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ATTN: TSCA 8(e) Coordinator



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Dear Sir or Madam:

On March 31, 1995, FMC Corporation ("FMC") notified EPA pursuant to TSCA §8(e) of preliminary information relative to an acute hen neurotoxicity study conducted with tricresyl phosphate, CAS Registry Number 1330-78-5. The audited report has just been made available and as indicated in that letter, FMC is providing the Agency with a copy of that report.

FMC makes no claims of confidentiality for this submission.

Sincerely yours,

Linda M. Clark

Linda M. Clark
Supervisor, Product Regulatory Affairs
215/299-6133



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Tricresyl Phosphate/Durad 125L Acute Delayed Neurotoxicity Study in Hens

GUIDELINE: 81, 82, and 83

AUTHOR: CHRISTINE FREEMAN

REPORT DATE: AUGUST 15, 1995

FMC CORPORATION
TOXICOLOGY LABORATORY
BOX 8
PRINCETON, NEW JERSEY 08543

STUDY NUMBER: I94-1925

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CERTIFICATION OF GOOD LABORATORY PRACTICE

This study was conducted in compliance with the EPA Good Laboratory Practice Standards, FDA Good Laboratory Practice Regulations, and OECD Principles of Good Laboratory Practice in all aspects with the following exceptions:

The positive control material was not analyzed for purity and stability.

The positive control dosing preparation was not analyzed for concentration, homogeneity, and stability of the positive control material in the vehicle.

Christine Freeman
Christine Freeman, B.S.
Laboratory Manager and
Study Director

8/15/95
Date

QUALITY ASSURANCE STATEMENT

The various phases of the study were inspected and the findings were reported to the study director and management as follows:

<u>PHASE</u>	<u>DATE INSPECTED</u>	<u>DATE REPORTED</u>
<u>IN LIFE:</u>		
ANIMAL RECEIPT	01-DEC-94	01-DEC-94
RANDOMIZATION	12-DEC-94	12-DEC-94
COMPOUND PREPARATION	13-DEC-94	13-DEC-94
DOSING	13-DEC-94	13-DEC-94
NTE/ACHE	15-DEC-94	15-DEC-94
BODY WEIGHTS	03-JAN-95	03-JAN-95
PERFUSION/NECROPSY	04-JAN-95	04-JAN-95
CLINICAL SIGNS	23-DEC-94	23-DEC-94
<u>AUDITS:</u>		
PROTOCOL	15-NOV-94	15-NOV-94
RAW DATA/TABLES	01-MAR to 03-MAR-95	03-MAR-95
HISTOPATHOLOGY	05-JUL-95	05-JUL-95
DRAFT REPORT	03-AUG to 07-AUG-95 09-AUG-95	07-AUG-95 09-AUG-95

The final report and raw data were reviewed for accuracy and compliance with FMC Toxicology Standard Operating Procedures, the study protocol, EPA Good Laboratory Practice Standards, FDA Good Laboratory Practice Regulations, and OECD Principles of Good Laboratory Practice. Findings were discussed with the study director and revisions were made where necessary.



William D. Barta, M.A.
Manager, Quality Assurance



11-August-95
Report Approval Date

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SUMMARY

White leghorn hens (11/group) received 2000 mg/kg of tricresyl phosphate/Durad 125L (test group), 500 mg/kg of tri-o-tolyl phosphate (positive control group), or corn oil (vehicle control group) as a single oral dose. Three animals from each group were sacrificed approximately 48 hours post-dosing for acetylcholinesterase (ACHE) measurements of the brains and neurotoxic esterase (NTE) measurements of the brains and spinal cords. The hens were observed for clinical signs hourly for five hours post-dosing on day 0, and once daily thereafter for 21 days (except those which were sacrificed at 48 hours post-dosing). Body weights were recorded on days 0, 7, 14, and 21; animals scheduled for 48-hour sacrifice for NTE and ACHE measurements were weighed on day 2, prior to sacrifice. Motor activity assessments were performed prior to dosing and twice weekly thereafter. Survivors from all groups were perfused and necropsied on study day 21 or 22. The brain, spinal cord, and tibial and sciatic nerves were evaluated for neuropathological lesions.

No deaths occurred during the study.

Test hens receiving tricresyl phosphate/Durad 125L displayed no treatment-related clinical signs, no significant differences in body weights or body weight gains, and no significant differences in brain cholinesterase levels when compared to vehicle control hens. Test hens displayed normal motor activity during the study. Brain and spinal cord NTE levels were significantly reduced (45% and 83%, respectively) among test hens when compared to vehicle control animals; the 83% reduction in spinal cord NTE levels was considered toxicologically significant. Neuropathologic examination of the nervous systems of test group hens revealed minimal to mild treatment-related lesions of the spinal cord.

Among positive control hens, the most significant clinical signs noted included ataxia, leaning on hocks, and staggered gait, which began on or about day 13 of the study and progressively worsened until study termination. No significant differences in body weights, body weight gains, or brain cholinesterase levels were noted among positive control hens when compared to vehicle controls. Significant reductions in brain and spinal cord NTE levels (90% and 96%, respectively) were noted among positive control hens when compared to vehicle control. Motor activity impairment was apparent on study day 10 among positive control hens and progressively worsened until study termination. Neuropathologic examination of the nervous systems of the positive control hens revealed lesions of the spinal cord, sciatic nerve, and the tibial nerve or nerve branches considered consistent with delayed neurotoxicity.

In conclusion, a single oral dose of 2000 mg/kg of tricresyl phosphate/Durad 125L produced toxicologically significant spinal cord NTE inhibition and spinal cord lesions without concomitant effects on clinical signs or motor activity. Under the conditions of this study, the No Observed Effect Level (NOEL) for acute delayed neurotoxicity for tricresyl phosphate/Durad 125L is less than 2000 mg/kg. A single oral dose of 2000 mg/kg of tricresyl phosphate/Durad 125L demonstrated a much weaker neurotoxic potential than did a single oral dose of 500 mg/kg of tri-o-tolyl phosphate, the positive control material.

REFERENCES

U.S. EPA Pesticide Assessment Guidelines - Subdivision F. Hazard Evaluation: Human and Domestic Animals. Addendum 10 - Neurotoxicity Series 81, 82 and 83; pp. 1-10; March 1991; (PB 91-154617).

PERSONNEL ASSIGNED TO STUDY

Study Director:	C Freeman
Pathologist:	L Brennecke, Pathology Associates, Inc., Frederick, MD
Data Processing Coordinator:	SS Corprew
Clinical Laboratory Consultant:	W Loeb, Ani Lytics, Inc., Gaithersburg, MD
FMC ACG Analytical Laboratory Personnel:	RM Herbst, FMC ACG Analytical Dept.
Sponsoring Toxicologist:	LA Kotkoskie
Technician in Charge:	FA Cooper

SPONSOR

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OBJECTIVE

To evaluate the potential of Tricresyl Phosphate/Durad 125L to produce neurotoxicity in hens following a single oral administration at a dosage level of 2000 mg/kg.

JUSTIFICATION FOR DOSE SELECTION

The maximum cut-off dose recommended in the guidelines is 2000 mg/kg. This dosage level was confirmed to be a non-lethal level as determined by the range-finding phase of this study.

MATERIALS AND METHODS

Experimental Design

The protocol is in Appendix A. The study was initiated on November 16, 1994, the date the study director signed the protocol. The following figure summarizes the experimental design.

Experimental Design					
<u>Group</u>	<u>Treatment</u>	<u>Dosage Level</u>	<u>Dosage Volume</u>	<u>Animals/Group NTE/ACHE</u>	<u>Pathology</u>
Vehicle Control	Corn Oil	Not Applicable	1.7 ml/kg	3	8
Test Group	Tricresyl phosphate / Durad 125L	2000 mg/kg	1.7 ml/kg	3	8
Positive Control	TOTP	500 mg/kg	2.0 ml/kg	3	8

TOTP = Tri-o-tolyl phosphate

Animal Husbandry and Maintenance

Forty-four white leghorn hens arrived from Spafas, Preston, CT on December 1, 1994. The animals were 8-14 months of age upon receipt and were ordered at a weight range of approximately 1.8 kg.

The hens were housed individually in stainless steel cages with solid bottoms in animal room 12. The animal room contained no other species or strain of test animal and no other test materials. Deorb bedding was used in the litter pans as direct bedding. The fluorescent light/dark cycle was 12 hours/12 hours. The average daily temperature was 65°F to 69°F and the average daily relative humidity was 49% to 63%. Animals received chicken scratch (as provided by the hen supplier to maintain consistency of the diet) *ad libitum* during the acclimation and study periods. Fresh feed was provided daily. Domestic water, untreated with additional HCl or chlorine, was supplied via an automatic watering system. Periodic water testing results are kept on file at the FMC Toxicology Laboratory.

Upon arrival, hens received temporary, sequential numbers. The animals were acclimated for 12 days, and were observed twice daily for mortality. Only healthy animals were assigned to study.

Randomization

On December 12, 1994, all hens released from acclimation were individually weighed. The body weights were analyzed in the following manner:

A computerized weight stratification/randomization program eliminated animals at the weight extremes until 33 animals remained. The program randomly allocated animals by weight to a specific cage and column. Each column of cages was randomly assigned to a treatment group. The randomization program ensured that the mean body weights of the groups were approximately equal. At the commencement of the study, the weight variation of the animals

did not exceed $\pm 20\%$ of the mean weight. Homogeneity of initial body weights was tested using analysis of variance. Following randomization, the animals were identified with leg bands containing permanent numbers, then placed into assigned cages. Each cage was labeled with a color-coded cage card.

Test Material

The test material used in the study was tricresyl phosphate/Durad 125L (batch 09238, laboratory # LDP304), a clear liquid assigned FMC-T# 1142. The test material was stored in a closed container at room temperature. Purity and 30-, 60-, and 90-day stability analyses of the test material were performed by the FMC ACG Analytical Department under study number P94-0072.

Positive Control Material

The positive control material, tri-o-tolyl phosphate, was purchased from the Eastman Kodak Company. It was a thin, clear liquid, CAS #78-30-8 1517, Lot Number 0420103361; the material was assigned FMC-T#1113. The material was not analyzed.

Preparation and Administration of the Test Material

All dosing preparations were made on the day of dosing. The test group received 1.7 ml/kg of undiluted tricresyl phosphate/Durad 125L; the vehicle control group received corn oil at a dosage volume of 1.7 ml/kg. The positive control dosing preparation was made as a 25% (w:v) solution in corn oil by adding corn oil to 50 g of tri-o-tolyl phosphate to yield a total volume of 200 ml; the material was dosed at a volume of 2.0 ml/kg. All dosing preparations, including vehicle control, were stirred for approximately 15 minutes prior to and during dosing.

The animals were fasted for at least fifteen hours prior to dosing. On study day 0, the animals were weighed; individual doses were calculated and verified. The appropriate dosing preparation was administered via oral gavage. Food was returned to the animals following dosing.

Mortality

Observations for mortality were conducted twice daily. The time of death or the time of the discovery of death was also noted.

Clinical Signs

The nature, onset and duration of all gross or visible toxicological or pharmacological effects were recorded hourly for five hours post-dosing and daily thereafter for 21 days. The observations were performed to note any changes associated with the eyes and mucous membranes, respiratory, circulatory and excretory systems, autonomic and central nervous

systems, somatomotor activity and behavior patterns with emphasis on locomotor coordination (i.e., ataxia or paralysis).

Body Weights

Body weights were recorded on the day of randomization, on day 0 (prior to dosing), and on days 7, 14, and 21. Animals designated for sacrifice for NTE/ACHE determinations were weighed prior to sacrifice on day 2.

Neurotoxic Esterase (NTE) and Acetylcholinesterase (ACHE) Measurements

Approximately 48 hours post-dosing, the last three hens in each group were sacrificed via carbon dioxide inhalation. The brain and spinal cord were removed and rinsed in ice cold buffer (50 mM Tris/0.2mM EDTA adjusted to pH 8.0 at approximately 25°C with HCl). The meninges and blood vessels were rapidly removed; the brain was blotted dry, weighed, and frozen in liquid nitrogen. The brains and spinal cords were stored frozen at approximately -75° C, then shipped on dry ice to Dr. Walter Loeb, Ani Lytics, Inc., Gaithersburg, MD for NTE and cholinesterase measurements.

Motor Activity

Animals designated for pathology underwent a period of forced motor activity twice weekly (Tuesdays and Fridays). Assessment involved grading each animal by evaluation of walking, running, perching, ladder climbing, and landing from a height of approximately 12 inches according to the rating system outlined below.

Motor Activity Assessment	
<u>Points</u>	<u>Ataxia Assessment</u>
0	No ataxia.
1	Slight incoordination; occasional stumbling or wing drooping, especially after exertion.
2	Staggering gait, tail and leg reflexes may be affected; bird lands awkwardly.
3	Continuous staggering gait, bird rests often; tail and leg reflexes usually noticeably affected.
4	Bird stands for short periods only; normally moves by shuffling on hocks; tail and leg reflexes usually noticeably affected.
5	Bird unable to stand, weak limb movements; tail and leg reflexes virtually non-existent.

Perfusion/Necropsy

Eight hens/group were sacrificed at termination via an intravenous injection of Pentothal®, followed by perfusion with heparinized saline and a glutaraldehyde/paraformaldehyde buffer solution. Following successful perfusion, animals underwent a gross necropsy. Each hen was then skinned and eviscerated. Personnel from Pathology Associates, Inc. (PAI) removed the brain, spinal cord, and the appropriate sections of the sciatic and tibial nerves, and placed them into perfusate in accordance with PAI SOPs. Following the removal of these tissues, the remaining intact leg of each animal was disarticulated from the carcass and stored in perfusate. After isolation of the tissues from the last successfully-perfused hen, the required tissues were transported to PAI for processing, slide preparation, and neuropathological examination. After 18-24 hours in perfusate, the isolated tissues were placed in refrigerated phosphate-buffered saline (PBS) until processing for neuropathological evaluation. At PAI, the tissues were cut, mounted, and stained as described on pages 7-8 of the protocol (see Appendix A).

Neuropathology

Nervous system tissues were cut, mounted, and stained with hematoxylin/eosin and/or appropriate myelin and axon specific stains (Luxol Fast Blue/PAS) for histopathological examination. The following tissues were examined:

- Brain with medulla (cross sections including the forebrain, the center of the cerebrum, the cerebellum and pons, and the medulla oblongata).
- Three sections of the spinal cord: rostral cervical, mid-thoracic and lumbosacral (cross and longitudinal sections).
- Left and right sciatic nerves (cross and longitudinal sections of each).
- Left and right tibial nerves (cross and longitudinal sections of each).
- Left and right medial and lateral tibial nerve branches (cross and longitudinal sections of each).

Statistical Analyses

Dunnett's test in the Toxstat™ System developed by Statistics Unlimited, Inc., Wellesley, MA, was used to perform statistical analyses of body weights, brain and spinal cord NTE, and brain ACHE data.

Data Storage

All raw data, tissue specimens and reports generated during this study are maintained by the FMC Toxicology Department, Box 8, Princeton, New Jersey 08543.

RESULTS

Test Material Analyses

The analytical report for the purity and stability of tricresyl phosphate/Durad 125L is located in Appendix B.

A summary of the results appears in the following figure. The composition of the test material is tricresyl phosphate with a purity of 98%. The results show that the test material is stable at room temperature for at least 90 days.

Room Temperature Stability of Tricresyl Phosphate/Durad 125L				
	<u>Initial (Day 0) Analysis</u>	<u>30-Day Analysis</u>	<u>61-Day Analysis</u>	<u>90-Day Analysis</u>
Total % tricresyl phosphate	98.0	97.9	97.4	97.3
% Change from Initial Analysis	NA	-0.1	-0.6	-0.7
NA = Not Applicable				

Mortality

Mortality data appear in Table 1.

No deaths occurred during the study. All hens survived until their scheduled termination date.

Clinical Signs

Summary and individual clinical signs are listed in Table 2.

The only clinical sign noted among hens receiving tricresyl phosphate/Durad 125L was a single isolated incident of decreased feces; this spurious observation was not considered treatment-related.

The most significant clinical signs noted among positive control hens were ataxia, leaning on hocks, and staggered gait; the onset of these signs began on day 13 and worsened as the study progressed. Additionally, these hens displayed decreased feces and decreased locomotion.

Body Weights

Summary and individual body weights are presented in Table 3.

No significant differences in body weights or body weight gains were noted among treated or positive control hens sacrificed two days following dosing for NTE and cholinesterase

determinations when compared to vehicle controls. Similarly, the remaining treated and positive control hens, sacrificed at study termination, exhibited no significant differences in weekly body weights when compared to vehicle control animals. Positive control hens exhibited a greater reduction in body weight gains when compared to control hens; this reduction was not, however, statistically significant.

NTE/Cholinesterase Measurements

Summary and individual NTE and cholinesterase measurements are located in Table 4.

Hens receiving tricresyl phosphate/Durad 125L displayed a statistically significant reduction in brain and spinal cord NTE levels (45% and 83%, respectively) when compared to vehicle control animals; the 83% reduction in spinal cord NTE levels among test hens was considered toxicologically significant. Brain cholinesterase measurements were not statistically different from vehicle control levels.

Positive control hens also displayed significant reductions in brain and spinal cord NTE levels (90% and 96%, respectively) when compared to vehicle control hens. Brain cholinesterase levels were reduced among positive control hens when compared to vehicle control animals; this reduction, however, was not statistically significant.

Motor Activity

Individual motor activity data appear in Table 5.

No effects on motor activity were noted among hens receiving tricresyl phosphate/Durad 125L at any time during the study.

Positive control hens exhibited mild to severe motor activity impairment. This impairment was first apparent on day 10 of testing and progressively worsened until study termination.

Perfusion/Necropsy

No treatment-related gross necropsy findings were noted. One hen receiving tricresyl phosphate/Durad 125L had an oval mass in the peritoneal cavity; this single incidence was considered a spurious finding, unrelated to treatment.

Neuropathology

The neuropathology report is located in Appendix C.

Test hens receiving tricresyl phosphate/Durad 125L displayed minimal to mild lesions (axonal degeneration) of the spinal cord.

STUDY NUMBER: 194-1925

Acute Delayed Neurotoxicity Study in Hens With Tricresyl Phosphate/Durad 125L

Positive control hens displayed varying degrees of axonal degeneration of the spinal cord, sciatic nerve, and tibial nerve or nerve branches; these findings were considered indicative of delayed neurotoxicity.

DISCUSSION

Administration of a single oral dose of 2000 mg/kg of tricresyl phosphate/Durad 125L did not result in any effects on mortality, clinical signs, body weights, motor activity, gross necropsy findings, or brain cholinesterase levels. Findings indicative of neurotoxicity were evidenced by toxicologically significant reductions in spinal cord NTE levels when compared to vehicle control animals and the presence of minimal to mild neuropathologic lesions of the spinal cord among test hens.

