3		22	72			

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Hematology Values
Exposure Termination - Females

Group mg/m^3	An.No.	HGB g%	HCT %	RBC 10^6/mm^3	PLAT 10^5/mm^3	WBC 10^3/mm^3	Differential - % WBC					
							Neutrophils					
							Mon	Lym	Seg	Ban	Eos	Bas
I	4624	19.4	54	7.11	3.42	12.1	4	15	78	0	3	0
0	4626	17.3	50	6.66	4.83	12.5	4	13	79	0	4	0
	Mean	18.4	52	6.89	4.13	12.3		14	79			
II	4621	16.3	45	6.03	3.94	8.4	2	21	67	0	10	0
10	4623	17.4	49	6.63	4.27	14.8	5	16	73	0	6	0
	Mean	16.9	47	6.33	4.11	11.6		19	70			
III	4622	19.7	55	7.42	2.83	7.5	0	15	82	0	3	0
100	4750	17.8	48	6.56	3.88	5.8	4	34	60	0	2	0
	Mean	18.8	52	6.99	3.36	6.7		25	71			

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Hematology Values
Exposure Recovery - Males

Group mg/m^3	An.No.	HGB g%	HCT %	RBC 10^6/mm^3	PLAT 10^5/mm^3	WBC 10^3/mm^3	Differential - % WBC					
							Neutrophils					
							Mon	Lym	Seg	Ban	Eos	Bas
I	4587	17.3	51	6.58	3.99	8.6	3	35	57	0	5	0
0	4585	17.3	51	6.36	3.18	8.7	0	22	70	1	7	0
	Mean	17.3	51	6.47	3.59	8.7		29	64			
II	4592	18.8	58	7.16	3.30	10.7	1	26	67	1	5	0
10	4593	16.0	49	6.36	3.89	8.2	0	30	63	0	7	0
	Mean	17.4	54	6.76	3.60	9.5		28	65			
III	4568	16.8	50	6.63	4.16	7.5	0	21	72	0	7	0
100	4573	17.2	52	6.55	4.29	7.0	1	25	72	0	2	0
	Mean	17.0	51	6.59	4.23	7.3		23	72			

Table 5 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Hematology Values
Exposure Recovery - Females

Group mg/m^3	An.No.	HGB g%	HCT %	RBC 10^6/mm^3	PLAT 10^5/mm^3	WBC 10^3/mm^3	Differential - % WBC					
							Neutrophils					
							Mon	Lym	Seg	Ban	Eos	Bas
I	4624	18.6	55	6.82	3.84	9.3	0	33	56	6	5	0
0	4626	18.3	56	6.91	5.10	9.0	0	30	62	1	7	0
	Mean	18.5	56	6.87	4.47	9.2		32	59			
II	4621	17.8	53	6.65	4.00	12.1	0	28	55	1	16	0
10	4623	19.8	61	7.57	4.39	8.7	0	37	61	1	1	0
	Mean	18.8	57	7.11	4.20	10.4		33	58			
III	4622	19.5	59	7.29	3.28	5.9	0	20	70	0	10	0
100	4750	18.6	56	6.99	4.07	9.8	0	38	48	0	14	0
	Mean	19.1	58	7.14	3.68	7.9		29	59			

Table 6
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Clinical Chemistry Values
Pretest 1 - Males

Group mg/m^3	An.No.	GLU mg/dl	BUN mg/dl	LDH IU	SGPT IU	ALK IU	CHOL mg/dl	T-BILI mg/dl	D-BILI mg/dl	Ca^{++} mg/dl	Na^+ mEq/l	K^+ mEq/l	T-PROT g/dl	ALB g/dl	GLOB g/dl	A/G
I	4587	96	14.8	29	18	60	137	0.26	0.11	10.5	148	4.5	6.5	3.2	3.3	1.0
0	4585	108	15.2	115	11	45	138	0.24	0.16	10.8	149	4.7	5.9	3.3	2.6	1.3
	Mean	102	15.0	72	15	53	138	0.25	0.14	10.7	149	4.6	6.2	3.3	3.0	1.2
II	4593	108	16.7	87	17	107	105	0.28	0.20	11.0	149	4.4	6.1	3.2	2.9	1.1
10	4592	98	15.6	43	19	60	103	0.28	0.17	10.8	149	4.3	6.2	3.2	3.0	1.1
	Mean	103	16.2	65	18	84	104	0.28	0.19	10.9	149	4.4	6.2	3.2	3.0	1.1
III	4573	104	14.5	25	21	95	142	0.29	0.06	11.4	157	4.9	6.7	3.7	3.0	1.2
100	4568	92	12.5	36	29	41	137	0.22	0.05	10.8	151	5.2	5.9	3.1	2.8	1.1
	Mean	98	13.5	31	25	68	140	0.26	0.06	11.1	154	5.1	6.3	3.4	2.9	1.2

Table 6 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Clinical Chemistry Values
Pretest 1 - Females

Group mg/m^3	An.No.	GLU mg/dl	BUN mg/dl	LDH IU	SGPT IU	ALK IU	CHOL mg/dl	T-BILI mg/dl	D-BILI mg/dl	Ca^{++} mg/dl	Na^+ mEq/l	K^+ mEq/l	T-PROT g/dl	ALB g/dl	GLOB g/dl	A/G
I	4624	84	7.3	87	15	36	165	0.35	0.21	10.9	155	4.5	6.0	3.2	2.8	1.1
0	4626	102	13.6	57	15	104	140	0.39	0.28	10.8	155	4.8	6.3	3.1	3.2	1.0
	Mean	93	10.5	72	15	70	153	0.37	0.25	10.9	155	4.7	6.2	3.2	3.0	1.1
II	4621	79	18.5	138	16	38	186	0.40	0.25	10.7	149	5.3	5.9	3.1	2.8	1.1
10	4623	72	12.1	162	20	35	81	0.29	0.14	11.3	151	5.2	6.1	3.3	2.8	1.2
	Mean	76	15.3	150	18	37	134	0.35	0.20	11.0	150	5.3	6.0	3.2	2.8	1.2
III	4750	103	12.5	73	13	148	184	0.19	0.10	13.6	152	4.8	6.3	3.5	2.8	1.3
100	4622	89	23.2	73	16	69	171	0.24	0.11	11.6	154	5.0	6.4	3.1	3.3	0.9
	Mean	96	17.9	73	15	109	178	0.22	0.11	12.6	153	4.9	6.4	3.3	3.1	1.1

Table 6 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Individual and Mean Clinical Chemistry Values
Pretest 2 - Males

Group mg/m^3	An.No.	GLU mg/dl	BUN mg/dl	LDH IU	SGPT IU	ALK IU	CHOL mg/dl	T-BILI mg/dl	D-BILI mg/dl	Ca^{++} mg/dl	Na^+ mEq/l	K^+ mEq/l	T-PROT g/dl	ALB g/dl	GLOB g/dl	A/G
I	4587	63	14.5	219	18	57	133	0.78	0.69	12.0	152	4.8	7.1	3.5	3.6	1.0
0	4585	85	12.3	125	14	54	132	0.37	0.29	14.6	153	4.5	7.2	4.7	2.5	1.9
	Mean	74	13.4	172	16	56	133	0.58	0.49	13.3	153	4.7	7.2	4.1	3.1	1.5
II	4592	56	15.6	213	22	57	118	1.33	1.73*	14.1	150	4.6	6.9	4.3	2.6	1.7
10	4593	72	14.7	297	16	103	118	0.46	0.39	13.1	149	4.7	6.8	3.6	3.2	1.1
	Mean	64	15.2	255	19	80	118	0.90	1.06	13.6	150	4.7	6.9	4.0	2.9	1.4
III	4568	86	14.2	105	27	42	163	0.49	0.42	14.6	147	5.3	6.7	4.2	2.5	1.7
100	4573	78	15.7	86	14	92	124	0.43	0.27	14.8	148	4.5	7.0	4.5	2.5	1.8
	Mean	82	15.0	96	21	67	144	0.46	0.35	14.7	148	4.9	6.9	4.4	2.5	1.8

*Direct bilirubin value is higher than total bilirubin due to hemolysis.

Table 6 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Clinical Chemistry Values
Pretest 2 - Females

Group mg/m^3	An.No.	GLU mg/dl	BUN mg/dl	LDH IU	SGPT IU	ALK IU	CHOL mg/dl	T-BILI mg/dl	D-BILI mg/dl	Ca^{++} mg/dl	Na^+ mEq/l	K^+ mEq/l	T-PROT g/dl	ALB g/dl	GLOB g/dl	A/G
I	4624	34	11.5	376	13	50	195	0.68	0.70*	12.9	148	6.4	3.7	2.7	2.7	1.4
0	4626	85	12.8	148	15	68	163	0.40	0.29	13.3	149	6.8	4.0	2.8	2.8	1.4
	Mean	60	12.2	262	14	59	179	0.54	0.50	13.1	149	6.6	3.9	2.8	2.8	1.4
II	4621	67	19.5	461	9	40	245	0.77	0.79*	13.3	146	5.1	6.3	3.8	2.5	1.5
10	4623	44	15.4	288	20	34	165	0.49	0.44	13.9	149	5.4	6.5	4.1	2.4	1.7
	Mean	56	17.5	375	15	37	205	0.63	0.62	13.6	148	5.3	6.4	4.0	2.5	1.6
III	4622	70	18.9	187	18	63	196	0.38	0.29	13.8	150	5.2	6.5	4.1	2.4	1.7
100	4750	82	13.0	377	18	148	172	0.61	0.66*	14.6	150	4.8	6.2	3.9	2.3	1.7
	Mean	76	16.0	282	18	106	184	0.50	0.48	14.2	150	5.0	6.4	4.0	2.4	1.7

*Direct bilirubin value is higher than total bilirubin due to hemolysis.

Table 6 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Individual and Mean Clinical Chemistry Values
Exposure Termination - Males

Group mg/m^3	An.No.	GLU mg/dl	BUN mg/dl	LDH IU	SGPT IU	ALK IU	CHOL mg/dl	T-BILI mg/dl	D-BILI mg/dl	Ca^{++} mg/dl	Na^+ mEq/l	K^+ mEq/l	T-PROT g/dl	ALB g/dl	GLOB g/dl	A/G
I	4587	109	14.4	46	21	102	187	0.38	0.26	11.4	148	4.3	7.5	3.7	3.8	1.0
0	4585	120	12.5	33	15	53	162	0.31	0.17	10.1	146	4.2	6.5	3.8	2.7	1.4
	Mean	115	13.5	40	18	78	175	0.35	0.22	10.8	147	4.3	7.0	3.8	3.3	1.2
II	4592	84	22.7	59	28	320	208	0.41	0.32	10.2	149	4.0	6.9	3.6	3.3	1.1
10	4593	112	15.6	36	16	158	213	0.31	0.22	10.6	145	4.0	7.8	4.1	3.7	1.1
	Mean	98	19.2	48	22	239	211	0.36	0.27	10.4	147	4.0	7.4	3.9	3.5	1.1
III	4568	102	19.0	61	18	296	299	0.43	0.27	10.6	145	4.6	7.6	4.1	3.5	1.2
100	4573	105	18.9	51	12	166	320	1.09	1.02	10.3	145	4.0	7.3	3.9	3.4	1.1
	Mean	104	19.0	56	15	231	310	0.76	0.65	10.5	145	4.3	7.5	4.0	3.5	1.2

Table 6 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Clinical Chemistry Values
Exposure Termination - Females

Group mg/m^3	An.No.	GLU mg/dl	BUN mg/dl	LDH IU	SGPT IU	ALK IU	CHOL mg/dl	T-BILI mg/dl	D-BILI mg/dl	Ca^{++} mg/dl	Na^+ mEq/l	K^+ mEq/l	T-PROT g/dl	ALB g/dl	GLOB g/dl	A/G
I	4624	88	19.6	58	15	47	353	0.32	0.17	10.2	145	4.2	7.3	3.6	3.7	1.0
0	4626	114	14.1	85	17	146	231	0.35	0.19	10.3	141	4.4	7.0	3.9	3.1	1.3
	Mean	101	16.9	72	16	97	292	0.34	0.18	10.3	143	4.3	7.2	3.8	3.4	1.2
II	4621	92	16.1	62	57	483	318	0.87	0.41	10.2	140	4.8	6.6	3.4	3.2	1.1
10	4623	79	12.4	44	21	108	291	0.27	0.14	10.7	144	4.6	7.4	4.1	3.3	1.2
	Mean	86	14.3	53	39	296	305	0.57	0.28	10.5	142	4.7	7.0	3.8	3.3	1.2
III	4622	85	28.9	58	14	195	429	0.47	0.33	10.4	145	4.5	7.4	4.1	3.3	1.2
100	7500	119	13.9	54	14	197	393	0.48	0.41	10.8	143	4.6	8.2	4.6	3.6	1.3
	Mean	102	21.4	56	14	196	411	0.48	0.37	10.6	144	4.6	7.8	4.4	3.5	1.3

Table 6 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Clinical Chemistry Values
Exposure Recovery - Males

Group mg/m^3	An.No.	GLU mg/dl	BUN mg/dl	LDH IU	SGPT IU	ALK IU	CHOL mg/dl	T-BILI mg/dl	D-BILI mg/dl	Ca^{++} mg/dl	Na^+ mEq/l	K^+ mEq/l	T-PROT g/dl	ALB g/dl	GLOB g/dl	A/G
I	4587	94	13.8	40	19	53	136	0.32	0.10	11.6	151	4.8	7.0	3.6	3.4	1.1
0	4585	126	11.4	44	13	35	126	0.35	0.20	12.2	145	4.7	6.5	3.8	2.7	1.4
	Mean	110	12.6	42	16	44	131	0.34	0.15	11.9	148	4.8	6.8	3.7	3.1	1.3
II	4592	105	14.8	45	43	140	137	0.42	0.23	11.9	147	4.4	6.9	3.5	3.4	1.0
10	4593	99	14.7	65	29	115	107	0.35	0.15	11.6	149	4.5	6.6	3.6	3.0	1.2
	Mean	102	14.8	55	36	128	122	0.39	0.19	11.8	148	4.5	6.8	3.6	3.2	1.1
III	4568	106	14.6	47	23	59	170	0.30	0.17	11.7	139	4.8	6.7	3.6	3.1	1.2
100	4573	114	15.6	66	22	89	161	0.37	0.24	11.6	145	3.9	6.9	4.0	2.9	1.4
	Mean	110	15.1	57	23	74	166	0.34	0.21	11.7	142	4.4	6.8	3.8	3.0	1.3

Table 6 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Clinical Chemistry Values
Exposure Recovery - Females

Group mg/m^3	An.No.	GLU mg/dl	BUN mg/dl	LDH IU	SGPT IU	ALK IU	CHOL mg/dl	T-BILI mg/dl	D-BILI mg/dl	Ca^{++} mg/dl	Na^+ mEq/l	K^+ mEq/l	T-PROT g/dl	ALB g/dl	GLOB g/dl	A/G
I	4624	83	11.9	210	15	39	241	0.44	0.27	12.2	143	4.4	6.8	3.7	3.1	1.2
0	4626	109	17.6	96	17	55	172	0.66	0.50	12.4	145	5.0	6.8	3.8	3.0	1.3
	Mean	96	14.8	153	16	47	207	0.55	0.39	12.3	144	4.7	6.8	3.8	3.1	1.3
II	4621	81	14.4	36*	62*	306*	178	0.61	0.37	11.0	139	4.3	6.1	3.2	2.9	1.1
10	4623	93	18.4	53	24	59	176	0.29	0.15	12.2	139	4.9	6.6	3.9	2.7	1.4
	Mean	87	16.4	53	24	59	177	0.45	0.26	11.6	139	4.6	6.4	3.6	2.8	1.3
III	4622	101	18.1	49	16	86	182	0.34	0.19	12.3	138	4.6	6.6	4.1	2.5	1.6
100	4750	102	11.6	35*	23	105*	161	0.29*	0.11*	12.7	144	5.1	7.3	4.5	2.8	1.6
	Mean	102	14.9	49	20	86	172	0.34	0.19	12.5	141	4.9	7.0	4.3	2.7	1.6

*Values for these parameters were reanalyzed from new samples drawn on 3-21-80, due to suspicious values obtained on 3-19-80. These reanalyzed samples were used for individual animals but not in mean calculations.

Table 7
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-/349

Individual and Mean Terminal Body Weights and Organ Weights

Group mg/m^3	An.No.	TBW (kg)	Brain Wt. (g)	Pituitary[a] Wt. (g)	Adrenal[a] Wt. (g)	Testes Wt. (g)	Thyroid[a] Wt. (g)	Kidney Wt. (g)	Heart Wt. (g)	Liver Wt. (g)
					Males					
I	4587	11.3	81.5	82	1490	17.3	820	67.4	102.9	304.8
0	4585	11.4	84.0	70	1310	14.3	850	76.3	106.1	323.0
	Mean	11.4	82.8	76	1400	15.8	835	71.9	104.5	313.9
II	4593	14.4	95.1	67	760	14.9	840	52.8	113.0	326.5
10	4623	10.8	89.3	70	1000	17.5	850	51.5	97.7	311.1
	Mean	12.6	92.2	69	880	16.2	845	52.2	105.4	318.8
III	4573	8.6	82.7	74	1140	14.8	480	61.8	87.0	329.0
100	4568	10.4	93.7	73	1200	14.1	870	67.2	102.0	398.3
	Mean	9.5	88.2	74	1170	14.5	675	64.5	94.5	363.7

[a]Weights multiplied by 10^3.

Table 7 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Terminal Body Weights and Organ Weights

Group mg/m^3	An.No.	TBW (kg)	Brain Wt. (g)	Pituitary[a] Wt. (g)	Adrenal[a] Wt. (g)	Ovaries[a] Wt. (g)	Thyroid[a] Wt. (g)	Kidney Wt. (g)	Heart Wt. (g)	Liver Wt. (g)
					Females					
I	4624	8.6	76.8	51	960	730	760	32.6	72.7	169.6
0	4626	9.7	80.5	70	930	1790	590	43.6	81.5	270.5
	Mean	9.2	78.7	61	945	1260	675	38.1	77.1	220.1
II	4621	9.9	69.9	66	1310	670	1030	38.8	86.5	232.8
10	4623	9.8	81.5	70	1150	760	630	47.0	80.5	280.0
	Mean	9.9	75.7	68	1230	715	830	42.9	83.5	256.4
III	4750	7.1	83.1	83	990	610	890	39.1	70.1	197.2
100	4622	8.2	70.0	48	1290	880	590	36.9	67.5	237.0
	Mean	7.7	76.6	66	1140	745	740	38.0	68.8	217.1

[a]Weights multiplied by 10^3.

Table 7 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Terminal Body Weights and Organ Weights Ratios - %

Group mg/m^3	An.No.	TBW (kg)	Brain Wt Ratio[a]	Pituitary Wt Ratio[a]	Adrenal Wt Ratio[a]	Testes Wt Ratio	Thyroid Wt Ratio[a]	Kidney Wt Ratio	Heart Wt Ratio	Liver Wt Ratio
					Males					
I	4587	11.3	0.721	0.726	13.186	0.153	7.2557	0.597	0.911	2.697
0	4585	11.4	0.737	0.614	11.491	0.125	7.4556	0.669	0.931	2.833
	Mean	11.4	0.729	0.670	12.339	0.139	7.3557	0.633	0.921	2.765
II	4593	14.4	0.660	0.465	5.278	0.103	5.8333	0.367	0.785	2.267
10	4592	10.8	0.827	0.648	9.259	0.162	7.8770	0.477	0.905	2.881
	Mean	12.6	0.744	0.557	7.269	0.133	6.8552	0.422	0.845	2.574
III	4573	8.6	0.962	0.860	13.256	0.172	5.5881	0.719	1.012	3.826
100	4568	10.4	0.901	0.702	11.538	0.136	8.3665	0.646	0.981	3.830
	Mean	9.5	0.932	0.781	12.397	0.154	6.9773	0.683	0.997	3.828

[a]Ratios multiplied by 10^3.

Table 7 (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

79-7349

Individual and Mean Terminal Body Weights and Organ Weights Ratios - %

Group mg/m^3	An.No.	TBW (kg)	Brain Wt Ratio[a]	Pituitary Wt Ratio[a]	Adrenal Wt Ratio[a]	Ovaries Wt Ratio	Thyroid Wt Ratio[a]	Kidney Wt Ratio	Heart Wt Ratio	Liver Wt Ratio
					Females					
I	4624	8.6	0.893	0.593	11.163	8.488	8.837	0.379	0.845	1.972
0	4626	9.7	0.830	0.722	9.588	18.454	6.082	0.449	0.840	2.789
	Mean	9.2	0.862	0.658	10.376	13.471	7.460	0.414	0.843	2.381
II	4621	9.9	0.706	0.667	13.232	6.768	10.404	0.392	0.874	2.352
10	4623	9.8	0.832	0.714	11.735	7.755	6.429	0.480	0.821	2.857
	Mean	9.9	0.769	0.691	12.484	7.262	8.417	0.436	0.848	2.605
III	4750	7.1	1.170	1.169	13.944	8.592	12.535	0.551	0.987	2.777
100	4622	8.2	0.854	0.585	15.732	10.732	7.195	0.450	0.823	2.890
	Mean	7.7	1.012	0.877	14.838	9.662	9.865	0.501	0.905	2.834

[a]Ratios multipled by 10^3.

Appendix A
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Methodology and References - General

Parameter	Reference or Description
Physical Examination:	Central Nervous System: examined - behavior movements, reactivity to stimuli, muscle tone. Autonomic Nervous System: examined- pupil size, secretions. Respiration: examined - nostrils, character, rate. Cardiovascular: examined - palpation. Gastrointestinal: examined - abdominal shape, feces consistency and color. Genitourinary: examined - penis, vulva, perineal region. Skin and Fur: examined - color, turgor, integrity. Mucous Membrane: examined - mouth, rectal, vaginal. Eye: examined - eyelids, eyeball, transparency. General Condition.
Neurological Examination:	The following reflexes were observed: righting reflex, standing, patellar, flexor, extensor, visual placing response, corneal and pupillary reflex.
Ophthalmoscopic Examination	Lids, lacrimal apparatus, conjunctiva examined grossly; cornea, anterior chamber, lens, vitreous humor, retina and optic disc examined by indirect ophthalmoscopy. Tropicamide (mydriacyl-Alcon) induced mydriasis.
Body Weight	Detecto scale (60-S) NV.
Organ Weights	Ohaus Triple Beam Balance, Model 700 - Liver. Mettler P162 - All other organs NV.

Appendix A (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Methodology and References - Hematology

Abbreviation	Parameter	Specimen	Reporting Units	Reference or Description
HGB	Hemoglobin	Whole Blood	g/dl	Laser Hematology Counter, ELT-8, Ortho Instruments.
			g%	Coulter Hemoglobinometer, Coulter Electronics, Inc.
HCT	Hematocrit	Whole Blood %	Percent	Laser Hematology Counter, ELT-8, Other Instruments.
			Percent	Directions for Use - Adams Mechanical Hematocrit Tube Reader. Clay Adams, Inc., 1960.
RBC	Erythrocytes	Whole Blood	$10^6/mm^3$	Laser Hematology Counter, ELT-8, Ortho Instruments,
			$10^6/mm^3$	Instruction Manual, Model Zf - Coulter Counter, Coulter Electronics, Inc.
PLAT	Platelets	Whole Blood	$10^5/mm^3$	Laser Hematology Counter, ELT-8, Ortho Instruments.
			$10^5/mm^3$	Unopette System. Becton-Dickinson, Division of Becton, Dickinson and Co.
WBC	Total Leukocytes	Whole Blood	$10^3/mm^3$	Laser Hematology Counter, ELT-8, Ortho Instruments.
			$10^3/mm^3$	Instruction Manual, Model Zf - Coulter Counter, Coulter Electronics, Inc.
	Differential Leukocytes and Erythrocyte Morphology	Whole Blood	% WBC	Schermer, S. The Blood Morphology of Laboratory Animals. 3rd Edition. Philadelphia: F.A. Davis Co., 1967.

Appendix A (cont.)
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Methodology and References - Clinical Chemistry

Abbreviation	Parameter	Specimen	Reporting Units	Reference or Description
GLU	Fasting Glucose	Serum	mg/dl	CentrifiChem System 400®; Hexokinase Method.
BUN	Blood Urea Nitrogen	Serum	mg/dl	CentrifiChem System 400®; Modified Urease Technique.
LDH	Lactic Dehydrogenase	Serum	IU	CentrifiChem System 400®; Lactate-pyruvate Technique.
SGPT	Serum Glutamic Pyruvic Transaminase	Serum	IU	CentrifiChem System 400®; Modified Wroblewski; and LaDue Technique.
ALK	Alkaline Phosphatase	Serum	IU	CentrifiChem System 400®; Modified Bessey-Lowry-Brock Technique.
CHOL	Cholesterol (Enzymatic)	Serum	mg/dl	CentrifiChem System 400®; Cholesterol esterase-cholesterol oxidase Method.
T BILI	Total Bilirubin	Serum	mg/dl	CentrifiChem System 400®; Modified Jendrassik and Nosslin Method.
D BILI	Direct Bilirubin	Serum	mg/dl	CentrifiChem System 400®; Modified Jendrassik and Nosslin Method.
Ca^{++}	Calcium	Serum	mg/dl	CentrifiChem System 400®; Alizarin Method.
Na^{+}	Sodium	Serum	mEq/l	Clinical Flame Photometer, Model 243. Instrumentation Laboratories, Inc.
K^{+}	Potassium	Serum	mEq/l	Clinical Flame Photometer, Model 243. Instrumentation Laboratories, Inc.
T PROT	Total Protein	Serum	g/dl	CentrifiChem System 400®; Biuret Technique.
ALB	Albumin	Serum	g/dl	CentrifiChem System 400®; Bromocresol Green Method.
GLOB	Globulin	Serum	g/dl	CentrifiChem System 400®; Total protein-albumin = globulin.

Appendix B

DETERMINATION OF TRIPHENYL PHOSPHINE (TPP) ON GLASS FIBER FILTERS FOR STUDY NO. 79-7349

Report No.: 79081

Sponsor: M & T Chemicals
Rahway, New Jersey 07065

Prepared by: Benjamin J. Gulizia Date: December 22, 1980

Issued by: Bio/dynamics Inc.
Department of Metabolism and Analytical Chemistry
Box 43, Mettlers Road
East Millstone, New Jersey 08873

SUMMARY

During a four-week inhalation toxicity study in dogs, glass fiber filters were used to trap chamber air samples containing Triphenyl Phosphine. Atmospheric concentrations were determined by desorption of the test material from the filters with acetonitrile and subsequent injection of the extracts on an HPLC, utilizing a uBondapak column. At least three samples were drawn from each exposure chamber each day, five days a week. Assays of the samples demonstrated mean chamber concentrations of 19.3 and 110.9 ug/l of air for Groups II and III, respectively.

The distribution of Triphenyl Phosphine in the chamber air was also determined prior to the start of the study. Assays of the samples demonstrated a mean chamber concentration of 21.3 and 66.8 ug/l of air for Groups II and III, respectively.

Benjamin J. Gulizia 12/22/80
Benjamin J. Gulizia
Manager, Analytical Service

Joseph S. O'Grodnick 12-12-80
Joseph S. O'Grodnick, Ph.D.
Director
Department of Metabolism and
Analytical Chemistry

1.0 INTRODUCTION

An inhalation toxicity study with dogs was conducted by the Inhalation Toxicology Department of Bio/dynamics Inc., using Triphenyl Phosphine as the test material. One group of dogs was exposed to an atmosphere containing approximately 10 ug/l of air of the test material and another group to an atmosphere containing approximately 100 ug/l of air of the test material for 6 hours/day, 5 days/week for 4 weeks. The purpose of this study was to determine daily chamber concentrations.

2.0 EXPERIMENTAL

2.1 Test Material

The test material was Triphenyl Phosphine (TPP) supplied by M & T Chemicals.

2.2 Sampling

Glass fiber filter papers were used to sample chamber air. At least three samples were drawn from each exposure chamber. The flow of air was controlled by a vacuum pump. The total volume of air pulled through the filters was recorded and used in the calculations.

Samples were forwarded to the Department of Metabolism and Analytical Chemistry in glass scintillation vials for analysis. The test material was desorbed from the glass fiber filter papers with a minimum of 1 ml of acetonitrile. Further dilutions of the samples were made, when necessary, for injection into the HPLC.

2.3 Desorption Efficiency

Desorption efficiency was determined by fortifying glass fiber filter papers with a known amount of Triphenyl Phosphine, desorbing the test material from the filter papers and subsequent analysis of the extract on an HPLC. The results of the assays demonstrated a mean recovery of 95%. Complete analytical results are listed in Table I.

2.4 High Pressure Liquid Chromatographic Analysis

A standard curve was generated by injecting known (varying) amounts of Triphenyl Phosphine. Standards were plotted as the peak area of Triphenyl Phosphine vs. ng of the test material injected. Sample amounts were determined by comparing peak areas of samples with the standard curve. Atmospheric concentrations (ug of test material per liter of air) are given by the following equation:

$$ug/l = \left[\frac{\frac{(ng\ found)}{(ul\ inj.)} \times \frac{10^3\ ul}{ml} \times ml\ final\ volume \times \frac{ug}{10^3\ ng}}{Total\ Air\ (l)}\right]$$

HPLC conditions are listed in Table II.

3.0 RESULTS AND CONCLUSIONS

Assays for Triphenyl Phosphine ranged from 2.7 to 58.0 ug/l of air with a mean chamber concentration of 19.3 ug/l of air for Group II, and from 0.6 to 852 ug/l air with a mean chamber concentration of 110.9 ug/l of air for Group III. As can be readily seen significant variation in analytical results occurred during the study. Complete results of daily analyses are listed in Table III.

79081

A chamber air distribution study of Triphenyl Phosphine was designed to determine if the test material would be equally diffused through the chamber air. Values ranged from 12.1 - 18.0 ug/l of air with an average of 14.4 ug/l of air for Group II and from 50.5 - 78.0 ug/l of air with an average of 66.8 ug/l of air for Group III. Complete results are listed in Table IV.

In addition, trial chamber samples were analyzed prior to and during the course of the study. Complete results are listed in Appendix A, Table I.

79081

TABLE I. FORTIFICATIONS OF TRIPHENYL PHOSPHINE (TPP) ON FIBER FILTER PAPERS FOR STUDIES NO. 79-7330 AND 79-7349

Date	Fortification Level (ng)	ng Found	% Recovery
1/15/80[a]	200000	150000	75
	200000	150000	75
	200000	150000	75
1/15/80	875000	875000	100
	875000	887500	101
	875000	887500	101
1/16/80	200000	175000	88
	200000	175000	88
	200000	162500	81
1/28/80	200000	197500	99
	200000	202500	101
			$\bar{x}$ = 95

a. Not included in mean.

TABLE II. TYPICAL HPLC CONDITIONS FOR TRIPHENYL PHOSPHINE (TPP)

Instrument:	Tracor 960 or equivalent
Column:	uBondapak, 30 cm x 3.9 mm
Precolumn:	Copell ODS
Mobil Phase:	100% ACN
Rate:	1.5 ml/minute
Reservoir Temperature:	25°C
Wavelength:	254 nm

Note: These parameters may be varied to achieve optimum performance.

