418538 01

Microfiche

010333

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

JUN 14 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Methyl Parathion - One-Year Study in Rats with Emphasis on Ocular and Nerve Effects

TO: Larry Schnaubelt PM 72
Special Review and Reregistration Division (H7508W)

FROM: K. Clark Swentzel K. Clark Swentzel 6/2/93
Toxicology Branch II
HED (H7509C)

THROUGH: Marcia van Gemert, Ph.D. M van Gemert 6/10/93
Branch Chief, Toxicology Branch II
HED (H7509C)

BARCODE: D174776
SUBMISSION: S412034
CASE: 818931
ID#: 053501
MRID#: 418538-01
CASWELL#: 372
REGISTRANT: MOBAY CORP.

Requested Action

None

Background

The registrant previously submitted a chronic toxicity/carcinogenicity study with methyl parathion in Sprague-Dawley CD rats (Study # 77-20-60; 12/22/83; Acc. # 252501) at dietary levels of 0, 0.5, 5, or 50 ppm (26 months for males; 28 months for females). The study was classified Core-minimum for carcinogenicity and Core-supplementary for chronic toxicity (EPA Memorandum, Backus to Zendzian, 7/12/84) because a NOEL for neurologic effects was not attained (degenerative changes in the sciatic nerve). Retinal atrophy was also observed in high-dose females at the termination of this study. Therefore, the registrant proposed a 1-year bridging study in rats which would focus on neurologic and ocular examinations. One of the objectives of this

study was to establish a NOEL for neurologic effects in order to upgrade the noted chronic toxicity/carcinogenicity study.

Current Submission

The subject study was reviewed by Clement International Corp., the DER is attached; a secondary review was performed by Dr. Sette (Science Analysis Branch) whose memorandum is also attached. Methyl parathion was administered to Sprague-Dawley rats for 12-13 months at dietary levels of 0, 0.5, 2.5, 12.5 or 50 ppm. The average intake values at these dietary levels were 0, 0.02, 0.107, 0.533 and 2.207 mg/kg/day for males and 0, 0.026, 0.138, 0.697 and 3.088 mg/kg/day for females.

The NOEL for systemic toxicity was 12.5 ppm and the LOEL was 50 ppm based on decreased body weight/body weight gain. The NOEL for cholinesterase inhibition was 2.5 ppm and the LOEL was 12.5 ppm.

Ocular toxicity:

The evaluation of potential ocular effects included the following examinations: ophthalmoscopy, fundus observations, electroretinography (ERG), electron microscopy and histopathologic observations. The reviewer concluded that the highly variable nature of ERG responses and the lack of a positive control limited the usefulness of that data. Under the conditions of this study, the NOEL for ocular toxicity was 50 ppm (HDT). Dr. Sette noted that EPA did not request positive control data, however, he indicated that further details of the method for the ERG measurements should be provided, a summary of the data performed (or their absence defended by appeal to the summary tables) to confirm the absence of an effect (see Sette memorandum for further details).

Neurotoxicity:

A number of histological methods were used to assay nervous tissues for signs of damage. These methods varied both in the degree of sensitivity and in the specificity of the assay for certain end points. The Clement reviewer noted that signs of damage occurred at doses as low as 0.5 ppm: increased myelin bubbles and Schwann cell proliferation in the proximal sciatic nerve of females and increased myelin phagocytosis in the tibial nerve of males as well as increased myelin ovoids in the sural nerves of males. However, Dr. Sette concluded that since the incidence and/or severity of the histopathologic effects observed at 0.5 and 2.5 ppm were minimal and not statistically significant and the most consistent effects were seen at 12.5 and 50 ppm, 2.5 should be regarded as the most defensible NOEL.

Electron microscopy of retina and optic nerve:

Both the Clement reviewer and Dr. Sette agree that electron microscopy of these tissues should not have been performed in the absence of light microscopy since "the scope of assessment is so

small in EM that it is not generally considered appropriate as a screening method and thus limits the confidence in negative findings." These tissues should be reexamined by light microscopy.

Summary and Conclusions

Ocular toxicity was not observed under the conditions of this study at the highest dose tested (50 ppm). However, as previously indicated, the ERG data as presented were difficult to interpret. The registrant should provide the information requested regarding the method and the data generated during this procedure.

The registrant should also reexamine the retina and optic nerve tissues, examined only by EM, by light microscopy.

The lowest level at which cholinesterase inhibition occurred was 12.5 ppm (plasma, RBC and brain).

Consistent neuropathologic effects were seen at 12.5 and 50 ppm, therefore, the NOEL for neurotoxicity in this study is considered to be 2.5 ppm. The implications of this conclusion for the Core-classification of Study No. 77-20-60 (chronic toxicity/carcinogenicity study in rats) can not be determined until 1) the information requested for this study has been evaluated and 2) the neurotoxicity data from both studies have been reviewed collectively (possibly by the peer review process) to determine an appropriate NOEL.

NOELS AND LOELS:

Systemic toxicity:	NOEL = 12.5 ppm; LOEL = 50 ppm
Cholinesterase inhibition:	NOEL = 2.5 ppm; LOEL = 12.5 ppm
Neurotoxicity:	NOEL = 2.5 ppm; LOEL = 12.5 ppm

Core classification: unacceptable; may be upgraded pending evaluation of requested information

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

010333

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

May 12, 1993
Methyl Parathion
Caswell No. 372

MEMORANDUM

SUBJECT: Methyl Parathion: 1 Year Rat Neurotoxicity Study
Secondary Review

TO: K. Clark Swentzel, Chief
Review Section II
Toxicology Branch II

FROM: William F. Sette, Ph.D.
Peer Review Section (H7509c)

THRU: Kerry Dearfield, Ph.D. Chief (Acting)
Peer Review Section
Science Analysis Branch

William Burnam, Chief
Science Analysis Branch

The purpose of this memo is to respond to your request to provide a secondary review of a one year rat neurotoxicity study of Methyl Parathion (MRID No. 418538-01). I have reviewed in detail the sections of the DER on neuropathology. I have more briefly reviewed the summary tables on AChEs and on clinical signs, and reviewed the results on the ERG and histopathology of the visual system.

This is a very complex neurotoxicity study and the reviewers have done a thorough job of reviewing this study. For the most part, I agree with their conclusions regarding the study. My major comments are summarized below, with more detailed comments on succeeding pages. I hope this meets your needs and would be willing to discuss these findings in more detail with the contractor or the registrant. My number is 305-6375.

Conclusions

1. In my judgment, 2.5 ppm should be considered as the NOEL for histopathology of the peripheral nerves and spinal cord for this study.

010333

2. Further details of the method for the ERG measurements should be provided, a summary table of the measures presented, and statistical analyses of the data performed (or their absence defended by appeal to the summary tables) to confirm the absence of an effect.

NOELs and LOELs

Systemic toxicity:	NOEL = 12.5 ppm	LOEL = 50 ppm
Cholinesterase Inhibition:	NOEL = 2.5 ppm	LOEL = 12.5 ppm
Neuropathology	NOEL = 2.5 ppm	LOEL = 12.5 ppm

Summary Discussion

I think that the most consistent effects on histopathology were seen at 12.5 ppm and 50 ppm and that, therefore, 2.5 ppm should be regarded as the most defensible NOEL. These peripheral nerve and spinal cord data provide an equivocal data set when it comes to making conclusions about a NOEL. At the two lowest dose levels, 0.5 and 2.5 ppm, in my view the incidence and/or severity of the effects are minimal and not statistically significant and so would be difficult to defend on strict scientific grounds. This is, in part, an issue of multiple comparisons. We have here a large number of measures on these animals, and by chance, some measures would be expected to be affected in some animals. Further, these measures are not all independent. That is, as noted in the review, multiple measures of neuronal integrity should be correlated with one another to some extent.

I think that the reviewer's point about the lack of positive controls for some effects should be tempered by the fact that EPA did not ask for them, and by the observation that often, i.e., in hen studies, positive controls are so positive that they offer little help in resolving less clear data sets. In any event, they would not help resolve the potential asserted confound between more little fibers vs. more regenerated fibers. I agreee, however, with their comments about electron microscopy in the absence of light microscopic examinations. The scope of assessment is so small in EM that it is not generally considered appropriate as a screening method and thus limits the confidence in negative findings. Are the tissues still available?

For ERG measurements, on the other hand, some demonstration of sensitivity seems a more cogent concern in that the issue relates to variability and sensitivity of the technique rather than the absence of effects. I recommend a more thorough description and analysis of this data to further explore this issue, and any historical control data on ERGs that the testing lab might have.

Last, this study is not a CORE grade study; so it should either be rated as "acceptable" or "unacceptable".

A detailed analysis of the histopathology data and comments on some other areas follows.

Analysis of Histopathology of Peripheral Nerve and Spinal Cord

I see no a priori reason why the data from males and females on histopathology could not be combined for purpose of analysis, and this is what I have done.

Proximal Sciatic nerve

First, in terms of peripheral nerve damage, there is evidence of sciatic nerve degeneration in 4/5 males at 13 months at 50 ppm. There is no effect in males at 12 months or females at 12 or 13 months.

Second, at 13 months, 4/5 males and 3/4 females show myelin bubbles in the sciatic nerves at 50 ppm. At 12 months, bubbles are seen at lower doses, with 1/10, 2/10, 5/10, and 5/10 at increasing doses. From these data, then, there is an effect here at 12.5 ppm.

For Schwann cell proliferation, we have 4/9 rats at 13 months, and at 12 months, 1/10, 2/10, 5/10, and 4/10 respectively. Similarly, 12.5 ppm is an effect level. Both changes are statistically significant by Fisher's Exact Test. (printout attached).

Lumbosacral spinal cord

In males, 1/5 rats at 13 months showed neuronal degeneration, grade 3, and grade 3 (moderate) loss of myelinated fibers, at the high dose of 50 ppm.

In females, there was loss of myelinated fibers in 1/4 lumbosacral cord rated slight/mild at the high dose at 13 months, with no evidence of neuronal degeneration.

Tibial/Peroneal Nerve

There was a significant increase in myelin bubbles in 50 ppm rats at 12 months, and some increase at 13 months. Schwann cell proliferation was significantly increased at 13 months, but inconsistent at 12 months. Regeneration in one animal at 50 ppm was seen, but no other consistent changes were seen. (printout of statistical tests attached).

Teased Sural Nerve

At 6 months, myelin ovoids were seen in 2/5 50 ppm females, along with focal demyelination in 3/10 rats(2/5 females, 1/5 males). At 12 months, 4/10 controls, and 4/10, 3/10, 5/10, and 9/10 rats showed demyelinated lengths, indicating a clear effect at 50 ppm. At 13 months, there was also a clear effect in males, but not females at this dose. Myelin ovoids were increased in high dose rats at 6 months,(0/10 vs 4/10), but less consistently increased at 12 or 13 months. No increased incidence of myelin bubbles were in these fibers.

Morphometry of Tibial Nerve

Fiber diameters

Effects were noted at 6 months as increases in the % of small fibers and a decrease in the % of larger fibers for 50 ppm females. This would be consistent with the idea of loss of big fibers and replacement with new smaller fibers. The reviewer noted an increase in larger diameter fibers in males given 12.5 ppm at 12 months, and considered it consistent with axonal swelling. These were cited by the reviewer as statistically significant. The study author also notes various changes.

Interpretation of these findings is made more difficult by the nature of these explanations as bi-directional: more larger fibers is axonal swelling; more smaller fibers is regenerating fibers.