Positive control animals, 500 mg/kg of tri-o-tolyl phosphate displayed clinical signs, motor activity impairment, significant reductions in brain and spinal cord NTE levels and neuropathologic lesions consistent with delayed neurotoxicity.

A single oral dose of 2000 mg/kg of tricresyl phosphate/Durad 125L demonstrated a much weaker neurotoxic potential than did a single oral dose of 500 mg/kg of tri-o-tolyl phosphate, the positive control material.

CONCLUSION

Under the conditions of this study, the No Observed Effect Level (NOEL) for acute delayed neurotoxicity for tricresyl phosphate/Durad 125L is less than 2000 mg/kg.

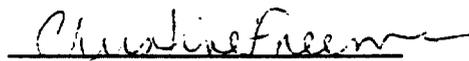
SCIENTIFIC STATEMENT

We, the undersigned, declare that this report constitutes a true record of the actions taken and the results obtained.

Report Prepared By:

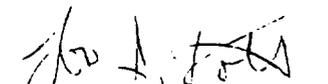

Bruce A. Watt, B.A.
Group Leader

8/14/95
Date

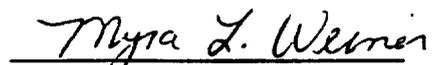

Christine Freeman, B.S.
Laboratory Supervisor and
Study Director

8/15/95
Date

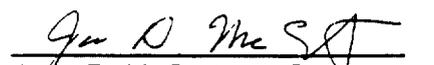
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Sponsoring Toxicologist

8-21-95
Date


Myra L. Weiner, Ph.D., D.A.B.T.
Manager, Toxicology Programs

8-21-95
Date


Jane D. McCarty, M.S.
Manager, Toxicology Programs

8/21/95
Date

STUDY NUMBER: I94-1925
 COMPOUND: TRICRESYL PHOSPHATE/DURAD 125L

TABLE 1
 ANIMAL FATE

VEHICLE CONTROL GROUP

ANIMAL NUMBER	TREATMENT	FATE
E0200F	VEHICLE CONTROL	TS (21)
E0201F	VEHICLE CONTROL	TS (21)
E0202F	VEHICLE CONTROL	TS (21)
E0203F	VEHICLE CONTROL	TS (21)
E0204F	VEHICLE CONTROL	TS (22)
E0205F	VEHICLE CONTROL	TS (22)
E0206F	VEHICLE CONTROL	TS (22)
E0207F	VEHICLE CONTROL	TS (22)
E0208F	VEHICLE CONTROL	NS (2)
E0209F	VEHICLE CONTROL	NS (2)
E0210F	VEHICLE CONTROL	NS (2)

TEST GROUP

ANIMAL NUMBER	TREATMENT	FATE
E0211F	TRICRESYL PHOSPHATE/DURAD 125L	TS (21)
E0212F	TRICRESYL PHOSPHATE/DURAD 125L	TS (21)
E0213F	TRICRESYL PHOSPHATE/DURAD 125L	TS (21)
E0214F	TRICRESYL PHOSPHATE/DURAD 125L	TS (21)
E0215F	TRICRESYL PHOSPHATE/DURAD 125L	TS (22)
E0216F	TRICRESYL PHOSPHATE/DURAD 125L	TS (22)
E0217F	TRICRESYL PHOSPHATE/DURAD 125L	TS (22)
E0218F	TRICRESYL PHOSPHATE/DURAD 125L	TS (22)
E0219F	TRICRESYL PHOSPHATE/DURAD 125L	NS (2)
E0220F	TRICRESYL PHOSPHATE/DURAD 125L	NS (2)
E0221F	TRICRESYL PHOSPHATE/DURAD 125L	NS (2)

POSITIVE CONTROL GROUP

ANIMAL NUMBER	TREATMENT	FATE
E0222F	TRI-O-TOLYL PHOSPHATE	TS (22)
E0223F	TRI-O-TOLYL PHOSPHATE	TS (22)
E0224F	TRI-O-TOLYL PHOSPHATE	TS (22)
E0225F	TRI-O-TOLYL PHOSPHATE	TS (22)
E0226F	TRI-O-TOLYL PHOSPHATE	TS (21)
E0227F	TRI-O-TOLYL PHOSPHATE	TS (21)
E0228F	TRI-O-TOLYL PHOSPHATE	TS (21)
E0229F	TRI-O-TOLYL PHOSPHATE	TS (21)
E0230F	TRI-O-TOLYL PHOSPHATE	NS (2)
E0231F	TRI-O-TOLYL PHOSPHATE	NS (2)
E0232F	TRI-O-TOLYL PHOSPHATE	NS (2)

TABLE 2. SUMMARY OF CLINICAL SIGNS

<u>FINDING</u>	<u>N</u>	<u>VEHICLE CONTROL</u>	<u>TEST GROUP</u>	<u>POSITIVE CONTROL</u>
ATAXIA	11	11	11	11
DARK COMB	0	0	0	39 (5)
DECREASED FECES	0	0	0	1 (1)
DECREASED LOCOMOTION	0	0	1 (1)	23 (4)
LEANING ON HOCKS	0	0	0	5 (2)
STAGGERED GAIT	0	0	0	16 (2)
48-HOUR SACRIFICE	0	0	0	28 (4)
TERMINAL SACRIFICE	3 (3)	3 (3)	3 (3)	3 (3)
	8 (8)	8 (8)	8 (8)	8 (8)

Numbers in this table represent the total number of times that a given finding occurred during this study. The numbers in parentheses represent the number of animals that had a particular finding.

TABLE 2. INDIVIDUAL CLINICAL SIGNS
VEHICLE CONTROL - CORN OIL

ANIMAL	OBSERVATION(S)	DAYS OBSERVED
E0200F	TERMINAL SACRIFICE	21
E0201F	TERMINAL SACRIFICE	21
E0202F	TERMINAL SACRIFICE	21
E0203F	TERMINAL SACRIFICE	21
E0204F	TERMINAL SACRIFICE	22
E0205F	TERMINAL SACRIFICE	22
E0206F	TERMINAL SACRIFICE	22
E0207F	TERMINAL SACRIFICE	22
E0208F	48-HOUR SACRIFICE	2
E0209F	48-HOUR SACRIFICE	2
E0210F	48-HOUR SACRIFICE	2

STUDY NO.: I94-1925
COMPOUND: TRICRESYL PHOSPHATE/DURAD 125L

09JAN95.08:01

TABLE 2. INDIVIDUAL CLINICAL SIGNS
TEST GROUP - TRICRESYL PHOSPHATE/DURAD 125L

ANIMAL	OBSERVATION(S)	DAYS OBSERVED
E0211F	TERMINAL SACRIFICE	21
E0212F	TERMINAL SACRIFICE	21
E0213F	DECREASED FECES	16
	TERMINAL SACRIFICE	21
E0214F	TERMINAL SACRIFICE	21
E0215F	TERMINAL SACRIFICE	22
E0216F	TERMINAL SACRIFICE	22
E0217F	TERMINAL SACRIFICE	22
E0218F	TERMINAL SACRIFICE	22
E0219F	48-HOUR SACRIFICE	2
E0220F	48-HOUR SACRIFICE	2
E0221F	48-HOUR SACRIFICE	2

TABLE 2. INDIVIDUAL CLINICAL SIGNS
 POSITIVE CONTROL - TRI-O-TOLYL PHOSPHATE

ANIMAL	OBSERVATION(S)	DAYS OBSERVED
E0222F	ATAXIA	18 19 20 21 22
	STAGGERED GAIT	18 19 20 21 22
	TERMINAL SACRIFICE	22
	TERMINAL SACRIFICE	22
	TERMINAL SACRIFICE	22
E0225F	DECREASED FECES	2 3 10 11 12 13
	TERMINAL SACRIFICE	22
	ATAXIA	13 14 15 16 17 18 19 20 21
	DARK COMB	21
E0227F	DECREASED FECES	18 19 20 21
	DECREASED LOCOMOTION	18 19 20 21
	LEANING ON HOCKS	14 15 16 17 18 19 20 21
	STAGGERED GAIT	14 15 16 17 18 19 20 21
	TERMINAL SACRIFICE	21
	ATAXIA	13 14 15 16 17 18 19 20 21
	DECREASED FECES	16 17 18 19 20 21
E0227F	DECREASED LOCOMOTION	21
	LEANING ON HOCKS	14 15 16 17 18 19 20 21
	STAGGERED GAIT	14 15 16 17 18 19 20 21
	STAGGERED GAIT	14 15 16 17 18 19 20 21

TABLE 2. INDIVIDUAL CLINICAL SIGNS
 POSITIVE CONTROL - TRI-O-TOLYL PHOSPHATE

ANIMAL	OBSERVATION(S)	DAYS OBSERVED										
E0227F (CONT.)	TERMINAL SACRIFICE											21
E0228F	ATAXIA											13 14 15 16 17 18 19 20 21
	TERMINAL SACRIFICE											21
E0229F	ATAXIA											15 16 17 18 19 20 21
	DECREASED FECES											15 16 17 18 19 20 21
	STAGGERED GAIT											15 16 17 18 19 20 21
	TERMINAL SACRIFICE											21
E0230F	48-HOUR SACRIFICE											2
E0231F	48-HOUR SACRIFICE											2
E0232F	48-HOUR SACRIFICE											2

STUDY NO.: I94-1925
COMPOUND: TRICRESYL PHOSPHATE/DURAD

PAGE: 1
DATE: 06/26/95
TIME: 13:29:19
PGM: DUNNETT

TABLE 3

A SUMMARY OF MEAN BODY WEIGHT VALUES (kg)
FOR FEMALES

STUDY DAY

	0	2	GAIN
VEHICLE CONTROL			
MEAN:	1.76	1.81	0.05
STD ERR:	0.049	0.052	0.039
N:	3	3	3
P:	0.534		
TEST GROUP			
MEAN:	1.80	1.77	-.04
STD ERR:	0.026	0.043	0.019
N:	3	3	3
P:		0.742	0.353
POSITIVE CONTROL			
MEAN:	1.84	1.82	-.02
STD ERR:	0.057	0.030	0.061
N:	3	3	3
P:		0.98	0.487

STUDY NO.: 194-1925
COMPOUND: TRICRESYL PHOSPHATE/DURAD

TABLE 3

PAGE: 1
DATE: 06/26/95
TIME: 13:29:38
PGM: DUNNETT

BODY WEIGHT DATA (kg) FOR FEMALES

STUDY DAY

GAIN

2

0

ANIMAL
NUMBER

VEHICLE CONTROL

E0208F	1.84	1.91	0.07
E0209F	1.77	1.74	-.03
E0210F	1.67	1.77	0.10
MEAN:	1.76	1.81	0.05
STD ERR:	0.049	0.052	0.039
N:	3	3	3
F:	0.534		

STUDY NO.: I94-1925
COMPOUND: TRICRESYL PHOSPHATE/DURAD

PAGE: 2
DATE: 06/26/95
TIME: 13:29:38
PGM: DUNNETT

TABLE 3

BODY WEIGHT DATA (kg) FOR FEMALES

STUDY DAY

TEST GROUP	ANIMAL NUMBER	0	2	GAIN
	E0219F	1.80	1.74	-.06
	E0220F	1.85	1.85	0.00
	E0221F	1.76	1.71	-.05
	MEAN:	1.80	1.77	-.04
	STD ERR:	0.026	0.043	0.019
	N:	3	3	3
	P:		0.742	0.353

STUDY NO.: I94-1925
COMPOUND: TRICRESYL PHOSPHATE/DUREAD

TABLE 3

PAGE: 3
DATE: 06/26/95
TIME: 13:29:38
PGM: DUNNETT

BODY WEIGHT DATA (kg) FOR FEMALES

ANIMAL NUMBER	STUDY DAY	
	0	2
POSITIVE CONTROL		
E0230F	1.95	1.83
E0231F	1.79	1.76
E0232F	1.77	1.86
MEAN:	1.84	1.82
STD ERR:	0.057	0.030
N:	3	3
P:		0.98
		GAIN
		--.12
		-.03
		0.09
		-.02
		0.061
		3
		0.487

STUDY NO.: I94-1925
 COMPOUND: TRICRESYL PHOSPHATE/DURAD

 TABLE 3

PAGE: 1
 DATE: 01/06/95
 TIME: 9:21:57
 PGM: DUNNETT

A SUMMARY OF MEAN BODY WEIGHT VALUES (kg)
 FOR FEMALES

	0	7	14	21	GAIN
	STUDY DAY				
VEHICLE CONTROL					
MEAN:	1.62	1.58	1.51	1.48	-.14
STD ERR:	0.027	0.040	0.042	0.041	0.038
N:	8	8	8	8	8
P:	0.737				
TEST GROUP					
MEAN:	1.60	1.58	1.55	1.52	-.08
STD ERR:	0.020	0.037	0.047	0.060	0.065
N:	8	8	8	8	8
P:		1	0.767	0.851	0.697
POSITIVE CONTROL					
MEAN:	1.60	1.54	1.44	1.33	-.27
STD ERR:	0.020	0.035	0.053	0.068	0.066
N:	8	8	8	8	8
P:		0.671	0.511	0.133	0.221

STUDY NO.: I94-1925
 COMPOUND: TRICRESYL PHOSPHATE/DURAD

 TABLE 3

PAGE: 1
 DATE: 01/06/95
 TIME: 9:22:35
 PGM: DUNNETT

BODY WEIGHT DATA (kg) FOR FEMALES

ANIMAL NUMBER	STUDY DAY					GAIN
	0	7	14	21	28	
VEHICLE CONTROL						
E0200F	1.69	1.65	1.45	1.42	1.27	
E0201F	1.58	1.46	1.43	1.43	-0.15	
E0202F	1.58	1.48	1.42	1.40	-0.18	
E0203F	1.50	1.42	1.35	1.34	-0.16	
E0204F	1.70	1.73	1.70	1.68	-0.02	
E0205F	1.56	1.60	1.61	1.62	0.06	
E0206F	1.71	1.69	1.60	1.52	-0.19	
E0207F	1.66	1.59	1.48	1.46	-0.20	
MEAN:	1.62	1.58	1.51	1.48	-0.14	
STD ERR:	0.027	0.040	0.042	0.041	0.038	
N:	8	8	8	8	8	
P:	0.737					

STUDY NO.: I94-1925
 COMPOUND: TRICRESYL PHOSPHATE/DURAD

 TABLE 3

PAGE: 2
 DATE: 01/06/95
 TIME: 9:22:35
 PGM: DUNNETT

BODY WEIGHT DATA (kg) FOR FEMALES

TEST GROUP	ANIMAL NUMBER	STUDY DAY				GAIN
		0	7	14	21	
	E0211F	1.54	1.42	1.33	1.25	-0.29
	E0212F	1.53	1.55	1.55	1.57	0.04
	E0213F	1.68	1.62	1.49	1.41	-0.27
	E0214F	1.55	1.53	1.61	1.60	0.05
	E0215F	1.60	1.69	1.71	1.74	0.14
	E0216F	1.63	1.73	1.70	1.71	0.08
	E0217F	1.63	1.59	1.57	1.54	-0.09
	E0218F	1.66	1.48	1.41	1.36	-0.30
	MEAN:	1.60	1.58	1.55	1.52	-0.08
	STD ERR:	0.020	0.037	0.047	0.060	0.065
	N:	8	8	8	8	8
	P:		1	0.767	0.851	0.697

STUDY NO.: I94-1925
 COMPOUND: TRICRESYL PHOSPHATE/DURAD

 TABLE 3

PAGE: 3
 DATE: 01/06/95
 TIME: 9:22:35
 PGM: DUNNETT

BODY WEIGHT DATA (kg) FOR FEMALES

ANIMAL NUMBER	STUDY DAY					GAIN
	0	7	14	21	21	
POSITIVE CONTROL						
E0222F	1.70	1.65	1.53	1.43	1.43	-0.27
E0223F	1.58	1.51	1.48	1.43	1.43	-0.15
E0224F	1.50	1.50	1.38	1.36	1.36	-0.14
E0225F	1.59	1.44	1.27	1.35	1.35	-0.24
E0226F	1.62	1.44	1.30	1.05	1.05	-0.57
E0227F	1.57	1.51	1.44	1.17	1.17	-0.40
E0228F	1.64	1.72	1.74	1.67	1.67	0.03
E0229F	1.59	1.53	1.36	1.19	1.19	-0.40
MEAN:	1.60	1.54	1.44	1.33	1.33	-0.27
STD ERR:	0.020	0.035	0.053	0.068	0.068	0.066
N:	8	8	8	8	8	8
P:		0.671	0.511	0.133	0.133	0.221

STUDY NO.: 194-1925
COMPOUND: TRICRESYL PHOSPHATE/DURAD

TABLE 4

PAGE: 1
DATE: 01/06/95
TIME: 8:41:04
PCN: DUNNETT

INDIVIDUAL NTE AND CHOLINESTERASE MEASUREMENTS

ANIMAL NUMBER	BRAIN NTE (NMOL/MIN/G TISS)	SC NTE (NMOL/MIN/G TISS)	BRAIN CHE (IU/G TISS)
VEHICLE CONTROL			
DAY-TERM			
E0208F	1834	111.3	5.4
E0209F	1512	117.0	5.1
E0210F	1707	77.1	4.9
MEAN:	1684	101.8	5.1
STD ERR:	93.64	12.5	0.15
N:	3	3	3

STUDY NO.: I94-1925
COMPOUND: TRICRESYL PHOSPHATE/DURAD

TABLE 4

PAGE: 2
DATE: 01/06/95
TIME: 8:41:04
PGM: DUNNETT

INDIVIDUAL NTE AND CHOLINESTERASE MEASUREMENTS

TEST GROUP	ANIMAL NUMBER	BRAIN NTE (NMOL/MIN/G TISS)	SC NTE (NMOL/MIN/G TISS)	BRAIN CHE (IU/G TISS)
DAY-TERM	E0219F	381	19.9	5.1
	E0220F	1166	19.2	5.4
	E0221F	1252	13.2	5.9
MEAN:	933	17.4	17.4	5.5
STD ERR:	277.11	2.1	2.1	0.23
N:	3	3	3	3
P:	0.04	.000	.000	0.619

STUDY NO.: I94-1925

COMPOUND: TRICRESYL PHOSPHATE/DURAD

TABLE 4

PAGE: 3
DATE: 06/26/95
TIME: 14:10:18
PGM: DUNNETT

INDIVIDUAL NTE AND CHOLINESTERASE MEASUREMENTS

ANIMAL NUMBER	BRAIN NTE (NMOL/MIN/G TISS)	SC NTE (NMOL/MIN/G TISS)	BRAIN CHE (IU/G TISS)
246		10.6	4.7
7		1.6	3.8
265		0.0	5.1
POSITIVE CONTROL			
DAY-TERM			
E0230P			
E0231P			
E0232P			
MEAN:	173	4.1	4.5
STD ERR:	83.01	3.3	0.38
N:	3	3	3
P:	0.002	.000	0.278