TABLE III. DETERMINATION OF TRIPHENYL PHOSPHINE (TPP) ON FIBER FILTER PAPERS FOR STUDY NO. 79-7349

Dose Group	Sample	Test Day	Date Sampled	Date Analyzed	ug/l Found
II	136	2	1/29/80	1/29/80	< 0.08
	137				< 0.08
	137-D				< 0.08
	138				< 0.08
III	139	2	1/29/80	1/29/80	38.50
	140				38.00
	141				51.00
II	148	3	1/30/80	1/30/80	5.20
	149				3.90
	150				19.00
III	151	3	1/30/80	1/30/80	< 0.50
	152				< 0.50
	153				< 0.50
II	160	4	1/31/80	1/31/80	24.00
	$161\text{-}D_2$				22.50
	$162\text{-}D_2$				132.00
III	163	4	1/31/80	1/31/80	26.60
	164				26.20
	165				79.00
II	172	5	2/1/80	2/1/80	23.20
	173				2.70
	174				10.20
III	175	5	2/1/80	2/1/80	0.60
	176-D				9.80
	177				37.00
II	184	6	2/2/80	2/3/80	15.50
	185				13.80
	186				38.00
III	187	6	2/2/80	2/3/80	31.00
	188				37.20
	189				245.00
II	196	8	2/4/80	2/4/80	58.00
	196D-1				52.20
	197				40.20
	198				13.30
III	199	8	2/4/80	2/4/80	126.00
	200				33.00
	200-D				17.00
	201				35.40

TABLE III. DETERMINATION OF TRIPHENYL PHOSPHINE (TPP) ON FIBER FILTER PAPERS FOR STUDY NO. 79-7349 - continued

Dose Group	Sample	Test Day	Date Sampled	Date Analyzed	ug/l Found
II	208	9	2/5/80	2/5/80	12.50
	209				41.30
	210				40.00
III	211	9	2/5/80	2/5/80	25.00
	211-D				91.50
	212				93.00
	213				72.00
II	208-A	10	2/6/80	2/6/80	26.50
	209-A				23.70
	210-A				26.50
III	211-A	10	2/6/80	2/6/80	62.00
	212-A				142.00
	213-A				186.00
II	220	11	2/7/80	2/11/80	15.00
	221				9.00
	222				8.70
III	223	11	2/7/80	2/11/80	81.00
	224				42.00
	225				48.00
II	235	12	2/8/80	2/11/80	20.20
	236				25.00
	237				3.00
III	238	12	2/8/80	2/11/80	160.80
	239				133.20
	240				68.00
II	247	15	2/11/80	2/12/80	23.80
	248				29.50
	249				22.50
III	250	15	2/11/80	2/12/80	<0.50
	251				0.60
	252				136.80
	253				123.00
	254				147.00
	255				130.80
II	265	16	2/12/80	2/12/80	35.50
	266				32.00
	267				26.20
III	268	16	2/12/80	2/12/80	121.20
	269				72.00
	270				64.00

79081

TABLE III. DETERMINATION OF TRIPHENYL PHOSPHINE (TPP) ON FIBER FILTER PAPERS FOR STUDY NO. 79-7349 - continued

Dose Group	Sample	Test Day	Date Sampled	Date Analyzed	ug/l Found
II	277	17	2/13/80	2/14/80	14.30
	278				30.00
	279				14.50
III	280	17	2/13/80	2/14/80	96.00
	281				5.70
	281-D				18.90
	281-D_2				141.00
	282				126.00
II	289	18	2/14/80	2/15/80	26.50
	290				26.00
	291				21.70
III	292	18	2/14/80	2/15/80	5.60
	292-D				18.90
	293				1.90
	293-D				5.50
	294				75.00
II	317	19	2/15/80	2/18/80	16.50
	318				10.80
	319				18.00
III	320	19	2/15/80	2/18/80	207.00
	320-D				94.00
	321				86.00
	322				162.00
II	329	22	2/18/80	2/19/80	22.30
	330				22.30
	331				19.00
III	332	22	2/18/80	2/19/80	212.50
	332-D				210.00
	332-D_2				238.00
	333				180.00
	333-D				177.50
	333-D_2				201.00
	334				186.00
	334-D				265.20
	334-D_2				177.50

79081

TABLE III. DETERMINATION OF TRIPHENYL PHOSPHINE (TPP) ON FIBER FILTER PAPERS FOR STUDY NO. 79-7349 - continued

Group	Sample	Test Day	Date Sampled	Date Analyzed	ug/l Found
II	341	23	2/19/80	2/19/80	6.50
	342				10.20
	343				7.30
III	344	23	2/19/80	2/19/80	78.00
	344-D				63.00
	344-D_2				75.00
	345				73.00
	345-D				61.00
	345-D_2				59.00
	346				64.00
	346-D				55.00
II	353	24	2/20/80	2/21/80	8.70
	354				7.50
	355				9.00
III	356	24	2/20/80	2/21/80	60.00
	356-D				58.00
	356-D_2				63.00
	357				42.00
	357-D				165.00
	357-D_2				114.00
	358				177.00
	358-D				96.00
	358-D_2				94.80
II	365	25	2/21/80	2/22/80	14.00
	366				9.80
	367				9.50
III	368	25	2/21/80	2/22/80	105.00
	368-D				852.00
	369			2/26/80	162.00
	369-D				105.00
	369-D_2			2/22/80	183.00
	370				138.00
	370-D				147.00
	370-D_2				129.00
II	377	26	2/22/80	2/25/80	7.00
	378				5.30
	379				2.80
III	380	26	2/22/80	2/25/80	105.00
	380-D				108.00
	380-D_2				97.20
	381				69.00
	381-D				22.50
	381-D_2				70.00
	382				39.00
	382-D				57.00
	382-D_2				416.00

79081

TABLE IV. CHAMBER AIR DISTRIBUTION OF TRIPHENYL PHOSPHINE (TPP) ON FIBER FILTER PAPERS FOR STUDY NO. 79-7349

Dose Group	Sample	Date Sampled	Date Analyzed	ug/l Found
II	H-108	1/25/80	1/27/80	10.6
	H-109			12.1
	H-110			13.3
	H-111			15.6
	H-112			15.4
	H-113			18.0
	H-114			15.6
III	H-115	1/25/80	1/27/80	78.0
	H-116			56.3
	H-117			73.8
	H-118			77.0
	H-119			68.0
	H-120			50.5
	H-121			63.8

APPENDIX A

79081

TABLE I. DETERMINATION OF TRIPHENYL PHOSPHINE (TPP) ON FIBER FILTER PAPERS (TRIAL SAMPLES) FOR STUDIES NO. 79-7330 AND 79-7349

Sample Identification No.		Date Sampled	Date Analyzed	ug/l Found
	A	11/12/79	12/10/79	155
	B[a]			170
	C[a]			-
	D			-
	E			41
	F			52
	G			44
	A	11/13/79	12/10/79	10
	B			11
	C			10
	A[b]	11/12/79	12/11/79	75
	B[b]			89
	C[b]		12/12/79	2
	D[b]		12/11/79	11
	E[b]		12/12/79	36
	F[b]			42
	G[b]		12/11/79	29
	A[b]	11/13/79	12/11/79	8
	B[b]			8
	C[b]		12/12/79	9
	A	12/27/79	12/28/79	57
	C			71
	G			77
III	N	1/2/80	1/21/80	50
	Q			53.8
	R			71.9
II	C	1/3/80	1/21/80	5.6
	D			6.9
	F			8.1
	11	1/8/80		2.7
	12			19.8
	13			7
	14			9.3
	21		1/22/80	8.2
	22			9.2
	26			9.3
	27			9.8

a. Samples not within standard curve range, therefore, no values could be obtained.
b. Re-assay of samples analyzed on 12/10/79.

APPENDIX A

79081

TABLE I. DETERMINATION OF TRIPHENYL PHOSPHINE (TPP) ON FIBER FILTER PAPERS (TRIAL SAMPLES) FOR STUDIES NO. 79-7330 AND 79-7349 - continued

	Sample Identification No.	Date Sampled	Date Analyzed	ug/l Found
III	40	1/9/80	1/22/80	127.5
	41			101.3
	42			59.3
	43			105
	44			95.5
	45			58.5
	46			76.3
	47			78.3
	48			66.3
79-7349				
II	301	2/15/80	2/16/80	22.0
	302			17.3
	303			< 3.3
	304			18.7
III	305	2/15/80	2/16/80	147.0
	306			165.0
	307			90.0
	308			99.0
III	389	2/27/80	2/28/80	90.0
	$389\text{-}D_1$			116.0
	$389\text{-}D_2$			73.0
	390			96.0
	$390\text{-}D_1$			66.0
	$390\text{-}D_2$			72.0
	391			52.0
	$391\text{-}D_1$			84.0
	$391\text{-}D_2$			64.0
	392			68.0
	$392\text{-}D_1$			72.0
	$392\text{-}D_2$			58.0
	393			58.0
	$393\text{-}D_1$			72.0
	$393\text{-}D_2$			66.0
III	409	2/28/80	2/28/80	6.9
	410			64.0
	$411\text{-}D_1$			59.0
	412			73.0
	413			74.0
	414			80.0
	421			94.0

Appendix C

M-11, 79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Terminal Sacrifice

79-7349

A Four Week Inhalation Toxicity Study of Triphenyl Phosphine in Dogs Pathology Report

TABLE OF CONTENTS

INTRODUCTION

MATERIALS AND METHODS
- Test Material
- Animals
- Test Material Administration
- Experimental Design
- Gross Postmortem Procedures
- Tissues Fixed
- Microscopic Methods

RESULTS AND DISCUSSION

SUMMARY

TABLE I
- Gross Postmortem Observations

TABLE II
- Microscopic Findings in Individual Animals

TABLE III
- Summary-Incidence of Microscopic Findings in Individual Animals

79-7349

A Four Week Inhalation Toxicity Study of Triphenyl Phosphine in Dogs Pathology Report

INTRODUCTION

This study was designed to evaluate the effects produced in dogs by daily inhalation exposure to Triphenyl Phosphine for four weeks.

MATERIALS AND METHODS

Test Material: Triphenyl Phosphine

Animals: 12 Marshall Research Beagle dogs* (6 males, 6 females), minimum age of 6 months, were randomly distributed into 3 groups (2/sex/group).

Test Material Administration: Inhalation

Randomly selected groups of 2 males and 2 females were exposed 6 hours/day, 5 days/weeks for 4 weeks to an aerosol of Triphenyl Phosphine. A similar control group were exposed to the vehicle only. Details regarding exposure procedures are presented in the antemortem report.

Experimental Design:

Group	Dose Concentration	Exposure Time	Number of Animals 1 Exposed	Necropsy	Histopathology
I	None (Chamber Control)	6 Hr/Day 5 Day/Wk 4 Weeks	4	4	4
II	10mg/m^3	"	4	4	4
III	100mg/m^3	"	4	4	4
		TOTALS	12	12	12

* Marshall Research Animals, Inc., North Rose, New York.
1 Equally divided male and female.
2 Scheduled sacrifices were performed 4 weeks after the last exposure date.

Gross Postmortem Procedures:

All animals survived the study and were sacrificed by exsanguination under sodium pentobarbitol anesthesia. The gross postmortem observations included examination of the external surface, all orifices, the cranial cavity,

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

MATERIALS AND METHODS (cont.)

Gross Postmortem Procedures: (cont.)

carcass, the external and cut surface of the brain and spinal cord, the thoracic, abdominal and pelvic cavities and their viscera, the cervical tissues and organs. Organs selected for weighing include the adrenals, brain, gonads, heart, kidneys, liver, pituitary, and thyroid (with parathyroid). All tissues were preserved in 10% neutral buffered formalin.

Tissues Fixed:

The following tissues, when present, were preserved for all animals:

abdominal aorta
adrenals (2)
bone and bone marrow (sternum)
brain (2 sections)
esophagus
eyes (2) with contiguous optic nerve
gallbladder
gonads
ovaries
testes with epididymides
heart (with coronary vessels)
intestine
colon
duodenum
ileum
kidneys (2)
liver (2 sections)
lungs[1] (2 sections, at least 2 lobes and mainstem bronchi)
lymph nodes (peribronchial and mesenteric)
mammary gland (right inguinal)
pancreas
pituitary
prostate
major salivary glands
sciatic nerve with muscle (both right and left, 3 sections each)
skin (inguinal, taken with mammary gland)
spinal cord (cervical and lumbar, 3 sections each)
spleen
stomach (2 sections)
thymus
trachea
thyroid/parathyroid
urinary bladder[3]
uterus (corpus and cervix uteri)
gross lesions (to include a border of normal tissue)
tissue masses or suspect tumors and regional lymph nodes

---

[1] Lungs were infused via the trachea with an amount of formalin approximately equal to 75% of the lung capacity.

[3] Urinary bladders were opened and examined prior to formalin fixation.

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

MATERIALS AND METHODS: (cont.)

Microscopic Methods:

Selected peripheral and central nervous system tissues, as listed below, were prepared for microscopic examination as follows:

After proper fixation selected tissues were trimmed, processed in the duo-autotechnicon, paraffin embedded, duplicate sets cut at 6u in the microtome, and placed on a slide. A complete set of all tissues was prepared with Hematoxylin and Eosin stain and a duplicate set, with the exception of the brain, was prepared with Luxol fast blue stain (counterstained with cresyl violet).

TISSUE	SECTIONS	H&E 1	L.F.B 2
Sciatic Nerve (R&L)	1 longitudinal section (on each)	X	X
	2 transverse sections (on each)	X	X
Cervical Spinal Cord	1 longitudinal section	X	X
	2 transverse sections	X	X
Lumbar Spinal Cord	1 longitudinal section	X	X
	2 transverse sections	X	X
Brain	2 transverse sections	X	

1 = hematoxylin and eosin stain
2 = Luxol fast blue stain
(R&L) = right and left

RESULTS AND DISCUSSION:

A tabulation of the gross postmortem observations is presented in Table I; Table II lists the microscopic findings in individual animals and Table III summarizes the incidence of microscopic findings by group and sex.

There was no pattern of gross lesions present which could have been interpreted as being associated with the exposure of the animals to the test material.

The examination of selected control and peripheral nervous tissue, how-

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

RESULTS AND DISCUSSION: (cont.)

ever, reveals a definite test material related abnormality as demonstrated in Tables II and III. The lesion consisted of patchy to segmental degeneration of myelin in the white tracts of the brain and spinal cord with no apparent abnormality in the peripheral tissue examined. The ventral and lateral white tracts of the cervical and lumbar spinal cords were the segmental areas predominantely affected by the test material; apparently there was a sparing effect of the dorsal spinal white tracts. There was a remarkable inconsistency of the degree of severity in Group II and III animals. The Group II animals displayed a mild to moderate degree of severity and all the Group III animals exhibited quite a severe reaction. In the Group II animals there was little glial reaction to the lesion, whereas, in Group III animals a moderate astrocytosis was present. The pattern of the lesion, dose response, and relative lack of glial response suggests a toxic etiology.

Special silver stains such as the Bodian stain would demonstrate abnormalities of the axon. Any such abnormalities in hematoxylin and eosin and Luxol fast blue are difficult to interpret, but the severity of the myelin degeneration is such that it is probable that the axons were affected adversely.

79-7349

A Four Week Inhalation Toxicity Study of Triphenyl Phosphine in Dogs Pathology Report

SUMMARY:

Six male and six female Beagle dogs were randomly distributed into three experimental groups (2/sex/group). Group I animals, controls, were exposed to an aerosol of the vehicle (xylene) at a level approximately equal to the level of vehicle in Group III. Groups II and III were exposed to an aerosol of Triphenyl Phosphine in inhalation chambers at levels of 10mg/m^3 and 100mg/m^3 respectively.

Four weeks post exposure the animals were sacrificed, a complete postmortem examination performed and selected central and peripheral nervous tissues chosen for microscopic examination.

No gross lesions related to the exposure of the animals to the test material were observed. Microscopic examination revealed test material related lesions in the central nervous system apparently sparing the peripheral nervous system. The lesions consisted of a mild to severe segmental myelin degeneration primarily affecting the ventral and lateral spinal white tracts of the lumbar spinal cords.

Fred A. Hines, D.V.M. 7/16/80

Fred A. Hines, D.V.M.
Pathologist

Table I

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Gross Postmortem Observaitons
Terminal Sacrifice

Table I

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

GENERAL KEY TO GROSS POSTMORTEM OBSERVATIONS

N.O.A.	=	No observable abnormalities
R	=	Right
L	=	Left
cm	=	centimeter
diam.	=	diameter
approx.	=	approximately

Table I
(cont.)

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Group	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
I	M	4585	80-2340	Ileum/Ileo-Colic Junction	All mucosa: slightly reddened.
				Brain	Cystic capsule in area of pituitary (optic chiasma)(0.3cm).
		4587	80-2341	Spleen	Raised, dark red area along head portion (3.0x0.5cm).
				Ileo-Colic Junction	Slight reddening of mucosa.
	F	4624	80-2342	Lungs	L median lobe: darkened areas on dorsal surface (approx. 0.5 cm in diam.); R posterior lobe: raised firm nodule on dorsal surface (approx. 1.0cm in diam.).
				Ileo-Colic Junction	Slight reddening of mucosa.
		4626	80-2343	Lungs	L posterior lobe: firm, red, non-elevated area on dorsal surface (1.0cm in diam.); L anterior lobe: irregular, tan, slightly elevated area; R mid and R anterior lobes: tan nodules scattered throughout (0.3cm in diam.); irregular tan area of R anterior lobe (0.3cm in diam.).
				Spleen	Large, raised, tan area (4.0x 0.3cm) along edge.
				Urinary Bladder	Trigone and proximal urethra slightly reddened.

Table I
(cont.)

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Group	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
II	M	4592	80-2344	-	N.O.A.
		4593	80-2345	Lungs	L median lobe: palpable nodule embedded deep in parenchyma (0.5cm).
	F	4621	80-2346	Lungs	L posterior lobe: red-brown area on dorsal surface (0.5cm).
				Trigone with Proximal Urethra	Reddened mucosa.
				Cecal Ridge	Mucosa: reddened.
		4623	80-2347	Lungs	R anterior lobe: aggregation of embedded, light tan nodules (0.2 to 0.3cm in diam.).
				Spleen	All surfaces: irregular.
				Urinary Bladder	Urethra: slightly reddened.
				Ileo-Colic Junction	Slightly reddened.

Table I
(cont.)

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Group	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
III	M	4568	80-2348	Spleen	Darkened, raised areas at head portion.
		4573	80-2349	Spleen	Apparent scar tissue at head region.
				Pyloric Junction	Mucosa: reddened area (approx. 0.8cm in diam.).
				Ileo-Colic Junction	Mucosa: slightly reddened.
				Nose	Scab on bridge of nose.
	F	4622	80-2350	Ileo-Colic Junction	Slightly reddened.
		4750	80-2351	Lungs	All lobes: multiple irregular red areas; R anterior lobe: non-elevated reddened area on dorsal surface (4.5x2.0cm).
				Spleen	Dark red, raised areas along edge of head portion.
				Peribronchiolar Lymph Nodes	Enlarged, reddened.
				Ileo-Colic Junction	Mucosa: slightly reddened.
				Proximal Urethra	Mucosa surface: slightly reddened.

Table II

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Microscopic Findings in Individual Animals
Terminal Sacrifice

Table II

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

GENERAL KEY TO HISTOPATHOLOGY TABLES

N	=	Not remarkable, within normal histologic limits
1	=	Minimal degree or amount of indicated change
2	=	Mild degree or amount of indicated change
3	=	Moderate degree or amount of indicated change
4	=	Marked degree or amount of indicated change
X	=	Indicated change present (not quantifiable)
*	=	Tissue not present or available
°	=	One of paired organs
U	=	Tissue insufficient
A	=	Autolysis precluded evaluation
H & E	=	Hematoxylin and eosin stain
L.F.B.	=	Luxol fast blue stain

Table II

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Microscopic Findings in Individual Animals
Terminal Sacrifice
Hematoxylin and Eosin

Animal Number	4 5 8 5	4 5 8 7	4 6 2 4	4 6 2 6	4 5 9 2	4 5 9 3	4 6 2 1	4 6 2 3	4 5 6 8	4 5 7 3	4 6 2 2	4 7 5 0
Sex	M	M	F	F	M	M	F	F	M	M	F	F
Pathology Number 80-	2 3 4 0	2 3 4 1	2 3 4 2	2 3 4 3	2 3 4 4	2 3 4 5	2 3 4 6	2 3 4 7	2 3 4 8	2 3 4 9	2 3 5 0	2 3 5 1
Group			I				II				III	
BRAIN												
-1st transverse section degeneration, myelin, patchy, characterized by a "status spongiosum" and swollen clear macrophages containing wisps of fibrillar eosinophilic material	N	N	N	N	1	N	N	N	2	2	2	2
-2nd transverse section degeneration, myelin, patchy, characterized by a "status spongiosum" and swollen clear macrophages containing wisps of fibrillar eosinophilic material	N	N	N	N	N	N	N	N	2	2	2	2
SCIATIC NERVE												
-Right transverse sections	N	N	N	N	N	N	N	N	*	N	N	N
-Right longitudinal section	N	N	N	N	N	N	*	N	*	N	N	N
-Left transverse sections	N	*	N	N	N	N	*	N	*	N	N	N
-Left longitudinal section	N	*	N	N	N	N	*	N	*	N	N	N
CERVICAL SPINAL CORD												
-longitudinal section degeneration, myelin, segmental, characterized by a "status spongiosum" and swollen clear macrophages containing wisps of fibrillar eosinophilic material	N	N	N	N	3	2	3	2	4	4	4	4
-transverse section degeneration, myelin, segmental, characterized by a "status spongiosum" and swollen clear macrophages containing wisps of fibrillar eosinophilic material	N	N	N	N	3	2	3	1	4	4	4	4

Table II
(cont.)

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Microscopic Findings in Individual Animals
Terminal Sacrifice
Luxol Fast Blue

Animal Number	4 5 8 5	4 5 8 7	4 6 2 4	4 6 2 6	4 5 9 2	4 5 9 3	4 6 2 1	4 6 2 3	4 5 6 8	4 5 7 3	4 6 2 2	4 7 5 0
Sex	M	M	F	F	M	M	F	F	M	M	F	F
Pathology Number 80-	2 3 4 0	2 3 4 1	2 3 4 2	2 3 4 3	2 3 4 4	2 3 4 5	2 3 4 6	2 3 4 7	2 3 4 8	2 3 4 9	2 3 5 0	2 3 5 1
Group	I				II				III			
SCIATIC NERVE												
-Right transverse sections	N	N	N	N	N	N	N	N	*	N	N	N
-Right longtitudinal section	N	N	N	N	N	N	N	N	*	N	N	N
-Left transverse sections	N	*	N	N	N	N	N	N	*	N	N	N
-Left longitudinal section	N	*	N	N	N	N	N	N	*	N	N	N
CERVICAL SPINAL CORD												
-longitudinal sections degeneration, myelin, segmental, characterized by a "status spongiosum" and swollen clear macrophages containing wisps of an L.F.B. positive material	N	N	N	N	3	2	2	2	4	4	4	4
-transverse section degeneration, myelin, segmental, characterized by a "status spongiosum" and swollen clear macrophages containing wisps of an L.F.B. positive material	N	N	N	N	3	2	2	2	4	4	4	4
LUMBAR SPINAL CORD												
-longitudinal section degeneration, myelin, segmental, characterized by a "status spongiosum" and swollen clear macrophages containing wisps of an L.F.B. positive material	N	N	N	N	3	2	2	2	4	4	4	*

Table II
(cont.)

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Microscopic Findings in Individual Animals
Terminal Sacrifice
Hematoxylin and Eosin

Animal Number	4 5 8 5	4 5 8 7	4 6 2 4	4 6 2 6	4 5 9 2	4 5 9 3	4 6 2 1	4 6 2 3	4 5 6 8	4 5 7 3	4 6 2 2	4 7 5 0
Sex	M	M	F	F	M	M	F	F	M	M	F	F
Pathology Number 80-	2 3 4 0	2 3 4 1	2 3 4 2	2 3 4 3	2 3 4 4	2 3 4 5	2 3 4 6	2 3 4 7	2 3 4 8	2 3 4 9	2 3 5 0	2 3 5 1
Group		I				II				III		
LUMBAR SPINAL CORD												
-longitudinal section	N	N	N	N								
degeneration, myelin, segmental, characterized by a "status spongiosum" and swollen clear macrophages containing wisps of fibrillar eosinophilic material					3	2	3	2				
degeneration, myelin, segmental, characterized by a "status spongiosum" and swollen clear macrophages contianing wisps of fibrillar eosinophilic material with diffuse astrocytosis									4	4	4	*
-transverse sections	N+	N	N	N								
degeneration, myelin, segmental, characterized by a "status spongiosum" and swollen clear macrophages containing wisps of fibrillar eosinophilic material					3	2	3	2				
degeneration, myelin, segmental, characterized by a "status spongiosum" and swollen clear macrophages contianing wisps of fibrillar eosinophilic material with diffuse astrocytosis									4	4	4	*

+ Only one section evaluated.

Table II
(cont.)

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Microscopic Findings in Individual Animals
Terminal Sacrifice
Luxol Fast Blue

Animal Number	4 5 8 5	4 5 8 7	4 6 2 4	4 6 2 6	4 5 9 2	4 5 9 3	4 6 2 1	4 6 2 3	4 5 6 8	4 5 7 3	4 6 2 2	4 7 2 0
Sex	M	M	F	F	M	M	F	F	M	M	F	F
Pathology Number 80-	2 3 4 0	2 3 4 1	2 3 4 2	2 3 4 3	2 3 4 4	2 3 4 5	2 3 4 6	2 3 4 7	2 3 4 8	2 3 4 9	2 3 5 0	2 3 5 1
Group	I				II				III			
LUMBAR SPINAL CORD (cont.)												
-transverse section degeneration, myelin, segmental, characterized by a "status spongionsum" and swollen clear macrophages containing wisps of a L.F.B. positive material	N+	N	N	N	3	2	3	2	4	4	4	*

+ Only one section evaluated

79-7349

Table III

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Summary-Incidence of Microscopic Findings in Individual Animals

Table III

79-7349

A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs
Pathology Report

Summary-Incidence of Microscopic Findings in Individual Animals
Terminal Sacrifice
Hematoxylin and Eosin/Luxol Fast Blue

ORGAN/TISSUE AND OBSERVATION	Group I M	Group I F	Group II M	Group II F	Group III M	Group III F
BRAIN						
-1st transverse section (frontal lobe) myelin degeneration	0/2	0/2	1/2	0/2	2/2	2/2
-2nd transverse section (parietal lobe) myelin degeneration	0/2	0/2	0/2	0/2	2/2	2/2
SCIATIC NERVE myelin degeneration	0/2	0/2	0/2	0/2	0/1	0/2
CERVICAL SPINAL CORD myelin degeneration	0/2	0/2	2/2	2/2	2/2	2/2
LUMBAR SPINAL CORD myelin degeneration	0/2	0/2	2/2	2/2	2/2	1/1

Appendix D
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Animal Termination History

Group mg/m^3	Males				Females			
	An. No.	Days on Test	Date of Death	Type of Death/Week	An. No.	Days on Test	Date of Death	Type of Death/Week
I	4587	57	3/24/80	T(9)	4624	57	3/24/80	T(9)
0	4585	57	3/24/80	T(9)	4626	58	3/25/80	T(9)
II	4593	57	3/24/80	T(9)	4621	57	3/24/80	T(9)
10	4592	58	3/25/80	T(9)	4623	58	3/25/80	T(9)
III	4573	57	3/24/80	T(9)	4750	57	3/24/80	T(9)
100	4568	57	3/24/80	T(9)	4622	58	3/25/80	T(9)

T = Terminal.

Appendix E
A Four Week Inhalation Toxicity Study
of Triphenyl Phosphine in Dogs

Analytical Exposure Concentrations (mg/m^3)

Day 1					Day 2			
1	2	3	4	Mean	1	2	3	Mean
265	240	173	187	216	180	155	150	162

Body Weights (kg)

An.No.	Sex	Pretest	Week 1	Week 2	Week 3
4730	M	11.7	10.3	11.5	11.5
4662	F	12.4	12.1	12.3	12.6

Absolute Organ Weights (g)

An.No.	Sex	TBW (kg)	Brain	Pituitary	Adrenal	Gonad	Thyroid	Kidney	Heart	Liver
4730	M	11.2	93.5	67[a]	1270[a]	20.4	1170[a]	48.1	82.4	293.1
4662	F	12.6	72.5	58[a]	960[a]	1150[a]	900[a]	44.2	96.5	285.6

Relative Organ Weights (% of Body Weight)

An.No.	Sex	TBW (kg)	Brain	Pituitary	Adrenal	Gonad	Thyroid	Kidney	Heart	Liver
4730	M	11.2	0.835	0.598[a]	11.339[a]	0.182	10.446[a]	0.429	0.736	2.617
4662	F	12.6	0.575	0.460[a]	7.619[a]	9.127[a]	7.143[a]	0.351	0.766	2.267

Clinical Chemistry

An.No.	Sex	GLU mg/dl	BUN mg/dl	LDH IU	SGPT IU	ALK IU	CHOL mg/dl	T-Bili mg/dl	D-Bili mg/dl	Ca^{++} mg/dl	Na^+ mEq/l	K^+ mEq/l	TP g/dl	ALB g/dl	GLOB g/dl	A/B
Pretest																
4730	M	124	15.8	33	19	32	165	.24	.12	11.7	148	4.4	6.5	3.4	3.1	1.1
4662	F	100	14.2	84	22	46	170	.27	.19	12.2	147	5.0	6.8	3.6	3.2	1.1
Post-Exposure																
4730	M	78	17.0	204(68)[b]	31	122(104)[b]	172	.64(.41)[b]	.52(.17)[b]	12.2	144	5.1	6.7	3.2	3.5	0.9
4662	F	75	13.7	168	28	75	181	.46	.27	13.1	146	5.2	7.0	3.6	3.4	1.1

Hematology / Differentials - % WBC Neutralphils

An.No.	Sex	HGB g%	HCT %	RBC $10^6/mm^3$	PLAT $10^5/mm^3$	WBC $10^3/mm^3$	Mon	Lym	Seg	Ban	Eos	Bas
Pretest												
4730	M	18.4	52	7.29	1.5	13.6	0	32	67	1	0	0
4662	F	17.7	52	6.76	3.5	11.6	3	33	63	1	0	0
Post-Exposure												
4730	M	17.3	51	7.01	3.55	11.7	0	19	76	2	3	0
4762	F	18.4	53	7.05	3.57	10.6	0	22	73	0	5	0

Animal Termination History

An.No.	Sex	Days on Test	Date of Death	Type of Death/Week
4730	M	28	5/28/80	Terminal (4)
4662	F	28	5/28/80	Terminal (4)

[a]Weights multiplied by 10^3.
[b]Sample redrawn from animal number 4730 on 5-28-80.

DETERMINATION OF TRIPHENYL PHOSPHINE (TPP) ON GLASS FIBER FILTERS FOR STUDY NO. 80-7380

Report No.: 80056

Sponsor: M & T Chemicals
Rahway, New Jersey 07065

Prepared by: Benjamin J. Gulizia Date: December 22, 1980

Issued by: Bio/dynamics Inc.
Department of Metabolism and Analytical Chemistry
Box 43, Mettlers Road
East Millstone, New Jersey 08873

SUMMARY

During an inhalation toxicity study in dogs, glass fiber filters were used to trap chamber air samples containing Triphenyl Phosphine. Atmospheric concentrations were determined by desorbtion of the test material with acetonitrile and subsequent injection of the extract on an HPLC, equipped with a uBondapak column. The average chamber concentration of the two-day study was 192.9 ug/l of air.

Benjamin J. Gulizia 12/22/80
Benjamin J. Gulizia
Manager
Analytical Services

Joseph S. O'Grodnick 12-22-80
Joseph S. O'Grodnick, Ph.D.
Director
Department of Metabolism and
Analytical Chemistry

1.0 INTRODUCTION

An inhalation toxicity study with dogs was conducted by the Inhalation Toxicology Department of Bio/dynamics Inc., using Triphenyl Phosphine as the test material. One group of animals was exposed to an atmosphere containing approximately 200 ug/l of air of Triphenyl Phosphine for 6 hours/day for two days. The purpose of this study was to determine daily chamber concentrations.

2.0 EXPERIMENTAL

2.1 Test Material

The test material was Triphenyl Phosphine supplied by M & T Chemicals.

2.2 Sampling

Glass fiber filters were used to sample chamber air. The flow of air pulled through the tubes was controlled by a vacuum pump. The total volume of air pulled through the tubes was recorded and used in the calculations.

Samples were forwarded to the Department of Metabolism and Analytical Chemistry in glass scintillation vials for analysis. The test material was desorbed from the filters with a minimum of 1 ml of acetonitrile. Further dilutions were made, when necessary, for injection into the HPLC.

2.3 High Pressure Liquid Chromatographic Analysis

A standard curve was generated by injecting known (varying) amounts of Triphenyl Phosphine. Standards were plotted as the peak

area of Triphenyl Phosphine vs. ng of test material injected. Sample amounts were determined by comparing peak areas of samples with the standard curve. Atmospheric concentrations (ug of test material per liter of air) are given by the following equation:

$$ug/l = \frac{(ng\ found)}{(ul\ inj.)} \times \frac{10^3\ ul}{ml} \times ml\ final\ volume \times \frac{ug}{10^3\ ng} \Big/ Total\ air\ (l)$$

HPLC conditions are given in Table I.

3.0 RESULTS AND CONCLUSIONS

Assays of Triphenyl Phosphine ranged from 150.0-265.0 ug/l of air with an average chamber concentration of 192.9 ug/l of air. Complete analytical results are listed in Table II.

In addition, trial chamber air samples were analyzed prior to the start of the study. Theoretical concentrations were 30 and 200 ug/l of air. Actual mean chamber concentrations were found to be 33.3 and 177.9 ug/l of air, respectively. Complete results are listed in Table III.

TABLE I. TYPICAL HIGH PRESSURE LIQUID CHROMATOGRAPHIC CONDITIONS FOR TRIPHENYL PHOSPHINE

Instrument:	Tracor 960
Column:	u Bondapak; 30 cm x 3.9 mm
Precolumn:	Copell ODS
Mobile Phase:	100% ACN
Reservoir Temperature:	25°C
Wavelength:	254 nm

Note: These parameters may be varied to achieve optimum performance.

80056

TABLE II. DETERMINATION OF TRIPHENYL PHOSPHINE (TPP) ON GLASS FIBER FILTERS FOR STUDY NO. 80-7380

Group	Sample	Test Day	Date Sampled	Date Analyzed	Chamber Concentration (ug/l of air found)
I	489	1	5/1/80	5/2/80	265.0
	490-D				240.0
	492				173.0
	493-D				187.0
I	495	2	5/2/80	5/5/80	180.0
	497-D				155.0
	498				150.0
					$\bar{x}$ = 192.9

TABLE III. TRIAL SAMPLES OF TRIPHENYL PHOSPHINE (TPP) ON GLASS FIBER FILTERS FOR STUDY NO. 80-7380

Concentration (mg/m^3)	Sample	Date Sampled	Date Analyzed	Chamber Concentration (ug/l of air found)
30	439	4/22/80	4/24/80	4.7
	442			< 0.5
	442-D			< 0.3
	443			35.6
	443-D			35.5
	444			22.0
	444-D			33.0
	445			33.0
	445-D			44.0
	446			35.5
	446-D			33.5
30	447	4/23/80	4/24/80	35.0
	447-D			29.5
	448			41.5
	448-D			41.5
	449			11.0
	449-D			7.0
	450			43.5
	450-D			15.0
	451			40.5
	451-D			42.0
	452			37.0
	452-D			38.5
	453			41.5
	453-D			36.5
	454			37.5
	454-D			47.5
	455			38.5
	455-D			38.0
200	456	4/24/80	4/24/80	180.0
	456-D			108.0
	457			122.0
	457-D			106.0
	458			270.0
	458-D			265.0
	459			200.0
	459-D			200.0
	460			255.0
	460-D			253.0
	461			270.0
	461-D			280.0
	462			270.0
	462-D			280.0
	463			270.0
	463-D			280.0

TABLE III. TRIAL SAMPLES OF TRIPHENYL PHOSPHINE (TPP) ON GLASS FIBER FILTERS FOR STUDY NO. 80-7380 - continued

Concentration (mg/m^3)	Sample	Date Sampled	Date Analyzed	Chamber Concentration (ug/l of air found)
200	464	4/28/80	4/29/80	112.0
	464-D			125.0
	465			145.0
	465-D			133.3
	466			131.7
	466-D			140.0
	467			147.5
	467-D			128.3
	468			140.0
	468-D			126.7
	469			148.3
	469-D			130.0
	470			125.0
	470-D			105.0
	471			125.0
	471-D			121.7

M-11, 80-7380

A 28 Day Inhalation Toxicity Study of Triphenyl Phosphine in the Dog Pathology Report Terminal Sacrifice

80-7380

A 28 Day Inhalation Toxicity Study of Triphenyl Phosphine in the Dog Pathology Report

TABLE OF CONTENTS

INTRODUCTION

MATERIALS AND METHODS
- Test Material
- Test Animals
- Test Material Administration
- Experimental Design
- Postmortem Procedures
- Tissues Fixed
- Microscopic Methods

RESULTS AND DISCUSSION

SUMMARY

TABLE I
- Gross Postmortem Observations

TABLE II
- Microscopic Findings

TABLE III
- Summary-Incidence of Microscopic Findings

80-7380

A 28 Day Inhalation Toxicity Study of Triphenyl Phosphine in the Dog Pathology Report Terminal Sacrifice

INTRODUCTION:

This study was designed to evaluate the effects produced in dogs by two inhalation exposures to Triphenyl Phosphine.