In addition, these findings are not very consistent with the other findings, and in males at 12 months, are not dose dependent.

A significantly (?) increased percentage of distal sciatic nerve occupied by myelinated nerve fibers was noted for high dose males at 13 months (60.56+/-7.489 vs. 67.02+/-4.027) in comparison to controls and some smaller increases in males in all dose groups at 12 months, but these changes seem somewhat small and I question how they could be statistically significant.

Internodal lengths

Somewhat shorter internodal lengths were noted by the study author for males at 12.5 or 50 ppm after 12 months; for females and lower dose males, changes were inconsistent.

At 6 and 13 months, somewhat shorter lengths in males at 50 ppm were noted by the reviewer and I concur. At 12 months, I find changes inconsistent.

Visual System Function (ERGs) and Structure

There appear to be no effects on the retinas in treated rats. With respect to the ERG data, however, it is difficult to review this data as presented. The description of the method used is too limited and insufficient. More importantly, there should be a summary table of the pre-exposure and test time means for each group and some statistical analysis of the data or at least some explanation of why no analyses are needed. It appears to this reviewer that no effect occurs in high dose males with respect to their own pre-exposure values.

With respect to the clinical signs noted, in my experience, aggressive behavior as noted in high dose rats is not commonly reported with cholinesterase inhibitors.

PROXIMAL STATE
MYELIN BUBBLES
12 MONTHS

010333

May 11, 1993

Fisher's Exact Test/Cochran-Armitage trend test

Methyl Parathion

DOSE()	0.0000	0.5000	2.5000	12.5000	50.0000
	0/10	1/10	2/10	5/10	6/10
	(0)	(10)	(20)	(50)	(60)
	p= 0.0012**	p= 0.5000	p= 0.2368	p= 0.0163*	p= 0.0054**

	CHI-SQUARE	DF	P VALUE	
LINEAR TREND (Ho: no trend)	9.3663	1	0.0012**	(one-sided)
DEPARTURE (Ho: linear)	3.9274	3	0.2685	(two-sided)

SCHWANN CELL PROLIFERATION
12 MONTHS

010333

May 11, 1993

Fisher's Exact Test/Cochran-Armitage trend test

Methyl Parathion

DOSE()	0.0000	0.5000	2.5000	12.5000	50.0000
	0/10	1/10	2/10	5/10	4/10
	(0)	(10)	(20)	(50)	(40)
	p= 0.0253*	p= 0.5000	p= 0.2368	p= 0.0163*	p= 0.0433*

	CHI-SQUARE	DF	P VALUE	
LINEAR TREND (Ho: no trend)	3.7295	1	0.0253*	(one-sided)
DEPARTURE (Ho: linear)	5.7003	3	0.1253	(two-sided)

MYELIN BUBBLES
12 MONTHS

May 11, 1993

010333

Fisher's Exact Test/Cochran-Armitage trend test

Methyl Parathion

DOSE()	0.0000	0.5000	2.5000	12.5000	50.0000
	0/10	0/10	3/10	2/10	7/10
	(0)	(0)	(30)	(20)	(70)
	p= 0.0001**	p= 1.0000	p= 0.1053	p= 0.2368	p= 0.0015**

	CHI-SQUARE	DF	P VALUE
LINEAR TREND (Ho: no trend)	15.4203	1	0.0001** (one-sided)
DEPARTURE (Ho: linear)	2.7815	3	0.4283 (two-sided)

MYELIN BUBBLES
13 MONTHS

May 11, 1993

010333

Fisher's Exact Test/Cochran-Armitage trend test

Methyl Parathion

DOSE()	0.0000	50.0000
	4/10	7/9
	(40)	(78)
	p= 0.0458*	p= 0.1149

	CHI-SQUARE	DF	P VALUE	
LINEAR TREND (Ho: no trend)	2.7732	1	0.0458*	(one-sided)
DEPARTURE (Ho: linear)				(two-sided)

*** Can not calculate departure with only 2 dose groups.

NASAL/PERIORAL
SCHWANN CELL PROLIFERATION
13 MONTHS

010333

May 11, 1993

Fisher's Exact Test/Cochran-Armitage trend test

Methyl Parathion

DOSE()	0.0000	50.0000
	0/10	4/9
	(0)	(44)
	p= 0.0084**	p= 0.0325*

	CHI-SQUARE	DF	P VALUE	
LINEAR TREND (Ho: no trend)	5.6296	1	0.0084**	(one-sided)
DEPARTURE (Ho: linear)				(two-sided)

*** Can not calculate departure with only 2 dose groups.

010333

DOC930220

FINAL

DATA EVALUATION REPORT

Methyl Parathion

Study Type: Chronic Toxicity in Rats
(with Emphasis on Ocular and Nerve Effects)

Prepared for:

Office of Pesticide Programs
Health Effects Division
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

July 1, 1992

Principal Reviewer Carrie Rabe Date 6/30/92
Carrie Rabe, Ph.D.

Independent Reviewer William L. McLellan Date 6/30/92
William McLellan, Ph.D.

QA/QC Manager Sharon A. Segal Date 6/30/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 1-99
Clement Number: 93-30
Project Officer: James E. Scott

010333

Approved by:
EPA Reviewer and Section Head:
Clark Swentzel, Review Section II
Toxicology Branch II, Health Effects Division

Signature: K. Clark Swentzel
Date: 7/21/92

DATA EVALUATION REPORT

STUDY TYPE: Chronic toxicity in rats (with emphasis on ocular and nerve effects)

TEST MATERIAL: Methyl parathion

TOX. CHEM. NUMBER: 372 P.C. NUMBER: 53501

SYNONYMS: E120, dimethyl parathion, metaphos

STUDY NUMBER: 87-3208 MRID NUMBER: 418538-01

SPONSOR: Mobay Corporation
A Bayer USA Inc. Company
17745 South Metcalf
Stillwell, Kansas 66085-9104

TESTING FACILITY: Bio/dynamics, Inc.
Mettlers Road
East Millstone, New Jersey 08873

TITLE OF REPORT: A Twelve Month Oral Toxicity Study of Methyl Parathion (E 120) in the Rat via Dietary Admixture with Special Focus on Ocular and Sciatic Nerve Effects

AUTHOR: Ira W. Daly

REPORT ISSUED: January 7, 1991

CONCLUSIONS: Methyl parathion was administered via the diet to Sprague-Dawley rats for 12-13 months at doses of 0, 0.5, 2.5, 12.5, and 50 ppm. The average daily intake values of methyl parathion at these dietary levels were 0, 0.02, 0.107, 0.533, and 2.207 mg/kg/day for males and 0, 0.026, 0.138, 0.697, and 3.088 mg/kg/day for females. No ocular toxicity was observed at any dose; therefore, the NOEL for ocular effects was 50 ppm. Systemic toxicity, as indicated by decreased body weight, body weight gain, and increased food consumption, occurred with a LOEL of 50 ppm; the NOEL was 12.5 ppm. Cholinesterase inhibition occurred with a LOEL of 12.5 ppm; the NOEL was 2.5 ppm. Histological evidence of neurotoxicity occurred with a LOEL of 0.5 ppm. No NOEL for histological evidence of neurotoxicity was found. The following treatment-related effects were observed:

0.5 ppm -- Equivalent to 0.020 mg/kg/day in males and 0.026 mg/kg/day in females. Increased myelin bubbles and Schwann cell proliferation in the proximal sciatic nerve of females and increased myelin phagocytosis in the tibial nerve of males at 12 months.

Increased myelin ovoids in the sural nerves of males at 12 months.

2.5 ppm -- Equivalent to 0.107 mg/kg/day in males and 0.138 mg/kg/day in females. Increased myelin bubbles and Schwann cell proliferation in the proximal sciatic nerve of males and females at 12 months. Increased myelin bubbles, myelin phagocytosis, and Schwann cell proliferation in the tibial nerve of males at 12 months. Increased myelin ovoids in the sural nerves of males at 12 months.

12.5 ppm -- Equivalent to 0.533 mg/kg/day in males and 0.697 mg/kg/day in females. Decreased plasma cholinesterase activity in males at 1 month and in females at 12 months. Decreased erythrocyte cholinesterase activity in males at 1 and 6 months and in females at 1, 9, and 12 months. Decreased brain cholinesterase activity in females at 12 months. Increased myelin bubbles and Schwann cell proliferation in the proximal sciatic nerve of males and females at 12 months. Increased myelin bubbles, myelin phagocytosis, and Schwann cell proliferation in the tibial nerve of males and increased myelin phagocytosis in the tibial nerve of females at 12 months. Morphometry also showed an increase in the percentage of large-diameter fibers in cross sections of the tibial nerve in males. Increased myelin ovoids in the sural nerve of males at 12 months.

50.0 ppm -- Equivalent to 2.207 mg/kg/day in males and 3.088 mg/kg/day in females. Decreased body weight and body weight gain in males and females. Increased food consumption in males and females. Decreased plasma, erythrocyte, and brain cholinesterase activity in males and females at all intervals tested (except erythrocyte cholinesterase activity in females at 3 months). Increased aggressiveness in males and increased aggressiveness, hyperactivity, tremors, abnormal gait, and lack of muscle tone in females. Increased tail problems and sores/scabs in females. Increased myelin ovoids and demyelinated lengths in the sural nerve in males and females at 6 months. Increased percentage of small-diameter fibers and decreased percentage of large-diameter fibers in cross sections of the tibial nerve of females at 6 months. Increased myelin bubbles and Schwann cell proliferation in the proximal sciatic nerve of males and females at 12 months. Increased myelin bubbles, myelin phagocytosis, and Schwann cell proliferation in the tibial nerve of males and increased myelin bubbles, myelin phagocytosis, and nerve regeneration in the tibial nerve of females at 12 months. Increased myelin ovoids in the sural nerve of males and increased demyelinated lengths in the sural nerve of males and females at 12 months. Increased myelin bubbles in the proximal sciatic nerve of males and females at 13 months. Increased neuronal degeneration and Schwann cell proliferation in the proximal sciatic nerve of males at 13 months. Increased neuronal degeneration in the lumbosacral spinal cord of males and loss of myelinated fibers in the lumbosacral spinal cord of males and females at 13 months. Increased myelin bubbles, myelin

phagocytosis, and Schwann cell proliferation in the tibial nerve of males and females at 13 months. Increased demyelinated lengths and myelin ovoids in the sural nerve of males at 13 months.

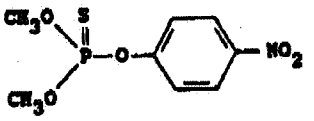
CORE CLASSIFICATION: This study is classified as Core Supplementary for chronic toxicity based on its design and intent. The study was designed to provide additional information for a chronic toxicity/oncogenicity study and was not intended to stand on its own.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Methyl parathion

Formula: $C_8H_{10}NO_5PS$: Phosphorothioic acid O,O-dimethyl O-(4-nitrophenyl) ester



Lot number: 233 690479

Purity: 94.6% active ingredient

Physical property: Brown liquid

Stability: Not presented in this report

2. Rationale for Dose Selection

No information was presented regarding the rationale for selection of the dietary levels of methyl parathion used in the current study. However, this study was performed for the purpose of upgrading a 1984 chronic toxicity/oncogenicity study in rats. Thus, it is likely that the doses used in the current study were based on results from the 1984 study. The dietary levels selected for the current study were 0, 0.5, 2.5, 12.5, and 50 ppm.