STUDY NUMBER: 194-1925
 COMPOUND: TRICRESYL PHOSPHATE/DURAD 125L

TABLE 5

INDIVIDUAL MOTOR ACTIVITY ASSESSMENT

VEHICLE CONTROL GROUP										
ANIMAL NUMBER	DAY 0	DAY 3	DAY 7	DAY 10	DAY 14	DAY 17	DAY 21			
E0200F	0	0	0	0	0	0	0			
E0201F	0	0	0	0	0	0	0			
E0202F	0	0	0	0	0	0	0			
E0203F	0	0	0	0	0	0	0			
E0204F	0	0	0	0	0	1	0			
E0205F	0	0	0	0	0	0	0			
E0206F	0	0	0	0	0	0	0			
E0207F	0	0	0	0	0	0	0			
TEST GROUP										
ANIMAL NUMBER	DAY 0	DAY 3	DAY 7	DAY 10	DAY 14	DAY 17	DAY 21			
E0211F	0	0	0	0	0	0	0			
E0212F	0	0	0	0	0	0	0			
E0213F	0	0	0	0	0	0	0			
E0214F	0	0	0	0	0	0	0			
E0215F	0	0	0	0	0	0	0			
E0216F	0	0	0	0	0	0	0			
E0217F	0	0	0	0	0	0	0			
E0218F	0	0	0	0	0	0	0			
POSITIVE CONTROL GROUP										
ANIMAL NUMBER	DAY 0	DAY 3	DAY 7	DAY 10	DAY 14	DAY 17	DAY 21			
E0222F	0	0	0	0	2	2	2			
E0223F	0	0	0	0	0	0	0			
E0224F	0	0	0	0	0	1	0			
E0225F	0	0	0	0	0	0	1			
E0226F	0	0	0	0	4	4	5			
E0227F	0	0	0	1	3	4	5			
E0228F	0	0	0	0	1	1	2			
E0229F	0	0	0	0	2	3	3			

SCORE EXPLANATION:

- POINTS ASSESSMENT
- 0 NO ATAXIA.
 - 1 SLIGHT INCOORDINATION; OCCASIONAL STUMBLING OR WING DROOPING, ESPECIALLY AFTER EXERTION.
 - 2 STAGGERING GAIT, TAIL AND LEG REFLEXES MAY BE AFFECTED; BIRD LANDS AWKWARDLY.
 - 3 CONTINUOUS STAGGERING GAIT, BIRD RESTS OFTEN, TAIL AND LEG REFLEXES USUALLY NOTICEABLY AFFECTED.
 - 4 BIRD STANDS FOR SHORT PERIODS ONLY, NORMALLY MOVES BY SHUFFLING ON HOCKS; TAIL AND LEG REFLEXES USUALLY NOTICEABLY AFFECTED.
 - 5 BIRD UNABLE TO STAND, WEAK LIMB MOVEMENTS; TAIL AND LEG REFLEXES VIRTUALLY NON-EXISTENT.

APPENDIX A

Protocol and Revisions/Deviations

PROTOCOL

TITLE

Acute Delayed Neurotoxicity Study in Hens with
Tricresyl Phosphate/Durad 125L

OBJECTIVE

To evaluate the potential of Tricresyl Phosphate/Durad 125L to produce neurotoxicity in hens following a single oral administration at a dosage level of 2000 mg/kg.

PROPOSED EXPERIMENTAL DATES

These are GLP defined dates. For detailed listing of dates, see proposed study schedule.

Experimental Start Date (the first date the test substance is applied to the system): December 13, 1994

Experimental Termination Date (the last date on which data are collected directly from the study): March 1, 1995

PERSONNEL ASSIGNED TO STUDY

Study Director:	C Freeman
Pathologist:	L Brennecke
Data Processing Coordinator:	SS Corprew
Clinical Laboratory Consultant:	W Loeb, AniLytics, Inc.
Technician in Charge:	FA Cooper
Analytical Laboratory:	RM Herbst, FMC ACG Analytical Dept.

TEST MATERIALS

	<u>TEST MATERIAL</u>	<u>POSITIVE CONTROL MATERIAL</u>	<u>VEHICLE CONTROL MATERIAL</u>
<u>Identity:</u>	Tricresyl Phosphate/ Durad 125L	Tri-o-tolyl Phosphate	Mazola Corn Oil
<u>Formulation:</u>	Not applicable	Not applicable	Not applicable
<u>Reference No.:</u>	Batch 09238 Laboratory # LDP 304	0420103361	To be documented at the time of use
<u>Purity:</u>	To be determined by FMC ACG Analytical Dept. under Study #P94-0072	Not determined	100%
<u>Chemical Composition:</u>	Tricresyl Phosphate	Tri-o-tolyl Phosphate	Not applicable

TEST MATERIALS

	<u>TEST MATERIAL</u>	<u>POSITIVE CONTROL MATERIAL</u>	<u>VEHICLE CONTROL MATERIAL</u>
<u>Physical State:</u>	Liquid	Liquid	Liquid
<u>Stability:</u>	To be determined by FMC ACG Analytical Dept. under Study #P94-0072	Not determined. Expiration date to be documented at time of use.	Not determined. Expiration date to be documented at time of use.
<u>Storage Requirements:</u>	Room temperature	Room temperature	Room temperature
<u>Safety Requirements:</u>	Prior to initiation of dosing, gloves, uniforms and safety glasses will be worn by personnel in the animal room at all times. Respirators, gloves, appropriate eye protection and tyvek suits will be worn by personnel during chemical preparation and at all times following the onset of dosing.		

AGENCY SUBMISSION

EPA (TSCA)

SPONSOR

FMC Corporation
 Process Additives Division
 Tenax Road
 Trafford Park
 Manchester M17 1WT
 United Kingdom

TESTING FACILITY

FMC Corporation
 Toxicology Laboratory
 Box 8
 Princeton, New Jersey 08543 USA

REFERENCE

US EPA Pesticide Assessment Guidelines - Subdivision F. Hazard Evaluation: Human and Domestic Animals. Addendum 10 - Neurotoxicity Series 81, 82 and 83; pp 1-10; March, 1991; (PB 91-154617).

TEST SYSTEM

Species: Domestic Laying Hen

Strain: Single comb white leghorn

Number/Sex: 44 Females
 Definitive - 33 (11/group)
 Range Finding - 2
 Extras - 9

Animals not assigned to study may be used for technician training as appropriate. All unassigned animals will be humanely sacrificed via CO₂ inhalation or IV injection of Pentothal®.

Source: Spafas
Preston, Conn.

Age: Adult (8-14 months)

Weight Range: Ordered at approximately 1.8 kg

Randomization: Upon receipt, the hens will be assigned sequential temporary animal numbers. The hens released from acclimation will be weighed individually. A computerized randomization program will be used to sequentially sort the body weights and assign animals to groups, 11 hens per group. Animals at the extreme ends of the weight ranges will be omitted from the population. The remaining animals will be weight stratified and randomly allocated to each of the groups and identified by leg bands. This ensures that each group contains a similar population of hens and the initial mean body weights are approximately equalized. These animals will be placed into assigned cages and identified by group by using color coded cards affixed to each animal's cage. At randomization, weight variation of the animals will not exceed $\pm 20\%$ of the mean weight. Homogeneity of initial body weights will be tested using analysis of variance.

Species Justification: This is the recommended laboratory animal for acute delayed neurotoxicity studies

Justification for Route of Administration: This route is a recommended route of administration for acute delayed neurotoxicity studies.

Justification for Dose Selection: 2000 mg/kg/day is the maximum cut-off dose recommended in the guidelines.

LABORATORY ANIMAL CARE:

Isolation: Upon receipt, animals will be isolated until their health status is determined. Any animal considered unhealthy will be immediately sacrificed.

Acclimation: Approximately 44 hens will be received. Thirty-three hens will be assigned to study. They will be assigned temporary sequential numbers and acclimated for a minimum of 7 days. The animals will be observed twice daily for mortality. Feed and water consumption will be visually checked daily. Clinical observations will

be recorded daily during acclimation. Only animals in apparent good health will be released from acclimation.

Identification: The animals selected for study will be identified by means of leg bands and color coded cage cards (by group).

Water: Domestic water supply (untreated with additional chlorine or HCl) which will be analyzed quarterly using parameters addressed in the EPA drinking water standards, via water hoppers (ad libitum). Water analyses records are on file in the Toxicology Laboratory.

Diet: Chicken scratch (supplied by hen supplier to keep food source consistent), will be offered to the animals ad libitum during the acclimation and study periods. Feed hoppers will be replaced every 2 weeks; fresh feed will be added daily. The levels of contaminants in the feed are expected to be negligible and will not interfere with the interpretation of the results of the study.

Housing: Hens will be housed individually, in suspended stainless steel cages, solid bottom with direct bedding (Deosorb). Cages will be oversized to allow for freedom of mobility in order to better assess motor activity and gait. The animals will be housed in an animal room which will contain no other species of animal or other studies.

Temperature: The temperature will be monitored continuously. The average daily temperature will be maintained between 61- 80°F.

Humidity: The humidity will be monitored continuously. The average daily humidity will be maintained between 45- 70%.

Lighting: 12-hour light/dark cycle, fluorescent lighting

PREPARATION OF TEST MATERIAL

The test material and vehicle control material will be administered undiluted. The positive control will be prepared as a 25% (w/v) preparation in corn oil. Dosing preparations will be stored at room temperature (including controls). They will be mixed on a stir plate for ~ 15 minutes prior to and during dosing (including controls). Retention samples will be taken from each dosing preparation (including controls).

TEST MATERIAL ANALYSES

The composition and 0, 30, 60, 90 day stability of the test material will be determined under study #: P94-0072.

RANGEFINDING

A rangefinding study will be conducted prior to the definitive study on two hens receiving 2000 mg/kg of undiluted Tricresyl Phosphate/Durad 125L. These range-finding animals will not follow the procedures outlined under the remainder of this protocol. They will be acclimated for one day. They will be fasted on 12/2/94 and dosed on 12/3/94. The test material will be administered once by oral intubation. Hens will be observed twice daily for mortality and once daily for clinical observations from 12/2/94 through 12/11/94. Hens which die will be necropsied only to verify that no gavage errors were made. Body weights will be recorded prior to dosing and prior to necropsy. Additional hens may be dosed if needed.

EXPERIMENTAL DESIGN

<u>Group</u>	<u>Treatment</u>	<u>Dosage Level</u> (mg/kg)	<u>Dosage</u> <u>Volume</u> (ml/kg)	<u>Animals/Group</u> NTE/ACHE Pathology	
Vehicle Control	Corn oil	NA	1.7	3	8
Test	Tricresyl Phosphate/ Durad 125L	2000*	1.7	3	8
Positive Control	Tri-o-tolyl Phosphate	500	2.0	3	8

*Density = 1.17 g/ml. If preliminary range-finding data indicate that a limit dose is not acceptable, the study will be conducted at the maximum, non-lethal dose as determined by the range-finding study.

TEST MATERIAL ADMINISTRATION

The animals will be fasted overnight prior to dosing (a minimum of 15 hours). The appropriate material will be administered once to each hen on day 0 by oral gavage, using a size 8 French flexible feeding tube.

OBSERVATIONS

Mortality: Observations for mortality will be conducted twice daily, once in the morning and once in the late afternoon (weekdays). During weekends and holidays, the observations will be made upon the arrival of the technician and immediately before leaving.

Clinical Signs: The nature, onset and duration of all gross or visible toxicological or pharmacological effects will be recorded hourly for 5 hours post dosing on day 0 and once daily thereafter. The observations will include changes associated with the eyes and mucous membranes, respiratory, circulatory and excretory systems, autonomic and central nervous systems, somatomotor activity and behavior pattern with emphasis on locomotor coordination (i.e., ataxia or paralysis). The time of death or discovery of death will also be noted.

Motor Activity: Animals (designated for Pathology) will be removed from their cages twice weekly (Tuesdays and Fridays) for a period of forced motor activity. Each animal will be graded for locomotor ataxia and paralysis according to the rating system as outlined below. Animals will be assessed while walking, running, perching, ladder climbing and jumping onto a crate.

<u>POINTS</u>	<u>ATAXIA ASSESSMENT</u>
0	No ataxia.
1	Slight incoordination; occasional stumbling or wing drooping, especially after exertion.
2	Staggering gait, tail and leg reflexes may be affected; bird lands awkwardly.
3	Continuous staggering gait, bird rests often, tail and leg reflexes usually noticeably affected.
4	Bird stands for short periods only, normally moves by shuffling on hocks; tail and leg reflexes usually noticeably affected.
5	Bird unable to stand, weak limb movements; tail and leg reflexes virtually non-existent.

Moribund Animals: Moribund animals will be weighed, sacrificed via CO₂ inhalation and necropsied as soon as possible after discovery of death only to verify that no gavage errors were made. No tissues will be saved unless deemed necessary.

Body Weights: Body weights will be recorded on the day of randomization and day 0 (prior to dosing), day 7, 14 and 21. Animals scheduled to be necropsied on day 22 will be weighed prior to sacrifice on day 22. Animals which die or are sacrificed prior to study termination will be weighed prior to sacrifice or upon discovery of death.

Neurotoxic Esterase (NTE) and Acetylcholinesterase (ACHE)

Measurements: Approximately 48 hours following the initial dose (day 2), the last 3 surviving hens in each group will be sacrificed by carbon dioxide inhalation. Body weights will be recorded. The brain and spinal cord will be removed from each animal and rinsed in ice cold buffer (50 mM Tris/0.2 mM EDTA adjusted to pH 8.0 with HCl). The meninges and blood vessels will be rapidly removed and the brain will be blotted dry and weighed. The brain and spinal cord will then be placed individually into separate, pre-labeled vials and frozen in liquid nitrogen. Prior to shipping, tissues will be stored on dry ice.

Tissues will be packed in styrofoam freezer boxes containing dry ice, then placed into cardboard shipping boxes. Tissues will be sent via express courier service to Dr. W. Loeb, AniLytics, Inc., Suite 200, 200 Girard Street, Gaithersburg, MD 20879, for NTE (Brain and Spinal Cord) and ACHE (Brain only) determinations.

POST-MORTEM EXAMINATION

Necropsy: Non-Survivors will be necropsied as soon as possible after discovery of death only to verify that no gavage errors were made. Moribund animals will be sacrificed via CO₂ inhalation and necropsied immediately after sacrifice only to verify that no gavage errors were made.

Surviving animals will be sacrificed on day 21 (4/group) or on day 22 (4/group) by an IV injection of Pentothal® into the wing vein, followed by perfusion with heparinized saline and a Gluteraldehyde/Paraformaldehyde buffer solution. Following a successful perfusion, a gross necropsy will be performed and each animal will be eviscerated and skinned.

Tissue Preservation: The tissues listed below will be removed following successful perfusion as selected by personnel from Pathology Associates, Inc. who will be present at FMC during necropsy. Personnel from Pathology Associates, Inc. (PAI), will remove the brain, spinal cord, and appropriate sections of the sciatic and tibial nerves, place them into pre-labeled cassettes and into perfusate (Glutaraldehyde/Paraformaldehyde buffer solution) in accordance with PAI SOP 608.5. These tissues will remain in perfusate for 18-24 hours. Following removal of the brain, spinal cord, and the sciatic and tibial nerves, the remaining intact leg will be disarticulated from the rest of the hen carcass, wrapped in gauze, and stored in a pre-labeled Ziplock® bag containing perfusate.

After 18-24 hours, the container holding the specimens of the brain, spinal cord, sciatic and tibial nerves in perfusate will be drained into an appropriate waste receptacle. Phosphate-buffered saline (PBS) will then be added to the containers. The tissue samples, submerged in PBS will be kept refrigerated or cooled until processing for histopathological examination.

After isolation of the brain, spinal cord, sciatic and tibial nerves of the last successfully perfused animal in the study, all tissues and extra legs stored in perfusate will be transported back to the laboratories of PAI for processing, slide preparation, and histopathological examination as required. The extra legs may be used as a source of additional tissue samples if deemed necessary by the pathologist.

Personnel from PAI will transport all isolated tissue samples and remaining hen carcasses to PAI for processing and histopathological evaluation.

The tissues will be cut, mounted, and stained as follows:

The brain with medulla (cross sections to include the forebrain, the center of the cerebrum, the cerebellum and pons, and the medula oblongata) and the spinal cord (cross and longitudinal sections from the rostral-cervical, mid-thoracic, and lumbosacral regions) will be embedded in paraffin blocks.

Two slides of the brain block (containing 4 tissues) and two slides from each of the spinal cord blocks (each containing two tissues) will be cut; one slide of each block will be cut at approximately 5 um and stained with Hematoxylin and Eosin. The other will be sectioned at approximately 8 um and stained with Luxol Fast Blue/PAS. The left and right sciatic nerves (cross and longitudinal sections of each), the left and right tibial nerves (cross and longitudinal sections of each), the left and right medial and lateral tibial nerve branches (cross and longitudinal sections of each) will be embedded in glycomethacrylate blocks. One slide from each block will be sectioned at 2-3 um and stained with Hematoxylin and Eosin.

Pathology: Dr. Lucas Brennecke of Pathology Associates Inc., will conduct histopathological evaluation of the slides of all saved organs and tissues designated for histopathological evaluation of all test, vehicle control and positive control hens.

STATISTICAL ANALYSIS

Body weights, brain and spinal cord NTE and brain ACHE data will be statistically analyzed using Dunnett's test in the ToxstatTM System developed by Statistics Unlimited. Detailed procedures for analyses will be included in the final report.

CHANGES OR REVISIONS TO PROTOCOL

Any change or revision to this approved protocol will be documented, approved by the Study Director and maintained and distributed with this protocol.

REPORT

The report will include:

1. A summary providing an evaluation independent of the report, and will contain:
 - a. A summary and analysis of the data
 - b. A statement of conclusions drawn therefrom
2. Description of test procedure, including:
 - a. Method and any deviations from applicable standards
 - b. Length of study, randomization, initiation and termination dates
 - c. Test material name, reference number, FMC-T number, physical state, purity and stability (if available), storage conditions, identification of vehicle and positive control materials
 - d. Species/strain, source of animal supply, number of animals per group, acclimation procedures, randomization method, age and condition of animals at beginning of study

- e. Means of identification, number of animals per cage, diet, caging description, bedding type, average daily temperature and humidity, photoperiod and source of water
 - f. Dosage levels, route of administration, duration of treatment (exposure), frequency and schedule of observations
 - g. Location of raw data and archives
 - h. References, where applicable
 - i. Statistical procedures used
3. Data including:
- a. Time of death during the study or whether animals survived to termination
 - b. Time of observation of each abnormal sign and its subsequent course
 - c. Necropsy findings if applicable
 - d. Detailed description and classification of all histopathological findings
 - e. Body weight data
 - f. NTE and ACHE data
 - g. Grading of ataxia
4. Evaluation of results will include the relationship between the dose of the test material and the incidence and presence or absence of abnormalities, including:
- a. Clinical signs
 - b. NTE/ACHE results
 - c. Body weights
 - d. Effects on mortality
 - e. Pathology results
 - f. Ataxia assessment
5. Name and address of the laboratory where the study was performed
6. Signature of the Study Director, Sponsoring Toxicologist and Managers of Toxicology Programs
7. Quality Assurance Statement with the signature of the Quality Assurance auditor
8. Statement of Compliance with the signature of the Study Director
9. Histopathology Report
10. Analytical Report

GOOD LABORATORY PRACTICE REGULATIONS/STANDARDS

Good Laboratory Practice Regulations will be followed assuring that the quality of the study will permit the study to be reproduced.