MATERIALS AND METHODS:

Test Material: Triphenyl Phosphine

Test Animals: 2 Beagle Dogs (1 male, 1 female)

Test Material Administratiion: Aerosol (inhalation)

Experimental Design: A male and a female Beagle dog comprised Group I which was exposed to an aerosol of Triphenyl Phosphine for two consecutive days at the concentration of 200/mg/m^3 for 6 hours/day. The animals were held for 26 days post exposure.

Group	Concentration	Exposure Time	Number of Animals [1] Exposed	Necropsy[2]	Histo-Pathology
I	200 mg/m^3	6 Hr/Day For 2 Days	2	2	2
		TOTALS	2	2	2

[1]Equally divided male and female.

[2]Scheduled sacrifices were performed 26 days after the last exposure date.

Postmortem Procedures: Both animals survived to the study termination date at which time they were exsanguinated under sodium pentabarbital anesthesia. A complete gross postmortem examination was performed on each animal and included the following: examination of the external surface, all orifices, the cranial cavity, carcass, the external and cut surfaces of the brain and spinal cord, the thoracic, abdominal and pelvic cavities and their viscera, the cervical tissues and organs. The following organs were weighed: adrenals, brain, gonads, kidneys, heart, liver, pituitary and thyroid with parathyroid.

80-7380

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report
Terminal Sacrifice

MATERIALS AND METHODS: (cont.)

Postmortem Procedures (cont.)

Tissues Fixed: The following tissues, were taken and preserved in 10% neutral buffered formalin:

abdominal aorta
adrenals (2)
bone and bone marrow (sternum)
brain (2 sections)
esophagus
eyes (2) with optic nerve
gall bladder
gonads
ovaries
testes with epididymides
heart (with major vessels)
intestine
colon
duodenum
ileum
kidneys (2)
liver (2 sections)
lungs[1] (2 sections, at least 2 lobes and mainstem bronchi)
lymph nodes (peribronchial and mesenteric)
mammary gland (right inguinal)
pancreas
pituitary
prostate
salivary glands (mandibular)
sciatic nerve with muscle (both right and left, 3 sections each)
skin (inguinal, taken with mammary gland)
spinal cord (four inch section of cervical and lumbar regions)
spleen
stomach (2 sections)
thymus
trachea
thyroid/parathyroid
urinary bladder[2]
uterus (corpus and cervix uteri)
gross lesions (to include a border of normal tissue)
tissue masses or suspect tumors and regional lymph nodes

---

[1] Lungs were infused via the trachea with an amount of formalin approximately equal to 75% of the lung capacity.

[2] Urinary bladders were opened and examined prior to formalin fixation.

Microscopic Methods: Selected nerve tissues, as outlined below, were trimmed, processed in a Duo-autotechnicon, paraffin embedded and duplicate sections, except the brain, cut at 6u in the microtome. The processed slides were then stained, one set with hematoxylin and eosin and one set with the exception of the brain, with Luxol fast blue stain counterstained with cresyl violet. The brain was stained only with hematoxylin and eosin.

80-7380

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report
Terminal Sacrifice

MATERIALS AND METHODS: (cont.)

Microscopic Methods: (cont.)

TISSUE	SECTIONS	H&E	L.F.B.
Sciatic Nerve (R/L)	1 longitudinal section (each)	X	X
	2 transverse sections (each	X	X
Cervical Spinal Cord	1 longitudinal secton	X	X
	2 transverse sections	X	X
Lumbar Spinal Cord	1 longitudinal section	X	X
	2 transverse sections	X	X
Brain	2 transverse sections	X	

R/L = Right and Left
H&E = hematoxylin and eosin stain
L.F.B. = Luxol fast blue stain

RESULTS AND DISCUSSION:

Gross postmortem observations are presented in Table I. The full significance of gross lesions can not be evaluated without microscopic examination, but the distribution and pattern of the observed gross lesions does not suggest a significant test-material related toxic effect. The lung and stomach gross lesions probably represent manifestatons of migrating nematode larva. Both animals exhibited reddening of the urinary bladder mucosa; the cause of this reddening could not be determined by gross examination alone.

The microscopic examination of selected nerve tissues, presented in Table II, does reveal minimal to mild myelin degeneration lesions primarily affecting the spinal white tracts of the cervical spinal cord. The lack of host glial response suggests a toxic effect of the test material rather than an immune mediated or infectious etiology. Whether the lesion manifests a progressive change (i.e. irreversible change) in the myelin, or a reversible healing stage could not be determined. A summary-incidence of microscopic findings is presented in Table III.

80-7380

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report
Terminal Sacrifice

SUMMARY:

Two Beagle dogs, one male, one female, were exposed to an aerosol of Triphenyl Phosphine at a concentration of 200 mg/m^3, 6 hours a day for two days. The animals were observed for twenty-six days after the last exposure where upon they were sacrificed, gross postmortem examinations performed, and selected perepheral and central nervous system tissues evaluated histopathologically utilizing hematoxylin and eosin and Luxol fast blue stains. No unequivocal test material related gross postmortem lesions were observed.

Microscopic evaluation of nerve tissues revealed a minimal to mild toxic degeneration of myelin in both animals. Whether the lesion represents a progressive degeneration of the myelin or the healing stage of a reversible test material related effect could not be determined from this experiment.

Fred A. Hines, D.V.M. DVM 7/15/80
Pathologist

Table I

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

80-7380

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report
Terminal Sacrifice

GENERAL KEY TO GROSS POSTMORTEM OBSERVATIONS

R	=	Right
L	=	Left
cm	=	centimeters
diam.	=	diameter

Table I

80-7380

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report

Gross Postmortem Observations
Terminal Sacrifice

Compound	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
TPP	M	4730	80-3066	Spleen	Raised, dark red areas on edge of head (0.3cm).
				Urinary Bladder	Scattered red foci on internal surface.
				Prostatic Urethra	Slightly reddened.
				Lungs	L diaphragmatic lobe, dorsal surface: irregular, circumscribed gray-white area (0.5cm in diam.).
				Ileo-Colic Junction	Slightly reddened.
TPP	F	4662	80-3067	Thyroid	R: small, flattened to point of transparency; L: slighlty enlarged.
				Lungs	L anterior lobe: raised tan area (1.9cm in diam.) with dark red center extending to opposite surface.
				Stomach	Few raised tan nodules (less than 0.2cm in diam.) on pyloric surface.
				Urinary Bladder	Moderately reddened.
				Ileo-Colic Junction	Brown areas with tan centers (0.3cm).

80-7380

Table II

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report

Microscopic Findings
Terminal Sacrifice

80-7380

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report

GENERAL KEY TO HISTOPATHOLOGY TABLES

N	=	Not remarkable, within normal histologic limits
1	=	Minimal degree or amount of indicated change
2	=	Mild degree or amount of indicated change
3	=	Moderate degree or amount of indicated change
4	=	Marked degree or amount of indicated change
H&E	=	hematoxylin and eosin stain
L.F.B.	=	Luxol fast blue stain

Table II

80-7380

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report

Microsopic Findingss
Terminal Sacrifice

	H&E		L.F.B.	
Animal Number	4 7 3 0	4 6 6 2	4 7 3 0	4 6 6 2
Sex	M	F	M	F
Pathology Number 80-	3 0 6 6	3 0 6 7	3 0 6 6	3 0 6 7
Group	I	I	I	I
BRAIN				
-1st transverse section	N	N		
-2nd transverse section	N	N		
SCIATIC NERVE				
-right transverse section	N	N	N	N
-right longitudinal section	N	N	N	N
-left transverse section	N	N	N	N
-left longitudinal section	N	N	N	N
CERVICAL SPINAL CORD				
-longitudinal section: degeneration, myelin, segmental, characterized by a status spongiosum and swollen clear macrophages containing fibrillar material	2	1	2	1
-transverse section: degeneration, myelin, segmental, characterized by a status spongiosum and swollen clear macrophages containing fibrillar material	2	1	2	2
LUMBAR SPINAL CORD				
-longitudinal section: degeneration, myelin, segmental, characterized by a status spongiosum and swollen clear macrophages containing fibrillar material	N	N		N
-transverse section: degeneration, myelin, segmental, characterized by a status spongiosum and swollen clear macrophages containing fibrillar material	N	N	1 2	N

Table III

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report

Summary-Incidence of Microscopic Findings
Terminal Sacrifice

Table III

80-7380

A 28 Day Inhalation Toxicity Study
of Triphenyl Phosphine in the Dog
Pathology Report

Summary-Incidence of Microscopic Findings
Terminal Sacrifice
Hematoxylin and Eosin and Luxol Fast Blue

Organ/Tissue and Microscopic Finding	Stain Sex/No. of Animals	H&E M(1)	H&E F(1)	L.F.B. M(1)	L.F.B. F(1)
SPINAL CORD (CERVICAL/LUMBAR)					
-degeneraton, myelin, segmental, characterized by a status spongiosum and swollen clear macrophages containing fibrillar material		1/1	1/1	1/1	1/1

Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO. 78-2317

A FOUR WEEK NEUROTOXICITY STUDY IN DOGS WITH TRIPHENYLPHOSPHINE AND TRIPHENYLPHOSPHINE OXIDE

Submitted to: M & T Chemicals, Inc.
Rahway, New Jersey 07065

Date: March 1, 1979

PROJECT 78-2317

A FOUR WEEK NEUROTOXICITY STUDY IN DOGS

ABSTRACT

This study was designed to evaluate the neurotoxic effects of triphenylphosphine (TPP) and triphenylphosphine oxide (TPPO) when administered orally to dogs in single doses of 500 mg/kg and repeated doses (fourteen days) of 300 mg/kg/day. Positive control dogs received a known neurotoxin, tri-o-tolyl-phosphate (TOTP), in single doses of 500 mg/kg or repeated doses of 100 mg/kg/ day. Vehicle control dogs received corn oil in single or repeated doses. Each single-dose group consisted of two males and two females; each repeated dose group consisted of three males and three females. The duration of study was four weeks, thus providing a two or four week post-dose observation period. Because of mortality observed after the first dose of TPP at 300 mg/kg subsequent repeated doses were lowered to 50 mg/kg/day. Because of mortality in all dogs receiving 300 and 500 mg/kg of TPPO, additional groups of two dogs each were later administered single doses of 100 and 50 mg/kg with post-dose observation periods of one and two weeks, respectively. Plasma cholinesterase activity was measured pretest and three hours after the last dose. Brain cholinesterase activity was measured terminally.

Triphenylphosphine oxide (TPPO) produced death in all dogs after single doses of 300 and 500 mg/kg. Death was preceded by violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming" movements, aggressive behavior, vocalization, excessve salivation, dyspnea and emesis. Two dogs receiving 100 mg/kg showed similar signs after dosing but recovered by the following day with no unusual signs for seven subsequent days. Two dogs receiving 50 mg/kg/day exhibited no unusual signs after dosing or during a fourteen day post-dose observation period. No impairment of reflexes was noted in dogs receiving 100 and 50 mg/kg for seven or fourteen days, respectively, after dosing. Slight body weight losses occurred in dogs receiving 50 mg/kg only. Cholinesterase values gave no clear indication of inhibition resulting from TPPO administration.

One of six dogs receiving 300 mg/kg of triphenylphosphine (TPP) died after dosing; predeath signs were similar to those seen in dogs which died after receiving TPPO. All four dogs receiving a single dose of 500 mg/kg survived. Dogs receiving 300 or 500 mg/kg of TPP exhibited convulsions, tremors and other signs described above or sedation and recumbancy. Animals which survived the initial dose showed recovery from initial signs within two days (300 mg/kg group) or three days (500 mg/kg group).

Dogs which received a single dose of 500 mg/kg of TPP exhibited no marked evidence of impaired reflexes or altered neurologic function during the four weeks after dosing. No body weight effect was apparent.

Dogs which received a single dose of 300 mg/kg of TPP with thirteen subsequent doses of 50 mg/kg exhibited signs of neurologic impairment beginning on Day 10 (nine days after the first dose). This was characterized by impaired reflexes and by evidence of front and/or hind limb weakness with inability to stand erect for normal periods of time. Maximum severity of impaired reflexes occurred on Day 14 or 21 with diminished severity of impairment of locomotion, standing and righting reflexes present in three of five dogs by Day 28. Body weights varied with only one animal showing a significant weight loss.

There was no evidence of cholinesterase inhibition in plasma or brain samples from animals treated with TPP.

Animals receiving tri-o-tolyl-phosphate in a single dose of 500 mg/kg or repeated doses of 100 mg/kg exhibited no mortality, no clear evidence of neurologic effects and no clear body weight effects. Loose stools and emesis occurred with greater frequency in this group than in the vehicle control group. Inhibition of plasma cholinesterase activity was evident in samples obtained after fourteen daily doses of 100 mg/kg, and a suggestion of brain cholinesterase inhibition was present terminally (two of six values were below control ranges). No evidence of cholinesterase inhibition was present in plasma and brain samples obtained from dogs receiving a single dose of 500 mg/kg of TOTP.

TABLE OF CONTENTS

I. INTRODUCTION..... 1

II. MATERIALS AND METHODS..... 1

A. Test Materials..... 1
B. Vehicle..... 1
C. Test Animals..... 1
D. Selection and Group Assignment..... 2
E. Animal Identification..... 2
F. Experimental Outline..... 3
G. Husbandry..... 4
H. Test Substance Administration..... 4
I. Observations..... 4
J. Body Weight..... 4
K. Laboratory Studies..... 5
L. Post-Mortem..... 5

III. RESULTS AND DISCUSSION 6

A. Mortality..... 6
B. Observations..... 6
C. Neurological Examinations..... 8
D. Body Weights..... 9
E. Cholinesterase Values..... 10

IV. SUMMARY..... 12

TABLES

I. Individual Body Weights and Mortality Data..... 15
II. Summary of Neurological Responses..... 17
III. Individual Plasma and Brain Cholinesterase Values..... 29

APPENDICES

A. Methodology and References..... A-1
B. Individual Neurological Evaluations..... B-1

78-2317

I. INTRODUCTION

The purpose of this study, conducted for M&T Chemicals, Inc., was to assess the potential of triphenylphosphine and triphenylphosphine oxide to produce neurotoxic or other adverse effects in Beagle dogs following single and repeated administration (14 consecutive daily doses) via gastric intubation. A known neurotoxic material (tri-o-tolyl-phosphate) was administered as a positive control. After 28 days, all surviving animals were sacrificed. This study was performed at Bio/dynamics, Inc., East Millstone, New Jersey.

II. MATERIALS AND METHODS

A. Test Materials

Test Substance	Description	Date Received	Label Information
Triphenyl-phosphine (TPP)	Off-white powder	26 September 1978	Triphenylphosphine WTVAH-352K
Triphenyl-phosphine oxide (TPPO)	Off-white powder	26 September 1978	T.P.P.O. VYVCD- IR#2
Tri-o-tolyl-phosphate (TOTP) (positive control)	Clear viscous liquid	25 October 1978	Tri-o-tolylphosphate practical Lot No. A7A (container #1) Lot No. B4A (container #2)

Supplier: TPP and TPPO from M&T Chemicals, Inc., Rahway, New Jersey; TOTP from Scientific Products, Edison, New Jersey.
Storage: All at room temperature.

B. Vehicle: Mazola® Corn Oil

C. Test Animals: Dogs

Strain: Beagle

Number of Animals: 44 (21 Male; 23 Female)

Supplier: (40 initial animals) Marshall Research Animals, Inc. North Rose, New York

Date Received: (40 initial animals) 7 November 1978

Age at Receipt: 5-7 months

(Note - The four additional animals used on this study were received in the following manner: Animals 4336M and 4352F were received on 9 October 1978 from White Eagle Laboratories, Doylestown, Pa.; Animal 4146F was received on 13 June 1978 and Animal 4104F on 4 April 1978 from Marshall Research Animals, Inc.)

Weight at Initiation of Treatment (kilograms):

	Mean	Range
Males	9.2	7.7-10.7
Females	7.6	6.0-11.3

Immunization: Against distemper, hepatitis, bordetella, leptospirosis and oral papillomas by supplier.

Parasites (intestinal): Anthelmintics (piperazine, thiabendazole) were routinely administered by supplier as required.

Physical Examination: Pretest examinations were conducted by Bio/dynamics' Staff Veterinarian (H. E. Blatt, V.M.D.) (Meth. and Ref., Appendix A)

D. Selection and Group Assignment: Dogs were ranked by body weight and distributed into five blocks of five animals per sex so that body weight means for each group were comparable. Groups were designated randomly as I, II, III, or IV. Two dogs per sex per group were placed on the single treatment study; the remaining 3 dogs per sex per group were placed on the repeated treatment study. Because of mortality which occurred at high doses (300 and 500 mg/kg) of TPPO, four additional dogs were selected from untreated animals equilibrated at Bio/dynamics and placed in groups as described in the Experimental Outline.

E. Animal Identification: Each dog was assigned a unique Bio/dynamics identification number which appeared on the animal's cage. In addition each animal bore an ear tatoo with a USDA number.

F. Experimental Outline

Single Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals M	F	Lab Studies[a]	Sacrifice/ Necropsy	Interval
IA	Corn Oil	0	2	2	All	All	29-30 days
IIA	Tri-ortho-tolyl-phosphate (TOTP)	500	2	2	All	All	29-30 days
IIIA	Triphenylphosphine (TPP)	500	2	2	All	All	29-30 days
IVA	Triphenylphosphine oxide (TPPO)	500	2	2	b	S.D.[c]	1-2 days
IVC	Triphenylphosphine oxide (TPPO)	50	-	2	All[d]	All	16 days
IVD	Triphenylphosphine oxide (TPPO)	100	1	1	All[e]	All	8-9 days

[a]Plasma cholinesterase was evaluated on the day prior to treatment and three hours after treatment; brain (medulla) cholinesterase was evaluated immediately after death.
[b]Animals died spontaneously on Day 1; three hour post treatment plasma cholinesterase evaluated in one animal.
[c]S.D. = Spontaneous Death
[d]Animals entered study on Day 15; only brain cholinesterase evaluated.
[e]Animals entered study on Day 22; only brain cholinesterase evaluated.

14-Day Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals M	F	Lab Studies[b]	Sacrifice/ Necropsy	Interval
IB	Corn Oil	0	3	3	All	All	29-30 days
IIB	Tri-ortho-tolyl-phosphate (TOTP)	100	3	3	All	All	29-30 days
IIIB	Triphenylphosphine (TPP)	50[a]	3	3	5[c]	5	29-30 days
IVB	Triphenylphosphine oxide (TPPO)	300	3	3	All[d]	S.D.[e]	1-2 days

[a]The initial dosing was performed at 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.
[b]Plasma cholinesterase was evaluated on the day prior to treatment and three hours following each animal's last treatment; brain (medulla) cholinesterase was evaluated immediately after death.
[c]One animal died spontaneously on Day 2 of the study and only pretest plasma cholinesterase was evaluated.
[d]One animal was killed in a moribund condition and 5 animals died spontaneously; only pretest plasma cholinesterase was evaluated.
[e]S.D. = Spontaneous Death of 5 animals; one animal was killed in a moribund condition.

G. Husbandry:

Acclimation Period:	7-29 November 1978
Housing:	Individually in elevated metal grid cages.
Food:	400 grams of Standard laboratory diet (Wayne Bite Size®)
Water:	ad libitum. By automated water system (Elizabethtown Water Company)
Environmental Conditions:	12 hour light/dark cycle.
	Temperature monitored twice daily.

H. Test Substance Administration:

Route:	Gastric Intubation
Method:	Appropriate mixtures of test substance in corn oil were administered. Individual dose volumes were adjusted by most recent weekly body weight.
Dose Volume:	Single Treatment: 5 ml/kg (except for single doses of 50 and 100 mg/kg of TPPO which were administered in volumes of 0.5 and 1.0 ml/kg, respectively)
	14-Day Repeated Treatment: 3 ml/kg (except for 50 mg/kg dose of TPPO which was administered in a volume of 0.5 ml/kg)
Frequency:	Single dose (30 November 1978)
	14-Day Repeated Treatment: Daily (7 days per week) (30 November - 13 December 1978)

I. Observations:

For Mortality and Gross Signs of Toxicologic or Pharmacologic Effects:	Daily
Neurologic:	Pretest and Days 0, 1, 4, 7, 10, 14, 21 and 28.(performed 3 hours after dosing)

J. Body Weight: Pretest, weekly during treatment and on Day 28. (Meth. and Ref., Appendix A)

K. Laboratory Studies: (Meth. and Ref.; Appendix A)

Blood was obtained from unanesthetized dogs via jugular venipuncture.

Number of Animals and Time Intervals: See Experimental Outline

Parameter Evaluation

Clinical Chemistry:

cholinesterase
brain
plasma

L. Post-Mortem:

Animals Dying Spontaneously: Complete gross-post-mortem examination.

Necropsy: 28-29 December 1978

Number of Animals: All survivors.

Sacrifice Method: Exsanguination under sodium pentobarbital anesthesia.

Tissues Preserved: All animals.

brain (2 transverse sections)
nerve (left, right sciatic - 1 longitudinal, 2 transverse)
spinal cord (cervical, lumbar - 1 longitudinal, 2 transverse)
all gross lesions

Preservatives: 10% neutral buffered formalin: all tissues

Stain: Hematoxylin and Eosin
Luxol-fast Blue
(Meth. and Ref.; Appendix A)

Tissue Examined Histopathologically: All nerve tissues listed above from surviving animals of the 14-Day Repeated treatment with corn oil, TOTP and TPP.

(To be forwarded as an addendum or separate report.)

III. RESULTS AND DISCUSSION

A. Mortality (Table I)

1. TPPO

All dogs receiving triphenylphosphine oxide (TPPO) at doses of 300 and 500 mg/kg died after a single dose. Death was preceded by violent convulsions and other signs of neurotoxic effect as described below. Administration of atropine to one animal which received 500 mg/kg (Female No. 4381) did not prevent death. Dogs receiving single doses of 50 and 100 mg/kg of TPPO survived throughout the subsequent observation period (14 and 7 days, respectively).

2. TPP

One dog (of six) died after a single dose of 300 mg/kg of triphenylphosphine (TPP); predeath signs were similar to those seen in dogs which died after receiving TPPO. The five other dogs survived an initial dose of 300 mg/kg of TPP and subsequent doses of 50 mg/kg/day for thirteen days. Four dogs which received a single dose of 500 mg/kg of TPP also survived.

3. Controls

No deaths occurred in the vehicle control group or in the positive control group, which received tri-o-tolyl-phosphate (TOTP) in a single dose of 500 mg/kg or in repeated doses of 100 mg/kg.

B. Observations

1. TPPO

Dogs which received TPPO in doses of 300 and 500 mg/kg exhibited violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming movements, aggressive behavior, excessive salivation, dyspnea, vocalization and occasional emesis, followed by death in all animals.

Two dogs which received a single dose of 100 mg TPPO per kg exhibited similar signs after dosing but recovered by the following day. These dogs exhibited no additional unusual signs (except for single occurrences of loose stools and emesis) during the seven-day post-dose observation period.

Two dogs which received a single dose of 50 mg TPPO/kg showed no unusual signs immediately after dosing or during a fourteen-day post-dose observation period.

2. TPP

Three of four dogs which received a single dose of 500 mg TPP per kg exhibited convulsions, tremors and other signs similar to those seen in dogs receiving high doses of TPPO; the fourth dog exhibited sedation and recumbancy. Dogs were generally lethargic on the day following dosing but had all recovered by Day 4 (three days after dosing). Occasional loose stools or emesis were the only unusual signs noted in these animals for the remainder of the four week post-dose observation period.

Five of six dogs receiving a single dose of 300 mg TPP per kg exhibited convulsions, tremors and other signs described previously after dosing; the sixth dog exhibited sedation and recumbancy. One dog was found dead the day after dosing; other dogs were lethargic or recumbant. (Because of the reaction to the first dose, subsequent doses were lowered to 50 mg/kg/day.) The five surviving dogs showed recovery from the initial signs by Day 3 (two days after the first dose). However, beginning on Day 13 or 14 of study, these dogs exhibited evidence of front and/or hind limb weakness with inability to stand erect for normal periods of time; anorexia was also noted in some animals. These signs persisted until termination of study. Other signs noted included occasional occurrences of loose stools or emesis.

3. Control

Animals in both control groups exhibited loose stools and emesis during the study. Incidence of both of these findings was higher in the TOTP-treated (positive control) group than in the vehicle (corn oil) control group. One TOTP-treated dog (No. 4376, female) showed excessive salivation and aggressive behavior prior to dosing on a single occasion (Day 6).

C. Neurological Examinations (Table II, Appendix B)

1. TPPO

All four dogs which were still alive at the time of neurological examinations on the day of dosing with 300 or 500 mg/kg of TPPO exhibited impaired visual placing, locomotion, standing and righting reflexes. Flexor and extensor reflexes were unaffected in three of the four dogs.

No evidence of impaired reflexes was present in dogs receiving single doses of 100 or 50 mg TPPO per kg for seven or fourteen days, respectively, after dosing.

2. TPP

Except for a clearly impaired righting reflex seen in one dog (No. 4387, female) on Day 4 (3 days post-dose), animals which received a single dose of 500 mg TPP per kg exhibited only doubtful or minimal impairment of reflexes during the four week post-dose observation period. At the final (28-day) observation, the only abnormalities noted were minimally-impaired righting reflexes in two of four dogs and minimally-impaired flexor and extensor reflexes in a single animal.

Of the animals which received one dose of 300 mg TPP per kg followed by thirteen daily doses of 50 mg/kg four out of five had normal reflexes on Days 1, 4 and 7, with one animal exhibiting minimal or doubtful impairment of the extensor and/or righting reflexes on Days 4 and 7. Impairment

(minimal to complete) of the locomotion and/or standing reflexes was evident in all dogs on Day 10, and impairment of the righting reflex was present in all dogs on Day 14. These reflexes remained impaired (except for a single dog on Day 21) for the remainder of the study. Maximum severity generally occurred on Day 14 or 21, with diminished severity present in three of the five dogs by Day 28. Impairment of the flexor, extensor and visual placing reflexes was seen in most dogs on Days 14, 21 and/or 28; incidence and severity of impairment of these reflexes was generally less than that seen for the righting, standing and locomotion reflexes. Because some decrease in severity of neurologic effects was noted on Day 28 (fourteen days after termination of dosing) it is possible that these neurologic changes are reversible.

3. Controls

One dog (No. 4355, male) receiving repeated doses of 100 mg/kg/day of TOTP had minimal or doubtful impairment of the righting and extensor reflexes on Day 14 only. Reflexes were normal on all other occasions for this dog and throughout the study for all other positive control and all negative control dogs.

D. Body Weights (Table I)

1. TPPO

Animals receiving 50 mg/kg of TPPO showed slight body weight losses one week after dosing with subsequent slight gains the following week. Animals receiving 100 mg/kg of TPPO showed slight gains one week after dosing. (Animals receiving 300 and 500 mg/kg of TPPO died prior to body weight measurement).

2. TPP

Animals receiving repeated doses of TPP (50 mg/kg/day after initial dose of 300 mg/kg) showed slight initial weight losses, with subsequent

slight gains. Overall weight gains were slightly less than vehicle control gains in two dogs and comparable to control gains in two dogs. One animal exhibited a substantial (1.6 kg) overall weight loss.

Body weight gains of dogs receiving a single 500 mg/kg dose of TPP were comparable to gains of vehicle control dogs.

3. Control

Body weight gains of dogs receiving a single 500 mg/kg dose of TOTP were comparable to weight gains of vehicle control dogs. Body weight gains of dogs receiving repeated (50 mg/kg/day) doses of TOTP were comparable to those of vehicle control dogs for four of six animals and slightly lower for two animals.

E. Cholinesterase Values (Table III)

1. TPPO

Because of spontaneous mortality at the 300 and 500 mg/kg doses of TPPO, only one plasma sample was obtained for analysis (No. 4370, male, 500 mg/kg). No inhibition of cholinesterase was evident.

The terminal brain cholinesterase activity of one of two dogs receiving a single dose of 50 mg/kg of TPPO was slightly lower than activity seen for vehicle control dogs. Because of the small difference noted and the small number of animals at this dose level, the significance of this finding is questionable. Terminal brain cholinesterase values for the two dogs receiving 100 mg/kg of TPPO were within ranges seen in vehicle control animals.

2. TPP

There was no evidence of cholinesterase inhibition in plasma or brain samples from animals receiving an initial dose of 300 mg/kg of TPP followed by thirteen daily doses of 50 mg/kg or in animals receiving a single dose of 500 mg/kg.

3. Controls

Plasma cholinesterase activity was inhibited in samples obtained three hours after the fourteenth daily dose of 100 mg/kg of TOTP in all dogs in this group. Terminal brain cholinesterase activity values were within control ranges for four of six dogs and slightly lower than control for two dogs.

Cholinesterase activity in plasma and brain samples obtained from dogs receiving a single dose of 500 mg/kg of TOTP was generally comparable to activity seen in vehicle control dogs.

IV. SUMMARY

This study was designed to evaluate the neurotoxic effects of triphenylphosphine (TPP) and triphenylphosphine oxide (TPPO) when administered orally to dogs in single doses of 500 mg/kg and repeated doses (fourteen days) of 300 mg/kg/day. Positive control dogs received a known neurotoxin, tri-o-tolyl-phosphate (TOTP), in single doses of 500 mg/kg or repeated doses of 100 mg/kg/ day. Vehicle control dogs received corn oil in single or repeated doses. Each single-dose group consisted of two males and two females; each repeated dose group consisted of three males and three females. The duration of study was four weeks, thus providing a two or four week post-dose observation period. Because of mortality observed after the first dose of TPP at 300 mg/kg subsequent repeated doses were lowered to 50 mg/kg/day. Because of mortality in all dogs receiving 300 and 500 mg/kg of TPPO, additional groups of two dogs each were later administered single doses of 100 and 50 mg/kg with post-dose observation periods of one and two weeks, respectively. Plasma cholinesterase activity was measured pretest and three hours after the last dose. Brain cholinesterase activity was measured terminally.

Triphenylphosphine oxide (TPPO) produced death in all dogs after single doses of 300 and 500 mg/kg. Death was preceded by violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming" movements, aggressive behavior, vocalization, excessve salivation, dyspnea and emesis. Two dogs receiving 100 mg/kg showed similar signs after dosing but recovered by the following day with no unusual signs for seven subsequent days. Two dogs receiving 50 mg/kg/day exhibited no unusual signs after dosing or during a fourteen day post-dose observation period. No impairment of reflexes was noted in dogs receiving 100 and 50 mg/kg for seven or fourteen days, respectively, after dos-

ing. Slight body weight losses occurred in dogs receiving 50 mg/kg only. Cholinesterase values gave no clear indication of inhibition resulting from TPPO administration.

One of six dogs receiving 300 mg/kg of triphenylphosphine (TPP) died after dosing; predeath signs were similar to those seen in dogs which died after receiving TPPO. All four dogs receiving a single dose of 500 mg/kg survived. Dogs receiving 300 or 500 mg/kg of TPP exhibited convulsions, tremors and other signs described above or sedation and recumbancy. Animals which survived the initial dose showed recovery from initial signs within two days (300 mg/kg group) or three days (500 mg/kg group).

Dogs which received a single dose of 500 mg/kg of TPP exhibited no marked evidence of impaired reflexes or altered neurologic function during the four weeks after dosing. No body weight effect was apparent.

Dogs which received a single dose of 300 mg/kg of TPP with thirteen subsequent doses of 50 mg/kg exhibited signs of neurologic impairment beginning on Day 10 (nine days after the first dose). This was characterized by impaired reflexes and by evidence of front and/or hind limb weakness with inability to stand erect for normal periods of time. Maximum severity of impaired reflexes occurred on Day 14 or 21 with diminished severity of impairment of locomotion, standing and righting reflexes present in three of five dogs by Day 28. Body weights varied with only one animal showing a significant weight loss.

There was no evidence of cholinesterase inhibition in plasma or brain samples from animals treated with TPP.

Animals receiving tri-o-tolyl-phosphate in a single dose of 500 mg/kg or repeated doses of 100 mg/kg exhibited no mortality, no clear evidence of neurologic effects and no clear body weight effects. Loose stools and emesis occurred with greater frequency in this group than in the vehicle control group. Inhibition of plasma cholinesterase activity was evident in samples obtained after fourteen daily doses of 100 mg/kg, and a suggestion of brain cholinesterase inhibition was present terminally (two of six values were below control ranges). No evidence of cholinesterase inhibition was present in plasma and brain samples obtained from dogs receiving a single dose of 500 mg/kg of TOTP.