3. Test Article Analyses for Purity and Stability

The purity of the test material was reported to be 94.6% and was reported to have been documented by the sponsor. Analysis of a sample of the technical grade material was performed by the testing facility following a report by pharmacy personnel that the test material was crystallizing. This analysis found that the test material was 98.5% pure. At that time, a sample of the test material was also sent to the sponsor for analysis. The results of that

analysis were not presented. Test diets were prepared assuming a purity of 100%.

Test diets were prepared by combining appropriate amounts of methyl parathion with 200 ml of acetone and mixing with rat chow to obtain the desired test levels. Control diets were prepared by mixing with the same amount of acetone. Fresh test diets were prepared weekly. No information was provided regarding storage of the diets prior to feeding during the study.

Stability of the test material in the diet at room temperature was measured after 4 days and when stored frozen for 7 days. No loss of test material was observed.

Homogeneity of sample batches of the test material in the diet was tested prior to the initiation of the study. The mixing procedure was modified until homogeneity was found to be acceptable. Homogeneity was tested again when the batch size was reduced and was also found to be acceptable at that time. The actual concentration of the test material in diets offered to the rats was measured at weeks 1, 2, 3, 4, 8, 13, 14 (due to problems with the extraction of group II at week 13), 18, 22, 26, 31, 35, 39, 44, 48, and 52. The average measured concentrations at each test level were as follows:

Nominal Concentration (ppm)	Measured Concentration (expressed as a % of the nominal concentration)
0.5	104% ± 6.87%
2.5	99.1% ± 4.72%
12.5	99.8% ± 6.81%
50.0	100% ± 4.81%

*Mean ± S.D., data from Appendix I

4. Animals

Rats (429 males and 435 females, Sprague-Dawley CD®) were received from Charles River Breeding Laboratories, Inc., Kingston, NY. The rats were 28 days old upon arrival and were caged 2 rats/cage for approximately 1 week. Thereafter, rats were individually caged in stainless steel cages with wire mesh floors. The animal room was operated on a 12-hour light/dark cycle, and temperature and relative humidity were maintained at 64°-78°F and 16%-82%, respectively. Water and food (Purina® Certified Rodent Chow #5002) were provided ad libitum. Following elimination of unsuitable rats (based on pretest body weight, ophthalmoscopic or physical examination), rats were randomized by body weight and allocated to study groups (70/sex/dose) using a computerized procedure such that all groups of the same sex had similar mean body weights. These groups were then subdivided into two subgroups (A, 50 rats/sex/dose; B, 20 rats/sex/dose). The

group A rats were primarily used to test for effects on nervous tissues other than the eye and group B rats were used to assess ocular effects. At the time of the first exposure to test diets, the rats were 49 days old, and males and females ranged in weight from 205 to 310 g and from 144 to 219 g, respectively. Rats were uniquely identified through the use of ear and cage tags.

5. Statistical Analyses

Body weight, body weight gain, food consumption, and cholinesterase activities were analyzed statistically. Data with equal variances (as determined using Bartlett's test) were analyzed using analysis of variance. If the result was significant ($p \leq 0.05$), Dunnett's test was used to analyze for differences between the control and treated groups. Data with unequal variances were analyzed using the Kruskal-Wallis test, and Dunn's summed rank test was used to analyze which groups differed from control. The limit for statistical significance was set at 0.05 for all tests.

6. General Observations

(a) Mortality/moribundity/survival

Animals were observed twice daily (once in the morning and once in the afternoon) for mortality/moribundity.

Mortality of the male and female treatment groups ranged from 0% to 5%. No treatment-related effects on mortality were observed.

(b) Clinical observations

Animals were reported to have been observed twice daily for overt adverse clinical signs. In addition, detailed clinical observations (including palpations) were reported to have been made weekly.

Only summary data were presented for clinical observations. With the exception of the signs associated with neurotoxicity, these data were presented on an "incidence/observation interval" basis such that determination of an overall incidence was impossible. Despite this limitation, increased incidences of tail problems, scabs, and ano-genital yellow staining were observed in high-dose females, primarily during the second half of the study. Incidences from selected intervals are shown in Table 1.

In contrast, signs associated with possible neurotoxicity were presented on a "per animal" basis. As seen in Table 2, increases in the incidence of aggressiveness were observed in high-dose males, and increases in the incidences of aggressiveness, tremors, hyperactivity, abnormal gait, and loss of motor tone were observed in high-dose females. Aggressiveness was observed in more males than females. These effects were observed primarily during the first 3-6 months of the study.

(c) Body weights/food consumption/feed efficiency/test article intake

Body weights--Individual body weights were determined weekly throughout the study.

010333

Body weight data from selected intervals are presented in Table 3. Body weights of the high-dose males were consistently significantly decreased relative to controls throughout the study. Similarly, body weights of the high-dose females were significantly decreased relative to controls during weeks 1-14 and sporadically thereafter. The decrease in male body weights ranged from 2.5% to 10% (average, 8.6%) below control values and the decrease in female body weights ranged from 2% to 12.2% (average 6.1%) below control values. At lower doses, occasional differences were observed with respect to controls, but these were incidental in nature.

Body weight gain data from selected intervals are presented in Table 4. Body weight gains for high-dose males were significantly decreased throughout the study. Body weight gains for the high-dose females were significantly decreased for weeks 1-16 and sporadically thereafter. Male body weight gains ranged from 13.3% to 22.8% (average, 16.3%) below control values. Female body weight gains ranged from 6.3% to 55.2% (average, 16.6%) below control values. At lower doses, occasional differences were observed with respect to controls, but these were incidental in nature.

Food consumption--Although fresh feed was presented to the rats twice weekly, individual food consumption values were determined weekly.

Food consumption data from selected intervals are presented in Table 5. Food consumption was reported to have been increased in males at doses as low as 2.5 ppm for the first 9 weeks of the study and in females at doses as low as 12.5 ppm during weeks 2-7. However, food consumption was significantly increased in treated males and females relative to controls prior to the administration of test material. In males and females, these pretreatment increases were in the range of 4%-6% above control and were observed in dose groups as low as 2.5 ppm and 0.5 ppm, respectively. Thus, the biological significance of comparable increases following administration of methyl parathion is doubtful. Only high-dose males and females showed substantial increases in food consumption above the increases observed pretreatment. In high-dose males, increases in food consumption ≥10% above control were observed during weeks 3, 5, 7, 8, 9, and 11. In high-dose females, increases ≥10% above control were observed during weeks 3-19, 23, 27, 31, 33, 34, 37, 38, 40-43, 45, 47, 49, 50, and 51.

Feed efficiency--Feed efficiency was not determined in this study.

Test article intake--Test article intake (mg methyl parathion/kg body weight/day) was calculated weekly. These values were calculated using the nominal dietary concentrations of methyl parathion.

The study author calculated that the average intake values of methyl parathion for male rats receiving diets containing 0.5, 2.5, 12.5, and 50.0 ppm were 0.02, 0.107, 0.533, and 2.207 mg/kg/day, respectively. The average intake values for female rats receiving these same dietary concentrations were 0.026, 0.138, 0.697, and 3.088 mg/kg/day, respectively.

(d) Ophthalmoscopic and related examinations

With the exception that all rats were examined by indirect ophthalmoscopy prior to the start of the study, eye examinations were conducted exclusively on the group B rats.

Ophthalmoscopy. All group B rats (20/sex/dose) were examined by indirect ophthalmoscopy prior to the start of the study and then again at 3, 6, 9, and 12 months. No treatment-related effects on the appearance of the eyes were observed.

Fundus observations. The method used for the fundus examinations was incompletely described but may have involved evaluation of photographs of the retina taken pretest and again at 3, 6, 9, and 12 months. The report indicates that control and high-dose rats (20/sex/dose) were evaluated and that no treatment-related effects were observed, but it is unclear if data from the fundus examinations were presented in the study (i.e., the results of the fundus examinations may have been presented in combination with the ophthalmoscopy results).

Electroretinography. Electroretinograms were obtained (using a LKC Systems electroretinograph) from 5 rats/sex/dose pretest and at 3, 6, 9, and 12 months. No treatment-related effects on the latency or amplitude of the electrical activity obtained in response to a white light stimulus were reported. However, the responses were highly variable, and several responses were noted to have been affected by the anesthesia used to obtain the recordings. No positive control rats were used in this study. Thus, it is unclear whether the method used had sufficient sensitivity to detect changes from control. Given the highly variable nature of the responses, the absence of positive control data severely limits the usefulness of this data.

7. Clinical Pathology

Hematological analyses and blood and urine analyses were not performed. Clinical chemistry analyses were limited to analyses of plasma, erythrocyte, and brain cholinesterase activity. Analyses of plasma and erythrocyte cholinesterase activity were performed pretest and at 1, 3, 6, 9, and 12 months. Brain cholinesterase activity was determined from brains obtained at the 12-month sacrifice. These

analyses were performed on 10 rats/sex/dose chosen from among the group A rats. Blood samples were obtained from the retroorbital sinus of rats that had been fasted overnight.

Table 6 presents cholinesterase activity data obtained at the specified intervals. The effects observed include:

Plasma ChE. Significantly decreased plasma cholinesterase activity at dietary levels as low as 12.5 ppm at 1 month and 50.0 ppm at all intervals in males and at dietary levels as low as 12.5 ppm at 12 months and 50.0 ppm at all intervals in females.

RBC ChE. Significantly decreased RBC cholinesterase activity at dietary levels as low as 12.5 ppm at 1 and 6 months and 50.0 ppm at all intervals in males and at dietary levels as low as 12.5 ppm at 1, 9, and 12 months and 50.0 ppm at all intervals in females.

Brain ChE. Significantly decreased brain cholinesterase activity at dietary levels of 50.0 ppm in males and 12.5 ppm and 50.0 ppm in females.

8. Sacrifice and Pathology

Animals were allocated for gross and selected histopathologic analyses according to the scheme shown in Figure 1. Except for the 5 rats/sex/dose that were designated for whole-body perfusion at 3, 6, 9, 12, and 13 months, all rats that died, that were sacrificed in extremis, or that were sacrificed as scheduled, received a complete gross examination. Tissues from these rats were preserved in buffered formaldehyde. No organs were weighed. Whole-body perfusion was performed on 5 rats/sex/dose at the specified intervals using a buffered 3% paraformaldehyde and 3% glutaraldehyde solution with secondary fixation in osmium tetroxide. Selected tissues from each rat were dissected at the scheduled sacrifices and designated for specific microscopic analyses as follows:

Group A -- Nonperfused

Brain -- Light microscopy of five sections (stained with Hematoxylin & Eosin).

Spinal cord (cervical, thoracic, lumbar levels) -- Light microscopy of sections (stained with Hematoxylin & Eosin).

Sciatic nerve with quadriceps femoris (right and left) -- Light microscopy of cross and longitudinal sections (stained with Hematoxylin & Eosin).

Group A -- Whole-body perfused

Brain -- Light microscopy of five duplicate paraffin-embedded sections (stained with either Hematoxylin & Eosin or Luxol Fast Blue Holmes for myelin evaluation).

Spinal cord (cervical, thoracic, lumbar) -- Light microscopy of duplicate paraffin-embedded sections (stained with either Hematoxylin & Eosin or Luxol Fast Blue Holmes for myelin evaluation).