QUALITY ASSURANCE

The Quality Assurance Unit will perform the following:

1. Conduct a sufficient number of in-progress inspections to ensure the integrity of the study.
2. Determine that no deviations from approved protocols or Standard Operating Procedures were made without proper authorization and documentation.
3. Review the final report to assure that the report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study.

PROTOCOL APPROVAL

This protocol was reviewed and approved by the FMC Toxicology Department Animal Care Review Committee. This study does not duplicate previous testing. All procedures will be conducted in a manner to minimize pain, discomfort and stress to test animals. No alternatives are available.

Furdy Ceskamps
QUALITY ASSURANCE AUDITOR

11/16/94
DATE

Christine Freeman
Christine Freeman
STUDY DIRECTOR

11-16-94
DATE

Lois A. Kotkoskie
Lois A. Kotkoskie
SPONSORING TOXICOLOGIST

11-17-94
DATE

Jane D. McCarty
Jane D. McCarty
MANAGER OF TOXICOLOGY PROGRAMS

11/16/94
DATE

Myra L. Weiner
Myra L. Weiner
MANAGER OF TOXICOLOGY PROGRAMS

11/17/94
DATE

FMC Toxicology Laboratory
Protocol Revisions/Deviations

Study Number: I94-1925

Protocol Revision/Deviation Number: 1

Study Director: Christine Freeman

Date Effective: December 21, 1994

The above named protocol is to be followed except for the following revision(s)/deviation(s):

Respirators and tyvek suits are no longer required in the animal room.

Reason: This is no longer required since dosing was completed on December 13, 1994.

This revision/deviation did not adversely affect the outcome of the study.

C. Freeman
Study Director

12/21/94
Date

cc: Raw Data (Original)
Laboratory Study File
Study Director
Quality Assurance Unit
517
8/13/93
T:\TOX\LAB\PROTOCOL\DEVIAT\NS\I1925.DOC

FMC Toxicology Laboratory
Protocol Revisions/Deviations

Study Number: I94-1925

Protocol Revision/Deviation Number: 2

Study Director: Christine Freeman

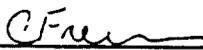
Date Effective: November 16, 1994

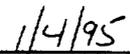
The above named protocol is to be followed except for the following revision(s)/deviation(s):

Dr. Mark Butt will replace Dr. Lucas Brennecke as pathologist.

Reason: PAI has requested that Dr. Butt replace Dr. Brennecke as pathologist for FMC.

This revision/deviation did not adversely affect the outcome of the study.


Study Director


Date

cc: Raw Data (Original)
Laboratory Study File
Study Director
Quality Assurance Unit
517
8/13/93
T:\TOX\LAB\PROTOCOL\DEVIATNS\211925.DOC

FMC Toxicology Laboratory
Protocol Revisions/Deviations

Study Number: I94-1925

Protocol Revision/Deviation Number: 3

Study Director: Christine Freeman

Date Effective: November 16, 1994

The above named protocol is to be followed except for the following revision(s)/deviation(s):

The section entitled Moribund Animals on page 6 of the protocol should read:

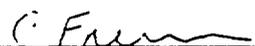
"Moribund animals will be weighed, sacrificed via CO₂ inhalation and necropsied as soon as possible after sacrifice only to verify that..."

rather than:

"Moribund animals will be weighed, sacrificed via CO₂ inhalation and necropsied as soon as possible after discovery of death only to verify..."

Reason: This was a wording error in the protocol.

This revision/deviation did not adversely affect the outcome of the study.


Study Director

7/19/95
Date

cc: Raw Data (Original)
Laboratory Study File
Study Director
Quality Assurance Unit
517
8/13/93
T:\TOX\LAB\PROTOCOL\DEVIATNS\311925.DOC

APPENDIX B

**Analytical Report for the Composition and Stability
of Tricresyl Phosphate/Durad 125L (P94-0072)**

Study Title: Composition and 90 Day Stability Analysis of Tricresyl Phosphate - **DURAD**[®] 125L

Sponsor Project No. P94-0072

Test Substance: Tricresyl Phosphate/DURAD 125L

Reference No. Batch 09238 LDP 304

Data Requirement TSCA

Author: Robert M. Herbst, Sr. Research Chemist

Study Dates:

Initiated: October 19, 1994

Terminated: January 23, 1995

Reported: June 30, 1995

This report supersedes the Analytical Technical Memo (ATM-0208) issued on March 21, 1995.

Performing Laboratory: FMC Corporation
Agricultural Products Group
Analytical Sciences
P.O. Box 8
Princeton, New Jersey 08543
(609) 951-3000

Laboratory Study No.: 703AF94283

DURAD[®] is a registered trademark of FMC Corporation

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Unpublished Work Protected by Copyright
FMC Corporation**

Good Laboratory Practice Compliance Statement

Study Number 703AF94283 entitled, "Composition and 90 Day Stability Analysis of Tricresyl Phosphate - DURAD® 125L," was conducted in compliance with the Good Laboratory Practice Standards as published in 40 CFR 792 where applicable to an analytical laboratory.

Study Director:



Robert M. Herbst
Sr. Research Chemist

June 30, 1995
Date

Sponsor:



Christine Freeman
Laboratory Supervisor

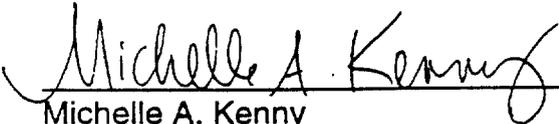
7/19/95
Date

QUALITY ASSURANCE STATEMENT

It is the intent of FMC Corporation that all studies sponsored by or conducted by our facility shall be of the highest quality in design and performance. Study No. 703AF94283, "Composition and 90 Day Stability Analysis of Tricresyl Phosphate - DURAD® 125L," reported herein, was inspected and the findings reviewed and signed by the Study Director and Management of FMC Corporation on the following dates.

<u>Inspection Date(s)</u>	<u>Signed by Study Director</u>	<u>Signed by Manager</u>	<u>Signed by Director</u>
11/30/94	12/02/94	12/14/94	12/16/94
12/19/94	12/21/94	12/23/94	12/27/94
12/22/94	01/13/95	01/24/95	01/26/95

This report and all records and raw data were audited and the report was found to be an accurate reflection of the study. All raw data will be maintained by FMC Corporation, P.O. Box 8, Princeton, NJ 08543, in the Quality Assurance Archives.


Michelle A. Kenny
Quality Assurance Specialist

7/7/95
Date

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I. SUMMARY AND INTRODUCTION

This report supersedes Technical Memo (ATM-0208) issued on March 21, 1995.

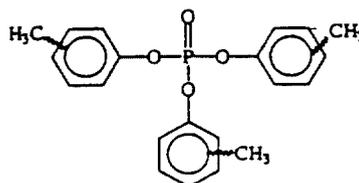
On October 17, 1994, we received a sample (Batch 09238, LDP 304) of tricresyl phosphate (DURAD 125L) from the Toxicology Department. The objective was to determine the composition and stability of tricresyl phosphate (DURAD 125L) at approximately 0, 30, 60, and 90 day intervals.

Composition of the sample was determined by spectroscopic and chromatographic techniques. Mass spectrometry and nuclear magnetic resonance spectroscopy indicated the material to be tricresyl phosphate. Gas chromatography was used to determine the area percent content of each tricresyl phosphate isomer in the sample.

To determine stability, we employed the same area percent gas chromatographic methodology. The sample was analyzed at intervals of 30, 61, and 90 days. The analysis dates and total isomer content for each interval appear in Section VI, Table 1. Isomer distribution appears in Section VI, Table 2.

Results verify that the tricresyl phosphate is stable for 90 days at room temperature.

The structure and CAS number of tricresyl phosphate follow:



CAS No. 1330-78-5

A complete Protocol and Protocol Modification can be found in Section VIII, Appendix A and B, respectively.

II. EXPERIMENTAL

We employed an area percent gas chromatography test method designated ACG No. 305, Section VIII, Appendix C for all quantitative analyses. No modifications were made to the test method during the course of this study.

Representative chromatograms of reference materials are presented in Section VI, Figures 1 and 2, and a typical sample chromatogram is shown in Section VI, Figure 3.

MS data were obtained using a Finnigan TSQ-70B Mass Spectrometer in both electron impact (EI) and chemical ionization (CI) modes. Proton and phosphorus NMR spectra were obtained using a Nicolet NT-300FT NMR Spectrometer. Typical spectra are presented in Section VI, Figures 4 to 7.

III. RESULTS AND DISCUSSION

We collected chromatographic data using Perkin-Elmer Corporation Access*Chrom[®] software, Version 1.9. We transferred the peak area percent data to a Microsoft[®] Excel, Version 5.0, spreadsheet, where we calculated the total area percent purity for the peaks of interest. We based the results on three sample preparations injected three times each.

A test material is considered stable if, over a specified time period, the assay results indicate no more than a 10% decrease from the initial assay. In this study, we analyzed the sample at intervals over 90 days of room temperature storage. After 90 days storage, the assay decreased 0.7%. This result verifies that the sample is stable for at least 90 days at room temperature. All results appear in Section VI, Tables 1 and 2.

MS data yielded the expected molecular weight (368 amu) for this compound. NMR spectra were consistent with the structure of an aromatic phosphate ester. All spectroscopic data support the identification of this material as tricresyl phosphate.

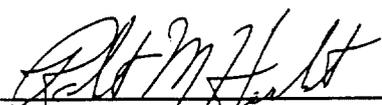
IV. CONCLUSION

DURAD 125L (Batch 09238, LDP304) was found to be stable for at least 90 days stored at room temperature. Its chemical identity as tricresyl phosphate was confirmed.

V. CERTIFICATION

We, the undersigned, hereby declare that this study was performed under our supervision according to the procedure herein described, and that this report provides a true and accurate record of the results obtained.

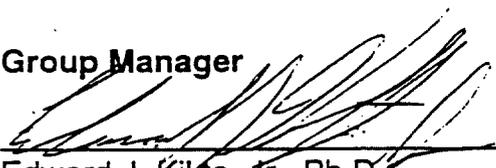
Study Director



Robert M. Herbst
Senior Research Chemist

6/30/95
Date

Group Manager



Edward J. Kikta, Jr., Ph.D.
Manager, APG Analytical Sciences

7/7/95
Date

Additional Personnel

Robert W. Creekmore, Sr. Research Associate
Robert E. Fisher, Sr. Instrument Specialist
Melissa Klein, Chemist
Noreen A. Klitus, Sr. Chemist
Ronald E. Shomo, Sr. Research Chemist

bc

VI. TABLES AND FIGURES

Index of Tables

<u>Table No.</u>	<u>Title</u>
1	Stability of Tricresyl Phosphate (DURAD® 125L), Batch 09238, LDP 304, at Room Temperature
2	Isomer Distribution in Tricresyl Phosphate (DURAD® 125L, Batch 09238, LDP304)

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<u>Figure No.</u>	<u>Title</u>
1	Typical Chromatogram of Tritolyl Phosphate ^a (Retention Time Marker)
2	Typical Chromatogram of Triphenyl Phosphate (Retention Time Marker)
3	Typical Chromatogram of DURAD® 125L Sample
4	Electron Impact Mass Spectrum of DURAD® 125 L
5	Chemical Ionization Mass Spectrum of DURAD® 125 L
6	Proton NMR Spectrum of DURAD® 125 L
7	Phosphorus NMR Spectrum of DURAD® 125 L

- a. Tritolyl phosphate is a synonym for tricresyl phosphate mixtures as supplied by the vendor.

A. Tables

Table 1

Stability of Tricresyl Phosphate (DURAD[®] 125L)
 Batch 09238, LDP 304, at Room Temperature

Sample Identification	Analysis Date	Elapsed Time (Days)	Total Isomer Composition (Percent)	Average ^(a) Composition (Percent)	Grand ^(a) Mean (Percent)	Percent ^(b) Change from Initial Analysis
<u>10/19/94</u>						
Weight 1/Inj 1		0	97.9	98.0	98.0	NA ^(c)
/Inj 2		(Initial)	98.1			
/Inj 3			98.0			
Weight 2/Inj 1		0	98.0	98.0		
/Inj 2		(Initial)	98.1			
/Inj 3			98.0			
Weight 3/Inj 1		0	98.0	98.0		
/Inj 2		(Initial)	98.0			
/Inj 3			98.0			
<u>11/18/94</u>						
Weight 1/Inj 1		30	97.7	97.8	97.9	-0.1
/Inj 2			98.0			
/Inj 3			97.9			
Weight 2/Inj 1		30	97.9	98.0		
/Inj 2			98.0			
/Inj 3			98.0			
Weight 3/Inj 1		30	97.9	97.9		
/Inj 2			97.9			
/Inj 3			97.9			

Continued.....

Table 1 (Continued)

Stability of Tricresyl Phosphate (DURAD® 125L)
 Batch 09238, LDP 304, at Room Temperature

Sample Identification	Analysis Date	Elapsed Time (Days)	Total Isomer Composition (Percent)	Average ^(a) Composition (Percent)	Grand ^(a) Mean (Percent)	Percent ^(b) Change from Initial Analysis
<u>12/19/94</u>						
Weight 1/Inj 1		61	97.4	97.3	97.4	-0.6
/Inj 2			97.3			
/Inj 3			97.4			
Weight 2/Inj 1		61	97.3	97.3		
/Inj 2			97.3			
/Inj 3			97.3			
Weight 3/Inj 1		61	97.3	97.5		
/Inj 2			97.5			
/Inj 3			97.6			
<u>01/17/95</u>						
Weight 1/Inj 1		90	97.2	97.3	97.3	-0.7
/Inj 2			97.3			
/Inj 3			97.3			
Weight 2/Inj 1		90	97.3	97.3		
/Inj 2			97.3			
/Inj 3			97.3			
Weight 3/Inj 1		90	97.3	97.3		
/Inj 2			97.3			
/Inj 3			97.5 ^(d)			

- a) Average composition and grand mean calculated on unrounded values.
 b) % Change from initial analysis based on the following equation:

$$\% \text{ Change from initial} = \frac{\text{Day (Elapsed) Value} - \text{Day 0 (Initial) Value}}{\text{Day 0 (Initial) Value}} \times 100$$

 c) NA = Not Applicable
 d) Outlier rejected because of poor peak integration. This data point was not used in calculations.

Table 2

Isomer Distribution in Tricresyl Phosphate
 (DURAD® 125L, Batch 09238,LDP304)

Sample Identification	Analysis Date	Isomers ^(a) (Area Percent)			
		1m 2p cresyl	2m 1p cresyl	3 m cresyl	3p cresyl
	<u>10/19/94</u>				
Weight 1/inj 1		33.4	39.5	15.6	9.5
/inj 2		33.5	39.5	15.6	9.5
/inj 3		33.4	39.5	15.6	9.5
Weight 2/inj 1		33.4	39.5	15.6	9.5
/inj 2		33.5	39.5	15.6	9.5
/inj 3		33.4	39.5	15.6	9.4
Weight 3/inj 1		33.5	39.5	15.6	9.5
/inj 2		33.4	39.5	15.6	9.5
/inj 3		33.4	39.5	15.6	9.5
	<u>11/18/94</u>				
Weight 1/inj 1		33.3	39.4	15.6	9.5
/inj 2		33.4	39.4	15.6	9.5
/inj 3		33.4	39.5	15.6	9.4
Weight 2/inj 1		33.4	39.5	15.6	9.4
/inj 2		33.4	39.5	15.6	9.5
/inj 3		33.4	39.5	15.6	9.5
Weight 3/inj 1		33.4	39.4	15.6	9.5
/inj 2		33.3	39.5	15.7	9.4
/inj 3		33.3	39.5	15.7	9.4

Continued

Table 2 (Continued)

Isomer Distribution in Tricresyl Phosphate
 (DURAD® 125L, Batch 09238, LDP304)