Carol S. Auletta

Carol S. Auletta, B.A.
Staff Toxicologist

William E. Rinehart

William E. Rinehart, Sc.D.
Vice President, Science and
Market Development

Prepared with the technical assistance of:
William G. Braun, M.S., Study Director
Jeffrey P. Lefrancois, B.S., Manager, Large Animal Section
Daniel J. Pisacreta, B.S., Supervisor
Doris H. Milligan, M.T., A.S.M.T., Manager, Clinical Chemistry and Histology Laboratories
Daniel T. LaRosa, Jr., B.A., Report Preparation

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4356 Sex: Male Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	1						
	Flexor Reflex		+	0	0	Animal found dead on Day 2.					
	Extensor Reflex		+	0	0						
	Visual Placing		+	0	1						
	Locomotion		+	0	2						
	Standing		+	0	2						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4361 Sex: Male Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0	Animal died spontaneously on Day 1.						
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4367 Sex: Male Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	3						
	Flexor Reflex		+	0	1	Animal killed in a moribund condition on Day 2.					
	Extensor Reflex		+	0	2						
	Visual Placing		+	0	2						
	Locomotion		+	0	3						
	Standing		+	0	3						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4377 Sex: Female Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0		Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4378 Sex: Female Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	1						
	Flexor Reflex		+	0	0	Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0	0						
	Visual Placing		+	0	2						
	Locomotion		+	0	2						
	Standing		+	0	2						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4391 Sex: Female Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0		Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4104 Sex: Female Group: IVC Dose: 50 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		0	1	0	1	0	0	Animal on test for 14 days only.	
	Flexor Reflex		0	0	0	0	0	0		
	Extensor Reflex		0	0	0	0	0	0		
	Visual Placing		0	0	0	0	0	0		
	Locomotion		0	0	0	0	0	0		
	Standing		0	0	0	0	0	0		

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4146 Sex: Female Group: IVC Dose: 50 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		0	0	0	0	0	0	Animal on test for 14 days only.	
	Flexor Reflex		0	0	0	0	0	0		
	Extensor Reflex		0	0	0	0	0	0		
	Visual Placing		0	0	0	0	0	0		
	Locomotion		0	0	0	0	0	0		
	Standing		0	0	0	0	0	0		

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4336 Sex: Male Group: IVD Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	23
Neurologic Examination:	Righting Reflex		0	0	0	0				
	Flexor Reflex		0	0	0	0	Animal on test for 7 days only.			
	Extensor Reflex		0	0	0	0				
	Visual Placing		0	0	0	0				
	Locomotion		0	0	0	0				
	Standing		0	0	0	0				

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4352 Sex: Female Group: IVD Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		0	0	0	0				
	Flexor Reflex		0	0	0	0	Animal on test for 7 days only.			
	Extensor Reflex		0	0	0	0				
	Visual Placing		0	0	0	0				
	Locomotion		0	0	0	0				
	Standing		0	0	0	0				

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

A FOUR WEEK NEUROTOXICITY STUDY IN DOGS WITH TRIPHENYLPHOSPHINE AND TRIPHENYLPHOSPHINE OXIDE

PATHOLOGY REPORT
TERMINAL SACRIFICE

78-2317

A Four Week Neurotoxicity Study in Dogs Pathology Report

Table of Contents

INTRODUCTION

MATERIAL AND METHODS

RESULTS

SUMMARY

Gross Postmortem Observations

Table I - Single Treatment

Table II - 14 Day Treatment

Microscopic Findings

Table III - 14 Day Treatment

A Four Week Neurotoxicity Study in Dogs Pathology Report

INTRODUCTION

The purpose of this study was to assess the neurotoxic potential of triphenylphosphine and triphenylphosphine oxide administered orally to dogs.

MATERIAL AND METHODS

A. Test Materials: Triphenylphosphine (TPP)
Triphenylphosphine oxide (TPPO)
Tri-o-tolyl-phosphate (TOTP-positive control)
Mazola Corn Oil (vehicle)

B. Route of Administration: Gastric intubation

C. Test Animals: 44 male and female Beagle dogs.

D. Experimental Design:

Single Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals Initial M	Initial F	Necropsy Interval M	Necropsy Interval F	
IA	Corn Oil	0	2	2	2	2	29-30 days
IIA	Tri-orotho-tolyl-phosphate (TOTP)	500	2	2	2	2	29-30 days
IIIA	Triphenylphosphine (TPP)	500	2	2	2	2	29-30 days
IVA	Triphenylphosphine oxide (TPPO)	500	2	2	2[a]	2[a]	1-2 days
IVC	Triphenylphosphine oxide (TPPO)	50	-	2	-	2	16 days
IVD	Triphenylphosphine oxide (TPPO)	100	1	1	1	1	8-9 days

[a]Both animals died spontaneously on day 1-2 of the study.

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

EXPERIMENTAL DESIGN (CONT.)

14 Day Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals Initial M	Initial F	Necropsy - Interval M	Necropsy - Interval F	Interval	Histopatholoc M	Histopatholoc F
IB	Corn Oil	0	3	3	3	3	29-30 days	3	3
IIB	Tri-ortho-tolyl-phosphate (TOTP)	100	3	3	3	3	29-30 days	3	3
IIIB	Triphenylphosphine (TPP)	50[a]	3	3[b]	3	3	29-30 days	3	2
IVB	Triphenylphosphine oxide (TPPO)	300	3[c]	3[c]	3	3	1-2 days	0	0

[a]The initial dose was 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.

[b]One animal died spontaneously on Day 2 of the study.

[c]All animals died spontaneously on days 1-2 of the study with the exception of one animal which was killed in a moribund condition.

E. Postmortem Methods:

A complete gross postmortem examination was performed on all animals those dying spontaneously or sacrificed at the end of the experiment. Animals were sacrificed by exsanguination under sodium pentobarbital anesthesia.

Following an external examination the cranial cavity was first opened and the brain removed. A representative section of medulla was dissected and placed on ice for cholinesterase evaluation. The remaining portions of the brain were preserved in 10% neutral buffered formalin for microscopic examination. Portions of the cervical and lumbar spinal cords and both sciatic nerves were dissected and preserved. Thoracic and abdominal cavities were opened and the viscera examined; tissues with grossly visible abnormalities were preserved.

Brain (3 sections) cervical and lumbar spinal cords (1 longitudinal and 2 transversal sections) and both sciatic nerves (1 longitudinal and two transversal sections) were processed and stained with hematoxylin and eosin, and Luxol Fast Blue for microscopic examination.

A Four Week Neurotoxicity Study in Dogs Pathology Report

RESULTS

I. Mortality

a. Single Treatment:

Four dogs given a single dose of 500 mg/kg of TPPO died spontaneously (three on day one, and one on day two of the study).

b. 14-Day Repeated Treatment

Six dogs given a dose of 300 mg/kg/day of TPPO died spontaneously or were sacrificed moribund (four died on day one, one on day two, and one was sacrificed moribund on day two).

One dog given 50 mg/kg/day of TPP died spontaneously on day two of the study.

II. Gross Postmortem Observations

a. Single Treatment

The most common gross observations were that of mottling, edema and congestion of lungs, (Table I).

b. Fourteen Day Treatment

The results of the gross postmortem examinations in individual animals are presented in Table II.

The most common gross observations noted in both control and treated groups were mottling edema and congestion of lungs. Pulmonary edema and congestion were limited to the animals dying spontaneously or killed *in extremis*.

III. Microscopic Findings

The results of the microscopic examination of brain, spinal cord and sciatic nerves are presented in Table III. No microscopic abnormalities were noted in the six dogs given corn oil and six dogs given Tri-ortho-tolyl-phosphate.

Minimal to moderate multifocal swelling and degeneration of axonal fibers in the white matter of the brain (predominantly in the sections of cerebellum) cervical and lumbar sections of the spinal cord and both sciatic nerves was noted in the five dogs given Triphenylphosphine.

A Four Week Neurotoxicity Study in Dogs Pathology Report

SUMMARY

Six dogs were treated, by gastric intubation with 50 mg/kg/day of Triphenylphosphine for fourteen days. One dog died spontaneously on day two of the study.

Microscopic examination of the brain, spinal cord and sciatic nerves revealed morphologic evidence of injury characterized by multifocal swelling and degeneration of axonal fibers.

No microscopic abnormality was noted in the nervous systems of dogs given corn oil (vehicle) or Tri-ortho-tolyl-phosphate (positive control).

A L Knezevich

Aleksandar L. Knezevich, Dr.Med.Vet.
Director of Pathology

Table I

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
Single Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IA (Control)	M	4357	-	-	N.O.A.
		4363	-	-	N.O.A.
	F	4379	-	-	N.O.A.
		4393	-	-	N.O.A.
IIA	M	4362	-	Lungs	All lobes: white mottling, multiple small, soft, palpable nodules on surfaces.
		4372	-	-	N.O.A.
	F	4380	-	-	N.O.A.
		4394	-	-	N.O.A.
IIIA	M	4371	-	-	N.O.A.
		4373	-	Lungs	All lobes: dark red spots (1-3mm in diam.)
	F	4387	-	-	N.O.A.
		4392	-	-	N.O.A.
IVA	M	4364	-	Lungs	All lobes: mottled dark red; on cut surface large amount of sanguineous fluid released.
				Stomach	Distended with gas, white frothy substance and food.
				Heart	Very firm in consistency.
		4370	-	Lungs	All lobes: mottled dark red; on cut surface moderate amount of sanguineous fluid released.
	F	4381	-	Lungs	All lobes: mottled dark red; on cut surface large amount of sanguineous fluid released.

Table I
(cont.)

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
Single Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IVA (cont.)	F	4385	-	Heart	Very firm in consistency.
IVC	F	4104	-	-	N.O.A.
		4146	-	-	N.O.A.
IVD	M	4336	-	-	N.O.A.
	F	4352	-	-	N.O.A.

Table II

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
14 Day Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IB	M	4358	79-654	Lungs	All lobes: mottled white and red.
		4360	79-655	Lung (L)	Mottled dark red.
		4365	79-656	-	N.O.A.
	F	4383	79-657	-	N.O.A.
		4384	79-658	-	N.O.A.
		4390	79-659	-	N.O.A.
IIB	M	4355	79-660	Lungs	All lobes: mottled white and red.
		4366	79-661	-	N.O.A.
		4369	79-662	-	N.O.A.
	F	4376	79-663	-	N.O.A.
		4382	79-664	Lungs	Red mottling on both diaphragmati surfaces.
		4386	79-665	-	N.O.A.
IIIB	M	4359	79-666	Lungs	All surfaces: mottled.
		4368	79-667	-	N.O.A.
		4374	79-668	Lungs	All lobes: mottled white and red.
	F	4375	79-669	-	N.O.A.
		4389	79-670	Lungs	All lobes: mottled red.
		4388*	-	Lungs	All lobes: mottled red; on cut surface large amount of sanguineou fluid released.
				Stomach	Distended with gas.

*Animal died spontaneously

Table II
(cont.)

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
14 Day Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IVB	M	4356*	-	Lungs	All lobes: mottled red; on cut surface moderate amount of sanguineous fluid released.
		4361*	-	Lungs Stomach	All lobes: mottled dark red. Distended with gas.
		4367**	-	Lungs	All lobes: mottled dark red; on cut surface large amount of sanguineous fluid released.
	F	4377*	-	Lungs	All lobes: mottled dark red; on cut surface sanguineous fluid released.
				Stomach	Distended with gas.
		4391*	-	Lungs	All lobes: mottled dark red; on cut surface sanguineous fluid released.
		4378*	-	Lungs	All lobes: mottled red and pin-point size red foci.
				Stomach	Distended with gas.
				Heart	Firm, contracted.

* Animal died spontaneously
** Animal killed in moribund condition.

A Four Week Neurotoxicity Study in Dogs Pathology Report

General Key to Histopathology Tables

N	=	Not remarkable, within normal histologic limits
1	=	Minimal degree or amount of indicated change
2	=	Mild degree or amount of indicated change
3	=	Moderate degree or amount of indicated change
4	=	Marked degree or amount of indicated change
H&E	=	hematoxylin and eosin stain
LFB	=	Luxol Fast Blue stain

Table III

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Microscopic Findings
14 Day Treatment

Animal Number	43581	43606	43655	43883	43884	43890	43955	43966	43969	43976	43982	43986	43595	43688	43674	43775	43789
Sex	M	M	M	F	F	F	M	M	M	F	F	F	M	M	M	F	F
Pathology Number 79-	65414	65515	65616	65717	65818	65919	66020	66121	66222	66323	66424	66525	66626	66727	66828	66929	67030
Group B-	I	I	I	I	I	I	II	II	II	II	II	II	III	III	III	III	III
BRAIN, three transverse sections (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N					
multifocal swelling and degeneration of axons													2	2	1	1	2
CERVICAL SPINAL CORD, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N					
multifocal swelling and degeneration of axons													2	3	1	1	2
LUMBAR SPINAL CORD, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N					
multifocal swelling and degeneration of axons													2	3	1	1	2
LEFT SCIATIC NERVE, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N			N	N	
multifocal swelling and degeneration of axons													1	1			1
focal mononuclear cell infiltrate													1				
RIGHT SCIATIC NERVE, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N			N	N	
multifocal swelling and degeneration of axons													1	1			1

Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO. 78-2317

A FOUR WEEK NEUROTOXICITY STUDY IN DOGS WITH TRIPHENYLPHOSPHINE AND TRIPHENYLPHOSPHINE OXIDE

Submitted to: M & T Chemicals, Inc.
Rahway, New Jersey 07065

Date: March 1, 1979

PROJECT 78-2317

A FOUR WEEK NEUROTOXICITY STUDY

IN DOGS

ABSTRACT

This study was designed to evaluate the neurotoxic effects of triphenylphosphine (TPP) and triphenylphosphine oxide (TPPO) when administered orally to dogs in single doses of 500 mg/kg and repeated doses (fourteen days) of 300 mg/kg/day. Positive control dogs received a known neurotoxin, tri-o-tolyl-phosphate (TOTP), in single doses of 500 mg/kg or repeated doses of 100 mg/kg/ day. Vehicle control dogs received corn oil in single or repeated doses. Each single-dose group consisted of two males and two females; each repeated dose group consisted of three males and three females. The duration of study was four weeks, thus providing a two or four week post-dose observation period. Because of mortality observed after the first dose of TPP at 300 mg/kg subsequent repeated doses were lowered to 50 mg/kg/day. Because of mortality in all dogs receiving 300 and 500 mg/kg of TPPO, additional groups of two dogs each were later administered single doses of 100 and 50 mg/kg with post-dose observation periods of one and two weeks, respectively. Plasma cholinesterase activity was measured pretest and three hours after the last dose. Brain cholinesterase activity was measured terminally.

Triphenylphosphine oxide (TPPO) produced death in all dogs after single doses of 300 and 500 mg/kg. Death was preceded by violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming" movements, aggressive behavior, vocalization, excessve salivation, dyspnea and emesis. Two dogs receiving 100 mg/kg showed similar signs after dosing but recovered by the following day with no unusual signs for seven subsequent days. Two dogs receiving 50 mg/kg/day exhibited no unusual signs after dosing or during a fourteen day post-dose observation period. No impairment of reflexes was noted in dogs receiving 100 and 50 mg/kg for seven or fourteen days, respectively, after dosing. Slight body weight losses occurred in dogs receiving 50 mg/kg only. Cholinesterase values gave no clear indication of inhibition resulting from TPPO administration.

One of six dogs receiving 300 mg/kg of triphenylphosphine (TPP) died after dosing; predeath signs were similar to those seen in dogs which died after receiving TPPO. All four dogs receiving a single dose of 500 mg/kg survived. Dogs receiving 300 or 500 mg/kg of TPP exhibited convulsions, tremors and other signs described above or sedation and recumbancy. Animals which survived the initial dose showed recovery from initial signs within two days (300 mg/kg group) or three days (500 mg/kg group).

Dogs which received a single dose of 500 mg/kg of TPP exhibited no marked evidence of impaired reflexes or altered neurologic function during the four weeks after dosing. No body weight effect was apparent.

Dogs which received a single dose of 300 mg/kg of TPP with thirteen subsequent doses of 50 mg/kg exhibited signs of neurologic impairment beginning on Day 10 (nine days after the first dose). This was characterized by impaired reflexes and by evidence of front and/or hind limb weakness with inability to stand erect for normal periods of time. Maximum severity of impaired reflexes occurred on Day 14 or 21 with diminished severity of impairment of locomotion, standing and righting reflexes present in three of five dogs by Day 28. Body weights varied with only one animal showing a significant weight loss.

There was no evidence of cholinesterase inhibition in plasma or brain samples from animals treated with TPP.

Animals receiving tri-o-tolyl-phosphate in a single dose of 500 mg/kg or repeated doses of 100 mg/kg exhibited no mortality, no clear evidence of neurologic effects and no clear body weight effects. Loose stools and emesis occurred with greater frequency in this group than in the vehicle control group. Inhibition of plasma cholinesterase activity was evident in samples obtained after fourteen daily doses of 100 mg/kg, and a suggestion of brain cholinesterase inhibition was present terminally (two of six values were below control ranges). No evidence of cholinesterase inhibition was present in plasma and brain samples obtained from dogs receiving a single dose of 500 mg/kg of TOTP.

TABLE OF CONTENTS

I. INTRODUCTION..... 1

II. MATERIALS AND METHODS..... 1

A. Test Materials..... 1
B. Vehicle..... 1
C. Test Animals..... 1
D. Selection and Group Assignment..... 2
E. Animal Identification..... 2
F. Experimental Outline..... 3
G. Husbandry..... 4
H. Test Substance Administration..... 4
I. Observations..... 4
J. Body Weight..... 4
K. Laboratory Studies..... 5
L. Post-Mortem..... 5

III. RESULTS AND DISCUSSION 6

A. Mortality..... 6
B. Observations..... 6
C. Neurological Examinations..... 8
D. Body Weights..... 9
E. Cholinesterase Values..... 10

IV. SUMMARY..... 12

TABLES

I. Individual Body Weights and Mortality Data..... 15
II. Summary of Neurological Responses..... 17
III. Individual Plasma and Brain Cholinesterase Values..... 29

APPENDICES

A. Methodology and References..... A-1
B. Individual Neurological Evaluations..... B-1

78-2317

I. INTRODUCTION

The purpose of this study, conducted for M&T Chemicals, Inc., was to assess the potential of triphenylphosphine and triphenylphosphine oxide to produce neurotoxic or other adverse effects in Beagle dogs following single and repeated administration (14 consecutive daily doses) via gastric intubation. A known neurotoxic material (tri-o-tolyl-phosphate) was administered as a positive control. After 28 days, all surviving animals were sacrificed. This study was performed at Bio/dynamics, Inc., East Millstone, New Jersey.

II. MATERIALS AND METHODS

A. Test Materials

Test Substance	Description	Date Received	Label Information
Triphenyl-phosphine (TPP)	Off-white powder	26 September 1978	Triphenylphosphine WTVAH-352K
Triphenyl-phosphine oxide (TPPO)	Off-white powder	26 September 1978	T.P.P.O. VYVCD- IR#2
Tri-o-tolyl-phosphate (TOTP) (positive control)	Clear viscous liquid	25 October 1978	Tri-o-tolylphosphate practical Lot No. A7A (container #1) Lot No. B4A (container #2)

Supplier: TPP and TPPO from M&T Chemicals, Inc., Rahway, New Jersey; TOTP from Scientific Products, Edison, New Jersey.
Storage: All at room temperature.

B. Vehicle: Mazola® Corn Oil

C. Test Animals: Dogs

Strain: Beagle

Number of Animals: 44 (21 Male; 23 Female)

Supplier: (40 initial animals) Marshall Research Animals, Inc. North Rose, New York

Date Received: (40 initial animals) 7 November 1978

Age at Receipt: 5-7 months

(Note - The four additional animals used on this study were received in the following manner: Animals 4336M and 4352F were received on 9 October 1978 from White Eagle Laboratories, Doylestown, Pa.; Animal 4146F was received on 13 June 1978 and Animal 4104F on 4 April 1978 from Marshall Research Animals, Inc.)

Weight at Initiation of Treatment (kilograms):

	Mean	Range
Males	9.2	7.7-10.7
Females	7.6	6.0-11.3

Immunization: Against distemper, hepatitis, bordetella, leptospirosis and oral papillomas by supplier.

Parasites (intestinal): Anthelmintics (piperazine, thiabendazole) were routinely administered by supplier as required.

Physical Examination: Pretest examinations were conducted by Bio/dynamics' Staff Veterinarian (H. E. Blatt, V.M.D.) (Meth. and Ref., Appendix A)

D. Selection and Group Assignment: Dogs were ranked by body weight and distributed into five blocks of five animals per sex so that body weight means for each group were comparable. Groups were designated randomly as I, II, III, or IV. Two dogs per sex per group were placed on the single treatment study; the remaining 3 dogs per sex per group were placed on the repeated treatment study. Because of mortality which occurred at high doses (300 and 500 mg/kg) of TPPO, four additional dogs were selected from untreated animals equilibrated at Bio/dynamics and placed in groups as described in the Experimental Outline.

E. Animal Identification: Each dog was assigned a unique Bio/dynamics identification number which appeared on the animal's cage. In addition each animal bore an ear tatoo with a USDA number.

F. Experimental Outline

Single Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals M	F	Lab Studies[a]	Sacrifice/ Necropsy - Interval
IA	Corn Oil	0	2	2	All	All 29-30 days
IIA	Tri-ortho-tolyl-phosphate (TOTP)	500	2	2	All	All 29-30 days
IIIA	Triphenylphosphine (TPP)	500	2	2	All	All 29-30 days
IVA	Triphenylphosphine oxide (TPPO)	500	2	2	b	S.D.[c] 1-2 days
IVC	Triphenylphosphine oxide (TPPO)	50	-	2	All[d]	All 16 days
IVD	Triphenylphosphine oxide (TPPO)	100	1	1	All[e]	All 8-9 days

[a]Plasma cholinesterase was evaluated on the day prior to treatment and three hours after treatment; brain (medulla) cholinesterase was evaluated immediately after death.
[b]Animals died spontaneously on Day 1; three hour post treatment plasma cholinesterase evaluated in one animal.
[c]S.D. = Spontaneous Death
[d]Animals entered study on Day 15; only brain cholinesterase evaluated.
[e]Animals entered study on Day 22; only brain cholinesterase evaluated.

14-Day Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals M	F	Lab Studies[b]	Sacrifice/ Necropsy - Interval
IB	Corn Oil	0	3	3	All	All 29-30 days
IIB	Tri-ortho-tolyl-phosphate (TOTP)	100	3	3	All	All 29-30 days
IIIB	Triphenylphosphine (TPP)	50[a]	3	3	5[c]	5 29-30 days
IVB	Triphenylphosphine oxide (TPPO)	300	3	3	All[d]	S.D.[e] 1-2 days

[a]The initial dosing was performed at 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.
[b]Plasma cholinesterase was evaluated on the day prior to treatment and three hours following each animal's last treatment; brain (medulla) cholinesterase was evaluated immediately after death.
[c]One animal died spontaneously on Day 2 of the study and only pretest plasma cholinesterase was evaluated.
[d]One animal was killed in a moribund condition and 5 animals died spontaneously; only pretest plasma cholinesterase was evaluated.
[e]S.D. = Spontaneous Death of 5 animals; one animal was killed in a moribund condition.

G. Husbandry:

Acclimation Period:	7-29 November 1978
Housing:	Individually in elevated metal grid cages.
Food:	400 grams of Standard laboratory diet (Wayne Bite Size®)
Water:	ad libitum. By automated water system (Elizabethtown Water Company)
Environmental Conditions:	12 hour light/dark cycle.
	Temperature monitored twice daily.

H. Test Substance Administration:

Route:	Gastric Intubation
Method:	Appropriate mixtures of test substance in corn oil were administered. Individual dose volumes were adjusted by most recent weekly body weight.
Dose Volume:	Single Treatment: 5 ml/kg (except for single doses of 50 and 100 mg/kg of TPPO which were administered in volumes of 0.5 and 1.0 ml/kg, respectively)
	14-Day Repeated Treatment: 3 ml/kg (except for 50 mg/kg dose of TPPO which was administered in a volume of 0.5 ml/kg)
Frequency:	Single dose (30 November 1978)
	14-Day Repeated Treatment: Daily (7 days per week) (30 November - 13 December 1978)

I. Observations:

For Mortality and Gross Signs of Toxicologic or Pharmacologic Effects:	Daily
Neurologic:	Pretest and Days 0, 1, 4, 7, 10, 14, 21 and 28.(performed 3 hours after dosing)

J. Body Weight: Pretest, weekly during treatment and on Day 28. (Meth. and Ref., Appendix A)

K. Laboratory Studies: (Meth. and Ref.; Appendix A)

Blood was obtained from unanesthetized dogs via jugular venipuncture.

Number of Animals and Time Intervals: See Experimental Outline

Parameter Evaluation

Clinical Chemistry:

cholinesterase
brain
plasma

L. Post-Mortem:

Animals Dying Spontaneously: Complete gross-post-mortem examination.

Necropsy: 28-29 December 1978

Number of Animals: All survivors.

Sacrifice Method: Exsanguination under sodium pentobarbital anesthesia.

Tissues Preserved: All animals.

brain (2 transverse sections)
nerve (left, right sciatic - 1 longitudinal, 2 transverse)
spinal cord (cervical, lumbar - 1 longitudinal, 2 transverse)
all gross lesions

Preservatives: 10% neutral buffered formalin: all tissues

Stain: Hematoxylin and Eosin
Luxol-fast Blue
(Meth. and Ref.; Appendix A)

Tissue Examined Histopathologically: All nerve tissues listed above from surviving animals of the 14-Day Repeated treatment with corn oil, TOTP and TPP.

(To be forwarded as an addendum or separate report.)

III. RESULTS AND DISCUSSION

A. Mortality (Table I)

1. TPPO

All dogs receiving triphenylphosphine oxide (TPPO) at doses of 300 and 500 mg/kg died after a single dose. Death was preceded by violent convulsions and other signs of neurotoxic effect as described below. Administration of atropine to one animal which received 500 mg/kg (Female No. 4381) did not prevent death. Dogs receiving single doses of 50 and 100 mg/kg of TPPO survived throughout the subsequent observation period (14 and 7 days, respectively).

2. TPP

One dog (of six) died after a single dose of 300 mg/kg of triphenylphosphine (TPP); predeath signs were similar to those seen in dogs which died after receiving TPPO. The five other dogs survived an initial dose of 300 mg/kg of TPP and subsequent doses of 50 mg/kg/day for thirteen days. Four dogs which received a single dose of 500 mg/kg of TPP also survived.

3. Controls

No deaths occurred in the vehicle control group or in the positive control group, which received tri-o-tolyl-phosphate (TOTP) in a single dose of 500 mg/kg or in repeated doses of 100 mg/kg.

B. Observations

1. TPPO

Dogs which received TPPO in doses of 300 and 500 mg/kg exhibited violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming movements, aggressive behavior, excessive salivation, dyspnea, vocalization and occasional emesis, followed by death in all animals.

Two dogs which received a single dose of 100 mg TPPO per kg exhibited similar signs after dosing but recovered by the following day. These dogs exhibited no additional unusual signs (except for single occurrences of loose stools and emesis) during the seven-day post-dose observation period.

Two dogs which received a single dose of 50 mg TPPO/kg showed no unusual signs immediately after dosing or during a fourteen-day post-dose observation period.

2. TPP

Three of four dogs which received a single dose of 500 mg TPP per kg exhibited convulsions, tremors and other signs similar to those seen in dogs receiving high doses of TPPO; the fourth dog exhibited sedation and recumbancy. Dogs were generally lethargic on the day following dosing but had all recovered by Day 4 (three days after dosing). Occasional loose stools or emesis were the only unusual signs noted in these animals for the remainder of the four week post-dose observation period.

Five of six dogs receiving a single dose of 300 mg TPP per kg exhibited convulsions, tremors and other signs described previously after dosing; the sixth dog exhibited sedation and recumbancy. One dog was found dead the day after dosing; other dogs were lethargic or recumbant. (Because of the reaction to the first dose, subsequent doses were lowered to 50 mg/kg/day.) The five surviving dogs showed recovery from the initial signs by Day 3 (two days after the first dose). However, beginning on Day 13 or 14 of study, these dogs exhibited evidence of front and/or hind limb weakness with inability to stand erect for normal periods of time; anorexia was also noted in some animals. These signs persisted until termination of study. Other signs noted included occasional occurrences of loose stools or emesis.

3. Control

Animals in both control groups exhibited loose stools and emesis during the study. Incidence of both of these findings was higher in the TOTP-treated (positive control) group than in the vehicle (corn oil) control group. One TOTP-treated dog (No. 4376, female) showed excessive salivation and aggressive behavior prior to dosing on a single occasion (Day 6).

C. Neurological Examinations (Table II, Appendix B)

1. TPPO

All four dogs which were still alive at the time of neurological examinations on the day of dosing with 300 or 500 mg/kg of TPPO exhibited impaired visual placing, locomotion, standing and righting reflexes. Flexor and extensor reflexes were unaffected in three of the four dogs.

No evidence of impaired reflexes was present in dogs receiving single doses of 100 or 50 mg TPPO per kg for seven or fourteen days, respectively, after dosing.

2. TPP

Except for a clearly impaired righting reflex seen in one dog (No. 4387, female) on Day 4 (3 days post-dose), animals which received a single dose of 500 mg TPP per kg exhibited only doubtful or minimal impairment of reflexes during the four week post-dose observation period. At the final (28-day) observation, the only abnormalities noted were minimally-impaired righting reflexes in two of four dogs and minimally-impaired flexor and extensor reflexes in a single animal.

Of the animals which received one dose of 300 mg TPP per kg followed by thirteen daily doses of 50 mg/kg four out of five had normal reflexes on Days 1, 4 and 7, with one animal exhibiting minimal or doubtful impairment of the extensor and/or righting reflexes on Days 4 and 7. Impairment

(minimal to complete) of the locomotion and/or standing reflexes was evident in all dogs on Day 10, and impairment of the righting reflex was present in all dogs on Day 14. These reflexes remained impaired (except for a single dog on Day 21) for the remainder of the study. Maximum severity generally occurred on Day 14 or 21, with diminished severity present in three of the five dogs by Day 28. Impairment of the flexor, extensor and visual placing reflexes was seen in most dogs on Days 14, 21 and/or 28; incidence and severity of impairment of these reflexes was generally less than that seen for the righting, standing and locomotion reflexes. Because some decrease in severity of neurologic effects was noted on Day 28 (fourteen days after termination of dosing) it is possible that these neurologic changes are reversible.

3. Controls

One dog (No. 4355, male) receiving repeated doses of 100 mg/kg/day of TOTP had minimal or doubtful impairment of the righting and extensor reflexes on Day 14 only. Reflexes were normal on all other occasions for this dog and throughout the study for all other positive control and all negative control dogs.

D. Body Weights (Table I)

1. TPPO

Animals receiving 50 mg/kg of TPPO showed slight body weight losses one week after dosing with subsequent slight gains the following week. Animals receiving 100 mg/kg of TPPO showed slight gains one week after dosing. (Animals receiving 300 and 500 mg/kg of TPPO died prior to body weight measurement).

2. TPP

Animals receiving repeated doses of TPP (50 mg/kg/day after initial dose of 300 mg/kg) showed slight initial weight losses, with subsequent

slight gains. Overall weight gains were slightly less than vehicle control gains in two dogs and comparable to control gains in two dogs. One animal exhibited a substantial (1.6 kg) overall weight loss.

Body weight gains of dogs receiving a single 500 mg/kg dose of TPP were comparable to gains of vehicle control dogs.

3. Control

Body weight gains of dogs receiving a single 500 mg/kg dose of TOTP were comparable to weight gains of vehicle control dogs. Body weight gains of dogs receiving repeated (50 mg/kg/day) doses of TOTP were comparable to those of vehicle control dogs for four of six animals and slightly lower for two animals.

E. Cholinesterase Values (Table III)

1. TPPO

Because of spontaneous mortality at the 300 and 500 mg/kg doses of TPPO, only one plasma sample was obtained for analysis (No. 4370, male, 500 mg/kg). No inhibition of cholinesterase was evident.

The terminal brain cholinesterase activity of one of two dogs receiving a single dose of 50 mg/kg of TPPO was slightly lower than activity seen for vehicle control dogs. Because of the small difference noted and the small number of animals at this dose level, the significance of this finding is questionable. Terminal brain cholinesterase values for the two dogs receiving 100 mg/kg of TPPO were within ranges seen in vehicle control animals.

2. TPP

There was no evidence of cholinesterase inhibition in plasma or brain samples from animals receiving an initial dose of 300 mg/kg of TPP followed by thirteen daily doses of 50 mg/kg or in animals receiving a single dose of 500 mg/kg.

3. Controls

Plasma cholinesterase activity was inhibited in samples obtained three hours after the fourteenth daily dose of 100 mg/kg of TOTP in all dogs in this group. Terminal brain cholinesterase activity values were within control ranges for four of six dogs and slightly lower than control for two dogs.

Cholinesterase activity in plasma and brain samples obtained from dogs receiving a single dose of 500 mg/kg of TOTP was generally comparable to activity seen in vehicle control dogs.

IV. SUMMARY

This study was designed to evaluate the neurotoxic effects of triphenylphosphine (TPP) and triphenylphosphine oxide (TPPO) when administered orally to dogs in single doses of 500 mg/kg and repeated doses (fourteen days) of 300 mg/kg/day. Positive control dogs received a known neurotoxin, tri-o-tolyl-phosphate (TOTP), in single doses of 500 mg/kg or repeated doses of 100 mg/kg/ day. Vehicle control dogs received corn oil in single or repeated doses. Each single-dose group consisted of two males and two females; each repeated dose group consisted of three males and three females. The duration of study was four weeks, thus providing a two or four week post-dose observation period. Because of mortality observed after the first dose of TPP at 300 mg/kg subsequent repeated doses were lowered to 50 mg/kg/day. Because of mortality in all dogs receiving 300 and 500 mg/kg of TPPO, additional groups of two dogs each were later administered single doses of 100 and 50 mg/kg with post-dose observation periods of one and two weeks, respectively. Plasma cholinesterase activity was measured pretest and three hours after the last dose. Brain cholinesterase activity was measured terminally.

Triphenylphosphine oxide (TPPO) produced death in all dogs after single doses of 300 and 500 mg/kg. Death was preceded by violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming" movements, aggressive behavior, vocalization, excessve salivation, dyspnea and emesis. Two dogs receiving 100 mg/kg showed similar signs after dosing but recovered by the following day with no unusual signs for seven subsequent days. Two dogs receiving 50 mg/kg/day exhibited no unusual signs after dosing or during a fourteen day post-dose observation period. No impairment of reflexes was noted in dogs receiving 100 and 50 mg/kg for seven or fourteen days, respectively, after dos-

ing. Slight body weight losses occurred in dogs receiving 50 mg/kg only. Cholinesterase values gave no clear indication of inhibition resulting from TPPO administration.