Sciatic nerve with quadriceps femoris (right and left) -- Light microscopy of duplicate paraffin-embedded cross and longitudinal sections of proximal sciatic nerve (stained with either Hematoxylin & Eosin or Luxol Fast Blue Holmes for myelin evaluation).

Light microscopy of 1-3 epoxy-embedded sections of tibial and peroneal extensions of the sciatic nerve (stained with Toluidine Blue for neuropathologic evaluation). Cross sections were also used for morphometry to determine cross-sectional area of myelinated nerves.

Light microscopy of teased nerve preparations of the sural extension of the sciatic nerve. Individual nerve fibers were also used for morphometry of internodal distances.

Eyes -- Electron microscopy of epoxy-embedded sections of retina and optic nerve (stained with uranyl acetate and lead citrate).

Group B - All nonperfused

Eyes -- Light microscopy of sections of retina and optic nerve (stained with Hematoxylin & Eosin).

(a) Macroscopic examination

A total of 45 rats/sex/dose were examined grossly. Very few gross lesions were observed in either the control or treated animals. Lesions that appeared to increase in treated rats were limited to sores/scabs and bumps on the tails of the high-dose female rats (Table 7). It is unclear whether the tail lesions may have been associated with nervous system effects (i.e., peripheral nerve damage, aggressiveness).

(c) Microscopic examination

As indicated above, a number of different histological methods were used to assay nervous tissues for signs of damage. These methods varied both in the degree of sensitivity and in the specificity of the assay for certain end points. Results from several of the methods indicated an increase in neuronal degeneration among treated rats with some changes observed in distal nerves of high-dose rats as early as 6 months. Comparison of the effects observed at all treatment levels at 12 months indicated that signs of damage occurred at doses as low as 0.5 ppm. The results of the individual assays are as follows:

Light microscopy of sections stained with Hematoxylin & Eosin from nonperfused rats from groups A and B. Examination of sections from control and high-dose rats from the 6- and 12-month sacrifices revealed no treatment-related increases in degeneration and/or demyelination. However, the method of tissue fixation and staining used is the least sensitive of the various methods (Spencer and Schaumburg 1980).

Light microscopy of paraffin-embedded sections from perfused rats from group A. Table 8 shows the incidence data for lesions observed in sections from control and treated rats. At 12 months, a treatment-related increase in the incidences of myelin bubbles (indicative of edema in the myelin sheath) and Schwann cell proliferation (seen along the axon at demyelinated sites) was observed in the proximal sciatic nerve. These effects were observed at doses as low as 2.5 ppm in males and as low as 0.5 ppm in females. In general, these lesions increased in incidence and/or severity with increasing doses. Similar effects were observed in high-dose rats at 13 months. In addition, loss of myelinated fibers was observed in the lumbosacral spinal cord in high-dose males and females, and neuronal degeneration was observed in the proximal sciatic nerve and the lumbosacral spinal cord in high-dose males.

Light microscopy of epoxy-embedded sections of the tibial/peroneal nerve from perfused rats from group A. Table 9 shows the incidence data for lesions observed in sections from control and treated rats. At 12 months, distal sections of nerves from males given diets containing 0.5 ppm and above showed myelin phagocytosis; at 2.5 ppm and above myelin bubbles and Schwann cell proliferation were observed. The incidence and/or severity of these findings did not clearly increase with dose. At 12 months, nerves from treated females showed myelin bubbles, myelin phagocytosis, regeneration, and Schwann cell proliferation. However, no clear dose response was observed. At 13 months, the only differences between controls and high-dose rats were an increase in Schwann cell proliferation in high-dose males and females and myelin phagocytosis in high-dose females. Myelin bubbles and phagocytosis were observed in both control and high-dose males, and myelin bubbles were observed in both control and high-dose females. The study author attributed the changes observed in the control rats in this study to age-related changes similar to those seen in other studies of rats housed in cages with wire mesh bottoms (Spencer and Schaumburg 1980).

Light microscopy of teased sural nerves from perfused rats from group A. Table 10 shows the incidence data for lesions observed in sections from control and treated rats. At 6 months, an increase in the incidences of demyelinated lengths and myelin ovoids (indicative of myelin degeneration) was observed in high-dose males and females. The incidence of demyelinated lengths appeared to be increased in high-dose males and females at 12 months and in high-dose males at 13 months relative to controls. In addition, the incidence of myelin ovoids appeared to increase

in males at doses as low as 0.5 ppm at 12 months and in high-dose males at 13 months. A relatively high incidence of demyelinated lengths and myelin ovoids were observed in controls. As indicated above, the study author attributed the degenerative changes in control rats to age-related changes.

Morphometry of cross-sectional area of myelinated nerves in epoxy-embedded sections of the tibial nerve from perfused rats from group A. Figures 2A-2C show the frequency distribution of nerve fiber areas in three random photomicrographs/nerve/rat taken from control and treated rats at 6 and 12 months. Nerve fiber area (μ^2) was measured using a Jandel Scientific Digitizer and computer. The only statistically significant changes in nerve fiber areas that appeared to be treatment related (statistical analysis, ANOVA and t-test, performed by reviewer) were an increase in the percentage of small fibers and a decrease in the percentage of large-diameter fibers in high-dose females at 6 months and an increase in the percentage of large diameter fibers in males receiving diets containing 12.5 ppm at 12 months. The increase in small fibers and loss of large diameter fibers in high-dose females at 6 months may be associated with increases in regenerating neurons (typically small diameter fibers) and selective loss of large diameter fibers (typically the first to undergo degeneration). The increase in large diameter fibers seen in 12.5-ppm males at 12 months may be associated with axonal swelling and/or myelin bubbles.

Based on the information on individual nerve fiber area, the percentage of the total area occupied by myelinated nerves was calculated. The only statistically significant difference between control and treated animals was observed at 13 months in high-dose males (statistical analysis, ANOVA and t-test, performed by reviewer). The high-dose males had a significant increase in percent area occupied by myelinated nerves (67.02 ± 4.03; n=5; mean ± standard deviation) above controls (60.56 ± 7.49; n=5; mean ± standard deviation). The study authors attributed this increase to axonal swelling and/or myelin bubbles. The significance of this finding is uncertain because the nerve fiber spectrum of high-dose males at 13 months showed no significant increase in large fibers.

Morphometry of internodal lengths in teased nerves from perfused rats from group A. Figures 3A-3C show the distribution of internodal lengths in teased nerves from control and treated rats. At 6 and 13 months, decreased internodal lengths were observed in high-dose males. At 12 months, decreased internodal lengths were observed in the 2.5-ppm and 50.0-ppm males; however, no clear dose response was evident. At 12 months, slightly decreased internodal lengths were observed in the high-dose females. However, interpretation of these results is limited because, for this analysis, all internodal lengths were combined irrespective of fiber diameter. Thus, the results do not distinguish between decreases in internodal length resulting from remyelination and decreases resulting from a predominance of

small diameter fibers that under normal conditions have smaller internodal distances than large diameter fibers. Since the selection of teased fibers used in this study may not have been random, increases or decreases in internodal length may simply represent variations in the nerve fiber selection process.

Electron microscopy of retina and optic nerve from perfused rats from group A. Electron micrographs of tissues obtained from males and females from the control and high-dose groups at the 12-month sacrifice showed only a few differences from the controls. These included the presence of two lymphocytes in the ganglion cell layer of the retina of one of the high-dose females and a distorted myelin sheath and a myelin sheath encircling multiple small axons in the optic nerve in high-dose females. These effects were reported to be incidental. However, a positive control was not used in this study. Thus, it is uncertain whether this method of analysis was adequately sensitive.

- -

A signed Good Laboratory Practice Compliance Statement, a signed Quality Assurance Statement, and a list of Quality Assurance Inspections were included. In addition, the study required flagging based on the systemic toxicity NOEL and the cholinesterase inhibition NOEL. A signed Flagging Statement was included.

B. DISCUSSION

This study was designed to upgrade a 1984 chronic toxicity/oncogenicity study in rats. Specifically, this study examined effects on survival, clinical signs, body weight, food consumption, ophthalmology, cholinesterase activity, and gross and microscopic ocular and nervous system tissue pathology. A number of techniques were used to assess ocular effects and examine microscopic changes in ocular and nervous system tissues. Individual animal data were available for all of these parameters except clinical signs. The data appeared to be accurately reported; however, body weight data from weeks 43 and 44 showed that males lost and regained approximately 250 grams and females lost and regained approximately 130 grams. No explanation for this was presented. Because of the specific design of this study, routine hematology, clinical chemistry, and urine analyses were not performed. Also, pathologic examination of tissues other than nervous system tissues was limited to routine macroscopic examinations.

Although the overall design of the study was adequate and the methods used were designed for optimal fixation of nervous tissues (whole-body perfusion with glutaraldehyde solution), several of the assays used to study ocular and nervous system pathology were poorly designed. For example, no positive controls were used. While positive controls are not absolutely required, evaluation of negative ocular toxicity or neurotoxicity results is aided if it can be shown that the assay system is sensitive enough to detect changes from control. This is particularly true for the electroretinography. Although this assay showed no

differences between controls and treated rats, the results of the electroretinography were highly variable. Also, electron microscopy of retinas and optic nerves were performed in the absence of light microscopy of these tissues from perfused rats. Thus, it is unclear what electron microscopic changes would have been detected if ocular toxicity had occurred. Data from positive controls would also have provided a standard of comparison to aid in the evaluation of the electron microscopic results.

Another limitation was found in the method of data analysis used for morphometry of internodal lengths. In normal animals, internodal distances vary in proportion to the fiber diameter. In regenerating myelin sheaths, the internodal length for individual fiber diameters is decreased below the normal range. The data from the current study was analyzed by grouping internodal lengths irrespective of fiber diameters. Thus, is was impossible to determine whether changes in internodal lengths corresponded to differences in nerve fiber diameters or whether they corresponded to on-going remyelination.

Interpretation of the microscopic analyses was also limited by the small number of animals investigated (5/sex/dose) in each assay at each time point. With an N of 5, the power to discern gradations in effect and distinguish between incidental and treatment-related effects is limited.

Despite these limitations, review of the final report and supporting data indicates that methyl parathion affects body weight, body weight gain, food consumption, and cholinesterase activity, and produces behavioral and histopathological evidence of neurotoxicity. No evidence of ocular toxicity was observed. Decreases in body weight and body weight gain and increases in food consumption were observed in both males and females; the NOEL was 12.5 ppm; the LOEL was 50 ppm. At doses as low as 12.5 ppm, at one or more of the intervals tested, decreases in plasma and erythrocyte cholinesterase activities were observed in males and decreases in plasma, erythrocyte, and brain cholinesterase activities were observed in females. Increases in aggressiveness in high-dose males and increases in aggressiveness, hyperactivity, and tremors in high-dose females were attributed to cholinesterase inhibition. Increases in tail problems and sores/scabs were observed in high-dose females both during examinations for clinical signs and at necropsy. It is unclear whether these effects were associated with neurological effects (i.e., behavioral changes [aggressiveness or hyperactivity] or possible sensory or motor deficits predisposing the tail to damage).