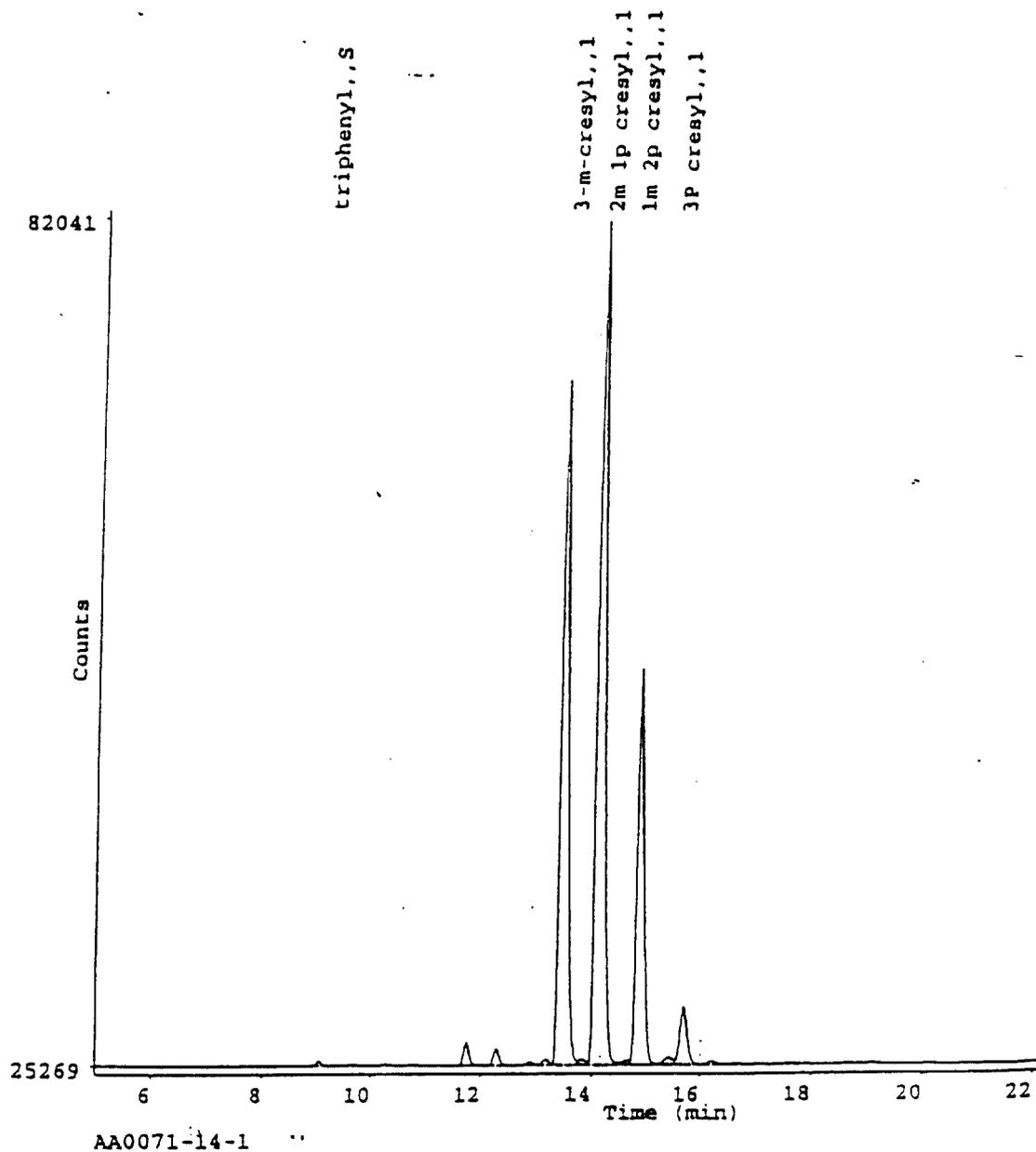
Sample Identification	Analysis Date	Isomers ^(a) (Area Percent)			
		1m 2p cresyl	2m 1p cresyl	3 m cresyl	3p cresyl
	<u>12/19/94</u>				
Weight 1/inj 1		33.0	39.2	15.6	9.5
/inj 2		33.0	39.2	15.7	9.5
/inj 3		33.0	39.2	15.6	9.5
Weight 2/inj 1		33.0	39.2	15.7	9.5
/inj 2		33.0	39.2	15.7	9.5
/inj 3		33.0	39.2	15.6	9.5
Weight 3/inj 1		33.0	39.2	15.6	9.5
/inj 2		33.1	39.4	15.7	9.4
/inj 3		33.1	39.4	15.7	9.4
	<u>1/17/95</u>				
Weight 1/inj 1		33.0	39.1	15.6	9.5
/inj 2		33.0	39.1	15.6	9.5
/inj 3		33.0	39.2	15.7	9.5
Weight 2/inj 1		33.0	39.1	15.6	9.5
/inj 2		33.0	39.1	15.7	9.5
/inj 3		33.0	39.2	15.7	9.5
Weight 3/inj 1		33.0	39.1	15.7	9.5
/inj 2		33.0	39.1	15.6	9.5
/inj 3 ^(b)		33.3	39.1	15.6	9.5

- a) 1m 2p cresyl = di-*p*-cresyl *m*-cresyl phosphate
 2m 1p cresyl = di-*m*-cresyl *p*-cresyl phosphate
 3 m cresyl = tri-*m*-cresyl phosphate
 3p cresyl = tri-*p*-cresyl phosphate

b) Outlier rejected because of poor peak integration.

B. Figures

Figure 1. Typical Chromatogram of Tritolyl Phosphate^(a)
(Retention Time Marker)



(a) Tritolyl phosphate is a synonym for tricresyl phosphate mixtures as supplied by the vendor.

Figure 2. Typical Chromatogram of Triphenyl Phosphate
(Retention Time Marker)

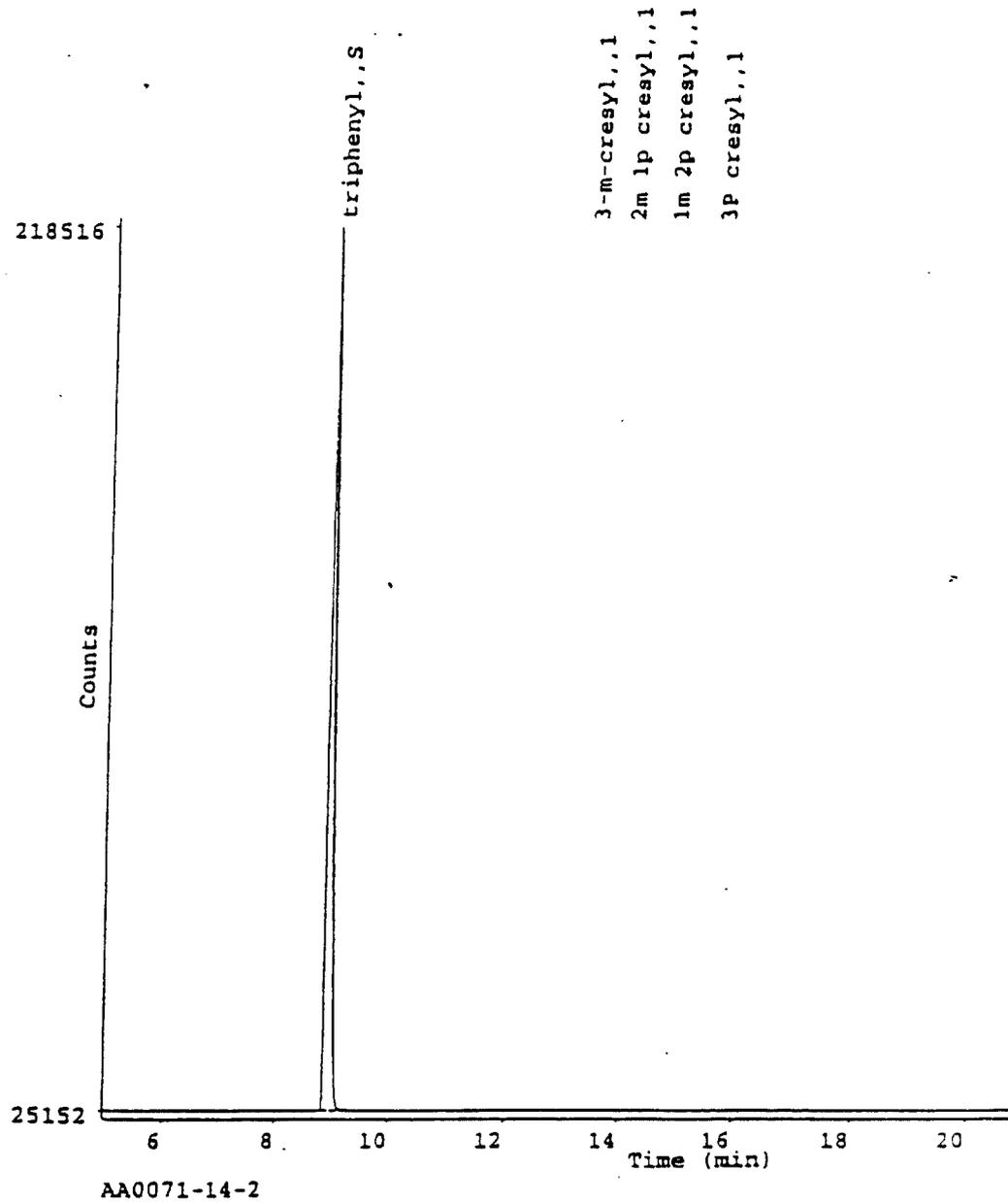


Figure 3. Typical Chromatogram of a DURAD® 125 L Sample

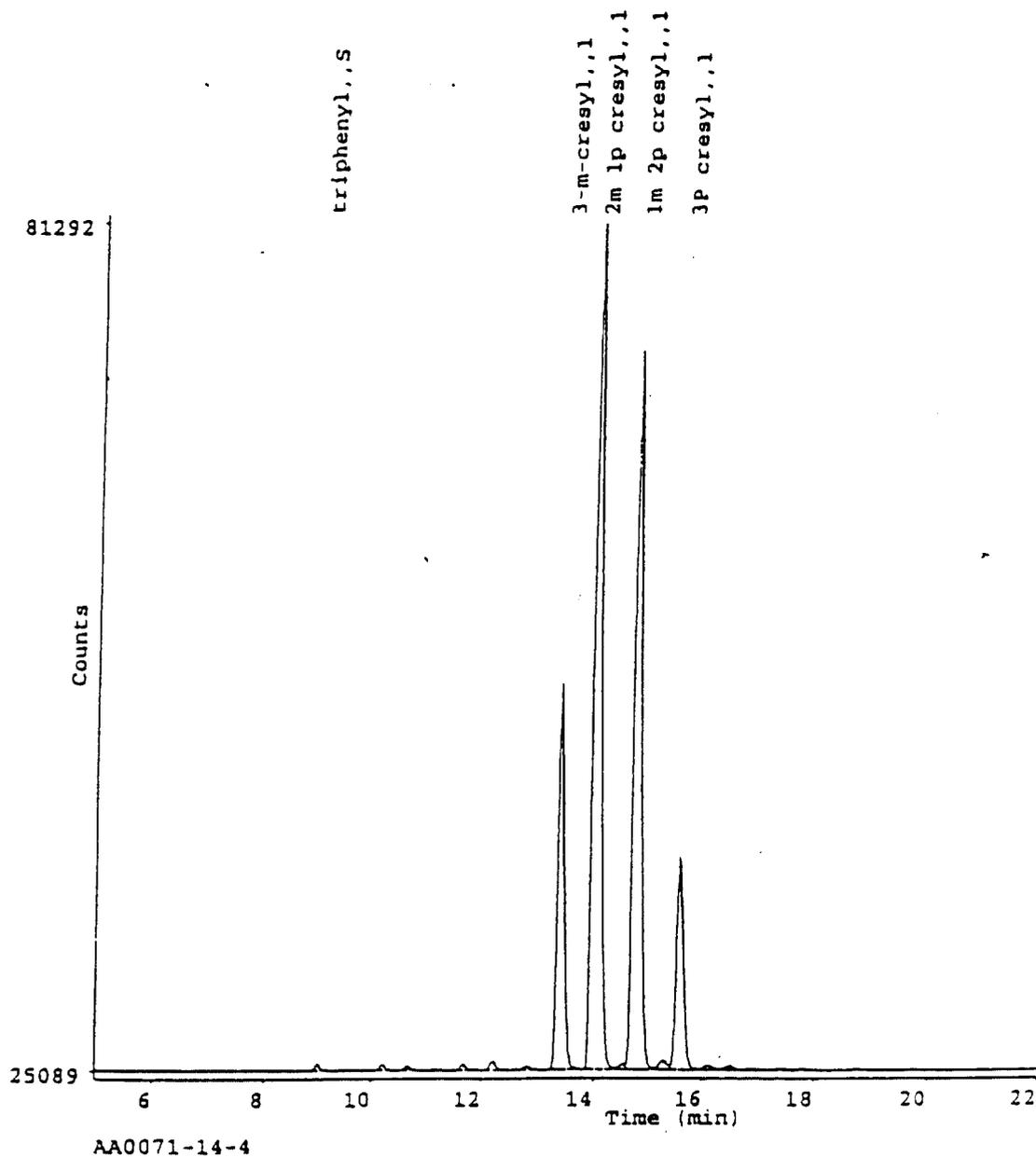
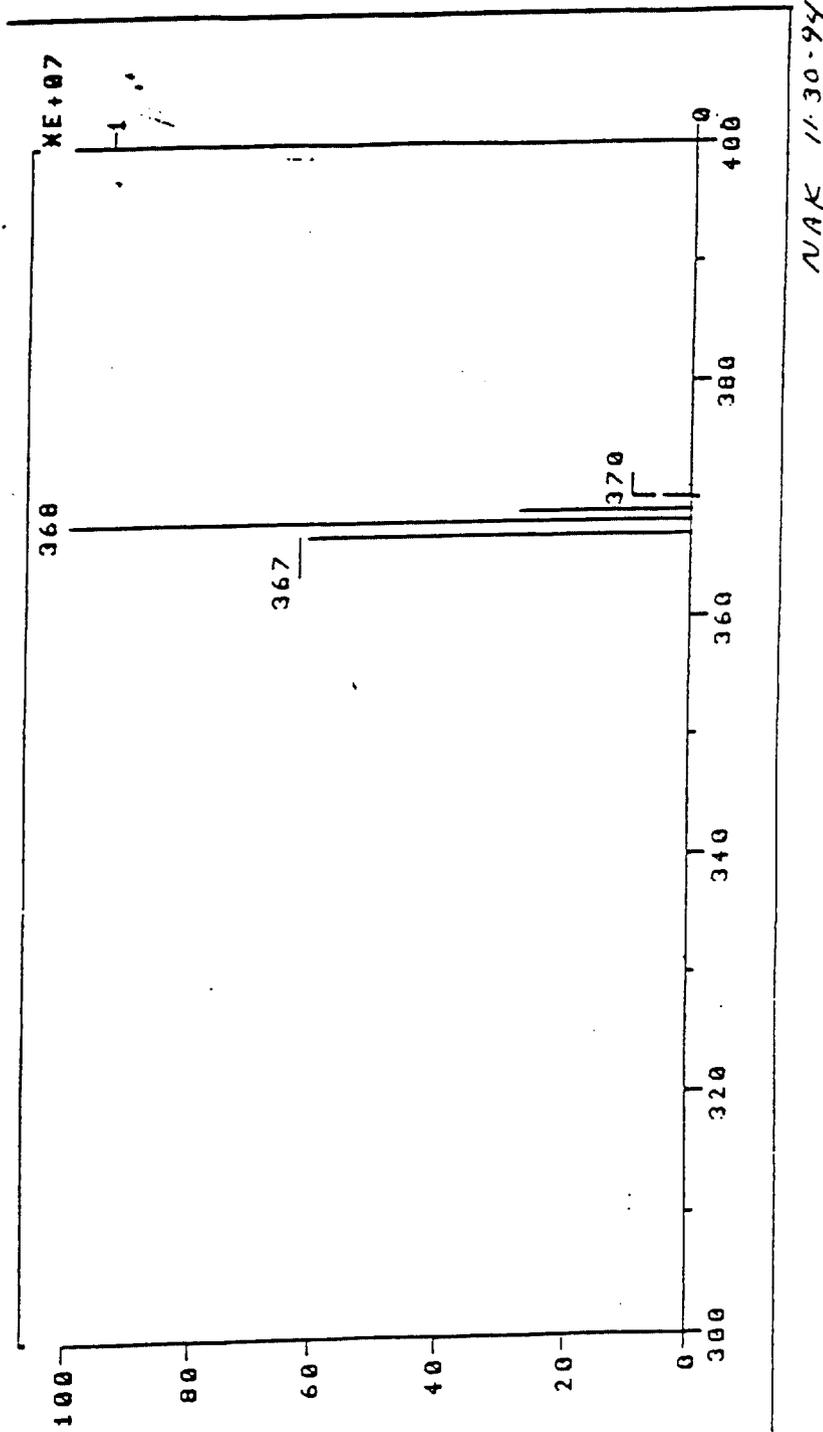
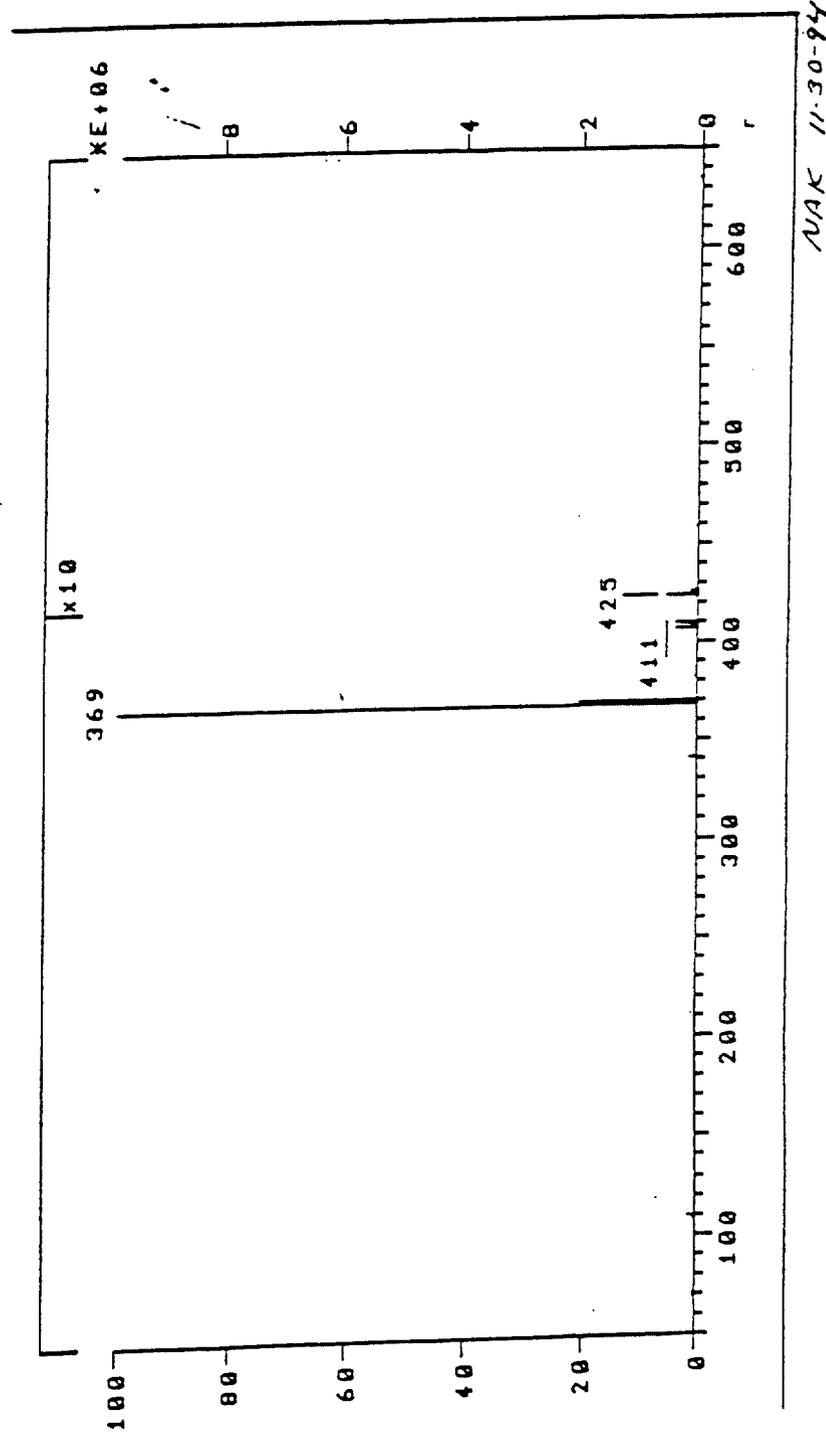