One of six dogs receiving 300 mg/kg of triphenylphosphine (TPP) died after dosing; predeath signs were similar to those seen in dogs which died after receiving TPPO. All four dogs receiving a single dose of 500 mg/kg survived. Dogs receiving 300 or 500 mg/kg of TPP exhibited convulsions, tremors and other signs described above or sedation and recumbancy. Animals which survived the initial dose showed recovery from initial signs within two days (300 mg/kg group) or three days (500 mg/kg group).

Dogs which received a single dose of 500 mg/kg of TPP exhibited no marked evidence of impaired reflexes or altered neurologic function during the four weeks after dosing. No body weight effect was apparent.

Dogs which received a single dose of 300 mg/kg of TPP with thirteen subsequent doses of 50 mg/kg exhibited signs of neurologic impairment beginning on Day 10 (nine days after the first dose). This was characterized by impaired reflexes and by evidence of front and/or hind limb weakness with inability to stand erect for normal periods of time. Maximum severity of impaired reflexes occurred on Day 14 or 21 with diminished severity of impairment of locomotion, standing and righting reflexes present in three of five dogs by Day 28. Body weights varied with only one animal showing a significant weight loss.

There was no evidence of cholinesterase inhibition in plasma or brain samples from animals treated with TPP.

Animals receiving tri-o-tolyl-phosphate in a single dose of 500 mg/kg or repeated doses of 100 mg/kg exhibited no mortality, no clear evidence of neurologic effects and no clear body weight effects. Loose stools and emesis occurred with greater frequency in this group than in the vehicle control group. Inhibition of plasma cholinesterase activity was evident in samples obtained after fourteen daily doses of 100 mg/kg, and a suggestion of brain cholinesterase inhibition was present terminally (two of six values were below control ranges). No evidence of cholinesterase inhibition was present in plasma and brain samples obtained from dogs receiving a single dose of 500 mg/kg of TOTP.

Carol S. Auletta

Carol S. Auletta, B.A.
Staff Toxicologist

William E Rinehart

William E. Rinehart, Sc.D.
Vice President, Science and
Market Development

Prepared with the technical assistance of:
William G. Braun, M.S., Study Director
Jeffrey P. Lefrancois, B.S., Manager, Large Animal Section
Daniel J. Pisacreta, B.S., Supervisor
Doris H. Milligan, M.T., A.S.M.T., Manager, Clinical Chemistry and Histology Laboratories
Daniel T. LaRosa, Jr., B.A., Report Preparation

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4356 Sex: Male Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	1						
	Flexor Reflex		+	0	0	Animal found dead on Day 2.					
	Extensor Reflex		+	0	0						
	Visual Placing		+	0	1						
	Locomotion		+	0	2						
	Standing		+	0	2						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4361 Sex: Male Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0		Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4367 Sex: Male Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	3						
	Flexor Reflex		+	0	1	Animal killed in a moribund condition on Day 2.					
	Extensor Reflex		+	0	2						
	Visual Placing		+	0	2						
	Locomotion		+	0	3						
	Standing		+	0	3						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4377 Sex: Female Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0		Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4378 Sex: Female Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	1						
	Flexor Reflex		+	0	0	Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0	0						
	Visual Placing		+	0	2						
	Locomotion		+	0	2						
	Standing		+	0	2						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4391 Sex: Female Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0		Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4104 Sex: Female Group: IVC Dose: 50 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		0	1	0	1	0	0	Animal on test for 14 days only.	
	Flexor Reflex		0	0	0	0	0	0		
	Extensor Reflex		0	0	0	0	0	0		
	Visual Placing		0	0	0	0	0	0		
	Locomotion		0	0	0	0	0	0		
	Standing		0	0	0	0	0	0		

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4146 Sex: Female Group: IVC Dose: 50 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		0	0	0	0	0	0	Animal on test for 14 days only.	
	Flexor Reflex		0	0	0	0	0	0		
	Extensor Reflex		0	0	0	0	0	0		
	Visual Placing		0	0	0	0	0	0		
	Locomotion		0	0	0	0	0	0		
	Standing		0	0	0	0	0	0		

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4336 Sex: Male Group: IVD Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	23
Neurologic Examination:	Righting Reflex		0	0	0	0				
	Flexor Reflex		0	0	0	0	Animal on test for 7 days only.			
	Extensor Reflex		0	0	0	0				
	Visual Placing		0	0	0	0				
	Locomotion		0	0	0	0				
	Standing		0	0	0	0				

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4352 Sex: Female Group: IVD Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		0	0	0	0				
	Flexor Reflex		0	0	0	0	Animal on test for 7 days only.			
	Extensor Reflex		0	0	0	0				
	Visual Placing		0	0	0	0				
	Locomotion		0	0	0	0				
	Standing		0	0	0	0				

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

A FOUR WEEK NEUROTOXICITY STUDY IN DOGS WITH TRIPHENYLPHOSPHINE AND TRIPHENYLPHOSPHINE OXIDE

PATHOLOGY REPORT
TERMINAL SACRIFICE

78-2317

A Four Week Neurotoxicity Study in Dogs Pathology Report

Table of Contents

INTRODUCTION

MATERIAL AND METHODS

RESULTS

SUMMARY

Gross Postmortem Observations

Table I - Single Treatment

Table II - 14 Day Treatment

Microscopic Findings

Table III - 14 Day Treatment

A Four Week Neurotoxicity Study in Dogs Pathology Report

INTRODUCTION

The purpose of this study was to assess the neurotoxic potential of triphenylphosphine and triphenylphosphine oxide administered orally to dogs.

MATERIAL AND METHODS

A. Test Materials: Triphenylphosphine (TPP)
Triphenylphosphine oxide (TPPO)
Tri-o-tolyl-phosphate (TOTP-positive control)
Mazola Corn Oil (vehicle)

B. Route of Administration: Gastric intubation

C. Test Animals: 44 male and female Beagle dogs.

D. Experimental Design:

Single Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals Initial M	Initial F	Necropsy Interval M	Necropsy Interval F	
IA	Corn Oil	0	2	2	2	2	29-30 days
IIA	Tri-orotho-tolyl-phosphate (TOTP)	500	2	2	2	2	29-30 days
IIIA	Triphenylphosphine (TPP)	500	2	2	2	2	29-30 days
IVA	Triphenylphosphine oxide (TPPO)	500	2	2	2[a]	2[a]	1-2 days
IVC	Triphenylphosphine oxide (TPPO)	50	-	2	-	2	16 days
IVD	Triphenylphosphine oxide (TPPO)	100	1	1	1	1	8-9 days

[a]Both animals died spontaneously on day 1-2 of the study.

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

EXPERIMENTAL DESIGN (CONT.)

14 Day Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals Initial M	Initial F	Necropsy - Interval M	Necropsy - Interval F	Interval	Histopatholoc M	Histopatholoc F
IB	Corn Oil	0	3	3	3	3	29-30 days	3	3
IIB	Tri-ortho-tolyl-phosphate (TOTP)	100	3	3	3	3	29-30 days	3	3
IIIB	Triphenylphosphine (TPP)	50[a]	3	3[b]	3	3	29-30 days	3	2
IVB	Triphenylphosphine oxide (TPPO)	300	3[c]	3[c]	3	3	1-2 days	0	0

[a]The initial dose was 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.

[b]One animal died spontaneously on Day 2 of the study.

[c]All animals died spontaneously on days 1-2 of the study with the exception of one animal which was killed in a moribund condition.

E. Postmortem Methods:

A complete gross postmortem examination was performed on all animals those dying spontaneously or sacrificed at the end of the experiment. Animals were sacrificed by exsanguination under sodium pentobarbital anesthesia.

Following an external examination the cranial cavity was first opened and the brain removed. A representative section of medulla was dissected and placed on ice for cholinesterase evaluation. The remaining portions of the brain were preserved in 10% neutral buffered formalin for microscopic examination. Portions of the cervical and lumbar spinal cords and both sciatic nerves were dissected and preserved. Thoracic and abdominal cavities were opened and the viscera examined; tissues with grossly visible abnormalities were preserved.

Brain (3 sections) cervical and lumbar spinal cords (1 longitudinal and 2 transversal sections) and both sciatic nerves (1 longitudinal and two transversal sections) were processed and stained with hematoxylin and eosin, and Luxol Fast Blue for microscopic examination.

A Four Week Neurotoxicity Study in Dogs Pathology Report

RESULTS

I. Mortality

a. Single Treatment:

Four dogs given a single dose of 500 mg/kg of TPPO died spontaneously (three on day one, and one on day two of the study).

b. 14-Day Repeated Treatment

Six dogs given a dose of 300 mg/kg/day of TPPO died spontaneously or were sacrificed moribund (four died on day one, one on day two, and one was sacrificed moribund on day two).

One dog given 50 mg/kg/day of TPP died spontaneously on day two of the study.

II. Gross Postmortem Observations

a. Single Treatment

The most common gross observations were that of mottling, edema and congestion of lungs, (Table I).

b. Fourteen Day Treatment

The results of the gross postmortem examinations in individual animals are presented in Table II.

The most common gross observations noted in both control and treated groups were mottling edema and congestion of lungs. Pulmonary edema and congestion were limited to the animals dying spontaneously or killed in extremis.

III. Microscopic Findings

The results of the microscopic examination of brain, spinal cord and sciatic nerves are presented in Table III. No microscopic abnormalities were noted in the six dogs given corn oil and six dogs given Tri-ortho-tolyl-phosphate.

Minimal to moderate multifocal swelling and degeneration of axonal fibers in the white matter of the brain (predominantly in the sections of cerebellum) cervical and lumbar sections of the spinal cord and both sciatic nerves was noted in the five dogs given Triphenylphosphine.

A Four Week Neurotoxicity Study in Dogs Pathology Report

SUMMARY

Six dogs were treated, by gastric intubation with 50 mg/kg/day of Triphenylphosphine for fourteen days. One dog died spontaneously on day two of the study.

Microscopic examination of the brain, spinal cord and sciatic nerves revealed morphologic evidence of injury characterized by multifocal swelling and degeneration of axonal fibers.

No microscopic abnormality was noted in the nervous systems of dogs given corn oil (vehicle) or Tri-ortho-tolyl-phosphate (positive control).

A L Knezevich

Aleksandar L. Knezevich, Dr.Med.Vet.
Director of Pathology

Table I

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
Single Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IA (Control)	M	4357	-	-	N.O.A.
		4363	-	-	N.O.A.
	F	4379	-	-	N.O.A.
		4393	-	-	N.O.A.
IIA	M	4362	-	Lungs	All lobes: white mottling, multiple small, soft, palpable nodules on surfaces.
		4372	-	-	N.O.A.
	F	4380	-	-	N.O.A.
		4394	-	-	N.O.A.
IIIA	M	4371	-	-	N.O.A.
		4373	-	Lungs	All lobes: dark red spots (1-3mm in diam.)
	F	4387	-	-	N.O.A.
		4392	-	-	N.O.A.
IVA	M	4364	-	Lungs	All lobes: mottled dark red; on cut surface large amount of sanguineous fluid released.
				Stomach	Distended with gas, white frothy substance and food.
				Heart	Very firm in consistency.
		4370	-	Lungs	All lobes: mottled dark red; on cut surface moderate amount of sanguineous fluid released.
	F	4381	-	Lungs	All lobes: mottled dark red; on cut surface large amount of sanguineous fluid released.

Table I
(cont.)

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
Single Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IVA (cont.)	F	4385	-	Heart	Very firm in consistency.
IVC	F	4104	-	-	N.O.A.
		4146	-	-	N.O.A.
IVD	M	4336	-	-	N.O.A.
	F	4352	-	-	N.O.A.

Table II

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
14 Day Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IB	M	4358	79-654	Lungs	All lobes: mottled white and red.
		4360	79-655	Lung (L)	Mottled dark red.
		4365	79-656	-	N.O.A.
	F	4383	79-657	-	N.O.A.
		4384	79-658	-	N.O.A.
		4390	79-659	-	N.O.A.
IIB	M	4355	79-660	Lungs	All lobes: mottled white and red.
		4366	79-661	-	N.O.A.
		4369	79-662	-	N.O.A.
	F	4376	79-663	-	N.O.A.
		4382	79-664	Lungs	Red mottling on both diaphragmatic surfaces.
		4386	79-665	-	N.O.A.
IIIB	M	4359	79-666	Lungs	All surfaces: mottled.
		4368	79-667	-	N.O.A.
		4374	79-668	Lungs	All lobes: mottled white and red.
	F	4375	79-669	-	N.O.A.
		4389	79-670	Lungs	All lobes: mottled red.
		4388*	-	Lungs	All lobes: mottled red; on cut surface large amount of sanguineou fluid released.
				Stomach	Distended with gas.

*Animal died spontaneously

Table II
(cont.)

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
14 Day Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IVB	M	4356*	-	Lungs	All lobes: mottled red; on cut surface moderate amount of sanguineous fluid released.
		4361*	-	Lungs Stomach	All lobes: mottled dark red. Distended with gas.
		4367**	-	Lungs	All lobes: mottled dark red; on cut surface large amount of sanguineous fluid released.
	F	4377*	-	Lungs	All lobes: mottled dark red; on cut surface sanguineous fluid released.
				Stomach	Distended with gas.
		4391*	-	Lungs	All lobes: mottled dark red; on cut surface sanguineous fluid released.
		4378*	-	Lungs	All lobes: mottled red and pin-point size red foci.
				Stomach	Distended with gas.
				Heart	Firm, contracted.

* Animal died spontaneously
** Animal killed in moribund condition.

A Four Week Neurotoxicity Study in Dogs Pathology Report

General Key to Histopathology Tables

N	=	Not remarkable, within normal histologic limits
1	=	Minimal degree or amount of indicated change
2	=	Mild degree or amount of indicated change
3	=	Moderate degree or amount of indicated change
4	=	Marked degree or amount of indicated change
H&E	=	hematoxylin and eosin stain
LFB	=	Luxol Fast Blue stain

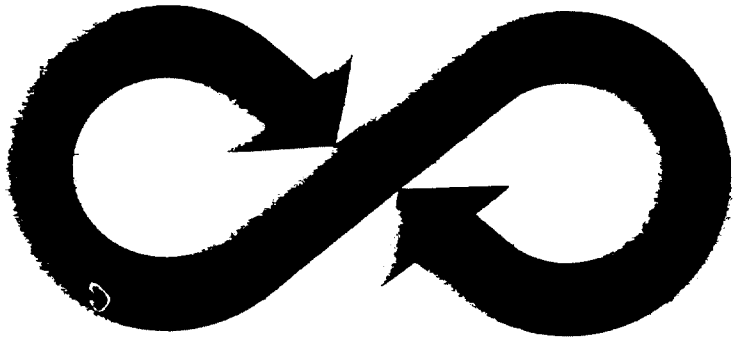
Table III

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Microscopic Findings
14 Day Treatment

Animal Number	43584	43605	43665	43683	43884	43904	43955	43966	43669	43767	43882	43866	43359	43568	43674	43775	43890
Sex	M	M	M	F	F	F	M	M	M	F	F	F	M	M	M	F	F
Pathology Number 79-	65454	65455	65456	65457	65458	65459	66060	66061	66062	66063	66064	66065	66666	66667	66668	66669	66770
Group B-	I	I	I	I	I	I	II	II	II	II	II	II	III	III	III	III	III
BRAIN, three transverse sections (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N					
multifocal swelling and degeneration of axons													2	2	1	1	2
CERVICAL SPINAL CORD, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N					
multifocal swelling and degeneration of axons													2	3	1	1	2
LUMBAR SPINAL CORD, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N					
multifocal swelling and degeneration of axons													2	3	1	1	2
LEFT SCIATIC NERVE, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N			N	N	
multifocal swelling and degeneration of axons													1	1			1
focal mononuclear cell infiltrate													1				
RIGHT SCIATIC NERVE, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N			N	N	
multifocal swelling and degeneration of axons													1	1			1



Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO. 78-2317

A FOUR WEEK NEUROTOXICITY STUDY IN DOGS WITH TRIPHENYLPHOSPHINE AND TRIPHENYLPHOSPHINE OXIDE

Submitted to: M & T Chemicals, Inc.
Rahway, New Jersey 07065

Date: March 1, 1979

PROJECT 78-2317

A FOUR WEEK NEUROTOXICITY STUDY

IN DOGS

ABSTRACT

This study was designed to evaluate the neurotoxic effects of triphenylphosphine (TPP) and triphenylphosphine oxide (TPPO) when administered orally to dogs in single doses of 500 mg/kg and repeated doses (fourteen days) of 300 mg/kg/day. Positive control dogs received a known neurotoxin, tri-o-tolyl-phosphate (TOTP), in single doses of 500 mg/kg or repeated doses of 100 mg/kg/ day. Vehicle control dogs received corn oil in single or repeated doses. Each single-dose group consisted of two males and two females; each repeated dose group consisted of three males and three females. The duration of study was four weeks, thus providing a two or four week post-dose observation period. Because of mortality observed after the first dose of TPP at 300 mg/kg subsequent repeated doses were lowered to 50 mg/kg/day. Because of mortality in all dogs receiving 300 and 500 mg/kg of TPPO, additional groups of two dogs each were later administered single doses of 100 and 50 mg/kg with post-dose observation periods of one and two weeks, respectively. Plasma cholinesterase activity was measured pretest and three hours after the last dose. Brain cholinesterase activity was measured terminally.

Triphenylphosphine oxide (TPPO) produced death in all dogs after single doses of 300 and 500 mg/kg. Death was preceded by violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming" movements, aggressive behavior, vocalization, excessve salivation, dyspnea and emesis. Two dogs receiving 100 mg/kg showed similar signs after dosing but recovered by the following day with no unusual signs for seven subsequent days. Two dogs receiving 50 mg/kg/day exhibited no unusual signs after dosing or during a fourteen day post-dose observation period. No impairment of reflexes was noted in dogs receiving 100 and 50 mg/kg for seven or fourteen days, respectively, after dosing. Slight body weight losses occurred in dogs receiving 50 mg/kg only. Cholinesterase values gave no clear indication of inhibition resulting from TPPO administration.

One of six dogs receiving 300 mg/kg of triphenylphosphine (TPP) died after dosing; predeath signs were similar to those seen in dogs which died after receiving TPPO. All four dogs receiving a single dose of 500 mg/kg survived. Dogs receiving 300 or 500 mg/kg of TPP exhibited convulsions, tremors and other signs described above or sedation and recumbancy. Animals which survived the initial dose showed recovery from initial signs within two days (300 mg/kg group) or three days (500 mg/kg group).

Dogs which received a single dose of 500 mg/kg of TPP exhibited no marked evidence of impaired reflexes or altered neurologic function during the four weeks after dosing. No body weight effect was apparent.

Dogs which received a single dose of 300 mg/kg of TPP with thirteen subsequent doses of 50 mg/kg exhibited signs of neurologic impairment beginning on Day 10 (nine days after the first dose). This was characterized by impaired reflexes and by evidence of front and/or hind limb weakness with inability to stand erect for normal periods of time. Maximum severity of impaired reflexes occurred on Day 14 or 21 with diminished severity of impairment of locomotion, standing and righting reflexes present in three of five dogs by Day 28. Body weights varied with only one animal showing a significant weight loss.

There was no evidence of cholinesterase inhibition in plasma or brain samples from animals treated with TPP.

Animals receiving tri-o-tolyl-phosphate in a single dose of 500 mg/kg or repeated doses of 100 mg/kg exhibited no mortality, no clear evidence of neurologic effects and no clear body weight effects. Loose stools and emesis occurred with greater frequency in this group than in the vehicle control group. Inhibition of plasma cholinesterase activity was evident in samples obtained after fourteen daily doses of 100 mg/kg, and a suggestion of brain cholinesterase inhibition was present terminally (two of six values were below control ranges). No evidence of cholinesterase inhibition was present in plasma and brain samples obtained from dogs receiving a single dose of 500 mg/kg of TOTP.

TABLE OF CONTENTS

I. INTRODUCTION..... 1

II. MATERIALS AND METHODS..... 1

A. Test Materials..... 1
B. Vehicle..... 1
C. Test Animals..... 1
D. Selection and Group Assignment..... 2
E. Animal Identification..... 2
F. Experimental Outline..... 3
G. Husbandry..... 4
H. Test Substance Administration..... 4
I. Observations..... 4
J. Body Weight..... 4
K. Laboratory Studies..... 5
L. Post-Mortem..... 5

III. RESULTS AND DISCUSSION 6

A. Mortality..... 6
B. Observations..... 6
C. Neurological Examinations..... 8
D. Body Weights..... 9
E. Cholinesterase Values..... 10

IV. SUMMARY..... 12

TABLES

I. Individual Body Weights and Mortality Data..... 15
II. Summary of Neurological Responses..... 17
III. Individual Plasma and Brain Cholinesterase Values..... 29

APPENDICES

A. Methodology and References..... A-1
B. Individual Neurological Evaluations..... B-1

78-2317

I. INTRODUCTION

The purpose of this study, conducted for M&T Chemicals, Inc., was to assess the potential of triphenylphosphine and triphenylphosphine oxide to produce neurotoxic or other adverse effects in Beagle dogs following single and repeated administration (14 consecutive daily doses) via gastric intubation. A known neurotoxic material (tri-o-tolyl-phosphate) was administered as a positive control. After 28 days, all surviving animals were sacrificed. This study was performed at Bio/dynamics, Inc., East Millstone, New Jersey.

II. MATERIALS AND METHODS

A. Test Materials

Test Substance	Description	Date Received	Label Information
Triphenyl-phosphine (TPP)	Off-white powder	26 September 1978	Triphenylphosphine WTVAH-352K
Triphenyl-phosphine oxide (TPPO)	Off-white powder	26 September 1978	T.P.P.O. VYVCD- IR#2
Tri-o-tolyl-phosphate (TOTP) (positive control)	Clear viscous liquid	25 October 1978	Tri-o-tolylphosphate practical Lot No. A7A (container #1) Lot No. B4A (container #2)

Supplier: TPP and TPPO from M&T Chemicals, Inc., Rahway, New Jersey; TOTP from Scientific Products, Edison, New Jersey.
Storage: All at room temperature.

B. Vehicle: Mazola® Corn Oil

C. Test Animals: Dogs

Strain: Beagle

Number of Animals: 44 (21 Male; 23 Female)

Supplier: (40 initial animals) Marshall Research Animals, Inc. North Rose, New York

Date Received: (40 initial animals) 7 November 1978

Age at Receipt: 5-7 months

(Note - The four additional animals used on this study were received in the following manner: Animals 4336M and 4352F were received on 9 October 1978 from White Eagle Laboratories, Doylestown, Pa.; Animal 4146F was received on 13 June 1978 and Animal 4104F on 4 April 1978 from Marshall Research Animals, Inc.)

Weight at Initiation of Treatment (kilograms):

	Mean	Range
Males	9.2	7.7-10.7
Females	7.6	6.0-11.3

Immunization: Against distemper, hepatitis, bordetella, leptospirosis and oral papillomas by supplier.

Parasites (intestinal): Anthelmintics (piperazine, thiabendazole) were routinely administered by supplier as required.

Physical Examination: Pretest examinations were conducted by Bio/dynamics' Staff Veterinarian (H. E. Blatt, V.M.D.) (Meth. and Ref., Appendix A)

D. Selection and Group Assignment: Dogs were ranked by body weight and distributed into five blocks of five animals per sex so that body weight means for each group were comparable. Groups were designated randomly as I, II, III, or IV. Two dogs per sex per group were placed on the single treatment study; the remaining 3 dogs per sex per group were placed on the repeated treatment study. Because of mortality which occurred at high doses (300 and 500 mg/kg) of TPPO, four additional dogs were selected from untreated animals equilibrated at Bio/dynamics and placed in groups as described in the Experimental Outline.

E. Animal Identification: Each dog was assigned a unique Bio/dynamics identification number which appeared on the animal's cage. In addition each animal bore an ear tatoo with a USDA number.

F. Experimental Outline

Single Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals M	F	Lab Studies[a]	Sacrifice/ Necropsy	Interval
IA	Corn Oil	0	2	2	All	All	29-30 days
IIA	Tri-ortho-tolyl-phosphate (TOTP)	500	2	2	All	All	29-30 days
IIIA	Triphenylphosphine (TPP)	500	2	2	All	All	29-30 days
IVA	Triphenylphosphine oxide (TPPO)	500	2	2	b	S.D.[c]	1-2 days
IVC	Triphenylphosphine oxide (TPPO)	50	-	2	All[d]	All	16 days
IVD	Triphenylphosphine oxide (TPPO)	100	1	1	All[e]	All	8-9 days

[a]Plasma cholinesterase was evaluated on the day prior to treatment and three hours after treatment; brain (medulla) cholinesterase was evaluated immediately after death.
[b]Animals died spontaneously on Day 1; three hour post treatment plasma cholinesterase evaluated in one animal.
[c]S.D. = Spontaneous Death
[d]Animals entered study on Day 15; only brain cholinesterase evaluated.
[e]Animals entered study on Day 22; only brain cholinesterase evaluated.

14-Day Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals M	F	Lab Studies[b]	Sacrifice/ Necropsy	Interval
IB	Corn Oil	0	3	3	All	All	29-30 days
IIB	Tri-ortho-tolyl-phosphate (TOTP)	100	3	3	All	All	29-30 days
IIIB	Triphenylphosphine (TPP)	50[a]	3	3	5[c]	5	29-30 days
IVB	Triphenylphosphine oxide (TPPO)	300	3	3	All[d]	S.D.[e]	1-2 days

[a]The initial dosing was performed at 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.
[b]Plasma cholinesterase was evaluated on the day prior to treatment and three hours following each animal's last treatment; brain (medulla) cholinesterase was evaluated immediately after death.
[c]One animal died spontaneously on Day 2 of the study and only pretest plasma cholinesterase was evaluated.
[d]One animal was killed in a moribund condition and 5 animals died spontaneously; only pretest plasma cholinesterase was evaluated.
[e]S.D. = Spontaneous Death of 5 animals; one animal was killed in a moribund condition.

G. Husbandry:

Acclimation Period:	7-29 November 1978
Housing:	Individually in elevated metal grid cages.
Food:	400 grams of Standard laboratory diet (Wayne Bite Size®)
Water:	ad libitum. By automated water system (Elizabethtown Water Company)
Environmental Conditions:	12 hour light/dark cycle.
	Temperature monitored twice daily.

H. Test Substance Administration:

Route:	Gastric Intubation
Method:	Appropriate mixtures of test substance in corn oil were administered. Individual dose volumes were adjusted by most recent weekly body weight.
Dose Volume:	Single Treatment: 5 ml/kg (except for single doses of 50 and 100 mg/kg of TPPO which were administered in volumes of 0.5 and 1.0 ml/kg, respectively)
	14-Day Repeated Treatment: 3 ml/kg (except for 50 mg/kg dose of TPPO which was administered in a volume of 0.5 ml/kg)
Frequency:	Single dose (30 November 1978)
	14-Day Repeated Treatment: Daily (7 days per week) (30 November - 13 December 1978)

I. Observations:

For Mortality and Gross Signs of Toxicologic or Pharmacologic Effects:	Daily
Neurologic:	Pretest and Days 0, 1, 4, 7, 10, 14, 21 and 28. (performed 3 hours after dosing)

J. Body Weight: Pretest, weekly during treatment and on Day 28. (Meth. and Ref., Appendix A)

K. Laboratory Studies: (Meth. and Ref.; Appendix A)

Blood was obtained from unanesthetized dogs via jugular venipuncture.

Number of Animals and Time Intervals: See Experimental Outline

Parameter Evaluation

Clinical Chemistry:

cholinesterase
brain
plasma

L. Post-Mortem:

Animals Dying Spontaneously: Complete gross-post-mortem examination.

Necropsy: 28-29 December 1978

Number of Animals: All survivors.

Sacrifice Method: Exsanguination under sodium pentobarbital anesthesia.

Tissues Preserved: All animals.

brain (2 transverse sections)
nerve (left, right sciatic - 1 longitudinal, 2 transverse)
spinal cord (cervical, lumbar - 1 longitudinal, 2 transverse)
all gross lesions

Preservatives: 10% neutral buffered formalin: all tissues

Stain: Hematoxylin and Eosin
Luxol-fast Blue
(Meth. and Ref.; Appendix A)

Tissue Examined Histopathologically: All nerve tissues listed above from surviving animals of the 14-Day Repeated treatment with corn oil, TOTP and TPP.

(To be forwarded as an addendum or separate report.)

III. RESULTS AND DISCUSSION

A. Mortality (Table I)

1. TPPO

All dogs receiving triphenylphosphine oxide (TPPO) at doses of 300 and 500 mg/kg died after a single dose. Death was preceded by violent convulsions and other signs of neurotoxic effect as described below. Administration of atropine to one animal which received 500 mg/kg (Female No. 4381) did not prevent death. Dogs receiving single doses of 50 and 100 mg/kg of TPPO survived throughout the subsequent observation period (14 and 7 days, respectively).

2. TPP

One dog (of six) died after a single dose of 300 mg/kg of triphenylphosphine (TPP); predeath signs were similar to those seen in dogs which died after receiving TPPO. The five other dogs survived an initial dose of 300 mg/kg of TPP and subsequent doses of 50 mg/kg/day for thirteen days. Four dogs which received a single dose of 500 mg/kg of TPP also survived.

3. Controls

No deaths occurred in the vehicle control group or in the positive control group, which received tri-o-tolyl-phosphate (TOTP) in a single dose of 500 mg/kg or in repeated doses of 100 mg/kg.

B. Observations

1. TPPO

Dogs which received TPPO in doses of 300 and 500 mg/kg exhibited violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming movements, aggressive behavior, excessive salivation, dyspnea, vocalization and occasional emesis, followed by death in all animals.

Two dogs which received a single dose of 100 mg TPPO per kg exhibited similar signs after dosing but recovered by the following day. These dogs exhibited no additional unusual signs (except for single occurrences of loose stools and emesis) during the seven-day post-dose observation period.

Two dogs which received a single dose of 50 mg TPPO/kg showed no unusual signs immediately after dosing or during a fourteen-day post-dose observation period.

2. TPP

Three of four dogs which received a single dose of 500 mg TPP per kg exhibited convulsions, tremors and other signs similar to those seen in dogs receiving high doses of TPPO; the fourth dog exhibited sedation and recumbancy. Dogs were generally lethargic on the day following dosing but had all recovered by Day 4 (three days after dosing). Occasional loose stools or emesis were the only unusual signs noted in these animals for the remainder of the four week post-dose observation period.

Five of six dogs receiving a single dose of 300 mg TPP per kg exhibited convulsions, tremors and other signs described previously after dosing; the sixth dog exhibited sedation and recumbancy. One dog was found dead the day after dosing; other dogs were lethargic or recumbant. (Because of the reaction to the first dose, subsequent doses were lowered to 50 mg/kg/day.) The five surviving dogs showed recovery from the initial signs by Day 3 (two days after the first dose). However, beginning on Day 13 or 14 of study, these dogs exhibited evidence of front and/or hind limb weakness with inability to stand erect for normal periods of time; anorexia was also noted in some animals. These signs persisted until termination of study. Other signs noted included occasional occurrences of loose stools or emesis.

3. Control

Animals in both control groups exhibited loose stools and emesis during the study. Incidence of both of these findings was higher in the TOTP-treated (positive control) group than in the vehicle (corn oil) control group. One TOTP-treated dog (No. 4376, female) showed excessive salivation and aggressive behavior prior to dosing on a single occasion (Day 6).

C. Neurological Examinations (Table II, Appendix B)

1. TPPO

All four dogs which were still alive at the time of neurological examinations on the day of dosing with 300 or 500 mg/kg of TPPO exhibited impaired visual placing, locomotion, standing and righting reflexes. Flexor and extensor reflexes were unaffected in three of the four dogs.

No evidence of impaired reflexes was present in dogs receiving single doses of 100 or 50 mg TPPO per kg for seven or fourteen days, respectively, after dosing.

2. TPP

Except for a clearly impaired righting reflex seen in one dog (No. 4387, female) on Day 4 (3 days post-dose), animals which received a single dose of 500 mg TPP per kg exhibited only doubtful or minimal impairment of reflexes during the four week post-dose observation period. At the final (28-day) observation, the only abnormalities noted were minimally-impaired righting reflexes in two of four dogs and minimally-impaired flexor and extensor reflexes in a single animal.

Of the animals which received one dose of 300 mg TPP per kg followed by thirteen daily doses of 50 mg/kg four out of five had normal reflexes on Days 1, 4 and 7, with one animal exhibiting minimal or doubtful impairment of the extensor and/or righting reflexes on Days 4 and 7. Impairment

(minimal to complete) of the locomotion and/or standing reflexes was evident in all dogs on Day 10, and impairment of the righting reflex was present in all dogs on Day 14. These reflexes remained impaired (except for a single dog on Day 21) for the remainder of the study. Maximum severity generally occurred on Day 14 or 21, with diminished severity present in three of the five dogs by Day 28. Impairment of the flexor, extensor and visual placing reflexes was seen in most dogs on Days 14, 21 and/or 28; incidence and severity of impairment of these reflexes was generally less than that seen for the righting, standing and locomotion reflexes. Because some decrease in severity of neurologic effects was noted on Day 28 (fourteen days after termination of dosing) it is possible that these neurologic changes are reversible.

3. Controls

One dog (No. 4355, male) receiving repeated doses of 100 mg/kg/day of TOTP had minimal or doubtful impairment of the righting and extensor reflexes on Day 14 only. Reflexes were normal on all other occasions for this dog and throughout the study for all other positive control and all negative control dogs.

D. Body Weights (Table I)

1. TPPO

Animals receiving 50 mg/kg of TPPO showed slight body weight losses one week after dosing with subsequent slight gains the following week. Animals receiving 100 mg/kg of TPPO showed slight gains one week after dosing. (Animals receiving 300 and 500 mg/kg of TPPO died prior to body weight measurement).