Lack of muscle tone and abnormal gait were also observed in high-dose females indicating possible neurotoxicity. Microscopic analyses of nervous system tissues supported the conclusion that methyl parathion produces nerve damage. In rats receiving whole-body perfusion with glutaraldehyde as a primary fixative and osmium tetroxide as a secondary fixative (optimum fixation for nervous system tissues), distal segments of the sciatic nerve (tibial and sural nerves) showed evidence of demyelination and neuronal regeneration as early as 6 months in the high-dose males and females. At later time points, evidence of myelin and neuronal degeneration was observed at more proximal sites along the sciatic nerve (12 and 13 months) and in the lumbosacral spinal cord

(13 months). The progression of observations follows the retrograde pattern of neuronal degeneration frequently observed in disorders of peripheral nerves. At 12 months, evidence of neuronal degeneration was observed in the proximal sciatic nerve at doses as low as 0.5 ppm in females and 2.5 ppm in males. The incidence and severity of these observations generally increased with dose.

At 12 months, evidence of nerve damage was observed in the distal segments of the sciatic nerves (tibial and sural nerves) of male rats at doses as low as 0.5 ppm. Similar effects were not observed in females at comparable doses and the effects did not clearly increase with dose. However, at 12 and 13 months, analysis of these distal nerve segments of the sciatic nerve was more complex. This was partially because of the appearance of evidence of peripheral nerve damage in sural nerves from controls at 12 and 13 months and in tibial nerves from controls at 13 months. The changes in control nerves were attributed by the study author to age-related changes similar to those observed in the distal peripheral nerves of rats housed in cages with wire mesh bottoms (Spencer and Schaumburg 1980). The analysis of distal nerve segments at 12 months was also complicated by the apparent lack of a relationship between incidence and/or severity of the lesions observed and the dose consumed. While this discrepancy may be explained by the retrograde appearance of lesions with time and the assumption that lower doses might have greater effects distally while higher doses might show greater effects proximally at later time points, it is not entirely clear that this was the case.

In summary, this study is Core Supplementary based on its design and intent. The following NOELs and LOELs were observed:

Systemic toxicity:	NOEL = 12.5 ppm; LOEL = 50 ppm (effects on body weight and food consumption)
Cholinesterase inhibition:	NOEL = 2.5 ppm; LOEL = 12.5 ppm (inhibition of plasma, RBC, and brain cholinesterase)
Neurotoxicity:	no NOEL observed; LOEL = 0.5 ppm (histopathologic evidence of neuronal damage)

TABLE 1. Incidence of Clinical Signs (Excluding Neurotoxic Signs) in Rats Given Diets Containing Methyl Parathion for 1 Year[a,b]

Parameter/ Dietary Level	Interval (weeks)					
	1	4	13-16	25-28	37-40	49-52
			Males			
Tail Problems						
Control	0/70	0/70	0/61	0/59	2/49	4/38
0.5 ppm	0/70	0/70	0/62	0/60	2/50	7/38
2.5 ppm	0/70	0/70	0/62	1/60	3/50	7/40
12.5 ppm	0/70	0/70	0/62	0/59	1/49	7/39
50 ppm	0/70	0/70	1/61	2/58	4/48	8/38
Scabs - General						
Control	0/70	0/70	0/61	2/59	3/49	4/38
0.5 ppm	0/70	0/70	1/62	1/60	2/50	7/38
2.5 ppm	0/70	1/70	0/62	1/60	3/50	8/40
12.5 ppm	0/70	0/70	0/62	0/59	1/49	6/39
50 ppm	0/70	0/70	1/62	2/58	4/48	9/38
Yellow Ano-Genital Stains						
Control	0/70	0/70	0/61	0/59	0/49	0/38
0.5 ppm	0/70	0/70	0/62	0/60	0/50	0/38
2.5 ppm	0/70	0/70	0/62	0/60	0/50	0/40
12.5 ppm	0/70	0/70	0/62	0/59	0/49	0/39
50 ppm	0/70	0/70	0/62	0/58	0/48	2/38

TABLE 1 (Continued)

Parameter/ Dietary Level	Interval (weeks) 1	4	13-16	25-28	37-40	49-52
			Females			
Tail Problems						
Control	0/70	0/70	0/62	0/60	0/48	0/38
0.5 ppm	0/70	0/70	0/62	0/59	0/49	0/38
2.5 ppm	0/70	0/70	0/62	0/60	0/50	0/40
12.5 ppm	0/70	0/70	0/62	0/60	0/49	1/39
50 ppm	0/69	0/70	1/62	9/59	27/49	13/38
Scabs - General						
Control	0/70	0/70	0/62	0/60	0/48	0/38
0.5 ppm	0/70	0/70	0/62	0/59	0/49	0/38
2.5 ppm	0/70	1/70	0/62	0/60	0/50	0/40
12.5 ppm	0/70	1/70	0/62	0/60	1/49	1/39
50 ppm	0/69	1/70	1/62	9/59	23/49	13/38
Yellow Ano-Genital Stains						
Control	0/70	0/70	0/62	0/60	0/48	0/38
0.5 ppm	0/70	0/70	0/62	0/59	0/49	0/38
2.5 ppm	0/70	0/70	0/62	0/60	0/50	0/40
12.5 ppm	0/70	0/70	0/62	0/60	0/49	0/39
50 ppm	0/69	1/70	0/62	5/59	13/49	2/38

[a]Data extracted from Study No. 87-3208, Appendix C.
[b]Author offered no explanation for the increase in the number of high-dose females between weeks 1 and 2.

TABLE 2. Overall Incidence of Clinical Signs Associated with Neurotoxicity in Rats Given Diets Containing Methyl Parathion for 1 Year[a]

Parameter	Dietary Level (ppm)				
	0.0	0.5	2.5	12.5	50
			Males		
Aggressive Behavior	0/70	0/70	0/70	1/70	39/70
			Females		
Aggressive Behavior	0/70	0/70	0/70	1/70	18/70
Tremors	0/70	0/70	0/70	0/70	10/70
Hyperactivity	0/70	0/70	0/70	0/70	17/70
Abnormal Gait	0/70	0/70	0/70	0/70	4/70
Lack of Muscle Tone	0/70	0/70	0/70	0/70	7/70

[a]Data extracted from Study No. 87-3208, Appendix C.

TABLE 3. Mean Body Weights (g ± S.D.) at Selected Intervals for Rats Given Diets Containing Methyl Parathion for 1 Year[a]

Dietary Level (ppm)	Study Week						
	1	7	13	20	30	40	51
				Males			
0.0	313.0±22.6	496.1±42.2	564.4±55.6	643.6±74.3	685.6±86.5	734.6±104.1	780.1±124.4
0.5	314.6±24.7	497.4±42.5	562.4±56.9	641.3±70.5	693.0±78.2	729.7±100.1	777.9±111.7
2.5	317.4±23.3	495.4±43.8	560.6±49.3	637.5±66.5	693.7±80.7	713.4±73.4	766.2±81.8
12.5	317.6±21.5	497.7±39.6	561.4±45.8	635.9±59.5	701.4±68.5	743.7±81.?	786.3±92.0
50.0	305.1±21.9	446.7±40.1**	515.2±45.7**	587.0±57.0**	624.2±66.3**	673.1±85.2*	705.1±92.2*
				Females			
0.0	195.8±13.6	270.6±24.5	297.1±25.7	334.1±33.5	367.1±38.1	398.7±50.4	436.8±61.4
0.5	200.9±11.9	280.1±22.5	302.3±28.9	340.5±37.8	373.1±52.1	409.6±66.1	437.5±66.4
2.5	200.6±12.7	278.0±23.6	304.6±26.6	344.7±36.9	374.9±45.8	406.3±54.9	438.9±61.6
12.5	200.1±12.2	275.3±24.2	304.3±29.6	343.7±39.0	372.3±41.8	403.1±53.6	439.6±58.4
50.0	187.6±12.5**	249.8±21.9**	283.5±31.0*	327.4±41.5	349.6±53.5	366.0±61.1	383.3±69.4**

[a]Data extracted from Study No. 87-3208, Appendix F.

*Significantly different from control value; p≤0.05.
**Significantly different from control value; p≤0.01.

010333

TABLE 4. Mean Body Weight Change (g ± S.D.) from Week 0 at Selected Intervals for Rats Given Diets Containing Methyl Parathion for 1 Year[a]

Dietary Level (ppm)	Study Week						
	1	7	13	20	30	40	51
				Males			
0.0	54.6±8.1	237.8±32.9	306.4±46.1	385.7±64.9	429.0±78.1	477.2±97.1	522.7±117.8
0.5	56.5±8.2	239.2±32.7	304.5±48.4	383.5±61.2	435.7±70.3	472.9±91.6	521.2±104.0
2.5	56.9±11.8	234.9±37.5	300.0±42.9	376.9±60.3	434.4±74.0	454.4±71.0	507.2±79.6
12.5	55.6±7.6	235.7±30.2	300.8±38.1	375.5±50.4	440.0±60.5	481.7±74.9	524.3±86.0
50.0	43.7±8.0**	185.4±30.3**	253.8±37.8**	325.3±48.9**	363.5±58.7**	410.0±77.4**	442.0±84.1**
				Females			
0.0	19.4±5.5	94.3±17.4	120.7±19.9	157.8±27.4	191.2±33.6	223.8±44.3	261.9±55.7
0.5	21.8±5.3*	101.0±17.3	123.8±24.6	162.0±33.6	195.5±48.5	231.8±62.7	259.8±63.4
2.5	22.3±4.8*	99.6±16.4	126.6±20.0	166.7±30.8'	197.1±40.2	229.4±49.9	262.0±57.7
12.5	23.2±5.0**	98.5±19.4	127.7±23.4	167.0±32.7	196.7±38.1	228.4±49.1	264.8±54.6
50.0	8.7±5.4**	70.9±17.4**	104.8±26.1**	148.5±36.2	171.1±47.9	188.5±58.1*	206.1±68.3**

[a]Data extracted from Study No. 87-3208, Appendix F.

*Significantly different from control value; p≤0.05.
**Significantly different from control value; p≤0.01.

010333

TABLE 5. Mean Food Consumption (g/kg/day ± S.D.) at Selected Intervals for Rats Given Diets Containing Methyl Parathion for 1 Year[a]

Dietary Level (ppm)	Study Week 0	1	7	13	27	38	51
				Males			
0.0	106.2±8.5	85.1±4.7	56.7±4.0	47.4±7.9	37.5±6.9	38.6±4.0	32.7±5.6
0.5	108.6±8.1	85.0±5.7	58.8±4.7	46.0±3.4	37.3±2.9	36.5±3.7	31.5±3.2
2.5	111.8±7.3**	86.8±8.9*	59.7±5.1**	46.7±4.2	38.9±5.3	37.5±3.9	33.3±3.3
12.5	112.3±7.7**	87.5±8.4*	60.1±4.8**	45.7±4.8	38.0±3.8	37.1±4.5	33 4±5.7
50.0	110.7±7.2**	87.2±6.5*	65.1±6.3**	48.6±5.9	40.4±9.7	36.3±5.6	33.0±4.2
				Females			
0.0	104.0±8.0	94.5±7.5	69.9±6.2	60.0±5.8	47.4±6.1	49.6±5.0	40.3±5.6
0.5	109.5±8.7**	96.7±6.9	72.0±6.7	60.6±7.5	50.0±7.2	51.0±5.8	40.6±8.9
2.5	109.6±6.2**	98.4±11.2	73.9±5.8**	61.0±6.7	49.4±6.5	48.9±5.2	42.3±6.7
12.5	110.1±7.1**	97.5±8.4	73.2±9.6**	60.9±6.7	50.9±5.6	48.1±6.6	41.4±7.1
50.0	109.8±5.8**	95.1±16.7	88.6±9.1**	72.1±11.6**	54.8±8.7**	54.9±8.0**	46.8±13.7**

[a]Data extracted from Study No. 87-3208, Appendix F.