Figure 4. Electron Impact Mass Spectrum of DURAD® 125 L



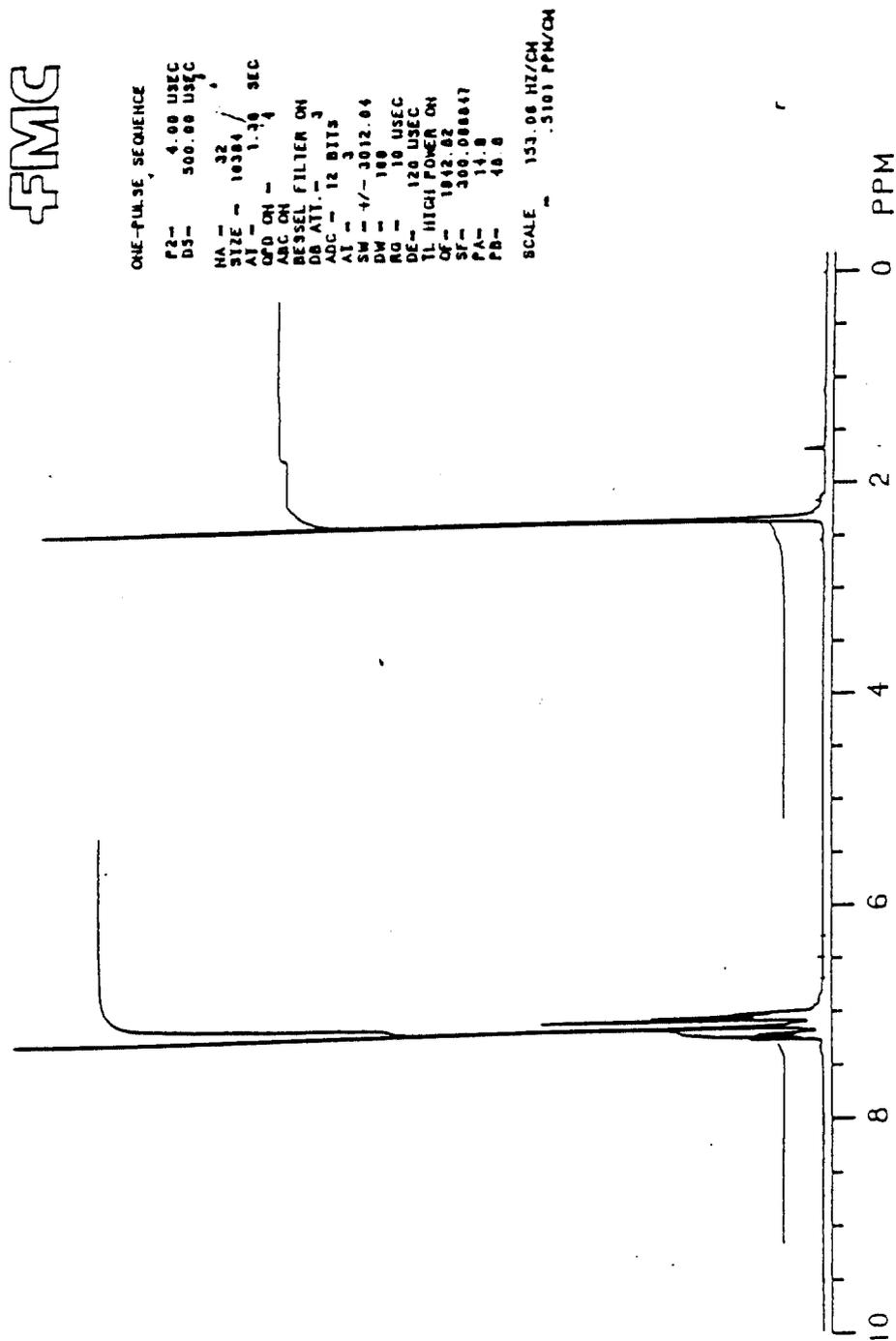
Reduced 74% of Original

Figure 5. Chemical Ionization Mass Spectrum of DURAD® 125 L



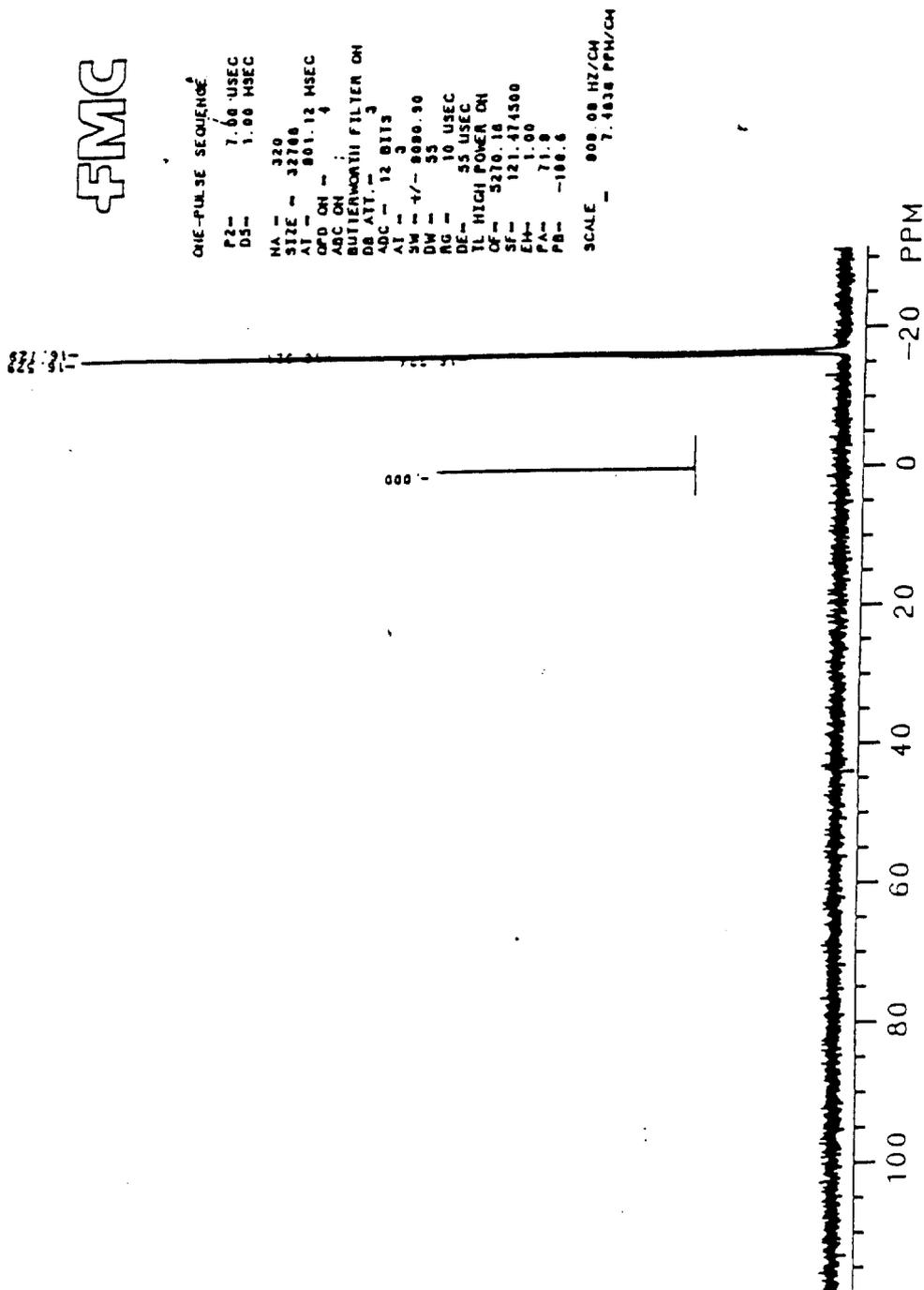
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Figure 6. Proton NMR Spectrum of DURAD® 125 L



Reduced 74% of Original

Figure 7. Phosphorus NMR Spectrum of DURAD® 125 L



Reduced 74% of Original

VII. REFERENCES

A. FMC References

1. FMC GLP Notebook AA0071, Melissa Klein, FMC Corporation, APG, Princeton, NJ 08543

VIII. APPENDICES

- A. Protocol
- B. Protocol Modification
- C. Test Method No. ACG 305

A. Protocol

FMC Corporation
Agricultural Chemical Group
ANALYTICAL SCIENCES

STUDY NO. 703AF94283

PROTOCOL

Page 1 of 5

STUDY NUMBER: 703AF94283

STUDY TITLE: Composition and 90 Day Stability Analysis of Tricresyl Phosphate-DURAD® 125 L

STUDY DIRECTOR: Robert M Herbst, Sr. Research Chemist

SPONSOR INTERNAL STUDY NO. P94-0072

OBJECTIVE: Determine the composition and stability of tricresyl phosphate/Durad 125L at approximately 0, 30, 60, and 90 days.

DATA REQUIREMENT: TSCA

SPONSOR FMC Corporation
Toxicology Department
301 College Road, East
Princeton, New Jersey 08543
(609) 951-3731
Representative: Christine Freeman
Laboratory Supervisor

ANALYSIS AND TESTING FACILITY FMC Corporation
Agricultural Chemical Group
Analytical Sciences
P.O. Box 8, Princeton, NJ 08543
(609) 951-3000

FMC Corporation
Agricultural Chemical Group
ANALYTICAL SCIENCES

STUDY NO. 703AF94283

PROTOCOL

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TEST SUBSTANCE

Identity: Name, Physical Description, Label Contents (Reference No.)

Tricresyl phosphate/Durad® 125 L, Batch 09238 LDP 304

REFERENCE SUBSTANCE (STANDARD): Not Applicable

TEST SYSTEM: DURAD® 125 L

CONTROL SUBSTANCE: Not Applicable

STABILITY/STORAGE REQUIREMENTS:

The sample(s) is/are assumed to be sent in the proper container and stable at room temperature. The samples will be stored at room temperature.

PROPOSED SCHEDULE

Experimental Start Date:	October 6, 1994
Experimental Termination Date:	January 11, 1995
Project Final Report Date:	February 25, 1995

ASSIGNED PERSONNEL (DELETIONS/ADDITIONS WILL BE NOTED IN STUDY FILE)

Robert M. Herbst, Sr. Research Chemist
Melissa Klein, Chemist
Ronald E. Shomo, Sr. Research Chemist
Noreen A. Klitus, Sr. Chemist
William R. Creekmore, Sr. Research Associate
Robert E. Fisher, Sr. Instrument Specialist

EQUIPMENT AND STANDARD OPERATING PROCEDURES TO INCLUDE BUT NOT LIMITED TO

HP5890 Gas Chromatograph, SOP AN5
TSQ-70B Mass Spec, SOP AN110
Nicolet NT300 FT NMR, SOP AN109

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Agricultural Chemical Group
ANALYTICAL SCIENCES

STUDY NO. 703AF94283

PROTOCOL

Page 3 of 5

TEST METHOD(S)

Method No., Name, or other unique identifying information.

Chromatographic methods will be developed as part of this study.

Mass spectrometry in appropriate mode to determine molecular weight of primary components.

^1H -NMR and ^{31}P -NMR to confirm that material is consistent with an aromatic phosphate ester.

Modifications to the methods, made in the course of a study, will be fully documented in the Laboratory Study File, and reported in the Final Report. The use of modified methods is contingent on Study Director approval.

SAFETY REQUIREMENTS

Safety requirements are outlined in MSDS 68952-35-2-1 (Attachment #1)

STATISTICAL METHODS

The statistical operations used to assess the data from this study will include, but not be limited to the following: mean, standard deviation and coefficient of variation.

PERSONNEL QUALIFICATION STATEMENT

Personnel conducting the study will have the education, training, and/or experience to perform their assigned function. Adequate supervision will be provided to assure proper function of personnel and equipment.

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ANALYTICAL SCIENCES

STUDY NO. 703AF94283

PROTOCOL

Page 4 of 5

LABORATORY STUDY FILE

Each individual responsible for the technical execution of the study will maintain a laboratory study file. This file will contain, but not be limited to, the following:

- a) Protocol
- b) Protocol Modification Documentation
- c) Raw Data
- d) Chain of Custody Log(s)
- e) The notebook that pertains to the Study
- f) Modified Test Methods when applicable
- g) SOP deviation documentation will include SOP#, what you are not following, what is being done and the reason for SOP deviation. This will be part of a notebook entry.

GLP COMPLIANCE STATEMENT

All applicable Standard Operating Procedures (SOPs) and/or modified SOPs and GLP Standards will be followed. SOP deviations will be documented as per Item g in the Laboratory Study File section.

FINAL REPORT

A Final Report will be written according to Standard Operating Procedure.

QUALITY ASSURANCE STATEMENT

The Quality Assurance Unit will review the raw data to ensure results reported are consistent with the raw data.

ARCHIVE

The ACG Research and Development Quality Assurance Unit will be responsible for retaining the protocol, all raw data, protocol modifications, interim reports, and the final report generated as a result of the study. The archives will be stored in such a way as to ensure expedient retrieval.

PROTOCOL MODIFICATIONS

Modifications to this Protocol will be documented, signed, and dated by the Sponsor, Study Director, Group Manager and Quality Assurance Unit Representative. Copies of the documentation for a Modification to this Protocol



Process Additives Division Tenax Road Trafford Park Manchester M17 1WT

ATTACHMENT #1-

Material Safety Data

DURAD 125L

EUROPEAN VERSION

Date Printed: 3/8/1994 CAS No: 68952-35-2* Revision No: 1
File Number: 967 Issue Date: 9407 EINECS No: 273-168-8

1. CHEMICAL PRODUCT

CHEMICAL CHARACTERISATION
Tricresyl Phosphate
PRODUCT: DURAD 125L
Product used as:

2. COMPOSITION

Tricresyl Phosphate
Harmful R21/22

3. HAZARDS IDENTIFICATION

R21/22 Harmful in contact with skin and if swallowed.

Other Hazards:

4. FIRST-AID MEASURES

RESPIRATORY SYSTEM

Remove to fresh air. If breathing difficulty or discomfort occurs and persists obtain medical attention.

INGESTION

Do not induce vomiting. Wash mouth out with water and obtain medical attention.

EYES

Flush with water for at least 15 minutes. If irritation occurs and persists, obtain medical attention.

SKIN

Wash with plenty of soap and water. Get medical attention if irritation occurs and persists.

5. FIRE-FIGHTING METHODS

Foam, carbon dioxide or dry power.

6. ACCIDENTAL RELEASE MEASURES

Contain and absorb in sand or earth. Ensure no material enters drains or water courses. Dispose of as solid waste in accordance with the relevant waste disposal regulations.

IN CASE OF EMERGENCY TELEPHONE 061 848 9797/EUROPE 4461 848 9797
PAGE 1



Process Additives Division Tenax Road Trafford Park Manchester M17 1WT

Material Safety Data

DURAD 125L

7. STORAGE AND HANDLING

Storage

- 1: Store in delivery pack in a cool dry place.
2:

HANDLING

At ambient temperatures wear gloves overalls and eye protection.
The work area should be adequately ventilated.
No eating drinking or smoking in the work area.

8. EXPOSURE CONTROL / PERSONAL PROTECTION

Exposure Standard: OES 3 mg/m³ (8hrTWA)
The substance with exposure limit is: Triphenyl Phosphate.
Notation 1: None

Exposure Standard:
2nd substance with exposure limit is:
Notation 2:

PERSONAL PROTECTION

Respiratory Protection:

Type of Cartridge (if Applicable) :

Eye Protection : Safety Spectacles
Hand Protection : Gloves butyl or nitrile rubber.
Industrial Hygiene : No eating drinking or smoking in the work area.

Ventilation : At room temperature handle in a well ventilated area. At elevated temperature use appropriate engineering control systems to remove fume or vapour from the atmosphere, eg, exhaust ventilation.

PROTECTION AGAINST FIRE AND EXPLOSION

Not flammable but combustible if exposed to external flames. No explosion hazard.

EC SAFETY PHRASES

S28 After contact with skin, wash immediately with plenty of soap and water.

IN CASE OF EMERGENCY TELEPHONE 061 848 9797/EUROPE 4461 848 9797
PAGE 2



Process Additives Division Tenax Road Trafford Park Manchester M17 1WT

Material Safety Data

DURAD 125L

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance : Liquid Colour: Colourless
Odour : None
Melting Point : (C)
Decomposition : > 300 (C)
Flash Point (COC) : > 220 (C)
Boiling Point : > 300 (C)
Density at 25C : 1.1 g/cm³
Specific Gravity (H₂O=1)(25C):

Solubility in Water (20C): Insoluble in water
Solubility (Other):
ph-Value: : NA

Vapour Pressure:
Concentration in air: (C)
Concentration in air: (C)

Viscosity : 70 mm²/sec 25(C)
Pour Point : -26 (C)

Explosion Limits - Upper:% Lower:%
Thermal Decomposition : > 300 (C)
Autoignition Temp : (C)

10. STABILITY AND REACTIVITY

HAZARDOUS REACTIONS
Hydrolysis in water produces phenols

Comments:

HAZARDOUS DECOMPOSITION PRODUCTS
Thermal decomposition and burning produces noxious fumes containing oxides of carbon and phosphorus.

IN CASE OF EMERGENCY TELEPHONE 061 848 9797/EUROPE 4461 848 9797
PAGE 3



Process Additives Division Tenax Road Trafford Park Manchester M17 1WT

Material Safety Data

DURAD 125L

11. TOXICOLOGICAL INFORMATION

LD50 Acute Oral Toxicity: > 5000:mg/Kg:Rat
LC50 Acute Inhalation :: mg/l :
Eye Irritation :Yes: Rabbit
Skin Irritation :Yes: Rabbit
Sensitisation : :
Further Information:

12. ECOLOGICAL INFORMATION

LC50 (96 Hour): mg/l
EC50 (48 Hour): mg/l
Biodegradability (Sturm Test):
Biodegradability (Coupled units):

Further Information:

WGK Classification: 1 Self-classified

13. DISPOSAL CONSIDERATIONS

Liquid material should be incinerated. Material absorbed on sand should be disposed of as hazardous solid waste.

14. TRANSPORT INFORMATION

IMDG No :Class 9
Packing Groups :III
UN No :3082
IATA-DGR Class No:9
TREM CARD :Not classified
RID/ADR Class No :Not classified
Poisons Schedule :
Non-Acute Hazard Code:

Other Information:

IN CASE OF EMERGENCY TELEPHONE 061 848 9797/EUROPE 4461 848 9797
PAGE 4



Process Additives Division Tenax Road Trafford Park Manchester M17 1WT

Material Safety Data

DURAD 125L

15. REGULATORY INFORMATION

Classification and labelling according to EC Directives 67/548
Harmful R21/22

EC RISK PHRASES

R21/22 Harmful in contact with skin and if swallowed.