2. TPP

Animals receiving repeated doses of TPP (50 mg/kg/day after initial dose of 300 mg/kg) showed slight initial weight losses, with subsequent

slight gains. Overall weight gains were slightly less than vehicle control gains in two dogs and comparable to control gains in two dogs. One animal exhibited a substantial (1.6 kg) overall weight loss.

Body weight gains of dogs receiving a single 500 mg/kg dose of TPP were comparable to gains of vehicle control dogs.

3. Control

Body weight gains of dogs receiving a single 500 mg/kg dose of TOTP were comparable to weight gains of vehicle control dogs. Body weight gains of dogs receiving repeated (50 mg/kg/day) doses of TOTP were comparable to those of vehicle control dogs for four of six animals and slightly lower for two animals.

E. Cholinesterase Values (Table III)

1. TPPO

Because of spontaneous mortality at the 300 and 500 mg/kg doses of TPPO, only one plasma sample was obtained for analysis (No. 4370, male, 500 mg/kg). No inhibition of cholinesterase was evident.

The terminal brain cholinesterase activity of one of two dogs receiving a single dose of 50 mg/kg of TPPO was slightly lower than activity seen for vehicle control dogs. Because of the small difference noted and the small number of animals at this dose level, the significance of this finding is questionable. Terminal brain cholinesterase values for the two dogs receiving 100 mg/kg of TPPO were within ranges seen in vehicle control animals.

2. TPP

There was no evidence of cholinesterase inhibition in plasma or brain samples from animals receiving an initial dose of 300 mg/kg of TPP followed by thirteen daily doses of 50 mg/kg or in animals receiving a single dose of 500 mg/kg.

3. Controls

Plasma cholinesterase activity was inhibited in samples obtained three hours after the fourteenth daily dose of 100 mg/kg of TOTP in all dogs in this group. Terminal brain cholinesterase activity values were within control ranges for four of six dogs and slightly lower than control for two dogs.

Cholinesterase activity in plasma and brain samples obtained from dogs receiving a single dose of 500 mg/kg of TOTP was generally comparable to activity seen in vehicle control dogs.

IV. SUMMARY

This study was designed to evaluate the neurotoxic effects of triphenylphosphine (TPP) and triphenylphosphine oxide (TPPO) when administered orally to dogs in single doses of 500 mg/kg and repeated doses (fourteen days) of 300 mg/kg/day. Positive control dogs received a known neurotoxin, tri-o-tolyl-phosphate (TOTP), in single doses of 500 mg/kg or repeated doses of 100 mg/kg/ day. Vehicle control dogs received corn oil in single or repeated doses. Each single-dose group consisted of two males and two females; each repeated dose group consisted of three males and three females. The duration of study was four weeks, thus providing a two or four week post-dose observation period. Because of mortality observed after the first dose of TPP at 300 mg/kg subsequent repeated doses were lowered to 50 mg/kg/day. Because of mortality in all dogs receiving 300 and 500 mg/kg of TPPO, additional groups of two dogs each were later administered single doses of 100 and 50 mg/kg with post-dose observation periods of one and two weeks, respectively. Plasma cholinesterase activity was measured pretest and three hours after the last dose. Brain cholinesterase activity was measured terminally.

Triphenylphosphine oxide (TPPO) produced death in all dogs after single doses of 300 and 500 mg/kg. Death was preceded by violent convulsions, tremors, uncoordinated body movements, "paddling" and "swimming" movements, aggressive behavior, vocalization, excessve salivation, dyspnea and emesis. Two dogs receiving 100 mg/kg showed similar signs after dosing but recovered by the following day with no unusual signs for seven subsequent days. Two dogs receiving 50 mg/kg/day exhibited no unusual signs after dosing or during a fourteen day post-dose observation period. No impairment of reflexes was noted in dogs receiving 100 and 50 mg/kg for seven or fourteen days, respectively, after dos-

ing. Slight body weight losses occurred in dogs receiving 50 mg/kg only. Cholinesterase values gave no clear indication of inhibition resulting from TPPO administration.

One of six dogs receiving 300 mg/kg of triphenylphosphine (TPP) died after dosing; predeath signs were similar to those seen in dogs which died after receiving TPPO. All four dogs receiving a single dose of 500 mg/kg survived. Dogs receiving 300 or 500 mg/kg of TPP exhibited convulsions, tremors and other signs described above or sedation and recumbancy. Animals which survived the initial dose showed recovery from initial signs within two days (300 mg/kg group) or three days (500 mg/kg group).

Dogs which received a single dose of 500 mg/kg of TPP exhibited no marked evidence of impaired reflexes or altered neurologic function during the four weeks after dosing. No body weight effect was apparent.

Dogs which received a single dose of 300 mg/kg of TPP with thirteen subsequent doses of 50 mg/kg exhibited signs of neurologic impairment beginning on Day 10 (nine days after the first dose). This was characterized by impaired reflexes and by evidence of front and/or hind limb weakness with inability to stand erect for normal periods of time. Maximum severity of impaired reflexes occurred on Day 14 or 21 with diminished severity of impairment of locomotion, standing and righting reflexes present in three of five dogs by Day 28. Body weights varied with only one animal showing a significant weight loss.

There was no evidence of cholinesterase inhibition in plasma or brain samples from animals treated with TPP.

Animals receiving tri-o-tolyl-phosphate in a single dose of 500 mg/kg or repeated doses of 100 mg/kg exhibited no mortality, no clear evidence of neurologic effects and no clear body weight effects. Loose stools and emesis occurred with greater frequency in this group than in the vehicle control group. Inhibition of plasma cholinesterase activity was evident in samples obtained after fourteen daily doses of 100 mg/kg, and a suggestion of brain cholinesterase inhibition was present terminally (two of six values were below control ranges). No evidence of cholinesterase inhibition was present in plasma and brain samples obtained from dogs receiving a single dose of 500 mg/kg of TOTP.

Carol S. Auletta

Carol S. Auletta, B.A.
Staff Toxicologist

William E Rinehart

William E. Rinehart, Sc.D.
Vice President, Science and
Market Development

Prepared with the technical assistance of:
William G. Braun, M.S., Study Director
Jeffrey P. Lefrancois, B.S., Manager, Large Animal Section
Daniel J. Pisacreta, B.S., Supervisor
Doris H. Milligan, M.T., A.S.M.T., Manager, Clinical Chemistry and Histology Laboratories
Daniel T. LaRosa, Jr., B.A., Report Preparation

Table I
A Four Week Neurotoxicity Study
in Dogs

Individual Body Weights and Mortality Data

Single Treatment

Group	Day: An. No.	0[a]	7	14	21	28	Weight Gain 0-28 Days (kg)	Death Mortality Type[b]	Day
		Body Weights (kg)							
IA	4363M	8.5	8.5	9.2	8.8	9.0	0.5	T	29
Corn Oil	4357M	8.7	8.7	8.6	8.9	8.9	0.2	T	29
(0 mg/kg)	4393F	6.4	6.5	6.8	6.6	7.0	0.6	T	30
	4379F	6.3	6.1	6.2	6.2	6.4	0.1	T	30
IIA	4362M	8.2	8.5	9.1	8.6	9.0	0.8	T	29
T.O.T.P.	4372M	8.3	8.6	9.3	8.9	9.5	1.2	T	29
(500 mg/kg)	4394F	6.8	7.1	7.4	7.1	7.8	1.0	T	30
	4380F	6.9	7.1	7.3	8.0	7.7	0.8	T	30
IIIA	4371M	8.5	8.8	9.3	9.1	9.7	1.2	T	29
T.P.P.	4373M	7.7	7.8	8.5	8.2	8.4	0.7	T	29
(500 mg/kg)	4387F	7.2	7.1	7.6	7.3	7.8	0.6	T	30
	4392F	6.5	6.5	6.7	6.9	7.3	0.8	T	30
IVA	4370M	9.0	-	-	-	-	-	F	2
T.P.P.O.	4364M	9.7	-	-	-	-	-	S	1
(500 mg/kg)	4385F	7.1	-	-	-	-	-	S	1
	4381F	6.0	-	-	-	-	-	S	1
	Day:			0[a]	7	14			
IVC	4104F			9.1	8.6	8.7	-0.4 (14-day	T	16
T.P.P.O.	4146F			11.3	11.0	11.3	0.0 inter-	T	16
(50 mg/kg)							val)		
	Day:				0[a]	7			
IVD	4336M				9.7	9.9	0.2 (7-day	T	8
T.P.P.O.	4352F				8.1	8.6	0.5 inter-	T	9
(100 mg/kg)							val)		

[a]Day 0 represents day prior to dosing for all animals.
[b]S = Spontaneous Death
F = Found Dead
T = Terminal Sacrifice

Table I (cont.)
A Four Week Neurotoxicity Study
in Dogs

Individual Body Weights and Mortality Data

14-Day Repeated Treatment

Group	Day: An.No.	0[a]	7	14	21	28	Weight Gain 0-28 Days (kg)	Death Type[b]	Death Day
		Body Weights (kg)							
IB	4360M	10.2	10.0	10.2	10.2	10.4	0.2	T	29
Corn Oil	4358M	9.8	10.1	10.2	9.9	10.2	0.4	T	29
(0 mg/kg)	4365M	8.8	8.7	9.4	9.1	9.3	0.5	T	29
	4384F	8.2	8.2	8.5	8.6	8.9	0.7	T	30
	4390F	7.7	7.7	7.8	7.8	7.9	0.2	T	30
	4383F	7.3	7.2	7.5	7.2	7.6	0.3	T	30
IIB	4355M	10.5	9.8	9.0	9.4	10.2	-0.3	T	29
T.O.T.P.	4366M	9.0	8.8	9.2	9.1	9.8	0.8	T	29
(100 mg/kg)	4369M	8.0	7.7	8.5	8.6	8.7	0.7	T	29
	4376F	9.0	8.6	8.8	8.9	9.0	0.0	T	30
	4382F	6.8	6.8	6.5	6.9	7.2	0.4	T	30
	4386F	6.7	6.3	6.2	6.5	7.2	0.5	T	30
IIIB[c]	4359M	11.2	10.5	9.3	8.9	9.6	-1.6	T	29
T.P.P.	4374M	9.1	9.0	9.0	8.7	9.3	0.2	T	29
(50 mg/kg)	4368M	8.9	8.9	8.5	8.3	8.9	0.0	T	29
	4375F	8.2	7.6	8.0	8.0	8.3	0.1	T	30
	4389F	7.6	7.4	6.8	7.4	8.3	0.7	T	30
	4388F	7.1	-	-	-	-	-	F	2
IVB	4361M	10.3	-	-	-	-	-	F	1
T.P.P.O.	4367M	10.7	-	-	-	-	-	M	2
(300 mg/kg)	4356M	7.7	-	-	-	-	-	F	2
	4378F	8.6	-	-	-	-	-	S	1
	4391F	7.6	-	-	-	-	-	S	1
	4377F	7.6	-	-	-	-	-	S	1

[a]Day 0 represents day prior to dosing for all animals.
[b]S = Spontaneous Death
F = Found Dead
T = Terminal Sacrifice
M = Killed in Moribund Condition
[c]The initial dosing was performed at 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.

78-2317

Table II
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Interval: Pretest (One Week Prior to Treatment)

Single Treatment

Group:	IA			IIA			IIIA			IVA		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	4	0	0	4	0	0	4	0	0	4	0	0
Flexor Reflex	4	0	0	4	0	0	4	0	0	4	0	0
Extensor Reflex	4	0	0	4	0	0	4	0	0	4	0	0
Visual Placing	4	0	0	4	0	0	4	0	0	4	0	0
Locomotion	4	0	0	4	0	0	4	0	0	4	0	0
Standing	4	0	0	4	0	0	4	0	0	4	0	0

Numbers represent number of animals (out of 4) exhibiting indicated response.

14-Day Repeated Treatment

Group:	IB			IIB			IIIB			IVB		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	6	0	0	6	0	0	6	0	0	6	0	0
Flexor Reflex	6	0	0	6	0	0	6	0	0	6	0	0
Extensor Reflex	6	0	0	6	0	0	6	0	0	6	0	0
Visual Placing	6	0	0	6	0	0	6	0	0	6	0	0
Locomotion	6	0	0	6	0	0	6	0	0	6	0	0
Standing	6	0	0	6	0	0	6	0	0	6	0	0

Numbers represent number of animals (out of 6) exhibiting indicated response.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

78-2317

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Interval: Day 0 (Day Prior to Treatment)

Single Treatment

Group:	IA			IIA			IIIA			IVA		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	4	0	0	4	0	0	4	0	0	4	0	0
Flexor Reflex	4	0	0	4	0	0	4	0	0	4	0	0
Extensor Reflex	4	0	0	4	0	0	4	0	0	4	0	0
Visual Placing	4	0	0	4	0	0	4	0	0	4	0	0
Locomotion	4	0	0	4	0	0	4	0	0	4	0	0
Standing	4	0	0	4	0	0	4	0	0	4	0	0

Numbers represent number of animals (out of 4) exhibiting indicated response.

14-Day Repeated Treatment

Group:	IB			IIB			IIIB			IVB		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	6	0	0	6	0	0	6	0	0	6	0	0
Flexor Reflex	6	0	0	6	0	0	6	0	0	6	0	0
Extensor Reflex	6	0	0	6	0	0	6	0	0	6	0	0
Visual Placing	6	0	0	6	0	0	6	0	0	6	0	0
Locomotion	6	0	0	6	0	0	6	0	0	6	0	0
Standing	6	0	0	6	0	0	6	0	0	6	0	0

Numbers represent number of animals (out of 6) exhibiting indicated response.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Interval: Day 1

Single Treatment

Group:	IA			IIA			IIIA			IVA[a]		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	4	0	0	4	0	0	3	1	0	0	1	0
Flexor Reflex	4	0	0	4	0	0	4	0	0	1	0	0
Extensor Reflex	4	0	0	4	0	0	4	0	0	1	0	0
Visual Placing	4	0	0	4	0	0	3	1	0	0	0	1
Locomotion	4	0	0	4	0	0	3	1	0	0	0	1
Standing	4	0	0	4	0	0	3	1	0	0	1	0

Numbers represent number of animals (out of 4) exhibiting indicated response.

14-Day Repeated Treatment

Group:	IB			IIB			IIIB			IVB[a]		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	6	0	0	6	0	0	6	0	0	0	2	1
Flexor Reflex	6	0	0	6	0	0	6	0	0	2	1	0
Extensor Reflex	6	0	0	6	0	0	6	0	0	2	0	1
Visual Placing	6	0	0	6	0	0	6	0	0	0	1	2
Locomotion	6	0	0	6	0	0	6	0	0	0	0	3
Standing	6	0	0	6	0	0	6	0	0	0	0	3

Numbers represent number of animals (out of 6) exhibiting indicated response.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

[a]Three animals died spontaneously prior to this evaluation.

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Interval: Day 4

Single Treatment

Group:	IA			IIA			IIIA			IVA		
Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	4	0	0	4	0	0	3	0	1			
Flexor Reflex	4	0	0	4	0	0	4	0	0	All Animals Dead		
Extensor Reflex	4	0	0	4	0	0	4	0	0			
Visual Placing	4	0	0	4	0	0	4	0	0			
Locomotion	4	0	0	4	0	0	4	0	0			
Standing	4	0	0	4	0	0	4	0	0			

Numbers represent number of animals (out of 4) exhibiting indicated response.

14-Day Repeated Treatment

Group:	IB			IIB			IIIB[a]			IVB		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	6	0	0	6	0	0	5	0	0			
Flexor Reflex	6	0	0	6	0	0	5	0	0	All Animals Dead		
Extensor Reflex	6	0	0	6	0	0	4	1	0			
Visual Placing	6	0	0	6	0	0	5	0	0			
Locomotion	6	0	0	6	0	0	5	0	0			
Standing	6	0	0	6	0	0	5	0	0			

Numbers represent number of animals (out of 6) exhibiting indicated response.
[a]One animal died spontaneously on Day 2 of this study.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Interval: Day 7

Single Treatment

Group:	IA			IIA			IIIA			IVA		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	4	0	0	4	0	0	4	0	0	All Animals Dead		
Flexor Reflex	4	0	0	4	0	0	4	0	0			
Extensor Reflex	4	0	0	4	0	0	4	0	0			
Visual Placing	4	0	0	4	0	0	4	0	0			
Locomotion	4	0	0	4	0	0	4	0	0			
Standing	4	0	0	4	0	0	4	0	0			

Numbers represent number of animals (out of 4) exhibiting indicated response.

14-Day Repeated Treatment

Group:	IB			IIB			IIIB[a]			IVB		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	6	0	0	6	0	0	4	1	0	All Animals Dead		
Flexor Reflex	6	0	0	6	0	0	5	0	0			
Extensor Reflex	6	0	0	6	0	0	4	1	0			
Visual Placing	6	0	0	6	0	0	5	0	0			
Locomotion	6	0	0	6	0	0	5	0	0			
Standing	6	0	0	6	0	0	5	0	0			

Numbers represent number of animals (out of 6) exhibiting indicated response.
[a]One animal died spontaneously on Day 2 of the study.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

78-2317

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Interval: Day 10

Single Treatment

Group:	IA			IIA			IIIA			IVA		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	4	0	0	4	0	0	4	0	0	All Animals Dead		
Flexor Reflex	4	0	0	4	0	0	4	0	0			
Extensor Reflex	4	0	0	4	0	0	4	0	0			
Visual Placing	4	0	0	4	0	0	4	0	0			
Locomotion	4	0	0	4	0	0	1	3	0			
Standing	4	0	0	4	0	0	3	1	0			

Numbers represent number of animals (out of 4) exhibiting indicated response.

14-Day Repeated Treatment

Group:	IB			IIB			IIIB[a]			IVB		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	6	0	0	6	0	0	5	0	0	All Animals Dead		
Flexor Reflex	6	0	0	6	0	0	5	0	0			
Extensor Reflex	6	0	0	6	0	0	4	1	0			
Visual Placing	6	0	0	6	0	0	5	0	0			
Locomotion	6	0	0	6	0	0	0	2	3			
Standing	6	0	0	6	0	0	1	2	2			

Numbers represent number of animals (out of 6) exhibiting indicated response.
[a]One animal died spontaneously on Day 2 of the study.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

78-2317

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Interval: Day 14

Single Treatment

Group:	IA			IIA			IIIA			IVA
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0 1 2 or 3
Observation										
Righting Reflex	4	0	0	4	0	0	4	0	0	All Animals Dead
Flexor Reflex	4	0	0	4	0	0	4	0	0	
Extensor Reflex	4	0	0	4	0	0	4	0	0	
Visual Placing	4	0	0	4	0	0	4	0	0	
Locomotion	4	0	0	4	0	0	4	0	0	
Standing	4	0	0	4	0	0	4	0	0	

Numbers represent number of animals (out of 4) exhibiting indicated response.

14-Day Repeated Treatment

Group:	IB			IIB			IIIB[a]			IVB
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0 1 2 or 3
Observation										
Righting Reflex	6	0	0	5	1	0	0	1	4	All Animals Dead
Flexor Reflex	6	0	0	6	0	0	3	2	0	
Extensor Reflex	6	0	0	5	1	0	1	3	1	
Visual Placing	6	0	0	6	0	0	2	2	1	
Locomotion	6	0	0	6	0	0	0	0	5	
Standing	6	0	0	6	0	0	0	1	4	

Numbers represent number of animals (out of 6) exhibiting indicated response.
[a]One animal died spontaneously on Day 2 of the study.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

78-2317

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Interval: Day 21

Single Treatment

Group:	IA			IIA			IIIA			IVA		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	4	0	0	4	0	0	2	2	0	All Animals Dead		
Flexor Reflex	4	0	0	4	0	0	2	2	0			
Extensor Reflex	4	0	0	4	0	0	2	2	0			
Visual Placing	4	0	0	4	0	0	4	0	0			
Locomotion	4	0	0	4	0	0	4	0	0			
Standing	4	0	0	4	0	0	4	0	0			

Numbers represent number of animals (out of 4) exhibiting indicated response.

14-Day Repeated Treatment

Group:	IB			IIB			IIIB[a]			IVB		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	6	0	0	6	0	0	0	1	4	All Animals Dead		
Flexor Reflex	6	0	0	6	0	0	1	2	2			
Extensor Reflex	6	0	0	6	0	0	1	3	1			
Visual Placing	6	0	0	6	0	0	2	2	1			
Locomotion	6	0	0	6	0	0	1	0	4			
Standing	6	0	0	6	0	0	1	0	4			

Numbers represent number of animals (out of 6) exhibiting indicated response.
[a]One animal died spontaneously on Day 2 of the study.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

78-2317

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Interval: Day 28

Single Treatment

Group:	IA			IIA			IIIA			IVA		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	4	0	0	4	0	0	2	2	0	All Animals Dead		
Flexor Reflex	4	0	0	4	0	0	3	1	0			
Extensor Reflex	4	0	0	4	0	0	3	1	0			
Visual Placing	4	0	0	4	0	0	4	0	0			
Locomotion	4	0	0	4	0	0	4	0	0			
Standing	4	0	0	4	0	0	4	0	0			

Numbers represent number of animals (out of 4) exhibiting indicated response.

14-Day Repeated Treatment

Group:	IB			IIB			IIIB[a]			IVB		
Neurological Score:	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3	0	1	2 or 3
Observation												
Righting Reflex	6	0	0	6	0	0	0	2	3	All Animals Dead		
Flexor Reflex	6	0	0	6	0	0	1	3	1			
Extensor Reflex	6	0	0	6	0	0	1	4	0			
Visual Placing	6	0	0	6	0	0	1	3	1			
Locomotion	6	0	0	6	0	0	0	1	4			
Standing	6	0	0	6	0	0	0	1	4			

Numbers represent number of animals (out of 6) exhibiting indicated response.
[a]One animal died spontaneously on Day 2 of the study.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Single Treatment

Interval: Pre

Group:	IV-C			IV-D		
Neurological Score:	0	1	2 or 3	0	1	2 or 3
Observation						
Righting Reflex	2	0	0	2	0	0
Flexor Reflex	2	0	0	2	0	0
Extensor Reflex	2	0	0	2	0	0
Visual Placing	2	0	0	2	0	0
Locomotion	2	0	0	2	0	0
Standing	2	0	0	2	0	0

Numbers represent number of animals (out of 2) exhibiting indicated response.

Interval: Day 1

Group:	IV-C			IV-D		
Neurological Score:	0	1	2 or 3	0	1	2 or 3
Observation						
Righting Reflex	2	0	0	2	0	0
Flexor Reflex	2	0	0	2	0	0
Extensor Reflex	2	0	0	2	0	0
Visual Placing	2	0	0	2	0	0
Locomotion	2	0	0	2	0	0
Standing	2	0	0	2	0	0

Numbers represent number of animals (out of 2) exhibiting indicated response.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

78-2317

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Single Treatment

Interval: Day 4

Group:	IV-C			IV-D		
Neurological Score:	0	1	2 or 3	0	1	2 or 3
Observation						
Righting Reflex	2	0	0	2	0	0
Flexor Reflex	2	0	0	2	0	0
Extensor Reflex	2	0	0	2	0	0
Visual Placing	2	0	0	2	0	0
Locomotion	2	0	0	2	0	0
Standing	2	0	0	2	0	0

Numbers represent number of animals (out of 2) exhibiting indicated response.

Interval: Day 7

Group:	IV-C			IV-D		
Neurological Score:	0	1	2 or 3	0	1	2 or 3
Observation						
Righting Reflex	1	1	0	2	0	0
Flexor Reflex	2	0	0	2	0	0
Extensor Reflex	2	0	0	2	0	0
Visual Placing	2	0	0	2	0	0
Locomotion	2	0	0	2	0	0
Standing	2	0	0	2	0	0

Numbers represent number of animals (out of 2) exhibiting indicated response.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Table II (cont.)
A Four Week Neurotoxicity Study
in Dogs

Summary of Neurological Responses

Single Treatment

Interval: Day 10

Group:	IV-C			IV-D		
Neurological Score:	0	1	2 or 3	0	1	2 or 3
Observation						
Righting Reflex	2	0	0	Animals on test for 7 days only		
Flexor Reflex	2	0	0			
Extensor Reflex	2	0	0			
Visual Placing	2	0	0			
Locomotion	2	0	0			
Standing	2	0	0			

Numbers represent number of animals (out of 2) exhibiting indicated response.

Interval: Day 14

Group:	IV-C			IV-D		
Neurological Score:	0	1	2 or 3	0	1	2 or 3
Observation						
Righting Reflex	2	0	0	Animals on test for 7 days only		
Flexor Reflex	2	0	0			
Extensor Reflex	2	0	0			
Visual Placing	2	0	0			
Locomotion	2	0	0			
Standing	2	0	0			

Numbers represent number of animals (out of 2) exhibiting indicated response.

Scoring Key: 0 = Normal
1 = Responds sluggishly (doubtful or minimal impairment)
2 = Reflex clearly impaired
3 = Reflex absent

Table III
A Four Week Neurotoxicity Study
in Dogs

Individual Plasma and Brain
Cholinesterase Values

Single Treatment

Group	An.No.	Plasma (uM/min)[a]		Brain (uM/min)
		Pretest	3-Hours Post Dose	Terminal
IA	4363M	3.4	2.8	10.0
Corn Oil	4357M	2.7	2.5	8.5
(0 mg/kg)				
	4393F	2.3	2.2	8.5
	4379F	3.1	2.6	10.0
IIA	4362M	3.2	2.0	8.5
T.O.T.P.	4372M	3.0	2.8	9.0
(500 mg/kg)				
	4394F	2.5	2.8	9.0
	4380F	3.3	2.5	8.0
IIIA	4371M	3.0	2.4	8.0
T.P.P.	4373M	3.1	2.3	8.5
(500 mg/kg)				
	4387F	3.7	3.1	9.0
	4392F	2.7	2.4	8.5
IVA	4370M	3.0	3.1	b
T.P.P.O.	4364M	2.5	b	b
(500 mg/kg)				
	4385F	2.3	b	b
	4381F	2.7	b	b
IVC	4104F	b	b	8.0
T.P.P.O.	4146F	b	b	9.5
(50 mg/kg)[c]				
IVD	4336M	b	b	8.0
T.P.P.O.	4352F	b	b	6.0
(100 mg/kg)[d]				

[a]Activity expressed as moles of substrate hydrolyzed per minute per ml of plasma or per gram of brain tissue.
[b]Cholinesterase values not evaluated since the animal died prior to the indicated interval.
[c]Animals entered study on Day 14.
[d]Animals entered study on Day 21.

Table III (cont.)
A Four Week Neurotoxicity Study
in Dogs

Individual Plasma and Brain
Cholinesterase Values

14-Day Repeated Treatment

Group	An.No.	Plasma (μM/min)[a]		Brain (μM/min)
		Pretest	3 Hours Following Final Treatment	Terminal
IB	4360M	2.9	2.5	7.5
Corn Oil	4358M	2.5	2.4	7.5
(0 mg/kg/)	4365M	2.4	2.1	8.5
	4384F	2.7	2.4	9.5
	4390F	2.7	2.3	7.0
	4383F	2.6	2.4	10.0
IIB	4355M	2.5	1.7	6.0
T.O.T.P.	4366M	2.5	0.9	5.5
(100 mg/kg)	4369M	3.5	1.0	8.0
	4376F	2.4	1.0	8.0
	4382F	3.0	0.9	8.0
	4386F	3.1	1.0	7.5
IIIB	4359M	3.2	2.9	8.5
T.P.P.	4374M	2.5	2.2	8.5
(50 mg/kg)[b]	4368M	2.7	2.6	9.0
	4375F	2.3	2.2	11.5
	4389F	2.9	2.6	9.5
	4388F	2.5	c	c
IVB	4361M	2.6	c	c
T.P.P.O.	4367M	2.5	c	c
(300 mg/kg)	4356M	2.5	c	c
	4378F	3.1	c	c
	4391F	2.9	c	c
	4377F	3.0	c	c

[a]Activity expressed as moles of substrate hydrolyzed per minute per ml of plasma or per gram of brain tissue.
[b]The initial dosing was performed at 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.
[c]Cholinesterase values not evaluated since the animal died prior to the indicated interval.

78-2317

Appendix A
A Four Week Neurotoxicity Study in Dogs

Methodology and References

General

Parameter	Reference or Description
Physical Examination	Examined pretest for each animal: Central Nervous System - behavior movements, reactivity to stimuli and muscle tone. Autonomic Nervous System - pupil size and secretions. Respiration - nostrils, character and rate. Cardiovascular - palpation. Gastrointestinal - abdominal shape and feces consistency and color. Genitourinary - penis/vulva and perineal region. Skin and Fur - color, turgor and integrity. Mucous Membranes - conjunctiva, mouth, rectal and vaginal. Eye - eyelids, eyeball and transparency. Other - general condition.
Body Weight	Detecto Scale (60-S)
Stain-Luxol-fast Blue	Armed Forces Institute. Manual for Histologic Technics (Modified). 2nd Edition. New York: McGraw-Hill Book Co., Inc. 1949.

Appendix A (cont.)
A Four Week Neurotoxicity Study
in Dogs

Methodology and References

Clinical Chemistry

Parameter	Specimen	Reporting Units	Reference or Description
Cholinesterase	Plasma	µm/ml/min	Modification of Nabb. D.P. and F. Whitefield Determination of Cholinesterase by an automated pH-stat. Method. Arch. Environmental Health Vol. 15, August 1967, pp. 147-154.
	Brain	um/g/min	

Appendix B
Individual Neurological Evaluations

Species: Dog Animal No: 4357 Sex: Male Group: IA Dose: 0 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4363 Sex: Male Group: IA Dose: 0 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4379 Sex: Female Group: IA Dose: 0 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4393 Sex: Female Group: IA Dose: 0 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4358 Sex: Male Group: IB Dose: 0 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4360 Sex: Male Group: IB Dose: 0 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4365 Sex: Male Group: IB Dose: 0 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4383 Sex: Female Group: IB Dose: 0 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4384 Sex: Female Group: IB Dose: 0 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4390 Sex: Female Group: IB Dose: 0 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4362 Sex: Male Group: IIA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4372 Sex: Male Group: IIA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4380 Sex: Female Group: IIA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4394 Sex: Female Group: IIA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4355 Sex: Male Group: IIB Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	1	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	1	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4366 Sex: Male Group: IIB Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4369 Sex: Male Group: IIB Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4376 Sex: Female Group: IIB Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4382 Sex: Female Group: IIB Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4386 Sex: Female Group: IIB Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4371 Sex: Male Group: IIIA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	1	1
	Flexor Reflex		+	0	0	0	0	0	0	1	1
	Extensor Reflex		+	0	0	0	0	0	0	1	1
	Visual Placing		+	0	1	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	1	0	0	0
	Standing		+	0	0	0	0	1	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4373 Sex: Male Group: IIIA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	0	1	1
	Flexor Reflex		+	0	0	0	0	0	0	1	0
	Extensor Reflex		+	0	0	0	0	0	0	1	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	1	0	0	1	0	0	0
	Standing		+	0	1	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4387 Sex: Female Group: IIIA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	2	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	1	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4392 Sex: Female Group: IIIA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	1	0	0	0	0	0	0
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	0	0	0
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	0	0	0	0
	Standing		+	0	0	0	0	0	0	0	0

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4359 Sex: Male Group: IIIB Dose: 50 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	3	3	3
	Flexor Reflex		+	0	0	0	0	0	1	2	2
	Extensor Reflex		+	0	0	0	0	0	0	1	1
	Visual Placing		+	0	0	0	0	0	1	1	2
	Locomotion		+	0	0	0	0	2	3	3	3
	Standing		+	0	0	0	0	2	3	3	3

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4368 Sex: Male Group: IIIB Dose: 50 mg/kg[a]

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	3	3	2
	Flexor Reflex		+	0	0	0	0	0	0	1	1
	Extensor Reflex		+	0	0	0	0	1	1	1	1
	Visual Placing		+	0	0	0	0	0	2	1	1
	Locomotion		+	0	0	0	0	2	3	2	2
	Standing		+	0	0	0	0	2	3	2	2

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

[a]The initial dosing was performed at 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4374 Sex: Male Group: IIIB Dose: 50 mg/kg[a]

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	1	2	1
	Flexor Reflex		+	0	0	0	0	0	0	1	1
	Extensor Reflex		+	0	0	0	0	0	1	1	1
	Visual Placing		+	0	0	0	0	0	0	0	0
	Locomotion		+	0	0	0	0	2	2	2	2
	Standing		+	0	0	0	0	1	1	2	2

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4375F Sex: Female Group: IIIB Dose: 50 mg/kg[a]

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	0	0	2	1	1
	Flexor Reflex		+	0	0	0	0	0	0	0	0
	Extensor Reflex		+	0	0	0	0	0	1	0	0
	Visual Placing		+	0	0	0	0	0	0	0	1
	Locomotion		+	0	0	0	0	1	2	0	1
	Standing		+	0	0	0	0	0	2	0	1

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

[a]The initial dosing was performed at 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4389 Sex: Female Group: IIIB Dose: 50 mg/kg[a]

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0	0	1	0	3	3	2
	Flexor Reflex		+	0	0	0	0	0	1	3	1
	Extensor Reflex		+	0	0	0	0	0	3	3	1
	Visual Placing		+	0	0	0	0	0	1	1	1
	Locomotion		+	0	0	0	0	1	3	3	2
	Standing		+	0	0	0	0	1	3	3	2

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4388 Sex: Female Group: IIIB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	0						
	Flexor Reflex		+	0	0	Animal found dead on Day 2.					
	Extensor Reflex		+	0	0						
	Visual Placing		+	0	0						
	Locomotion		+	0	0						
	Standing		+	0	0						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

[a]The initial dosing was performed at 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4364 Sex: Male Group: IVA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0		Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4370 Sex: Male Group: IVA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	1						
	Flexor Reflex		+	0	0	Animal found dead on Day 2.					
	Extensor Reflex		+	0	0						
	Visual Placing		+	0	2						
	Locomotion		+	0	2						
	Standing		+	0	1						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4381 Sex: Female Group: IVA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0	Animal died spontaneously on Day 1.						
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4385 Sex: Female Group: IVA Dose: 500 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0	Animal died spontaneously on Day 1.						
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4356 Sex: Male Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	1						
	Flexor Reflex		+	0	0	Animal found dead on Day 2.					
	Extensor Reflex		+	0	0						
	Visual Placing		+	0	1						
	Locomotion		+	0	2						
	Standing		+	0	2						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4361 Sex: Male Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0		Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4367 Sex: Male Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	3						
	Flexor Reflex		+	0	1	Animal killed in a moribund condition on Day 2.					
	Extensor Reflex		+	0	2						
	Visual Placing		+	0	2						
	Locomotion		+	0	3						
	Standing		+	0	3						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4377 Sex: Female Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0		Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4378 Sex: Female Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0	1						
	Flexor Reflex		+	0	0	Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0	0						
	Visual Placing		+	0	2						
	Locomotion		+	0	2						
	Standing		+	0	2						

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4391 Sex: Female Group: IVB Dose: 300 mg/kg

Organ System:	Observation	Day:	Pre	0	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		+	0							
	Flexor Reflex		+	0		Animal died spontaneously on Day 1.					
	Extensor Reflex		+	0							
	Visual Placing		+	0							
	Locomotion		+	0							
	Standing		+	0							

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4104 Sex: Female Group: IVC Dose: 50 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		0	1	0	1	0	0	Animal on test for 14 days only.	
	Flexor Reflex		0	0	0	0	0	0		
	Extensor Reflex		0	0	0	0	0	0		
	Visual Placing		0	0	0	0	0	0		
	Locomotion		0	0	0	0	0	0		
	Standing		0	0	0	0	0	0		

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4146 Sex: Female Group: IVC Dose: 50 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		0	0	0	0	0	0	Animal on test for 14 days only.	
	Flexor Reflex		0	0	0	0	0	0		
	Extensor Reflex		0	0	0	0	0	0		
	Visual Placing		0	0	0	0	0	0		
	Locomotion		0	0	0	0	0	0		
	Standing		0	0	0	0	0	0		

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

78-2317

Appendix B (cont.)
Individual Neurological Evaluations

Species: Dog Animal No: 4336 Sex: Male Group: IVD Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	23
Neurologic Examination:	Righting Reflex		0	0	0	0				
	Flexor Reflex		0	0	0	0	Animal on test for 7 days only.			
	Extensor Reflex		0	0	0	0				
	Visual Placing		0	0	0	0				
	Locomotion		0	0	0	0				
	Standing		0	0	0	0				

Key: + = Normal (Pretest)
0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

Species: Dog Animal No: 4352 Sex: Female Group: IVD Dose: 100 mg/kg

Organ System:	Observation	Day:	Pre	1	4	7	10	14	21	28
Neurologic Examination:	Righting Reflex		0	0	0	0				
	Flexor Reflex		0	0	0	0	Animal on test for 7 days only.			
	Extensor Reflex		0	0	0	0				
	Visual Placing		0	0	0	0				
	Locomotion		0	0	0	0				
	Standing		0	0	0	0				

Key: 0 = Normal
1 = responds sluggishly (doubtful or minimal impairment)
2 = reflex clearly impaired
3 = reflex absent

A FOUR WEEK NEUROTOXICITY STUDY IN DOGS WITH TRIPHENYLPHOSPHINE AND TRIPHENYLPHOSPHINE OXIDE

PATHOLOGY REPORT
TERMINAL SACRIFICE

A Four Week Neurotoxicity Study in Dogs Pathology Report

Table of Contents

INTRODUCTION

MATERIAL AND METHODS

RESULTS

SUMMARY

Gross Postmortem Observations
- Table I - Single Treatment
- Table II - 14 Day Treatment

Microscopic Findings
- Table III - 14 Day Treatment

A Four Week Neurotoxicity Study in Dogs Pathology Report

INTRODUCTION

The purpose of this study was to assess the neurotoxic potential of triphenylphosphine and triphenylphosphine oxide administered orally to dogs.