*Significantly different from control value; p≤0.05.
**Significantly different from control value; p≤0.01.

TABLE 6. Mean Cholinesterase Activity (IU/mL ± S.D.) in Rats Given Diets Containing Methyl Parathion for 1 Year[a,b]

Parameter/ Month	Dietary Level (ppm) 0.0	0.5	2.5	12.5	50
Males					
Plasma Cholinesterase					
1	0.380±0.089	0.342±0.079 (-10.0%)	0.384±0.080 (+1.1%)	0.286±0.043 * (-24.7%)	0.183±0.038 ** (-51.8%)
3	0.492±0.132	0.412±0.077 (-16.3%)	0.483±0.135 (-1.8%)	0.354±0.049 (-28.0%)	0.210±0.038 ** (-57.3%)
6	0.603±0.283	0.498±0.129 (-17.4%)	0.519±0.149 (-13.9%)	0.394±0.124 (-34.7%)	0.234±0.041 ** (-61.2%)
9	0.563±0.281	0.485±0.135 (-13.9%)	0.554±0.150 (-1.6%)	0.362±0.114 (-35.7%)	0.239±0.042 ** (-57.5%)
12	0.661±0.235	0.602±0.174 (-8.9%)	0.681±0.143 ** (+3.0%)	0.470±0.162 (-28.9%)	0.246±0.057 ** (-62.8%)
Erythrocyte Cholinesterase					
1	8.7±0.4	8.1±1.1 (-6.9%)	8.0±0.9 (-8.0%)	7.6±0.8 * (-12.6%)	7.0±0.8 ** (-19.5%)
3	8.4±0.8	8.6±0.3 (+2.4%)	8.4±0.5 (0.0%)	7.8±0.7 (-7.1%)	7.1±0.7 ** (-14.3%)
6	7.0±0.4	7.1±0.3 (+1.4%)	6.8±0.4 (-2.9%)	6.6±0.4 * (-5.7%)	6.1±0.3 ** (-12.9%)
9	5.9±0.5	5.9±0.5 (0.0%)	5.8±0.5 (-1.7%)	5.4±0.7 (-8.5%)	5.0±0.4 ** (-15.3%)
12	7.1±0.7	7.0±0.7 (-1.4%)	6.8±0.6 (-4.2%)	6.9±0.6 (-2.8%)	6.1±0.6 * (-14.1%)
Brain Cholinesterase					
12	8.2±0.6	7.8±0.7 (-4.9%)	7.9±0.3 (-3.7%)	7.9±0.4 (-3.7%)	3.5±0.2 ** (-57.3%)

TABLE 6 (Continued)

Parameter/ Month	Dietary Level (ppm) 0.0	0.5	2.5	12.5	50
Plasma Cholinesterase			**Females**		
1	1.547±0.315	1.616±0.687 (+4.5%)	1.260±0.263 (-18.6%)	1.036±0.306 (-33.0%)	0.404±0.102 ** (-73.9%)
3	2.130±0.619	2.539±1.112 (+19.2%)	1.951±0.486 (-8.4%)	1.441±0.452 (-32.3%)	0.583±0.190 ** (-72.6%)
6	2.623±0.674	2.940±1.104 (+12.1%)	2.213±0.540 (-15.6%)	1.896±0.573 (-27.7%)	0.556±0.118 ** (-78.8%)
9	2.462±0.543	2.743±1.130 (+11.4%)	1.910±0.738 (-22.4%)	1.640±0.606 (-33.4%)	0.571±0.127 ** (-76.7%)
12	2.484±0.621	2.609±0.920 (+5.0%)	2.247±0.786 (-9.5%)	1.671±0.435 * (-32.7%)	0.746±0.437 ** (-70.0%)
Erythrocyte Cholinesterase					
1	7.0±0.6	7.2±0.4 (+2.9%)	6.8±0.3 (-2.9%)	6.3±0.6 * (-10.0%)	6.0±0.4 ** (-14.3%)
3	7.7±0.6	7.4±0.8 (-3.9%)	7.5±1.1 (-2.6%)	7.5±1.1 (-2.6%)	6.8±0.4 (-11.7%)
6	6.8±0.4	7.0±0.7 (+2.9%)	6.9±0.4 (+1.5%)	6.2±0.6 (-8.8%)	5.9±0.5 ** (-13.2%)
9	7.1±0.4	7.2±0.3 (+1.4%)	7.0±0.4 (-1.4%)	6.5±0.3 ** (-8.5%)	6.1±0.3 ** (-14.1%)
12	7.7±0.4	7.5±0.4 (-2.6%)	7.2±0.4 (-6.5%)	6.8±0.7 ** (-11.7%)	6.2±0.6 ** (-19.5%)
Brain Cholinesterase					
12	8.7±0.5	8.1±0.6 (-6.9%)	8.6±0.3 (-1.1%)	6.5±0.8 ** (-25.3%)	2.2±0.1 ** (-74.7%)

[a]Data extracted from Study No. 87-3208, Appendix G.
[b]Values in parentheses represent percent difference from control value.

*Significantly different from control; p≤0.05.
**Significantly different from control; p≤0.01.

010333

TABLE 7. Incidence of Macroscopic Lesions in Rats Given Diets Containing Methyl Parathion for 1 Year[a]

Parameter	Dietary Level (ppm)				
	0.0	0.5	2.5	12.5	50
Males					
Tail					
Sores/Scabs	5/45	1/45	4/45	1/45	4/45
Bumps	5/45	1/45	4/45	1/45	3/45
Females					
Tail					
Sores/Scabs	0/45	0/45	0/45	0/45	13/46
Bumps	0/45	0/45	0/45	0/45	16/46

[a]Data extracted from Study No. 87-3208, Table IIA.

TABLE 8. Incidence (Frequency and Grade) of Lesions in Rats Given Diets Containing Methyl Parathion[a] (Detected by Light Microscopy of Paraffin-Embedded Sections)

Parameter/ Interval	Dietary Level (ppm)									
	0.0		0.5		2.5		12.5		50.0	
	F[b]	G[c]	F	G	F	G	F	G	F	G
Males										
Proximal Sciatic Nerve - Myelin Bubbles										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	1/5	2	2/5	2	3/5	1.3
13-Month	1/5	1	---	---	---	---	---	---	4/5	3
Proximal Sciatic Nerve - Neuronal Degeneration										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	0/5	---	0/5	---	0/5	---
13-Month	0/5	---	---	---	---	---	---	---	4/5	2.75
Proximal Sciatic Nerve - Schwann Cell Proliferation										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	1/5	1	2/5	2.5	3/5	1.3
13-Month	0/5	---	---	---	---	---	---	---	4/5	2.25
Lumbosacral Spinal Cord - Loss of Myelinated Fibers										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	0/5	---	0/5	---	0/5	---
13-Month	0/5	---	---	---	---	---	---	---	1/5	3
Lumbrosacral Spinal Cord - Neuronal Degeneration										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	0/5	---	0/5	---	0/5	---
13-Month	0/5	---	---	---	---	---	---	---	1/5	3

010333

TABLE 8 (Continued)

Parameter/ Interval	Dietary Level (ppm)									
	0.0		0.5		2.5		12.5		50.0	
	F	G	F	G	F	G	F	G	F	G
Females										
Proximal Sciatic Nerve - Myelin Bubbles										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	1/5	1	1/5	3	3/5	3	3/5	1.7
13-Month	0/5	---	---	---	---	---	---	---	3/4	2
Proximal Sciatic Nerve - Neuronal Degeneration										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	0/5	---	0/5	---	0/5	---
13-Month	0/5	---	---	---	---	---	---	---	0/4	---
Proximal Sciatic Nerve - Schwann Cell Proliferation										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	1/5	2	1/5	3	3/5	2.3	1/5	1
13-Month	0/5	---	---	---	---	---	---	---	0/4	---
Lumbosacral Spinal Cord - Loss of Myelinated Fibers										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	0/5	---	0/5	---	0/5	---
13-Month	0/5	---	---	---	---	---	---	---	1/4	2
Lumbrosacral Spinal Cord - Neuronal Degeneration										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	0/5	---	0/5	---	0/5	---
13-Month	0/5	---	---	---	---	---	---	---	0/4	---

[a]Data extracted from Study No. 87-3208.
[b]F = frequencey
[c]G = grade; mean grade calculated by the reviewer; grading of lesions was as follows: 1 = minimal; 2 = slight/mild; 3 = moderate; 4 = moderately severe; 5 = severe/high.

TABLE 9. Incidence (Frequency and Grade) of Lesions in Rats Given Diets Containing Methyl Parathion[a] (Detected by Light Microscopy of Epoxy Sections of the Tibial/Peroneal Nerve)

Parameter/ Interval	Dietary Level (ppm)									
	0.0		0.5		2.5		12.5		50.0	
	F[b]	G[c]	F	G	F	G	F	G	F	G
Males										
Myelin Bubbles										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	1/5	2	2/5	3	4/5	1.25
13-Month	3/5	1	---	---	---	---	---	---	4/5	1.25
Myelin Phagocytosis										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	3/5	1.7	2/5	2	1/5	2	1/5	2
13-Month	3/5	1	---	---	---	---	---	---	3/5	1.4
Regeneration										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	0/5	---	0/5	---	0/5	---
13-Month	0/5	---	---	---	---	---	---	---	0/5	---
Schwann Cell Proliferation										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	1/5	2	2/5	3	1/5	2
13-Month	0/5	---	---	---	---	---	---	---	1/5	2

TABLE 9 (Continued)

Parameter/ Interval	Dietary Level (ppm)									
	0.0		0.5		2.5		12.5		50.0	
	F	G	F	G	F	G	F	G	F	G
Females										
Myelin Bubbles										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	2/5	2.5	0/5	---	3/5	1.33
13-Month	1/5	1	---	---	---	---	---	---	3/4	1
Myelin Phagocytosis										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	0/5	---	1/5	2	2/5	1.5
13-Month	0/5	---	---	---	---	---	---	---	2/4	2
Regeneration										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	0/5	---	0/5	---	1/5	1
13-Month	0/5	---	---	---	---	---	---	---	0/4	---
Schwann Cell Proliferation										
6-Month	0/5	---	---	---	---	---	---	---	0/5	---
12-Month	0/5	---	0/5	---	2/5	2.5	0/5	---	0/5	---
13-Month	0/5	---	---	---	---	---	---	---	3/4	1.3

[a]Data extracted from Study No. 87-3208.
[b]F = frequency
[c]G = grade; mean grade calculated by reviewer; grading of lesions was as follows: 1 = minimal; 2 = slight/mild; 3 = moderate; 4 = moderately severe; 5 = severe/high.