Other Hazards

EC SAFETY PHRASES

S28 After contact with skin, wash immediately with plenty of soap and water.

16. OTHER INFORMATION

PRODUCT SAFETY

FMC Corporation (UK) Ltd Process Additives Division manufacturing high quality research based products designed and marketed for specific applications which are described in our Technical Bulletin already in your possession.

For safety reasons, it is IMPERATIVE that customers :-

1. Ensure that all those within their control who use the products are supplied with all relevant information contained within the Material Safety Data Sheet and Technical Bulletin concerning the applications for which the product is designed and any instructions or warnings contained therein.

2. Consult with FMC Process Additives Division before using or supplying the product for any other application.

A US/Canadian version of this Safety Data Sheet is available.

IN CASE OF EMERGENCY TELEPHONE 061 848 9797/EUROPE 4461 848 9797
PAGE 5

B. Protocol Modification

FMC Agricultural Chemical Group
ANALYTICAL SCIENCES

Page 1 of 1

Protocol Modification

Study Number: 703AF94283

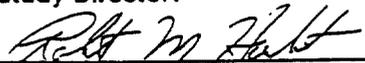
Modification # 1

Study Title: Composition and 90 Day Stability Analysis of Tricresyl
Phosphate-DURAD® 125L

Effective Date: 10/19/94

Experimental start date changed from 10/6/94 to 10/19/94 due to the logistics of routing the Protocol for signatures and prior commitments of resources. Experimental completion date changed from 1/11/95 to 1/24/95.

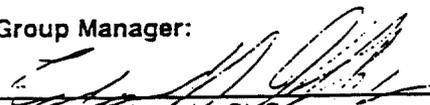
Study Director:



Robert M. Herbst
Senior Research Chemist

11/8/94
Date

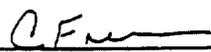
Group Manager:



Edward J. Kikte, Jr., Ph.D.
Manager, ACG Analytical Sciences

11/9/94
Date

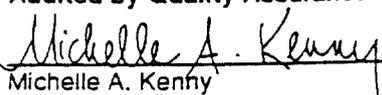
Reviewed and Accepted by Sponsor:



Christine Freeman
Laboratory Supervisor

11-11-94
Date

Audited by Quality Assurance Unit



Michelle A. Kenny
Quality Assurance Specialist

11/15/94
Date

Date Generated: 11/8/94

C. Test Method ACG 305

FMC Corporation
Agricultural Chemical Group
Box 8
Princeton, New Jersey 08543
609 951 3000



Issued: January 12, 1994

TEST METHOD ACG NO. 305

305 GC PROCEDURE FOR THE ASSAY OF TRICRESYL PHOSPHATE
(DURAD[®] 125L)

305.1 INTRODUCTION

This method describes a gas chromatographic (GC) procedure for the fractionation and purity determination of tricresyl phosphate (DURAD 125L). A crossbonded 100% dimethyl polysiloxane column, Restek[®] Rtx[™]-1, is employed for the fractionation. This method employs area percent techniques with retention time markers provided by reference materials.

Tricresyl phosphate (CAS No. 68952-35-2) includes these isomers:

tri-*m*-cresyl phosphate
di-*m*-cresyl *p*-cresyl phosphate
di-*p*-cresyl *m*-cresyl phosphate
tri-*p*-cresyl phosphate

305.2 SAFETY

This procedure does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use. Standard safety practices should be applied at all times during the implementation of this procedure. Exposure to all reagents, samples, and reference materials should be reduced to the lowest levels by the best practical means. Safety practices should be applied when handling all glassware and operating analytical instrumentation. Gloves and disposable lab coats are recommended anytime the technical material is transferred between containers and when manual sample injections are performed.

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All laboratory personnel involved in the use of this procedure should be familiar with safety information and data available on all chemicals employed. Safety information for tricresyl phosphate is documented in the FMC Material Safety Data Sheet for DURAD[®] 125L.

305.3 PRECISION AND ACCURACY

Preliminary studies of precision indicate that this method can generally provide results with a coefficient of variation of $\pm 0.5\%$. Standard measures of accuracy are inappropriate for evaluating methods which use area percent calculations.

305.4 REAGENTS AND REFERENCE MATERIALS

Equivalents may be substituted as necessary.

Acetone, J. T. Baker Product No. 9254-03

Tritolyl phosphate (CAS No. 1330-78-5), Aldrich Chemical Co. Product No. 26,8917-7.

Triphenyl phosphate (CAS No. 115-86-6), Aldrich Chemical Co. Product No. 24,128-8.

305.5 INSTRUMENT AND SPECIFICATIONS

Instrument: Hewlett Packard HP5890A GC, equipped with flame ionization detector (FID), or equivalent.

Data Reduction, PE/Nelson Access[®]Chrom[®], or equivalent

Analysis Time, Approximately 36 minutes

Semi-Microanalytical Balance, A&D Engineering Model HA202M or equivalent, capable of ± 0.01 mg readability.

OPERATING CONDITIONS

Note: Conditions may be changed as necessary to optimize resolution.

Column

Manufacturer:	Restek
Catalog Number:	10123
Phase:	Rtx-1
Film Thickness:	0.25 micron
Dimensions:	30 M x 0.25 mm
Carrier:	Helium
Column Pressure:	70 kPa

Injection Volume:	0.5 μ L
Mode:	Split

Flows

Column Flow	0.7 cc/min
Purge vent:	2.2 cc/min
Split vent:	60 cc/min

Temperatures

Injector:	325 $^{\circ}$ C
Detector (FID):	325 $^{\circ}$ C

Oven Temperature Program

Temperature ($^{\circ}$ C)	Hold time (min)	Rate ($^{\circ}$ C/min)
250	25.0	70.0
325	10.0	0

305.6 PREPARATION OF REFERENCE MATERIAL SOLUTIONS

Prepare a solution of each reference material (tritolyl phosphate and triphenyl phosphate) at approximately 5% in acetone. For example, weigh 0.5 g of tritolyl phosphate into a 10 mL volumetric flask, then dilute to volume with acetone. Mix each solution thoroughly and transfer to a sealed vial for storage.

305.7 SAMPLE PREPARATION AND ANALYSIS

Prepare sample solutions, approximately 5% in acetone, as described for reference materials. Mix each solution thoroughly and transfer to a sealed vial for storage.

Fill the autosampler vials with the reference material and sample solutions and load the autosampler tray.

Inject the reference material or sample solution onto the GC column. Rinse the syringe thoroughly with acetone between sample and/or reference material solution injections.

Record the peak areas for each sample analyzed.

All solutions should be analyzed in duplicate, at a minimum.

305.8 CALCULATIONS

Calculation of purity is a two-step process:

1. Identify the peaks of interest (tri-*m*-cresyl phosphate, di-*m*-cresyl *p*-cresyl phosphate, di-*p*-cresyl *m*-cresyl phosphate, and tri-*p*-cresyl phosphate) by comparing retention times and elution patterns to the chromatograms shown in Figures 1, 2, and 3, and the retention times of reference materials in the current data set.
2. For each sample injection, add the area percent values for the peaks of interest to obtain a total purity value.

305.9 DISCUSSION

NOTES

1. Follow instructions provided by instrument manufacturers for proper set-up and operation of chromatographic equipment and accessories.
2. Between injections of different sample or reference material solutions, rinse the syringe thoroughly with acetone.
3. It may be useful to stop integration of peaks at the beginning of the temperature ramp (approximately 26 minutes). This will prevent the inclusion of peak area which does not represent true impurities.
4. Figure 1 is a GC chromatogram of tritolyl phosphate reference material. Figure 2 is a GC chromatogram of triphenyl phosphate reference material. Figure 3 is a typical GC chromatogram of a DURAD[®] 125L sample.

305.10 REFERENCES

FMC Notebook Reference, E7569:103, R. M. Herbst

FMC Literature:

"Analytical Method: CDP or TCP Phosphate Ester Distribution by GLC," issued by FMC Process Additives Division, Research Department, Physical Analysis Group (Trafford Park, Manchester, U.K.). Document Reference No. PAG-66, issued 09-08-94.

Prepared By: Melissa Klein 1/12/95
Melissa Klein Date

Approved By: Robert M Herbst 12 Jan 95
Robert M. Herbst Date

Approved By: Edward J. Kikta, Jr. 1/12/95
Edward J. Kikta, Jr., Ph.D. Date

Figure 1

GC Chromatogram of Tritolyl Phosphate

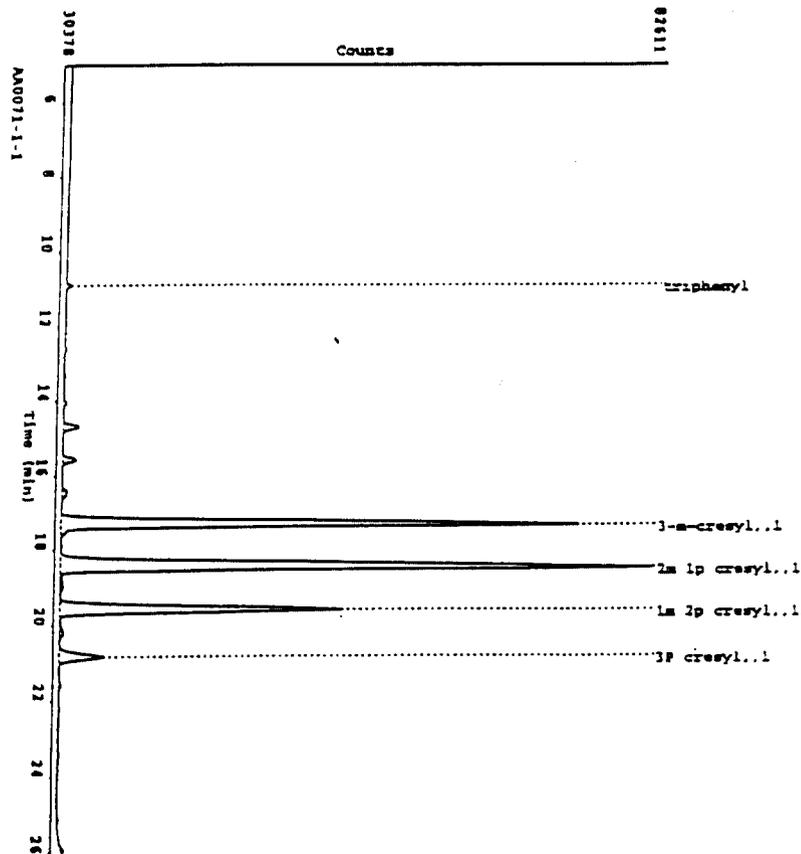


Figure 2

GC Chromatogram of Triphenyl Phosphate

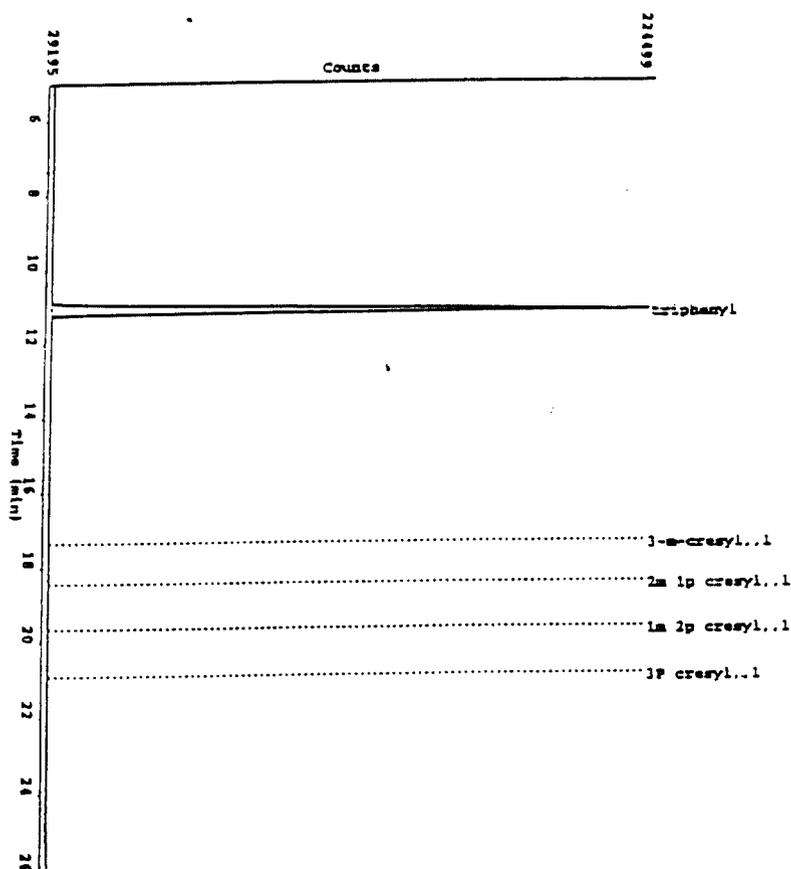
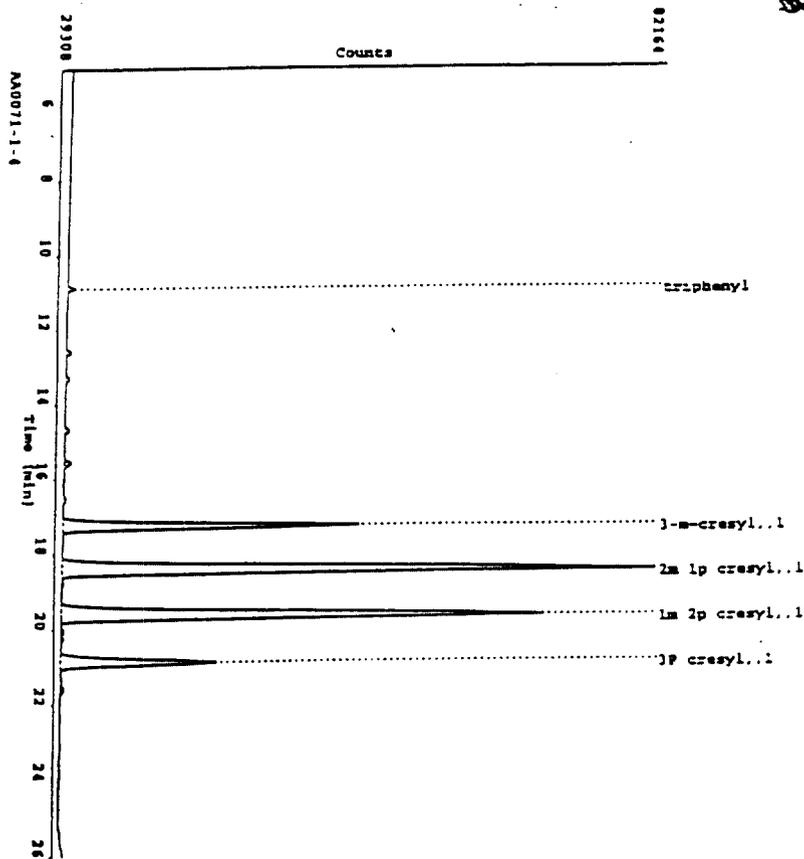


Figure 3
GC Chromatogram of DURAD® 125L Sample



APPENDIX C

Neuropathology Report



Pathology Associates, Inc.

Suite I
15 Worman's Mill Court
Frederick, MD 21701
(301) 663-1644
(301) 663-8994 FAX

NEUROHISTOPATHOLOGY REPORT

FOR

**ACUTE DELAYED NEUROTOXICITY STUDY IN HENS WITH
TRICRESYL PHOSPHATE/DURAD 125L**

FMC STUDY NUMBER I94-1925

PREPARED FOR

FMC CORPORATION

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I. NEUROHISTOPATHOLOGY REPORT

NEUROHISTOPATHOLOGY REPORT

Acute Delayed Neurotoxicity Study in Hens with Tricresyl Phosphate/Durad 125L

FMC Study I94-1925

Introduction

This study was conducted to evaluate the potential of Tricresyl Phosphate/Durad 125L to produce neurotoxicity in hens following a single oral administration at a dosage level of 2000 mg/kg. This report, prepared by Pathology Associates, Inc. (PAI), Frederick, MD 21701, presents the results of histopathologic evaluation of selected central nervous system (CNS) and peripheral nervous system (PNS) tissues from hens tested in accordance with the provisions of the study protocol. The report is prepared for FMC Corporation, Chemical Research and Development Center, Box 8, Princeton, NJ 08543.

Methods and Study Design

For the purpose of neuropathologic evaluation, a total of 24 hens were assigned to 3 groups (8 hens per group). Hens in the Vehicle Control group received a single dose of Corn oil (dose volume of 1.7 ml/kg) by oral intubation. Hens in the Test group received a single dose of Tricresyl Phosphate/Durad 125L (2000 mg/kg; dose volume of 1.7 ml/kg) by oral intubation. Hens in the Positive Control group received a single dose of Tri-o-tolyl Phosphate (500 mg/kg; dose volume of 2.0 ml/kg) by oral intubation. At the end of the study (Study days 21 or 22), surviving animals were anesthetized with an IV injection of Pentothal[®] into the wing vein and perfused with heparinized saline and a Glutaraldehyde/paraformaldehyde buffer solution. A gross necropsy and evisceration was performed after each successful perfusion.

Following a successful perfusion, the following tissues were prepared for histopathologic evaluation in accordance with the provisions of the study protocol and Pathology Associates, Inc. (PAI) Standard Operating Procedures (SOPs): brain, spinal cord, and appropriate sections of the sciatic and tibial nerves. The tissues were trimmed, processed through and embedded in paraffin (brain and spinal cord) or glycomethacrylate (sections of sciatic and tibial nerves, including branches), and slides were prepared and evaluated microscopically. The tissues examined were: brain (forebrain, center of the cerebrum, cerebellum and pons, and medulla oblongata), spinal cord (cross and longitudinal sections from the rostral-cervical, mid-thoracic, and lumbosacral regions), left and right sciatic nerves, left and right tibial nerves, and left and right medial and lateral tibial nerve branches.

Results and Discussion

One hen in the Test group (#E0218F) and one hen in the Positive Control group (#E0229F) were not successfully perfused and no tissues were processed or evaluated from these animals.

No gross lesions of the central nervous system (CNS) or peripheral nervous system (PNS) were noted in any animal at necropsy or during trimming.

The results of the neurohistopathologic evaluation are presented in the Tabulated Animal Data tables in Section II. The Project Summary tables (Section III) summarize the data by group. The Reports Code Table (Appendix 1) lists the codes and symbols used in the data tables and any abbreviations used in the tables are listed in the Abbreviations Table (Appendix 2).

Two hens in the Vehicle control group (#E0200F and #E0202F) had minimal, focal areas of chronic inflammation in the left medial tibial nerve branch but these lesions were considered to be spontaneous, not indicative of a neuropathologic effect, and of no significance to the study. There was no evidence of axonal degeneration or other neuropathologic change in any hen in the Vehicle Control group.