MATERIAL AND METHODS

A. Test Materials: Triphenylphosphine (TPP)
Triphenylphosphine oxide (TPPO)
Tri-o-tolyl-phosphate (TOTP-positive control)
Mazola Corn Oil (vehicle)

B. Route of Administration: Gastric intubation

C. Test Animals: 44 male and female Beagle dogs.

D. Experimental Design:

Single Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals Initial M	Initial F	Necropsy Interval M	Necropsy Interval F	
IA	Corn Oil	0	2	2	2	2	29-30 days
IIA	Tri-orotho-tolyl-phosphate (TOTP)	500	2	2	2	2	29-30 days
IIIA	Triphenylphosphine (TPP)	500	2	2	2	2	29-30 days
IVA	Triphenylphosphine oxide (TPPO)	500	2	2	2[a]	2[a]	1-2 days
IVC	Triphenylphosphine oxide (TPPO)	50	-	2	-	2	16 days
IVD	Triphenylphosphine oxide (TPPO)	100	1	1	1	1	8-9 days

[a]Both animals died spontaneously on day 1-2 of the study.

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

EXPERIMENTAL DESIGN (CONT.)

14 Day Treatment

Group	Test Substance	Dose Level mg/kg	Number of Animals Initial M	Initial F	Necropsy - Interval M	Necropsy - Interval F	Interval	Histopathology M	Histopathology F
IB	Corn Oil	0	3	3	3	3	29-30 days	3	3
IIB	Tri-ortho-tolyl-phosphate (TOTP)	100	3	3	3	3	29-30 days	3	3
IIIB	Triphenylphosphine (TPP)	50[a]	3	3[b]	3	3	29-30 days	3	2
IVB	Triphenylphosphine oxide (TPPO)	300	3[c]	3[c]	3	3	1-2 days	0	0

[a]The initial dose was 300 mg/kg on Day 1; subsequent dosing was administered as 50 mg/kg/day.

[b]One animal died spontaneously on Day 2 of the study.

[c]All animals died spontaneously on days 1-2 of the study with the exception of one animal which was killed in a moribund condition.

E. Postmortem Methods:

A complete gross postmortem examination was performed on all animals those dying spontaneously or sacrificed at the end of the experiment. Animals were sacrificed by exsanguination under sodium pentobarbital anesthesia.

Following an external examination the cranial cavity was first opened and the brain removed. A representative section of medulla was dissected and placed on ice for cholinesterase evaluation. The remaining portions of the brain were preserved in 10% neutral buffered formalin for microscopic examination. Portions of the cervical and lumbar spinal cords and both sciatic nerves were dissected and preserved. Thoracic and abdominal cavities were opened and the viscera examined; tissues with grossly visible abnormalities were preserved.

Brain (3 sections) cervical and lumbar spinal cords (1 longitudinal and 2 transversal sections) and both sciatic nerves (1 longitudinal and two transversal sections) were processed and stained with hematoxylin and eosin, and Luxol Fast Blue for microscopic examination.

A Four Week Neurotoxicity Study in Dogs Pathology Report

RESULTS

I. Mortality

a. Single Treatment:

Four dogs given a single dose of 500 mg/kg of TPPO died spontaneously (three on day one, and one on day two of the study).

b. 14-Day Repeated Treatment

Six dogs given a dose of 300 mg/kg/day of TPPO died spontaneously or were sacrificed moribund (four died on day one, one on day two, and one was sacrificed moribund on day two).

One dog given 50 mg/kg/day of TPP died spontaneously on day two of the study.

II. Gross Postmortem Observations

a. Single Treatment

The most common gross observations were that of mottling, edema and congestion of lungs, (Table I).

b. Fourteen Day Treatment

The results of the gross postmortem examinations in individual animals are presented in Table II.

The most common gross observations noted in both control and treated groups were mottling edema and congestion of lungs. Pulmonary edema and congestion were limited to the animals dying spontaneously or killed in extremis.

III. Microscopic Findings

The results of the microscopic examination of brain, spinal cord and sciatic nerves are presented in Table III. No microscopic abnormalities were noted in the six dogs given corn oil and six dogs given Tri-ortho-tolyl-phosphate.

Minimal to moderate multifocal swelling and degeneration of axonal fibers in the white matter of the brain (predominantly in the sections of cerebellum) cervical and lumbar sections of the spinal cord and both sciatic nerves was noted in the five dogs given Triphenylphosphine.

A Four Week Neurotoxicity Study in Dogs Pathology Report

SUMMARY

Six dogs were treated, by gastric intubation with 50 mg/kg/day of Triphenylphosphine for fourteen days. One dog died spontaneously on day two of the study.

Microscopic examination of the brain, spinal cord and sciatic nerves revealed morphologic evidence of injury characterized by multifocal swelling and degeneration of axonal fibers.

No microscopic abnormality was noted in the nervous systems of dogs given corn oil (vehicle) or Tri-ortho-tolyl-phosphate (positive control).

A L Knezevich

Aleksandar L. Knezevich, Dr.Med.Vet.
Director of Pathology

Table I

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
Single Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IA (Control)	M	4357	-	-	N.O.A.
		4363	-	-	N.O.A.
	F	4379	-	-	N.O.A.
		4393	-	-	N.O.A.
IIA	M	4362	-	Lungs	All lobes: white mottling, multiple small, soft, palpable nodules on surfaces.
		4372	-	-	N.O.A.
	F	4380	-	-	N.O.A.
		4394	-	-	N.O.A.
IIIA	M	4371	-	-	N.O.A.
		4373	-	Lungs	All lobes: dark red spots (1-3mm in diam.)
	F	4387	-	-	N.O.A.
		4392	-	-	N.O.A.
IVA	M	4364	-	Lungs	All lobes: mottled dark red; on cut surface large amount of sanguineous fluid released.
				Stomach	Distended with gas, white frothy substance and food.
				Heart	Very firm in consistency.
		4370	-	Lungs	All lobes: mottled dark red; on cut surface moderate amount of sanguineous fluid released.
	F	4381	-	Lungs	All lobes: mottled dark red; on cut surface large amount of sanguineous fluid released.

Table I
(cont.)

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
Single Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IVA (cont.)	F	4385	-	Heart	Very firm in consistency.
IVC	F	4104	-	-	N.O.A.
		4146	-	-	N.O.A.
IVD	M	4336	-	-	N.O.A.
	F	4352	-	-	N.O.A.

Table II

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
14 Day Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IB	M	4358	79-654	Lungs	All lobes: mottled white and red.
		4360	79-655	Lung (L)	Mottled dark red.
		4365	79-656	-	N.O.A.
	F	4383	79-657	-	N.O.A.
		4384	79-658	-	N.O.A.
		4390	79-659	-	N.O.A.
IIB	M	4355	79-660	Lungs	All lobes: mottled white and red.
		4366	79-661	-	N.O.A.
		4369	79-662	-	N.O.A.
	F	4376	79-663	-	N.O.A.
		4382	79-664	Lungs	Red mottling on both diaphragmatic surfaces.
		4386	79-665	-	N.O.A.
IIIB	M	4359	79-666	Lungs	All surfaces: mottled.
		4368	79-667	-	N.O.A.
		4374	79-668	Lungs	All lobes: mottled white and red.
	F	4375	79-669	-	N.O.A.
		4389	79-670	Lungs	All lobes: mottled red.
		4388*	-	Lungs	All lobes: mottled red; on cut surface large amount of sanguineou fluid released.
				Stomach	Distended with gas.

*Animal died spontaneously

Table II
(cont.)

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Gross Postmortem Observations
14 Day Treatment

Group/ Dose	Sex	Animal Number	Pathology Number	Organ/ Tissue	Observations
IVB	M	4356*	-	Lungs	All lobes: mottled red; on cut surface moderate amount of sanguineous fluid released.
		4361*	-	Lungs	All lobes: mottled dark red.
				Stomach	Distended with gas.
		4367**	-	Lungs	All lobes: mottled dark red; on cut surface large amount of sanguineous fluid released.
	F	4377*	-	Lungs	All lobes: mottled dark red; on cut surface sanguineous fluid released.
				Stomach	Distended with gas.
		4391*	-	Lungs	All lobes: mottled dark red; on cut surface sanguineous fluid released.
		4378*	-	Lungs	All lobes: mottled red and pin-point size red foci.
				Stomach	Distended with gas.
				Heart	Firm, contracted.

* Animal died spontaneously
** Animal killed in moribund condition.

A Four Week Neurotoxicity Study in Dogs Pathology Report

General Key to Histopathology Tables

N	=	Not remarkable, within normal histologic limits
1	=	Minimal degree or amount of indicated change
2	=	Mild degree or amount of indicated change
3	=	Moderate degree or amount of indicated change
4	=	Marked degree or amount of indicated change
H&E	=	hematoxylin and eosin stain
LFB	=	Luxol Fast Blue stain

Table III

78-2317

A Four Week Neurotoxicity Study
in Dogs
Pathology Report

Microscopic Findings
14 Day Treatment

Animal Number	43584	43605	43656	43833	43884	43900	43955	43966	43969	43976	43982	43886	43596	43568	43674	43775	43889
Sex	M	M	M	F	F	F	M	M	M	F	F	F	M	M	M	F	F
Pathology Number 79-	65454	65545	65656	65757	65858	65959	66050	66161	66262	66363	66464	66565	66666	66767	66868	66969	67070
Group B-	I	I	I	I	I	I	II	II	II	II	II	II	III	III	III	III	III
BRAIN, three transverse sections (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N					
multifocal swelling and degeneration of axons													2	2	1	1	2
CERVICAL SPINAL CORD, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N					
multifocal swelling and degeneration of axons													2	3	1	1	2
LUMBAR SPINAL CORD, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N					
multifocal swelling and degeneration of axons													2	3	1	1	2
LEFT SCIATIC NERVE, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N			N	N	
multifocal swelling and degeneration of axons													1	1			1
focal mononuclear cell infiltrate													1				
RIGHT SCIATIC NERVE, two transverse and one longitudinal section (H&E and LFB)	N	N	N	N	N	N	N	N	N	N	N	N			N	N	
multifocal swelling and degeneration of axons													1	1			1

Contains No CBI

Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO.: 6218-80

M-446

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine (TPP)

Submitted to: M&T Chemicals, Inc.
Rahway, New Jersey

Date: June 30, 1980

I. INTRODUCTION

This study was conducted for M&T Chemicals Inc. in order to evaluate the acute oral toxicity of Triphenylphosphine. The study was performed at Bio/dynamics, Inc., Mettlers Road, East Millstone, New Jersey, 08873, using procedures based on the methods described in 40 CFR Part 163.81-1. (ENVIRONMENTAL PROTECTION AGENCY PESTICIDE PROGRAMS. Proposed Guidelines for Registering Pesticides in the U.S.; Hazard Evaluation: Humans and Domestic Animals. Acute Oral Toxicity Study.)

II. MATERIALS AND METHODS

A. Dates of Study

LD_{50} Determination

Animal Receipt:	April 8, 1980
Initiation (Dosing):	April 23, 1980
Termination:	May 7, 1980

B. Study Personnel

Study Director:	George R. Lankas, Ph.D.
Laboratory Supervisor:	Donna L. Blaszcak, B.S.
Technician in Charge:	Gary Trimmer, B.S.
Study Monitor (Report Preparation):	Carol Puma, B.S.

C. Test Animals

Albino Rats

Strain:	Sprague-Dawley CD^R
Reason for Selection:	Standard laboratory animal
Supplier:	Charles River Breeding Laboratories, Inc. Wilmington, Massachusetts 01887

LD_{50} Determination: Sixty (five/sex/dose level)

II. MATERIALS AND METHODS (cont.)

Age:	Young adults
Weight: (prefasted)	Males : 267 to 329 grams Females: 220 to 288 grams
Equilibration Period:	15 days
Observations:	All animals were checked for viability twice daily during the equilibration period. Prior to assignment to study all animals received a physical examination.
Husbandry:	
Housing:	Group-housed (six/cage) during equilibration. Individually-housed during study.
Cages:	Suspended, stainless steel with wire mesh bottoms.
Environmental Conditions:	Temperature: monitored twice daily Temperature Range: 64°F to 72°F Humidity: monitored daily Light cycle: 12 hours light, 12 hours dark
Food:	Purina Laboratory Rodent Diet ad libitum.
Water:	Automatic watering system, ad libitum. Municipal water supply (Elizabethtown Water Co.)
Identification:	Each animal was identified with a monel ear tag bearing a unique number prior to testing.
Selection:	Animals considered unsuitable for study because of poor health or outlying body weights were excluded from selection. Animals for study were selected from those remaining; each animal from a group-housed unit was placed in a different test group.

D. Test Material:

Label Information:	Triphenylphosphine 500 grams for 6218-80
Lot. No.:	PNVAH-6KF
Description:	Cream, white colored granular powder
Date of Receipt:	April 11, 1980

II. MATERIALS AND METHODS (cont.)

Received from: M&T Chemicals Inc.

Storage: Ambient temperature

Analysis: Detailed analysis of triphenylphosphine performed by M&T Chemicals Inc., is presented in Appendix A.

Preparation of Dosing Material Mixtures:

LD_{50}:

Concentration: 0.2 g/ml (20%)

Vehicle: Peanut oil

Procedure: Prior to mixing, the test material was ground with a mortor and mestle until extremely fine. 30 grams of the test material were placed in an appropriate container and vehicle was added to achieve a total volume of 150 ml. The mixture was then stirred to achieve a homogeneous mixture.

Temperature of dosing mixture: room temperature

E. Test Design:

LD 5 0			
No. of Animals		Dose	Dose Volume*
M	F	(mg/kg)	(ml/kg)
5	5	Vehicle Control	7.12
5	5	350	1.75
5	5	497	2.49
5	5	706	3.53
5	5	1002	5.01
5	5	1423	7.12

*Concentration 0.2 g/ml

Vehicle Control:

Ten animals (five/sex) were dosed with 7.12 ml/kg of peanut oil.

II. MATERIALS AND METHODS (cont.)

F. Administration of Test Material:

Animals were fasted overnight (for approximately 17 hours) prior to dosing. The test and control material was administered by oral intubation, using a ball-tipped intubation needle. Doses were calculated using pre-fasted body weights.

G. Experimental Evaluation (In-Life):

Viability Check: Twice Daily

Observations of Pharmacologic and Toxicologic Signs:

Approximately 1, 2, and 4 hours after dosing and daily thereafter for fourteen days.

Body Weights:

Pre-fast (weights used for calculation of doses)
Post-fast (just prior to dosing)
Day 7 and Day 14
Terminal: Any animals which did not survive for 14 days were weighed at the time of death or at the time they were found dead.

H. Post Mortem:

Gross post mortem examinations were performed on all animals which died during the study. All animals surviving at termination of the observation period (Day 14) were killed by carbon dioxide inhalation and examined grossly. All abnormalities were recorded but no tissues were saved.

I. Raw Data Storage:

All raw data and the original study protocol will be retained permanently on file in the Bio/dynamics Archives.

J. References-LD_{50} Calculations:

Miller, Lloyd C. and M.L. Tainter., Estimation of the ED_{50} and Its Error by Means of Logarithmic-Probit Graph Paper, Proc. Soc. Exp. Bio. Med 57: 261-264 (1944).

III. RESULTS

Individual body weights are presented in Table 1. A summary of in-life observations is presented in Table II. Necropsy observations are presented in Table III.

A. Mortality

Dose levels, mortality data and LD_{50} calculations for the LD_{50} study were as follows:

Dose Level mg/kg	Mortality Male	Female	Total
Vehicle Control	0/5	0/5	0/10
350	0/5	0/5	0/10
497	0/5	0/5	0/10
706	0/5	1/5[a]	1/10[a]
1002	2/5	3/5	5/10
1423	3/5	4/5	7/10
LD_{50}:	1200mg/kg	980mg/kg	1150 mg/kg
95% Confidence Limits (mg/kg):	828 to 1572	805 to 1155	991 to 1309

[a]Accidental death, test material found in thoracic cavity.

LD_{50} calculations and dose response curves and slopes are presented in Appendix B.

B. Body Weights (Table I)

Those animals dying prior to termination of the study exhibited body weight losses. The animals surviving the fourteen-day post-dose observation period exhibited body weight gains. However, a few animals in the 2 highest dose groups exhibited weight losses at Day 7.

III. RESULTS (cont.)

C. In-Life Observations (Table II)

Fecal staining and soft stool were exhibited by several animals at all dose levels usually within the first four hours after dosing due to the cathartic effect of the vehicle. At the higher dose levels (706, 1002, and 1423 mg/kg) ataxia, fine or coarse tremors, red ocular discharge, irregular breathing and/or respiratory rate decrease, motor activity decrease and hypothermia were exhibited by several animals. Other signs noted sporadically are outlined in Table II.

IV. DISCUSSION

The results presented here can be contrasted with an earlier acute study (Bio/dynamics Project No. 2575-75) in which the LD_{50} for triphenylphosphine was approximately 30-fold greater than in the present study. This difference can be attributed to the fact that in the earlier study the test material was administered orally as an aqueous, particulate suspension in 1% Methocel. The relatively larger volume used coupled with the insolubility of the test substance in an aqueous medium would likely render a substantial fraction unavailable for absorption, thereby increasing the LD50.

George R. Lankas 6/30/80

George R. Lankas, Ph.D. Date
Study Director

William E. Rinehart 6/30/80

William E. Rinehart, Sc.D. Date
Vice-President

TABLE I

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
Vehicle Control	2860 M	268	244				321	53	347	79
	2901 M	270	239				322	52	361	91
	2880 M	279	255				329	50	363	84
	2875 M	271	245				322	51	354	83
	2845 M	296	263				346	50	377	81
	2924 F	244	222				263	19	286	42
	2952 F	244	223				265	21	269	25
	2916 F	260	244				287	27	294	34
	2931 F	240	218				252	12	260	20
	2919 F	255	241				280	25	289	34

[a]Change from prefasted weight (grams).

TABLE I (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level mg/kg	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
350	2867 M	283	257				339	56	373	90
	2886 M	293	271				325	32	367	74
	2893 M	286	255				335	49	370	84
	2838 M	290	270				337	47	373	83
	2842 M	323	290				381	58	426	103
	2966 F	250	228				265	15	278	28
	2929 F	248	227				276	28	280	32
	2920 F	235	215				253	18	247	12
	2951 F	251	230				276	25	288	37
	2943 F	253	230				277	24	289	36

[a]Change from prefasted weight (grams).

TABLE I (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level mg/kg	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
497	2883 M	304	280				350	46	408	104
	2900 M	306	275				350	44	387	81
	2834 M	288	265				325	37	364	76
	2862 M	276	250				308	32	352	76
	2878 M	292	265				329	37	367	75
	2946 F	256	232				280	24	290	34
	2941 F	254	236				272	18	286	32
	2912 F	250	233				274	24	280	30
	2965 F	248	235				262	14	272	24
	2928 F	220	201				245	25	249	29

[a]Change from prefasted weight (grams).

TABLE I (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level mg/kg	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
706	2879 M	317	287				345	28	338	21
	2892 M	286	254				300	14	355	69
	2876 M	295	266				321	26	366	71
	2852 M	279	247				323	44	389	110
	2859 M	292	255				315	23	349	57
	2909 F	287	258	238	2	-49				
	2940 F	256	235				266	10	301	45
	2942 F	263	244				269	6	278	15
	2903 F	262	240				273	11	277	15
	2947 F	288	260				319	31	342	54

[a]Change from prefasted weight (grams).

TABLE I (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level mg/kg	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
1002	2897 M	268	243	231	2	-37				
	2841 M	287	263				277	-10	330	43
	2856 M	280	253				302	22	356	76
	2868 M	267	242				276	9	286	19
	2884 M	285	260	245	3	-40				
	2917 F	250	226	213	2	-37				
	2914 F	262	241	224	1	-38				
	2934 F	233	211				229	-4	254	21
	2922 F	236	216				256	20	266	30
	2959 F	234	217	205	3	-29				

[a]Change from prefasted weight (grams).

TABLE I (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

BODY WEIGHTS (GRAMS) AND DAY OF DEATH

Dose Level mg/kg	Animal No. & Sex	Pretest Weights		Interim Deaths			Survivors			
		Pre-fast	Post-fast	Terminal Weight	Day of Death	Chg[a]	Day 7	Chg[a]	Day 14	Chg[a]
1423	2836 M	329	301				332	3	400	71
	2866 M	286	255				280	-6	336	50
	2839 M	308	280	258	3	-50				
	2887 M	296	270	240	4	-56				
	2858 M	281	257	237	2	-44				
	2963 F	243	225				248	5	267	24
	2930 F	250	230	213	3	-37				
	2907 F	243	226	219	3	-24				
	2902 F	244	225	211	2	-33				
	2936 F	248	225	203	3	-45				

[a]Change from prefasted weight (grams).

TABLE II

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose	Interval: Day 1 Hrs. 1 M/F	2 M/F	4 M/F	24 M/F	2 M/F	3 M/F	4 M/F	5-7 M/F	8-14 M/F	Time last Observed
Fecal Staining	Vehicle Control	1/0	0	0/1	0	0	0	0	0	0	4 Hours
Soft Stool		2/0	2/1	1/1	0	0	0	0	0	0	4 Hours

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Interval: Dose (mg/kg)	Day 1 Hrs. 1 M/F	2 M/F	4 M/F	24 M/F	2 M/F	3 M/F	4 M/F	5-7 M/F	8-14 M/F	Time last Observed
Fecal Staining	350	0	0	1/0	0	0	0	0	0	0	4 Hours
Soft Stool		0	0	1/0	0	0	0	0	0	0	4 Hours
Alopecia		0	0	0	0	0/1	0/1	0/1	0/1	0/1	Day 14

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex exhibiting sign one or more times during interval.

Biodynamics Inc.
6218-80

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose (mg/kg)	Interval: Day Hrs. 1 1 M/F	1 2 M/F	1 4 M/F	1 24 M/F	2 M/F	3 M/F	4 M/F	5-7 M/F	8-14 M/F	Time last Observed
Urinary Staining	497	0	0	0	0/1	0	0	0	0	0	24 Hours
Fecal Staining		0	0/1	0/1	0	0	0	0	0	0	4 Hours
Soft Stool		0	0/1	0/1	0	0	0	0	0	0	4 Hours
Piloerection		0	0	0	0	0/1	0/1	0	0	0	Day 3
Abdominal Griping		0	0	0	0	0/1	0	0	0	0	Day 2
Motor Activity Decrease		0	0	2/0	0	0	0	0	0	0	4 Hours
Alopecia (Ano-Genital Area)		0	0	0	0/1	0	0	0	0	0	24 Hours

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Interval: Day Hrs. Dose (mg/kg)	1 1 M/F	1 2 M/F	1 4 M/F	1 24 M/F	2 M/F	3 M/F	4 M/F	5-7 M/F	8-14 M/F	Time last Observed
Dead	706	0	0	0	0	0/1	0/1	0/1	0/1	0/1	
Fine Tremors		0	0	0	0	0/1	0	0	0	0	Day 2
Red Ocular Discharge		0	0	0	0/1	0	0	0	0	0	24 Hours
Urinary Staining		0	0	0	0	0/1	0	0	0	0	Day 2
Fecal Staining		0	1/0	0	0	0	0	0	0	0	2 Hours
General Poor Condition		0	0	0	0	0	0/1	0	0	0	Day 3
Soft Stool		0	1/0	0	0	0	0	0	0	0	2 Hours
Motor Activity Decrease		0	0	0	1/1	0	0	0	0	0	24 Hours
Hypothermia		0	0	0	0/1	0	0	0	0	0	24 Hours
Irregular Breathing		0	0	0	0/1	0	0	0	0	0	24 Hours
Alopecia (Ano-Genital Area)		0	0	0	0/1	0	0	0	0	0	24 Hours
Unthrifty		0	0	0	0	0/1	0	0	0	0	Day 2

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex or of those surviving exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose (mg/kg)	Interval: Day	1				2	3	4	5-7	8-14	Time last Observed
		Hrs.	1	2	4	24						
			M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F	M/F	
Dead	1002		0	0	0	0/1	1/2	2/3	2/3	2/3	2/3	
Fine Tremors			0	0	0	0	2/0	1/0	0	0	0	Day 3
Red Nasal Discharge (slight, dry)			0	0	0	0	0	0	0/1	0	0	Day 4
Respiratory Rate Decrease			0	0	1/0	0/1	0	0	0	0	0	24 Hours
Red Ocular Discharge			0	0	0	0	0/1	0	0	0	0	Day 2
Fecal Staining			0	0/1	0/1	0	0	0	0	1/0	0	Day 7
Soft Stool			0	0/1	0/1	0	0	0	0	0	0	4 Hours
Piloerection			0	0	0	0	0/1	0/1	0	0	0	Day 3
Abdominal Griping			0	0	0	0	1/1	0	0	0	0	Day 2
Motor Activity Increase			0	0	0	0	0	0	0	0/1	0/1	Day 12
Motor Activity Decrease			0	0	2/1	0	0	0	0	0	0	4 Hours
Prostration			0	0	0	1/1	0	0	0	0	0	24 Hours
Hypothermia			0	0	0	1/1	0	0	0	0	0	24 Hours
Food Consumption Decrease			0	0	0	0	0	0	0	0/1	0	Day 5

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex or of those surviving exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose (mg/kg)	Interval: Day Hrs. 1 1 M/F	1 2 M/F	1 4 M/F	1 24 M/F	2 M/F	3 M/F	4 M/F	5-7 M/F	8-14 M/F	Time last observed
Dead	1423	0	0	0	0	1/1	2/4	3/4	3/4	3/4	
Ataxia		0	0	0	0	1/0	0	0	0	0	Day 2
Convulsions		0	0	0	0	0/1	0	0	0	0	Day 2
Coarse Tremors		0	0	0	0	1/1	0	0	0	0	Day 2
Red Nasal Discharge		0	0	0	0	0	1/0	1/1	0	0	Day 4
Respiratory Rate Decrease		1/0	1/2	0	1/1	0/1	0	0	0	0	Day 2
Irregular Breathing		0	0	0/1	0	0	0	0	0	0	4 Hours
Wet Rales		0/1	0	0	0	0	0	0	0	0	1 Hours
Clear Oral Discharge		1/0	0/1	0	0	0	0	0	0	0	2 Hour
Red Ocular Discharge		0	0	0	1/0	0	0	0	0	0	24 Hours
Urinary Staining		0	0	0	0/1	0	0	0	0	0	24 Hours
Fecal Staining		0	1/1	0/1	0	0	0	0/1	0	0	Day 4
Soft Stool		0	1/1	0/1	0	0	0	0	0	0	24 Hours
Piloerection		0	0	0/2	1/0	1/2	0	0	0	0	Day 2

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex or of those surviving exhibiting sign one or more times during interval.

TABLE II (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

SUMMARY OF IN-LIFE OBSERVATIONS[a]

Observation	Dose (mg/kg)	Interval: Day 1 Hrs. 1 M/F	2 M/F	4 M/F	24 M/F	2 M/F	3 M/F	4 M/F	5-7 M/F	8-14 M/F	Time last Observed
Abdominal Griping	1423	0	0	0	1/0	1/2	0	0	0	0	Day 2
Motor Activity Decrease	(cont.)	0	0	2/2	0	2/0	1/1	1/0	1/0	0	Day 6
Hypothermia		0	0	0	1/1	0	0	0	0	0	24 Hours
Aggressive		0	0	0	0	0	0	0	1/0	0	Day 5
Alopecia (Ano-Genital Area)		0	0	0	1/2	0/2	0	0	0	0	Day 2
Prostration		0	0	0	1/1	1/1	1/0	0	0	0	Day 3
Unthrifty		0	0	0	0	1/0	0	0	0	0	Day 2

[a]Numbers represent numbers of males and females (M/F), out of 5 per sex or of those surviving exhibiting sign one or more times during interval.

dynamics Inc.

TABLE III

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
Vehicle Control	2860 M	14	T	Lungs: mottled dark red 75%; spleen: roughened; kidneys: mottled pale 10%; adrenals: dark red foci.
	2901 M	14	T	Lungs: mottled dark red 75%; spleen: roughened; kidneys: mottled pale; adrenals: dark red foci.
	2880 M	14	T	Lungs: mottled dark red 100%; spleen: roughened; adrenals: brown red foci.
	2875 M	14	T	Lungs: mottled dark red 40%, dark red foci throughout; adrenals: dark red foci.
	2845 M	14	T	Lungs: pale dark red foci 40%; kidneys: mottled dark red; adrenals: dark red.
	2924 F	14	T	Lungs: mottled dark red 60%; adrenals: dark red foci.
	2952 F	14	T	Lungs: mottled dark red 50%; spleen: roughened; adrenals: dark red foci.
	2916 F	14	T	Lungs: pale, dark red areas 50%; spleen: roughened.
	2931 F	14	T	Lungs: dark red patches 40%; spleen: slightly roughened; kidneys: mottled pale; adrenals: dark red foci.
	2919 F	14	T	Lungs: mottled dark red 90%, dark red foci 60%; spleen: roughened; adrenals: dark red foci.

[a]T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
350	2867 M	14	T	Lungs: mottled dark red 40%; spleen: slightly roughened; kidneys: mottled pale; adrenals: mottled red.
	2886 M	14	T	Lungs: mottled pale, dark red foci 100%; spleen: slightly roughened; adrenals: dark red foci.
	2893 M	14	T	Lungs: pale; spleen: roughened; kidneys: mottled pale 50%; adrenals: brown red foci.
	2838 M	14	T	Lungs: pale, dark red foci throughout; spleen: roughened; kidneys: mottled pale; adrenals: dark red foci.
	2842 M	14	T	Lungs: mottled pale with dark red foci 100%; spleen: slightly roughened; adrenals: dark red.
	2966 F	14	T	Lungs: pale, dark red foci throughout; spleen: slightly roughened; kidneys: mottled pale; adrenals: dark red foci.
	2929 F	14	T	Alopecia: stomach, left groin area; lungs: dark red foci 100%; spleen: dark, slightly roughened.
	2920 F	14	T	Lungs: mottled dark red 40%; spleen: roughened; adrenals: dark red foci.
	2951 F	14	T	Lungs: mottled pale 20%; spleen: slightly roughened; adrenals: dark red foci.
	2943 F	14	T	Lungs: dark red areas; stomach: vascularized; spleen: slightly roughened; kidneys: mottled tan 25%.