TABLE 10. Incidence of Lesions in Rats Given Diets Containing Methyl Parathion[a] (Detected by Light Microscopy of Teased Sural Nerves)

Parameter/ Interval	Dietary Level (ppm)									
	0.0		0.5		2.5		12.5		50.0	
	M[b]	F[c]	M	F	M	F	M	F	M	F
Demyelinated Lengths										
6-Month	0/5	0/5	---	---	---	---	---	---	1/5	2/5
12-Month	2/5	2/5	3/5	1/5	3/5	0/5	3/5	2/5	4/5	5/5
13-Month	1/5	4/5	---	---	---	---	---	---	5/5	3/5
Myelin Ovoids										
6-Month	0/5	0/5	---	---	---	---	---	---	2/5	2/5
12-Month	1/5	2/5	3/5	1/5	3/5	0/5	3/5	2/5	2/5	0/5
13-Month	1/5	1/5	---	---	---	---	---	---	5/5	0/5
Myelin Bubbles										
6-Month	0/5	0/5	---	---	---	---	---	---	0/5	0/5
12-Month	0/5	0/5	0/5	0/5	1/5	0/5	1/5	0/5	0/5	0/5
13-Month	0/5	0/5	---	---	---	---	---	---	0/5	0/5

[a]Data extracted from Study No. 87-3208.
[b]M - male
[c]F - female

41

010333

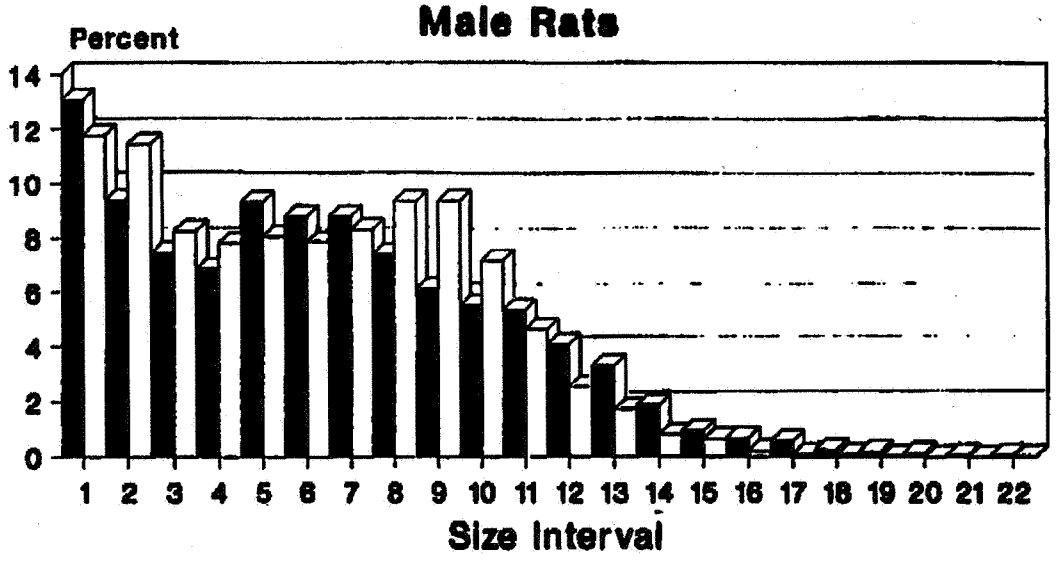
41

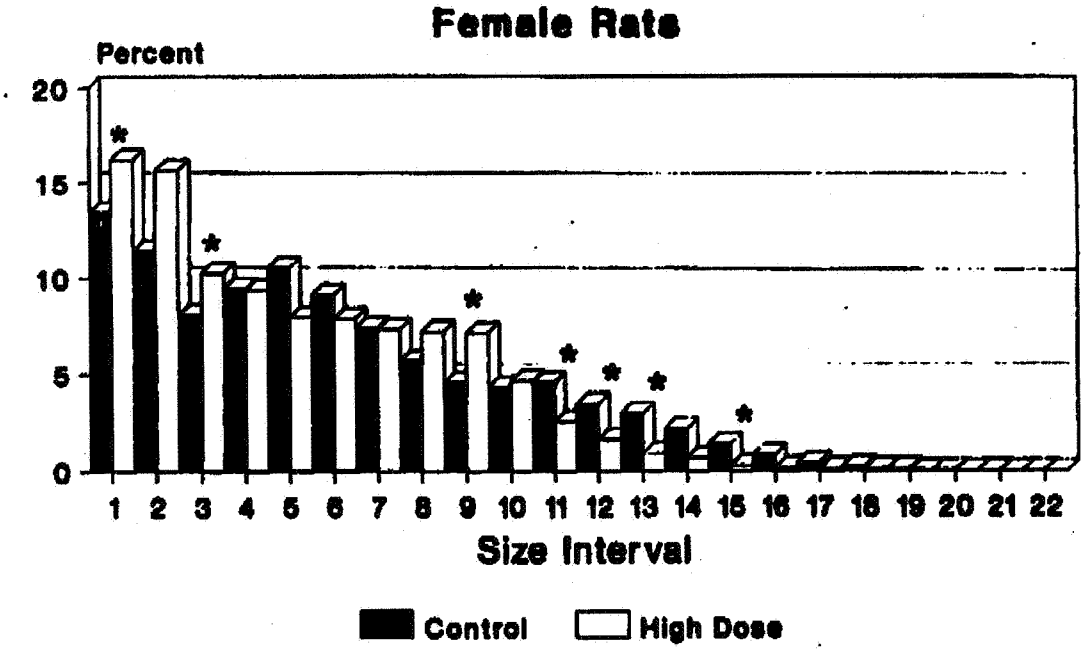
010333

Figure 2A

Nerve Fiber Spectrum

6-Month Sacrifice





*Significantly different from control value; p≤0.05.

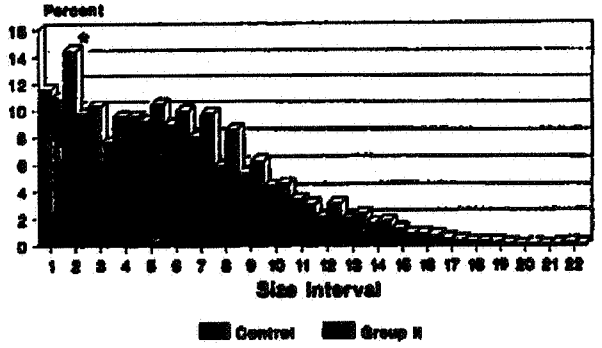
Figure 2B

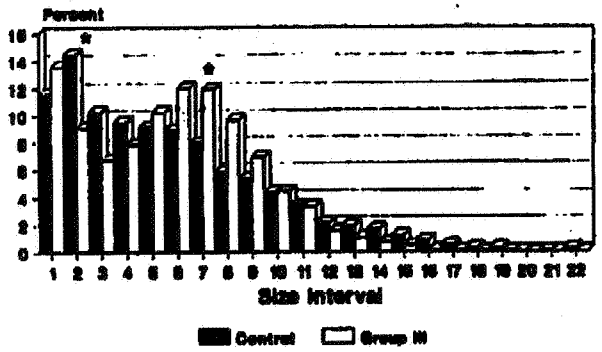
Nerve Fiber Spectrum

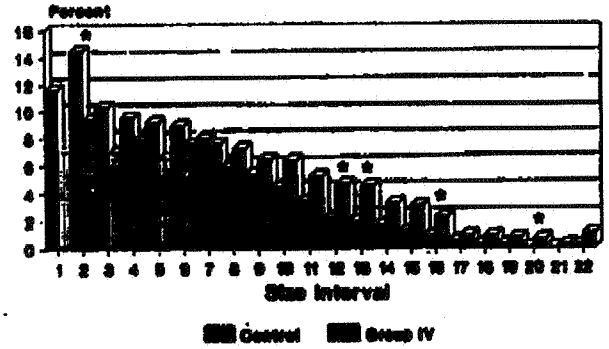
010333

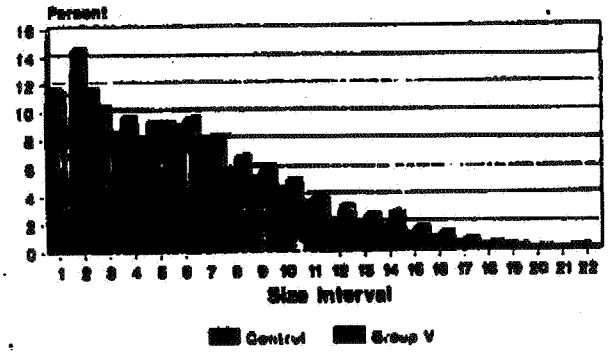
12-Month Sacrifice

Male Rats

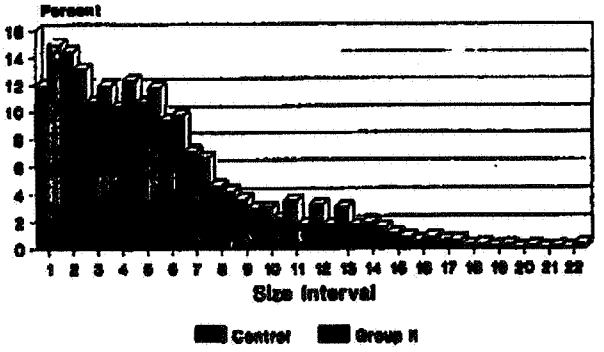


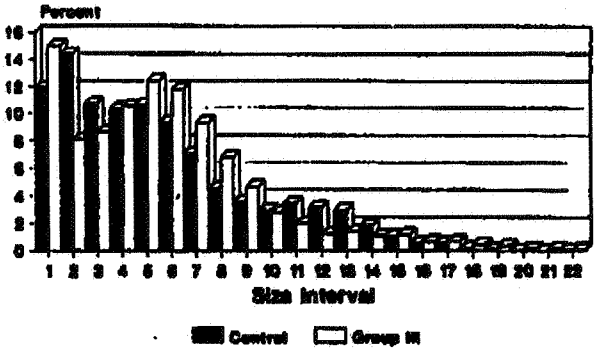


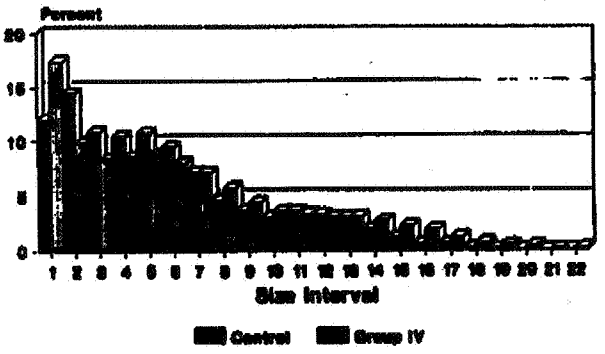


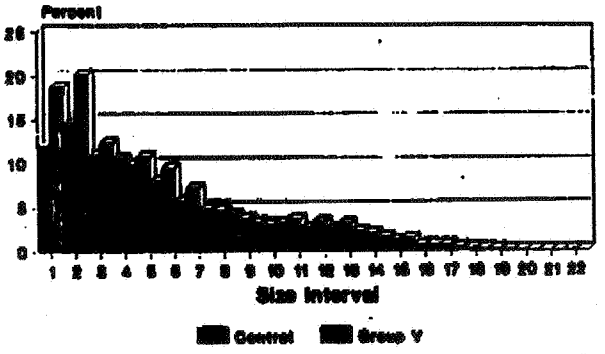


Female Rats







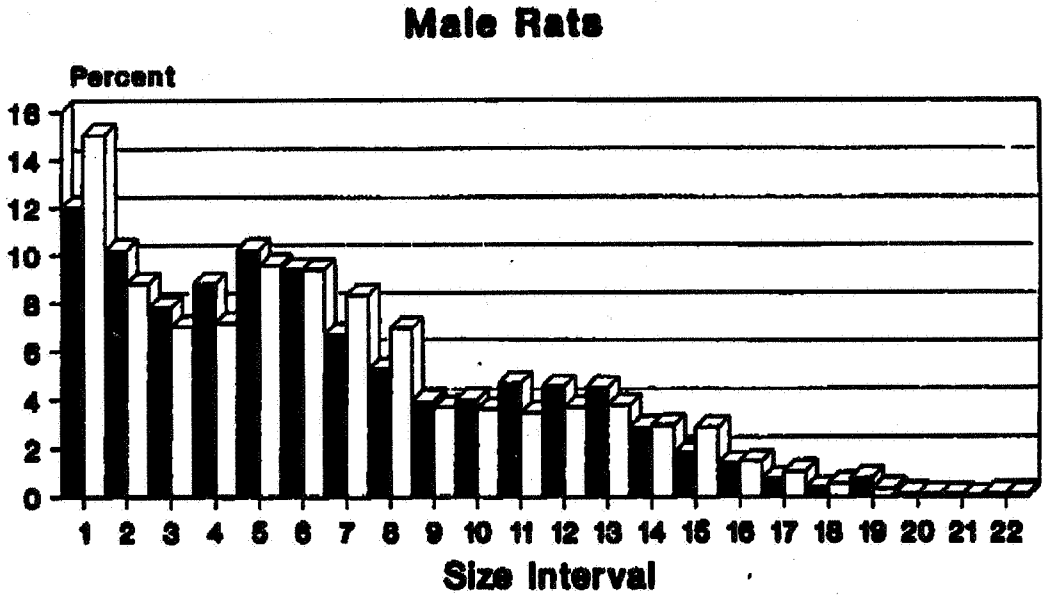


*Significantly different from control value; p≤0.05.