In the Positive Control group, 5/7 hens had microscopic lesions indicating neuropathologic changes related to the single administration of Tri-o-tolyl phosphate. The severity and location of these changes varied among the individual hens. All affected hens had axonal degeneration of at least one region of the spinal cord and the cervical spinal cord was the most consistently affected region (5/5 of the affected hens). Axonal degeneration in the spinal cord was characterized by axonal swelling (spheroid formation), dilatation of the myelin sheaths and occasional phagocytic cells (Gitter cells) within the dilated myelin sheaths. The lesions were consistently within the peripheral white matter (axonal) tracts in the spinal cord, an area corresponding to the ascending spinocerebellar tracts of the spinal cord. Axonal degeneration was present in the sciatic nerves of only 1/7 hens. Axonal degeneration of the tibial nerve or its branches was present in 4/7 hens. The axonal degeneration in the peripheral nerves was characterized by axonal debris, dilatation of myelin sheaths and phagocytic cells within the dilated myelin sheaths. The distribution of changes and the character of those changes seen in affected hens in the Positive Control group were consistent with those reported to occur in chickens with organophosphate neurotoxicity (1).

Neurohistopathologic changes were observed in 4/7 hens in the Test group, the group which received a single oral dose of Tricresyl phosphate/Durad 125L. These changes were limited to the spinal cord and were consistently minimal to mild. Two hens had axonal degeneration in the cervical region of the spinal cord and 2 hens had axonal degeneration in the lumbosacral region of the spinal cord. The axonal degeneration in the spinal cord was characterized by axonal swelling (spheroid formation), dilated myelin sheaths and a very occasional phagocytic cell (Gitter cell) in the dilated myelin sheaths. When present, the axonal degeneration was consistently within the peripheral white matter (axonal) tracts in the spinal cord, an area corresponding to the ascending

spinocerebellar tracts of the spinal cord. The degeneration was similar in character and location (although in general less severe) to the axonal degeneration seen in the Positive Control group. No lesions were observed in the peripheral nerves of any hen in the Test group.

Conclusion

Hens administered a single oral dose (1.7 ml/kg) of Corn oil (vehicle control) did not develop neuropathologic changes by 21 or 22 days post-dosing. Five of seven hens administered a single oral dose of 500 mg/kg of Tri-o-tolyl phosphate did develop neuropathologic lesions consistent with organophosphate neurotoxicity when sacrificed 21 or 22 days post-dosing. These findings verified the accuracy of the test system.

Hens receiving a single oral dose of 2000 mg/kg of Tricresyl phosphate/Durad 125L developed compound-related, minimal to mild axonal degeneration in the spinal cord by days 21 or 22 post-dosing but this dose was not associated with detectable axonal degenerative changes within the peripheral nerves.

Mark T. Butt

Mark T. Butt, DVM
Diplomate, ACVP
July 24, 1995

References

- 1) Jacobs JM, Le Quesne PM (1992). Toxic disorders, in Greenfield's Neuropathology, Adams JH and Duchen LW, eds. Oxford University Press, New York, NY, pg. 924.

FMC Toxicology Laboratory
Protocol Revisions/Deviations

Study Number: I94-1925

Protocol Revision/Deviation Number: 2

Study Director: Christine Freeman

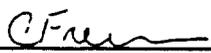
Date Effective: November 16, 1994

The above named protocol is to be followed except for the following revision(s)/deviation(s):

Dr. Mark Butt will replace Dr. Lucas Brennecke as pathologist.

Reason: PAI has requested that Dr. Butt replace Dr. Brennecke as pathologist for FMC.

This revision/deviation did not adversely affect the outcome of the study.


Study Director

1/4/95
Date

cc: Raw Data (Original)
Laboratory Study File
Study Director
Quality Assurance Unit
517
8/13/93
T:\TOXLAB\PROTOCOL\DEVIATNS\211925.DOC

APPENDIX 1. REPORTS CODE TABLE

PATHOLOGY ASSOCIATES, INC.

ACUTE DELAYED NEUROTOXICITY STUDY IN HENS WITH
TRICRESYL PHOSPHATE/DURAD 125L
FMC STUDY NO. I94-1925

REPORTS CODE TABLE

N Tissues within normal histological limits
A Autolysis precluding adequate evaluation
O Paired Organ Missing
U Unavailable/unsuitable for examination
S Tissues not applicable to animal
R Recut
* Not examined/not required by protocol
Ex Number examined

1 minimal
2 mild
3 moderate
4 marked
() focal
[] diffuse
< > multifocal
P Present
B Neoplasm, Benign
M Neoplasm, Malignant without Metastasis
C Neoplasm, Malignant with Metastasis
X Metastatic Site (+)
I Bilateral
L Unilateral
- No data entered

APPENDIX 2. ABBREVIATIONS TABLE

Abbreviations Used in the Data Tables

TIB.....Tibial
NE.....Nerve
VEH CONT.....Vehicle Control
TEST MAT.....Test Material
POS CONT.....Positive Control
R.....Right
L.....Left
CERE/FORE.....Cerebrum, Forebrain
ACUTE NEUROTOX HENS.....Acute Delayed Neurotoxicity Study in Hens with Tricresyl
Phosphate/Durad 125L

II. TABULATED ANIMAL DATA TABLES

PATHOLOGY ASSOCIATES, INC.
 ACUTE DELAYED NEUROTOXICITY STUDY IN HENS WITH
 TRICRESYL PHOSPHATE/DURAD 125L
 FMC STUDY NUMBER I94-1925

TABULATED ANIMAL DATA

STUDY ID : ACUTE NEUROTOX HENS
 FATE: Terminal sacrifice
 DAYS ON TEST: 0-22

STUDY NUMBER: 941925
 GROUP: VEH CONT: MAZOLA CORN OIL
 SEX: FEMALE

ANIMAL ID:	E0200F	E0201F	E0202F	E0203F	E0204F	E0205F	E0206F
BRAIN, CERE/FORE	N	N	N	N	N	N	N
BRAIN, CEREBELLUM	N	N	N	N	N	N	N
BRAIN, MEDULLA OBLONGATA	N	N	N	N	N	N	N
BRAIN, PONS	N	N	N	N	N	N	N
CERVICAL SPINAL CORD	N	N	N	N	N	N	N
THORACIC SPINAL CORD	N	N	N	N	N	N	N
LUMBOSACRAL SPINAL CORD	N	N	N	N	N	N	N
SCIATIC NERVE (RIGHT)	N	N	N	N	N	N	N
SCIATIC NERVE (LEFT)	N	N	N	N	N	N	N
TIBIAL NERVE (RIGHT)	N	N	N	N	N	N	N
TIBIAL NERVE (LEFT)	N	N	N	N	N	N	N
MEDIAL TIB NE BRANCH (R)	N	N	N	N	N	N	N
MEDIAL TIB NE BRANCH (L)	N	N	N	N	N	N	N
INFLAMMATION, CHRONIC	(1)	-	(1)	-	-	-	-
LATERAL TIB NE BRANCH (R)	N	N	N	N	N	N	N
LATERAL TIB NE BRANCH (L)	N	N	N	N	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
 ACUTE DELAYED NEUROTOXICITY STUDY IN HENS WITH
 TRICRESYL PHOSPHATE/DURAD 125L
 FMC STUDY NUMBER I94-1925

TABULATED ANIMAL DATA

STUDY ID : ACUTE NEUROTOX HENS
 FATE: Terminal sacrifice
 DAYS ON TEST: 0-22

STUDY NUMBER: 941925
 GROUP: VEH CONT: MAZOLA CORN OIL
 SEX: FEMALE

ANIMAL ID:	E0207F
BRAIN, CERE/FORE	N
BRAIN, CEREBELLUM	N
BRAIN, MEDULLA OBLONGATA	N
BRAIN, PONS	N
CERVICAL SPINAL CORD	N
THORACIC SPINAL CORD	N
LUMBOSACRAL SPINAL CORD	N
SCIATIC NERVE (RIGHT)	N
SCIATIC NERVE (LEFT)	N
TIBIAL NERVE (RIGHT)	N
TIBIAL NERVE (LEFT)	N
MEDIAL TIB NE BRANCH (R)	N
MEDIAL TIB NE BRANCH (L)	N
LATERAL TIB NE BRANCH (R)	N
LATERAL TIB NE BRANCH (L)	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
 ACUTE DELAYED NEUROTOXICITY STUDY IN HENS WITH
 TRICRESYL PHOSPHATE/DURAD 125L
 FMC STUDY NUMBER I94-1925

TABULATED ANIMAL DATA

STUDY ID : ACUTE NEUROTOX HENS

STUDY NUMBER: 941925

FATE: Terminal sacrifice

GROUP: TEST MAT: TRICRESYL PHOSPHATE/DURAD 125L 2000MG/KG

DAYS ON TEST: 0-22

SEX: FEMALE

ANIMAL ID:	E0211F	E0212F	E0213F	E0214F	E0215F	E0216F	E0217F
BRAIN, CERE/FORE	N	N	N	N	N	N	N
BRAIN, CEREBELLUM	N	N	N	N	N	N	N
BRAIN, MEDULLA OBLONGATA	N	N	N	N	N	N	N
BRAIN, PONS	N	N	N	N	N	N	N
CERVICAL SPINAL CORD AXONAL DEGENERATION	N	N	N	N		N	
	-	-	-	-	1	-	1
THORACIC SPINAL CORD	N	N	N	N	N	N	N
LUMBOSACRAL SPINAL CORD AXONAL DEGENERATION	N	N		N	N		N
	-	-	2	-	-	1	-
SCIATIC NERVE (RIGHT)	N	N	N	N	N	N	N
SCIATIC NERVE (LEFT)	N	N	N	N	N	N	N
TIBIAL NERVE (RIGHT)	N	N	N	N	N	N	N
TIBIAL NERVE (LEFT) PERINEURIAL FAT, INFLAMMATION, CHRONIC	N	N	N	N	N	N	
	-	-	-	-	-	-	(1)
MEDIAL TIB NE BRANCH (R)	N	N	N	N	N	N	N
MEDIAL TIB NE BRANCH (L)	N	N	N	N	N	N	N
LATERAL TIB NE BRANCH (R)	N	N	N	N	N	N	N
LATERAL TIB NE BRANCH (L)	N	N	N	N	N	N	N

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
 ACUTE DELAYED NEUROTOXICITY STUDY IN HENS WITH
 TRICRESYL PHOSPHATE/DURAD 125L
 FMC STUDY NUMBER I94-1925

TABULATED ANIMAL DATA

STUDY ID : ACUTE NEUROTOX HENS
 FATE: Terminal sacrifice
 DAYS ON TEST: 0-22

STUDY NUMBER: 941925
 GROUP: POS CONT: TRI-O-TOLYL PHOSPHATE 500 MG/KG
 SEX: FEMALE

ANIMAL ID:	E0222F	E0223F	E0224F	E0225F	E0226F	E0227F	E0228F
BRAIN, CERE/FORE	N	N	N	N	N	N	N
BRAIN, CEREBELLUM	N	N	N	N	N	N	N
BRAIN, MEDULLA OBLONGATA	N	N	N	N	N	N	N
BRAIN, PONS	N	N	N	N	N	N	N
CERVICAL SPINAL CORD AXONAL DEGENERATION	3	2	-	-	1	3	2
THORACIC SPINAL CORD AXONAL DEGENERATION	1	-	-	-	2	2	-
LUMBOSACRAL SPINAL CORD AXONAL DEGENERATION	2	-	-	-	-	-	-
SCIATIC NERVE (RIGHT) AXONAL DEGENERATION	N	N	N	N	1	N	N
SCIATIC NERVE (LEFT) AXONAL DEGENERATION	N	N	N	N	1	N	N
TIBIAL NERVE (RIGHT) AXONAL DEGENERATION	1	-	-	-	1	-	-
TIBIAL NERVE (LEFT) AXONAL DEGENERATION	1	-	-	-	1	1	-
PERINEURIAL FAT, GRANULOMA	-	-	-	(2)	-	-	-
MEDIAL TIBIAL NERVE BRANCH (R) AXONAL DEGENERATION	2	-	-	-	-	1	-

See Reports Code Table for Symbol Definitions

PATHOLOGY ASSOCIATES, INC.
 ACUTE DELAYED NEUROTOXICITY STUDY IN HENS WITH
 TRICRESYL PHOSPHATE/DURAD 125L
 FMC STUDY NUMBER I94-1925

TABULATED ANIMAL DATA

STUDY ID : ACUTE NEUROTOX HENS

STUDY NUMBER: 941925

FATE: Terminal sacrifice

GROUP: POS CONT: TRI-O-TOLYL PHOSPHATE 500 MG/KG

DAYS ON TEST: 0-22

SEX: FEMALE

ANIMAL ID:	E0222F	E0223F	E0224F	E0225F	E0226F	E0227F	E0228F
MEDIAL TIB NE BRANCH (L)			N	N			N
AXONAL DEGENERATION	2	1	-	-	1	2	-
LATERAL TIB NE BRANCH (R)		N	N	N			N
AXONAL DEGENERATION	2	-	-	-	2	1	-
LATERAL TIB NE BRANCH (L)		N	N	N			N
AXONAL DEGENERATION	1	-	-	-	2	1	-

See Reports Code Table for Symbol Definitions

III. PROJECT SUMMARY TABLES

PATHOLOGY ASSOCIATES, INC.
ACUTE DELAYED NEUROTOXICITY STUDY IN HENS WITH
TRICRESYL PHOSPHATE/DURAD 125L
FMC STUDY NUMBER I94-1925

PROJECT SUMMARY

STUDY ID : ACUTE NEUROTOX HENS
FATE: Terminal sacrifice
DAYS ON TEST: 0-22

STUDY NUMBER: 941925

SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:	VEH CONT		TEST MAT		POS CONT	
	(1)		(2)		(3)	
NUMBER OF ANIMALS:	8		7		7	
	#	%	#	%	#	%
BRAIN, CERE/FORE	# EX	8	7		7	
BRAIN, CEREBELLUM	# EX	8	7		7	
BRAIN, MEDULLA OBLONGATA	# EX	8	7		7	
BRAIN, PONS	# EX	8	7		7	
CERVICAL SPINAL CORD	# EX	8	7		7	
AXONAL DEGENERATION		0 0.0	2 29.0		5 71.0	
THORACIC SPINAL CORD	# EX	8	7		7	
AXONAL DEGENERATION		0 0.0	0 0.0		3 43.0	
LUMBOSACRAL SPINAL CORD	# EX	8	7		7	
AXONAL DEGENERATION		0 0.0	2 29.0		1 14.0	
SCIATIC NERVE (RIGHT)	# EX	8	7		7	
AXONAL DEGENERATION		0 0.0	0 0.0		1 14.0	
SCIATIC NERVE (LEFT)	# EX	8	7		7	
AXONAL DEGENERATION		0 0.0	0 0.0		1 14.0	
TIBIAL NERVE (RIGHT)	# EX	8	7		7	
AXONAL DEGENERATION		0 0.0	0 0.0		2 29.0	
TIBIAL NERVE (LEFT)	# EX	8	7		7	
AXONAL DEGENERATION		0 0.0	0 0.0		3 43.0	
PERINEURIAL FAT, GRANULOMA		0 0.0	0 0.0		1 14.0	
PERINEURIAL FAT, INFLAMMATION, CHRONIC		0 0.0	1 14.0		0 0.0	

Incidence Calculated by No. of Tissues Scored
(1) - MAZOLA CORN OIL

(2) - TRICRESYL PHOSPHATE/DURAD 125L 2000MG/KG
(3) - TRI-O-TOLYL PHOSPHATE 500 MG/KG

PATHOLOGY ASSOCIATES, INC.
ACUTE DELAYED NEUROTOXICITY STUDY IN HENS WITH
TRICRESYL PHOSPHATE/DURAD 125L
FMC STUDY NUMBER I94-1925

PROJECT SUMMARY

STUDY ID : ACUTE NEUROTOX HENS
FATE: Terminal sacrifice
DAYS ON TEST: 0-22

STUDY NUMBER: 941925

SEX: FEMALE

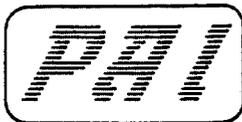
INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		VEH CONT		TEST MAT		POS CONT	
		(1)		(2)		(3)	
NUMBER OF ANIMALS:		8		7		7	
		#	%	#	%	#	%
MEDIAL TIB NE BRANCH (R)	# EX	8		7		7	
AXONAL DEGENERATION		0	0.0	0	0.0	2	29.0
MEDIAL TIB NE BRANCH (L)	# EX	8		7		7	
AXONAL DEGENERATION		0	0.0	0	0.0	4	57.0
INFLAMMATION, CHRONIC		2	25.0	0	0.0	0	0.0
LATERAL TIB NE BRANCH (R)	# EX	8		7		7	
AXONAL DEGENERATION		0	0.0	0	0.0	3	43.0
LATERAL TIB NE BRANCH (L)	# EX	8		7		7	
AXONAL DEGENERATION		0	0.0	0	0.0	3	43.0

Incidence Calculated by No. of Tissues Scored
(1) - MAZOLA CORN OIL

(2) - TRICRESYL PHOSPHATE/DURAD 125L 2000MG/KG
(3) - TRI-O-TOLYL PHOSPHATE 500 MG/KG

IV. QUALITY ASSURANCE STATEMENT



Pathology Associates, Inc.

Suite I
15 Worman's Mill Court
Frederick, MD 21701
(301) 663-1644
(301) 663-8994 FAX

QUALITY ASSURANCE STATEMENT

This neurohistopathology project has been inspected and audited by the PAI Quality Assurance Unit (QAU) as required by the Good Laboratory Practice (GLP) regulations promulgated by the U.S. Environmental Protection Agency. Results of these activities indicate that the portions of the study performed by PAI conformed with GLP regulations and applicable Standard Operating Procedures. The pathology narrative report is an accurate reflection of the recorded data. The following table is a record of the inspections/audits performed and reported by the QAU.

<u>Date of Inspection</u>	<u>Phase Inspected</u>	<u>Date Findings Reported to Management/ Study Pathologist</u>
03/22/95	Individual Animal Data	03/22/95
03/22/95	Draft Neurohistopathology Report	03/22/95
07/25/95	Final Neurohistopathology Report	07/25/95

Carol C. Hoffman
Director, Quality Assurance Division

July 25, 1995
Date

Study No. I94-1925
Study Title: Acute Delayed Neurotoxicity Study in Hens with Tricresyl Phosphate/Durad 125L

APPENDIX D

Miscellaneous - AALAC Accreditation

American Association

FOR

Accreditation of Laboratory Animal Care

FMC Corporation

Princeton, NJ

Is hereby accredited for demonstrating its compliance with the Association's standards.



October 4, 1984
DATE

Cory C. Christensen
CHAIRMAN
BOARD OF TRUSTEES