[a]T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
497	2883 M	14	T	Lungs: pale with dark red dots; spleen: roughened; adrenals: dark red foci.
	2900 M	14	T	Lungs: mottled pale 80%; spleen: slightly roughened; adrenals: red foci.
	2834 M	14	T	Lungs: pale dark red foci 80%; spleen: slightly roughened; kidneys: mottled pale; adrenals: red.
	2862 M	14	T	Lungs: dark red areas; spleen: roughened; kidneys: mottled pale 25%; left adrenal: dark red foci.
	2878 M	14	T	Lungs: pale dark red foci 30%; stomach: vascularized; kidneys: mottled pale; adrenals: dark red foci.
	2946 F	14	T	Lungs: mottled pale 30%; stomach: slightly vascularized; spleen: slightly roughened; adrenals: red foci; mesentery lymph nodes: dark red.
	2941 F	14	T	Lungs: mottled pale 40%; spleen: slightly roughened; adrenals: red foci.
	2912 F	14	T	Lungs: dark red foci throughout, mottled dark red 40%; spleen: roughened; kidneys: mottled pale; adrenals: dark red.
	2965 F	14	T	Spleen: roughened.
	2928 F	14	T	Lungs: pale mottled dark red 30%; liver: mottled pale 20%; spleen: slightly roughened; adrenals: dark red foci.

[a]T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
706	2879 M	14	T	Lungs: mottled pale with dark red foci 100%; stomach: pronounced vascularization; spleen: slightly roughened; adrenals: dark red foci; mesenteric lymph nodes: red.
	2892 M	14	T	Lungs: mottled pale 20%, dark red foci 100%; spleen: slightly roughened; adrenals: red; mesenteric lymph nodes: dark red.
	2876 M	14	T	Lungs: dark red foci 100%, mottled pale 40%; stomach: pronounced vascularization; spleen: slightly roughened.
	2852 M	14	T	Lungs: pale, dark red foci throughout; spleen: slightly roughened; kidneys: mottled pale; adrenals: dark red; mesentery lymph nodes: dark red.
	2859 M	14	T	Lungs: pale, with dark red foci; stomach: empty; spleen: roughened.
	2909 F	2	F	Red, dry ocular discharge; yellow dry oral discharge; brain: pronounced vascularization; hole in eosphagus and trachea; thoracic cavity: contained food and test material.
	2940 F	14	T	Spleen: dark, roughened; adrenals: dark red foci.
	2942 F	14	T	Lungs: mottled dark red 20%, mottled brown red 80%; stomach: vascularized; spleen: slightly roughened; adrenals: mottled red.
	2903 F	14	T	Lungs: mottled dark red 30%; liver: mottled pale 20%; adrenals: dark red foci.
	2947 F	14	T	Lungs: mottled pale, dark red areas; spleen: slightly roughened; adrenals: brown-red foci; uterus: blood vessels injected.

[a]F=Found dead (Day given is day found dead).
T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
1002	2897 M	2	F	Pale, brown, wet urinary staining; brain: pronounced vascularization; lungs: mottled dark red 20%, bright red 80%; stomach: pronounced vascularization, red foci 30%, distended 3 times normal; intestines: pronounced vascularization, viscous orange fluid; adrenals: red; testes: pronounced vascularization; urinary bladder: contains thin green fluid.
	2841 M	14	T	Lungs: mottled dark red 20%, mottled pale 10%, dark red foci 100%; spleen: slightly roughened; adrenals: pale red.
	2856 M	14	T	Lungs: mottled pale 70%; spleen: slightly roughened; kidneys: mottled pale; adrenals: pale red.
	2868 M	14	T	Thoracic cavity: contains test material surrounding heart, small white particles.
	2884 M	3	F	Red dry nasal discarge; brain: pronounced vascularization; lungs: mottled dark red 50%, brown red 20%; liver: mottled pale 100%; stomach: pyloric region - contains black material mixed with other contents of stomach; intestines: contain viscous black fluid with yellow suspension; spleen: diminished, slightly roughened; kidneys: pale; adrenals: pale red; urinary bladder: contains red-yellow fluid.

[a]F=Found dead (Day given is day found dead).
T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
1002 cont.	2917 F	2	F	Alopecia of the ano-genital region; brain: pronounced vascuarization; lungs: mottled extreme dark red 80%, caseous, pale red patches approx. 1x1cm; liver: clear edges; stomach: distended; intestines: contain viscous yellow fluid; spleen: diminished; adrenals: pale red.
	2914 F	1	F	Dry, dark red oral discharge; alopecia: ano-genital area; dry urinary staining; brain: pronounced vascularization; lungs: mottled dark red 40%; liver: mottled pale 30%; stomach: pronounced vascularization, contains viscous brown fluid; intestines: pronounced vascularization, contain viscous brown fluid; spleen: slightly roughened; adrenals: pale red.
	2934 F	14	T	Lungs: red foci 100%; stomach: pronounced vascularization; spleen: slightly roughened; adrenals: red foci.
	2922 F	14	T	Lungs: mottled pale 10%; stomach: pronounced vascularization of cardiac and pyloric region; spleen: slightly roughened; adrenals: mottled pale red; lymph nodes (left side): red.
	2959 F	3	F	Dry red nasal discharge; yellow brown urinary staining; brain: pronounced vascularization; lungs: mottled bright red 80%, mottled dark red 20%; liver: mottled pale 80%; stomach: pyloric region - black areas on inside wall, contains yellow substance mixed with green substance; intestines: contain red-yellow fluid, yellow suspension; spleen: slightly roughened; kidneys: pale; adrenals: pale red; urinary bladder: empty.

[a]F=Found dead (Day given is day found dead).
T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
1423	2836 M	14	T	Lungs: mottled pale 70%; adrenals: pale red.
	2866 M	14	T	Lungs: mottled pale 80%, dark red foci 100%; stomach: slightly vascularized; spleen: roughened; right kidney: mottled pale; adrenals: pale red.
	2839 M	3	F	Red dry oral and nasal discharge; brown dry urinary and fecal staining; lungs: mottled dark red 20%, mottled brown red 80%, dark red foci 100%; liver: mottled pale throughout, dark red foci 100%; stomach: pyloric region - inside wall has black areas; intestines: contain brown yellow fluid; spleen: slightly roughened; kidneys: pale; adrenals: pale red; testes: undescended.
	2887 M	4	F	Brown oral discharge; red dry nasal discharge; red-yellow urinary staining; brain: pronounced vascularization; lungs: mottled dark red 75%, mottled brown red 25%; liver: mottled pale 100%; stomach: pyloric region - inside wall, small black patches; intestines: distended with gas, contain yellow-brown fluid with yellow suspension; spleen: pale; kidneys: pale; adrenals: pale red; urinary bladder: contains red-yellow fluid.
	2858 M	2	F	Clear wet nasal discharge; dry, yellow urinary staining; brain: pronounced vascularization; lungs: mottled dark red 90%; liver: mottled pale; stomach: pronounced vascularization, pyloric region - dark black foci, distended with viscous brown substance; spleen: diminished; adrenals: mottled pale.

[a]F=Found dead (Day given is day found dead).
T=Terminal (Killed on Day 14).

TABLE III (cont.)

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

DAY OF DEATH AND NECROPSY OBSERVATIONS

Dose Level mg/kg	Animal No. and Sex	Day of Death	Type of Death[a]	Observations
1423 cont.	2963 F	14	T	Lungs: mottled pale 20%, dark red foci 80%; spleen: roughened; adrenals: mottled pale red.
	2930 F	3	F	Dry red oral and nasal discharge; brown yellow urinary and fecal staining; brain: pronounced vascularization; lungs: mottled dark red 75%; liver: pale 80%; stomach: contains green-yellow material; intestines: contain red-yellow fluid, yellow suspension; kidneys: pale; adrenals: pale red.
	2907 F	3	F	Brown dry oral discharge; red dry nasal discharge; brown wet urinary staining; lungs: mottled dark red 20%, brown red 80%; dark red foci 100%; liver: mottled pale 80%; stomach: black foci inside wall; intestines: viscous yellow fluid with yellow suspension; spleen: dark red patch at tip; adrenals: dark red foci; urinary bladder: empty.
	2902 F	2	F	Dry red nasal discharge; wet yellow urinary staining; lungs: mottled dark red 80%; liver: mottled pale 30%; stomach: distended with gas and pale green substance; intestines: slightly pronounced vascularization, contain viscous white creamy substance; adrenals: enlarged, pale red; uterus: greyish.
	2936 F	3	F	Red dry nasal discharge; brown yellow urinary staining; lungs: mottled dark red 90%; liver: pale 80%, yellow patch on lobe; stomach: cardiac region - distended with air, pyloric region - black areas on inside wall; intestines: contain red-yellow fluid, viscous; kidneys: pale; adrenals: pale red.

[a]F=Found dead (Day given is day found dead).
T=Terminal (Killed on Day 14).

APPENDIX A

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine

ANALYSIS OF TRIPHENYLPHOSPHINE

The analysis of the triphenylphosphine for Lot PNVAH-6KF (flaked) is as follows:

Triphenylphosphine	=	99.4%
Triphenylphosphine Oxide	=	0.6%
Loss on Drying (LOD)	=	0.1%
Light Transmission*	=	98% 650 nm
APHA Color*	=	50
Ash	=	25 ppm
Chlorides	=	9.9 ppm
Magnesium	=	1.4 ppm
Iron	=	1.4 ppm
Sodium	=	1.6 ppm

*measured for 25% acetone solution

APPENDIX B

PROJECT NO.: 6218-80

ACUTE ORAL TOXICITY STUDY IN RATS

TEST MATERIAL: Triphenylphosphine (TPP)

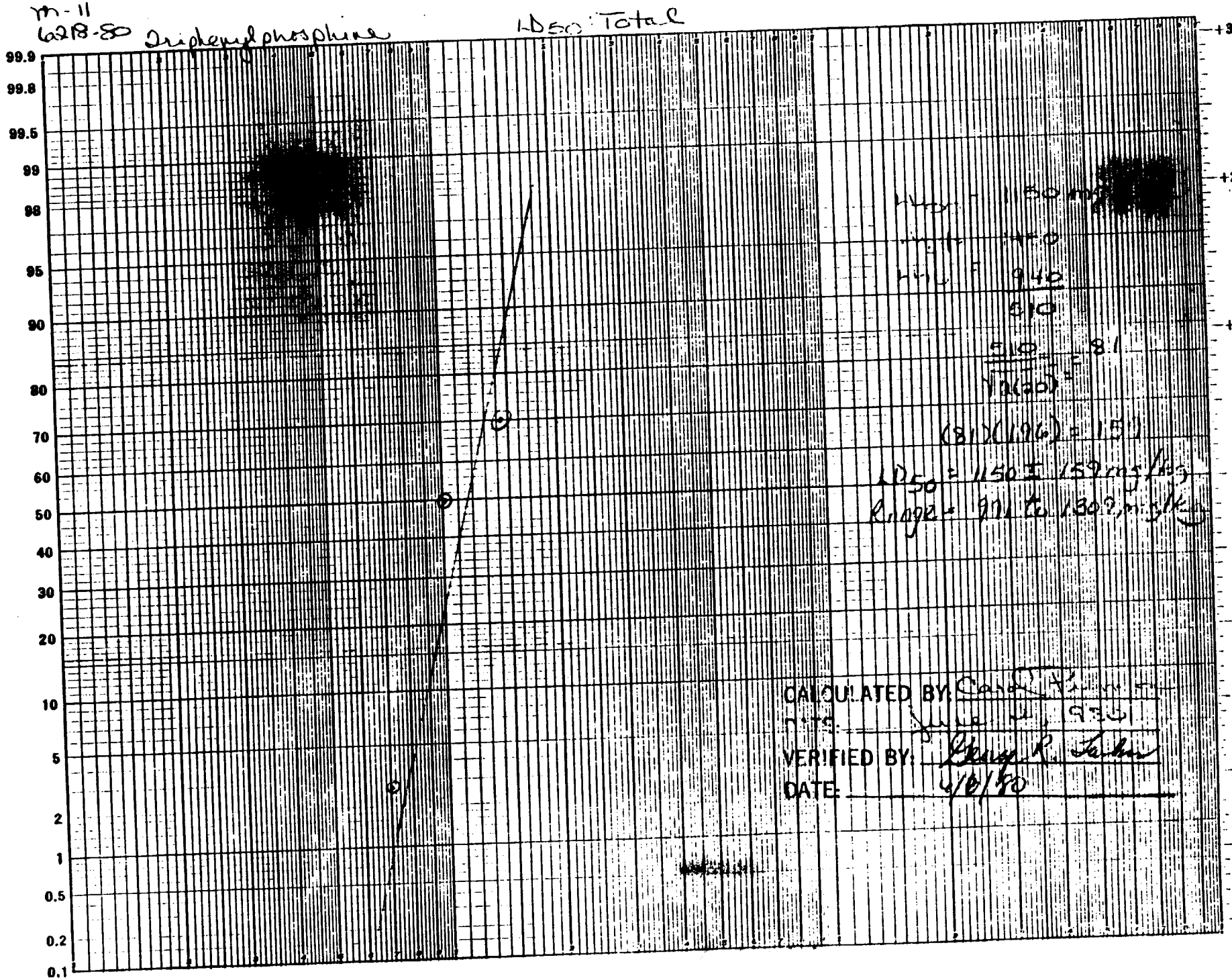
[redacted] CALCULATIONS, DOSE RESPONSE CURVES AND SLOPE

NO. 32,376. LOGARITHMIC NORMAL.

codex GRAPH PAPER ® IN STOCK DIRECT FROM ... PRINTED IN U.S.A.

m-11
6218-80 Triphenylphosphine

LD50: Total



LD50 = 1150 mg/kg

Range = 991 to 1309 mg/kg

CALCULATED BY: [illegible]

DATE: June 4, 1980

VERIFIED BY: [illegible]

DATE: 6/8/80

Bio dynamics Inc.

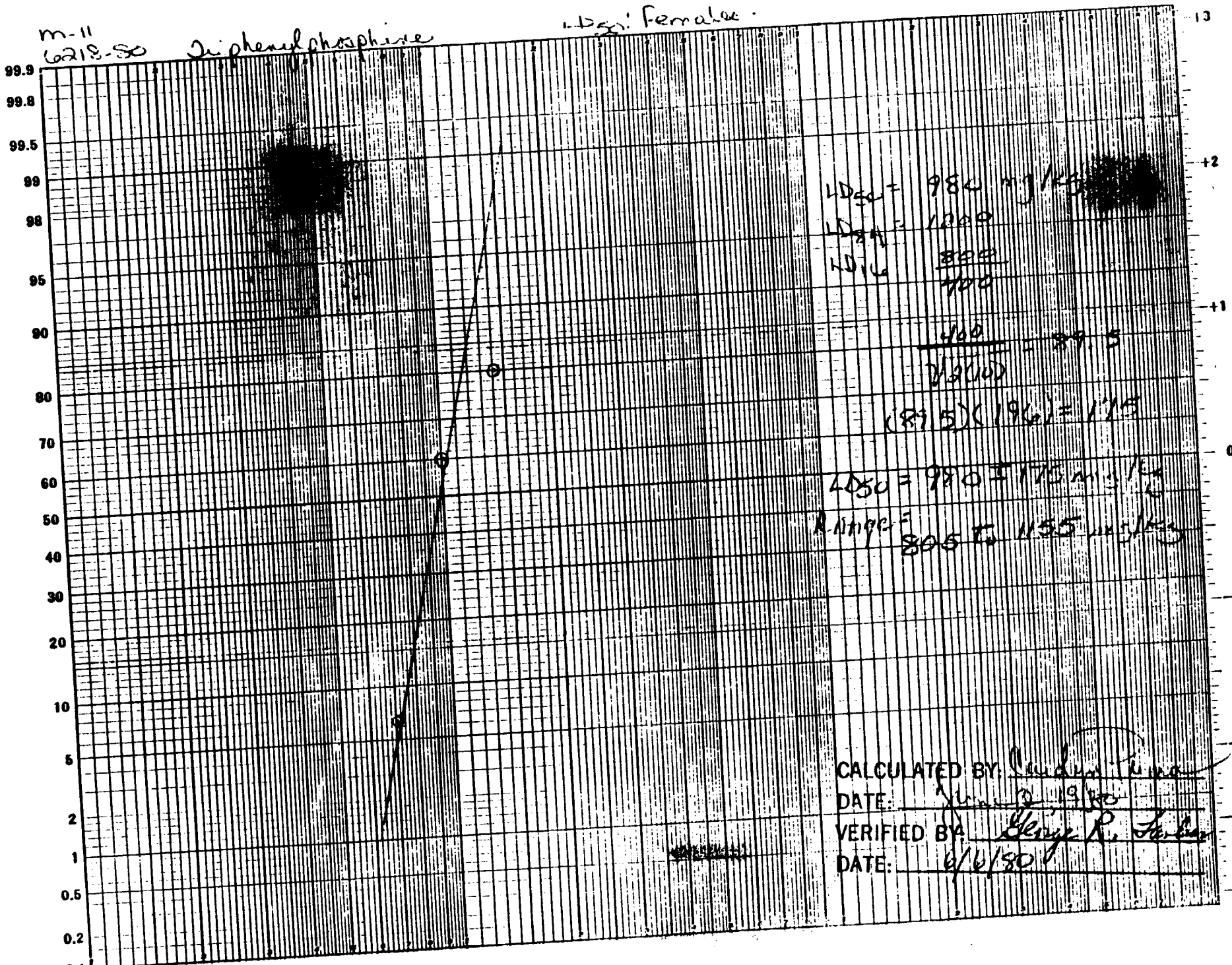
NO. 32.376. LOGARITHMIC NORMAL.

codex GRAPH PAPER

PRINTED IN U. S. A.

m-11
6218-80 Diphenylphosphine

LD50: Females.



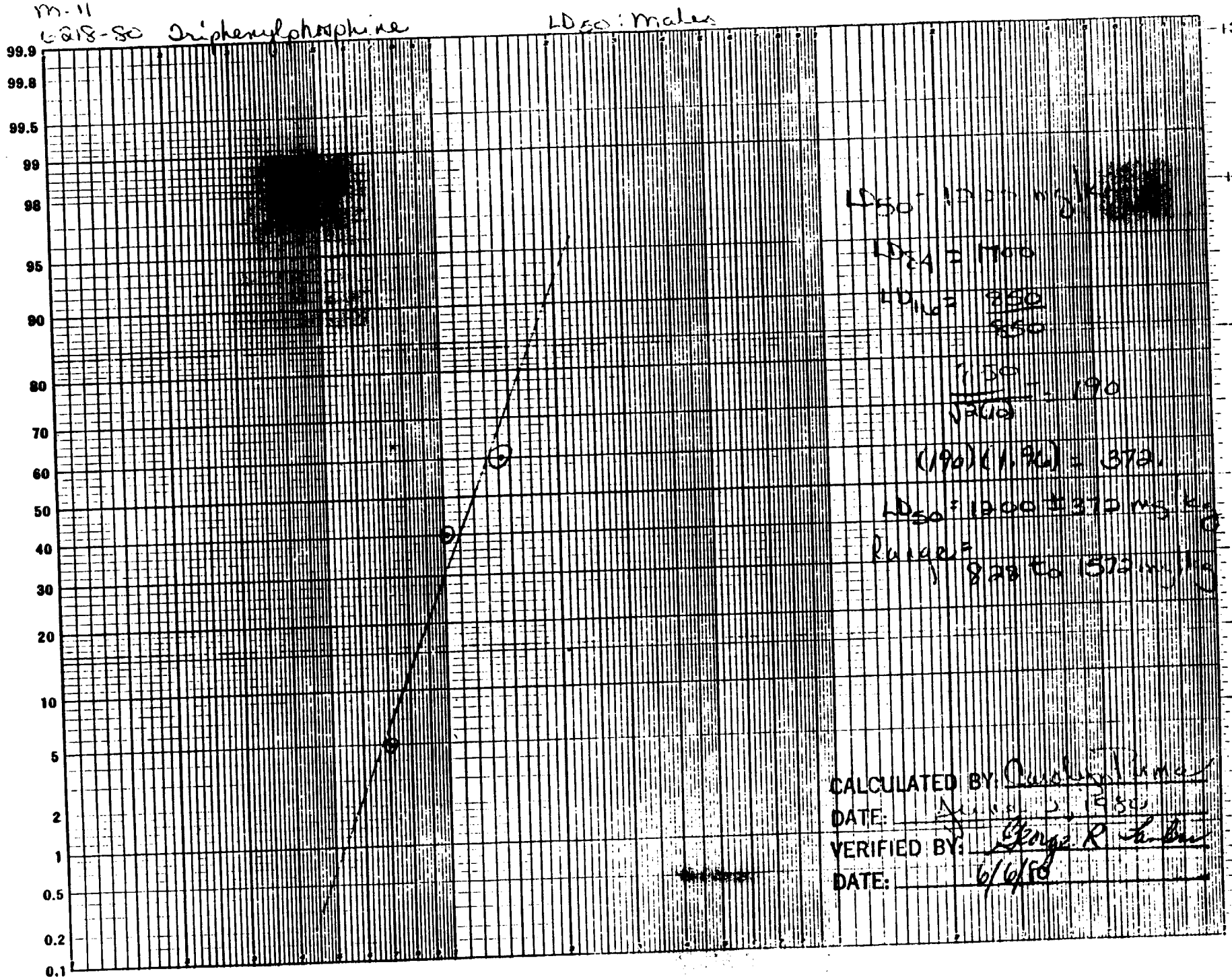
Bio-dynamics Inc.

NO. 32.376. LOGARITHMIC NORMAL. KEUFFEL & ESSER CO. GRAPH PAPER® PRINTED IN U.S.A.

M-11

C218-80 Triphenylphosphine

LD_{50}: Males



Biodynamics Inc.

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

C. H. Farr, PhD, DABT
Manager, Product Safety and Toxicology
Atochem North America, Inc.
900 First Avenue
P.O. Box 1536
King of Prussia, Pennsylvania 19406-0018

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

APR 0 6 1995

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information and **refer to the reverse side of this page for "EPA Information Requests"** .

All TSCA 8(e) submissions are placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA §8(e) policy statement (43 FR 11110, March 16, 1978). Confidential submissions received pursuant to the TSCA §8(e) Compliance Audit Program (CAP) should already contain information supporting confidentiality claims. This information is required and should be submitted if not done so previously. To substantiate claims, submit responses to the questions in the enclosure "Support Information for Confidentiality Claims". This same enclosure is used to support confidentiality claims for non-CAP submissions.

Please address any further correspondence with the Agency related to this TSCA 8(e) submission to:

Document Processing Center (7407)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan

Terry R. O'Bryan
Risk Analysis Branch

Enclosure 12588A

Recycled/Recyclable
Printed with Soy/Canola Ink on paper that contains at least 50% recycled fiber

Triage of 8(e) Submissions

Date sent to triage: 5/25/96

NON-CAP CAP

Submission number: 12588A

TSCA Inventory: Y N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO no eco study

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX	CTOX	EPI	RTOX	GTOX
STOX/ONCO	CTOX/ONCO	IMMUNO	CYTO	NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

~~THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY~~

For Contractor Use Only

entire document: 0 6 1 (2) **pages** _____ **pages** 1, 2, 3, TABS

Notes:

Contractor reviewer : PDK **Date:** 3/13/95

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CATS DATA:
Submission # 8EHQ-1092-12588 SEQ A

TYPE: INT. SUPP FLWP

SUBMITTER NAME: Elf Atochem North America, Inc.

SUB. DATE: 10/22/92 OTS DATE: 10/29/92 CSRAD DATE: 02/09/95

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)

DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
(0678) CAP NOTICE

VOLUNTARY ACTIONS:
(0400) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKERS/USERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

CHEMICAL NAME: CAS#

603-35-0

791-28-6

INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04
0202	ONCO (ANIMAL)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04
0204	MUTA (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04
(0207)	REPRO/TERATO (ANIMAL)	01 02 04
0208	NEURO (HUMAN)	01 02 04
(0209)	NEURO (ANIMAL)	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04
(0212)	ACUTE TOX. (ANIMAL)	01 02 04
(0213)	SUB ACUTE TOX (ANIMAL)	01 02 04
(0214)	SUB CHRONIC TOX (ANIMAL)	01 02 04
0215	CHRONIC TOX (ANIMAL)	01 02 04

INFORMATION TYPE:		P F C
0216	EPI/CLIN	01 02 04
0217	HUMAN EXPOS (PROD CONTAM)	01 02 04
0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04
0219	HUMAN EXPOS (MONITORING)	01 02 04
0220	ECO/AQUA TOX	01 02 04
0221	ENV. OCCC/REL/FATE	01 02 04
0222	EMER INCI OF ENV CONTAM	01 02 04
0223	RESPONSE REQEST DELAY	01 02 04
0224	PROD/COMP/CHEM ID	01 02 04
0225	REPORTING RATIONALE	01 02 04
0226	CONFIDENTIAL	01 02 04
0227	ALLERG (HUMAN)	01 02 04
0228	ALLERG (ANIMAL)	01 02 04
0239	METAB/PHARMACO (ANIMAL)	01 02 04
0240	METAB/PHARMACO (HUMAN)	01 02 04

INFORMATION TYPE:		P F C
0241	IMMUNO (ANIMAL)	01 02 04
0242	IMMUNO (HUMAN)	01 02 04
(0243)	CHEM/PHYS PROP	01 02 04
0244	CLASTO (IN VITRO)	01 02 04
0245	CLASTO (ANIMAL)	01 02 04
0246	CLASTO (HUMAN)	01 02 04
0247	DNA DAM/REPAIR	01 02 04
0248	PROD/USE/PROC	01 02 04
0251	MSDS	01 02 04
0299	OTHER	01 02 04

TRIAGE DATA:	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
	(YES)	YES (DROP/REFER)	DOG	LOW		
CAS SR	NO	NO (CONTINUE)	HEN RAT	MED		
	IN TRANSIT	REFER		HIGH		

COMMENTS: 8EHQ-1177-0015, 0978-0015, 1283-0015, 0379-0015, 0278-0055

CECATS\TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA:
Submission # 8EHQ- 1092 - 12588 SEQ. A

TYPE: (INT.) SUPP FLWP

SUBMITTER NAME: Elf Atochem North America, Inc.

SUB. DATE: 10/22/92 OTS DATE: 10/29/92 CSRAD DATE: 02/09/95

INFORMATION REQUESTED: FLWP DATE:
0501 NO INFO REQUESTED
0502 INFO REQUESTED (TECH)
0503 INFO REQUESTED (VOL ACTIONS)
0504 INFO REQUESTED (REPORTING RATIONALE)
DISPOSITION:
(0639) REFER TO CHEMICAL SCREENING
(0678) CAP NOTICE

VOLUNTARY ACTIONS:
(0400) NO ACTION REPORTED
0402 STUDIES PLANNED/UNDERWAY
0403 NOTIFICATION OF WORKER/USERS
0404 LABEL/MSDS CHANGES
0405 PROCESS/HANDLING CHANGES
0406 APP./USE DISCONTINUED
0407 PRODUCTION DISCONTINUED
0408 CONFIDENTIAL

CHEMICAL NAME: CAS#

603 - 35 - 0

791 - 28 - 6

INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C	INFORMATION TYPE:		P F C
0201	ONCO (HUMAN)	01 02 04	0216	EPI/CLIN	01 02 04	0241	IMMUNO (ANIMAL)	01 02 04
0202	ONCO (ANIMAL)	01 02 04	0217	HUMAN EXPOS (PROD CONTAM)	01 02 04	0242	IMMUNO (HUMAN)	01 02 04
0203	CELL TRANS (IN VITRO)	01 02 04	0218	HUMAN EXPOS (ACCIDENTAL)	01 02 04	(0243)	CHEM/PHYS PROP	01 02 04
0204	MUTA (IN VITRO)	01 02 04	0219	HUMAN EXPOS (MONITORING)	01 02 04	0244	CLASTO (IN VITRO)	01 02 04
0205	MUTA (IN VIVO)	01 02 04	0220	ECO/AQUA TOX	01 02 04	0245	CLASTO (ANIMAL)	01 02 04
0206	REPRO/TERATO (HUMAN)	01 02 04	0221	ENV. OCCC/REL/FATE	01 02 04	0246	CLASTO (HUMAN)	01 02 04
(0207)	REPRO/TERATO (ANIMAL)	01 02 04	0222	EMER INCI OF ENV CONTAM	01 02 04	0247	DNA DAM/REPAIR	01 02 04
0208	NEURO (HUMAN)	01 02 04	0223	RESPONSE REQEST DELAY	01 02 04	0248	PROD/USE/PROC	01 02 04
(0209)	NEURO (ANIMAL)	01 02 04	0224	PROD/COMP/CHEM ID	01 02 04	0251	MSDS	01 02 04
0210	ACUTE TOX. (HUMAN)	01 02 04	0225	REPORTING RATIONALE	01 02 04	0299	OTHER	01 02 04
0211	CHR. TOX. (HUMAN)	01 02 04	0226	CONFIDENTIAL	01 02 04			
(0212)	ACUTE TOX. (ANIMAL)	01 02 04	0227	ALLERG (HUMAN)	01 02 04			
(0213)	SUB ACUTE TOX (ANIMAL)	01 02 04	0228	ALLERG (ANIMAL)	01 02 04			
(0214)	SUB CHRONIC TOX (ANIMAL)	01 02 04	0239	METAB/PHARMACO (ANIMAL)	01 02 04			
0215	CHRONIC TOX (ANIMAL)	01 02 04	0240	METAB/PHARMACO (HUMAN)	01 02 04			

TRIAGE DATA: NON-CBI INVENTORY
(YES)
CAS SR NO
IN TERMINI

ONGOING REVIEW
YES (DROP/REFER)
NO (CONTINUE)
REFER

SPECIES
DOG
HEN
RAT

TOXICOLOGICAL CONCERN:
(LOW) ATOX
MED
(HIGH) SBTOX

USE:

PRODUCTION:

COMMENTS: 8ehq - 1177-0015, 0978-0015, 1283-0015, 0379-0015, 0278-0055

*COMMENT

8EHQ-1177-0015, 0978-0015, 1283-0015, 0379-0015, 0278-0055
SIGNS OF NEUROLOGICAL IMPAIRMENT WERE NOTED IN MALE DOGS RECEIVING TWENTY ORAL DOSES OF TPP AT 5, 10, OR 20 MG/KG, AND IN FEMALE DOGS RECEIVING 20 DOSES AT 1 MG/KG OR 2 DOSES AT 5 MG/KG. NEUROPATHOLOGY WAS APPARENT AT 20 MG/KG IN SOME ANIMALS. SIGNS OF NEUROTOXICITY WERE APPARENT IN HENS AFTER DOSING WITH 5 MG/KG HOWEVER THEY WERE MINOR. IN DOGS, A SINGLE ORAL DOSE OF 300 AND 500 MG/KG PRODUCED CLASSIC SIGNS OF CHOLINERGIC TOXICITY. DOGS RECEIVING 20 EXPOSURES OF 30 MG/M3 SHOWED NEUROTOXICITY (ATAXIA,LIMB,WEAKNESS). RATS GIVEN SINGLE ORAL DOSE OF 706, 1002, 1423 MG/KG SHOWED CLINICAL SIGNS OF NEUROTOXICITY.

12588A

L

Acute oral toxicity in rats is of low concern. Single oral gavage doses to Sprague-Dawley CD rats (5/sex/dose) at levels of 350, 497, 706, 1002, and 1423 mg/kg were lethal (0/10, 0/10, 0/9, 5/10, and 7/10, respectively). ~~The LD_{50} was 1200 mg/kg.~~ Clinical signs were observed at ≥706 mg/kg and included ataxia, fine or coarse tremors, red ocular discharge, irregular breathing, decreased respiratory rate, hypoactivity, and hypothermia.

H

Subacute oral toxicity in dogs is of high concern. Beagle dogs (1/sex/dose) received twenty oral gavage doses of 1, 5, 10, or 20 mg/kg/day over four weeks or two oral gavage doses of 1, 5, or 20 mg/kg/day. Signs of neurological impairment (altered reflexes, limb weakness/uncoordination, and "knuckling under" of paws) were noted in the male dogs that received twenty doses of 5, 10, or 20 mg/kg/day. Similar signs were also seen in female dogs that received two or twenty doses of 1 or 5 mg/kg/day. Microscopic evaluation revealed mild to moderate generalized vacuolative axonal lesions in the ventral and lateral funicle of the spinal cord in the male and female dogs that received twenty 20-mg/kg/day doses. Similar lesions, although minimal and focal, were noted at lower doses (1 and 10 mg/kg/day).

H

Subacute inhalation toxicity in dogs is of high concern. Beagle dogs (1/sex/dose) were exposed to 0.5, 3.2, 9.7, and 28 mg/m^3, 6 hours/day, 5 days/week for four weeks or 0.3, 3.6, and 24 mg/m^3, 6 hours/day for two days. Signs of neurological impairment (altered reflexes, tendency to "knuckle over," and uncoordination) were noted in 24-mg/m^3 males (two exposures) and 28-mg/m^3 males and females (20 exposures). Microscopic evaluation revealed moderate generalized vacuolative degeneration of the ventral and lateral funicle of the cervical and lumbar spinal cord in the male and female dogs that received twenty 28-mg/m^3 exposures. Similar lesions, although minimal and focal, were noted in 0.5- and 3.2-mg/m^3 dogs (20 exposures).

In a separate study, beagle dogs (2/sex/group for 4-week exposures; 1/sex/group for two-day exposures) were exposed to 18.0 or 94.8 mg/m^3, 6 hours/day, 5 days/week for four weeks or 162 and 216 mg/m^3, 6 hours/day for two days. Visual evidence of locomotor disfunctions were observed at 94.8 mg/m^3 after two weeks of exposure; there was no reversal during the recovery period. Elevated serum alkaline phosphatase activities and cholesterol concentrations were seen in both groups receiving twenty exposures. Patchy to segmental degeneration of the myelin in the white tracts of the brain and spinal cord were also noted in these animals. Histopathological evidence of myelin degeneration was also found in animals exposed for only two days, although these dogs did not exhibit signs of locomotor impairment.