010333

Figure 2C

Nerve Fiber Spectrum
13-Month Sacrifice



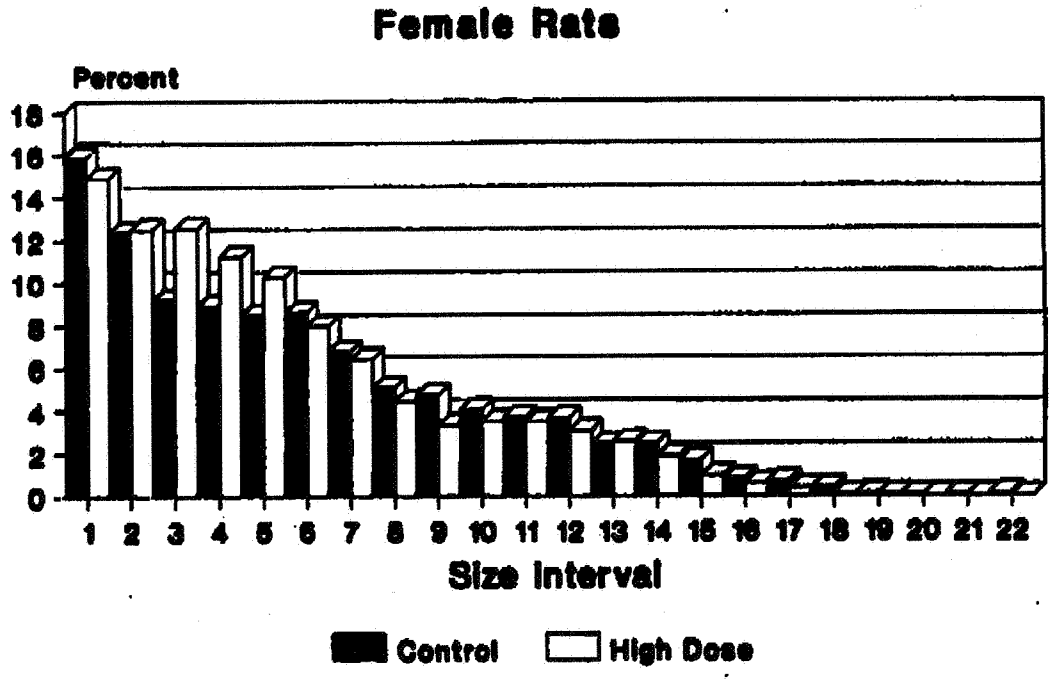
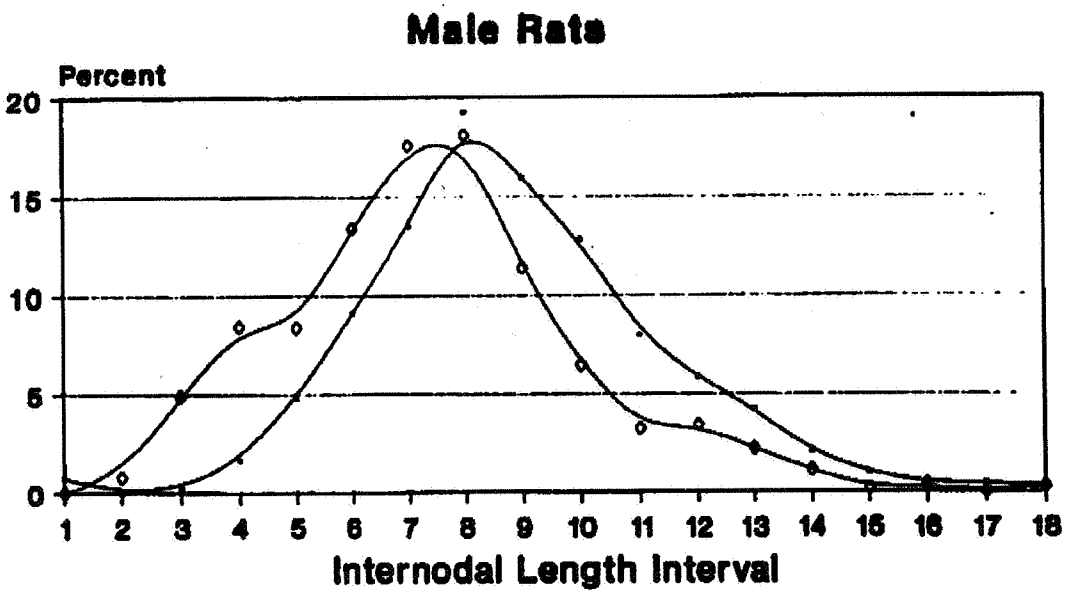


Figure 3A

010333

Teased Nerves
6-Month Sacrifice



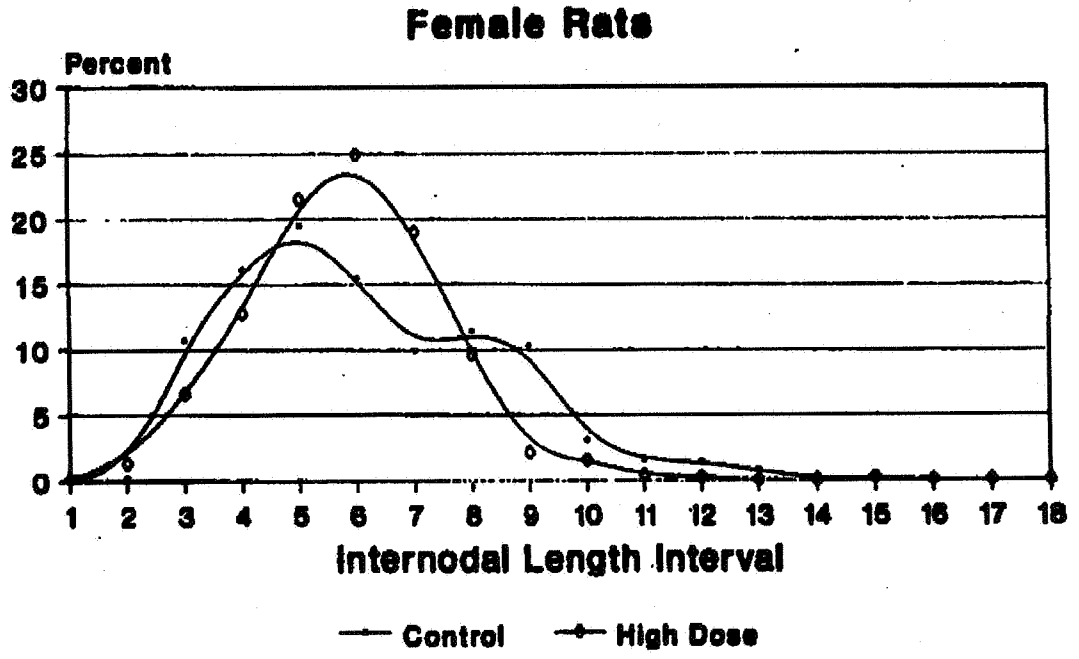
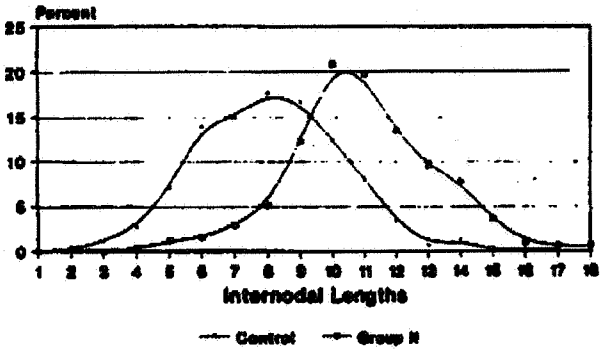


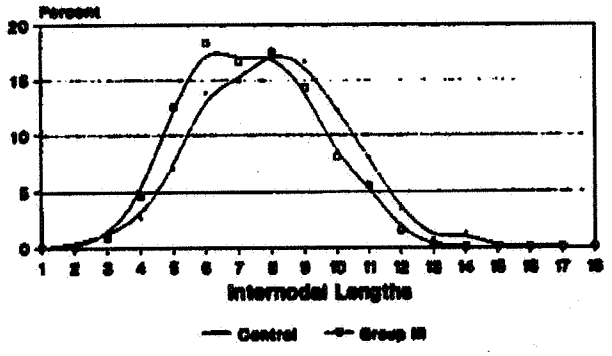
Figure 3B

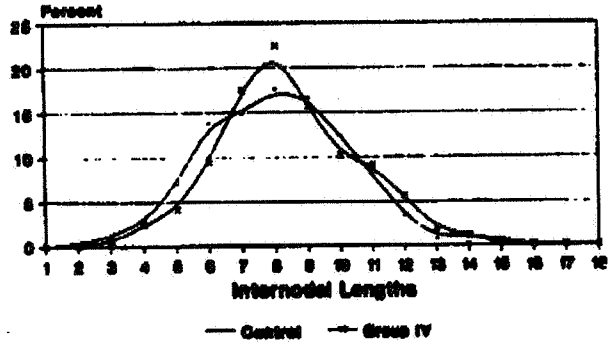
010333

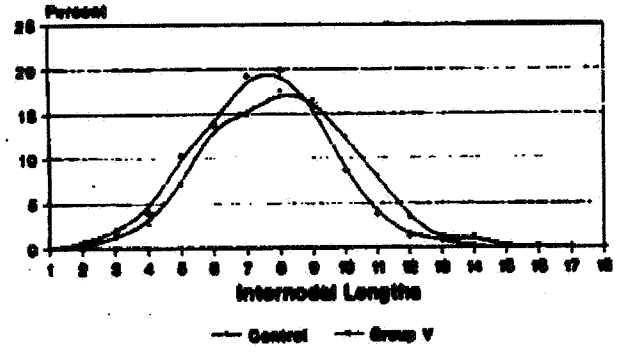
Teased Nerves
12-Month Sacrifice

Male Rats

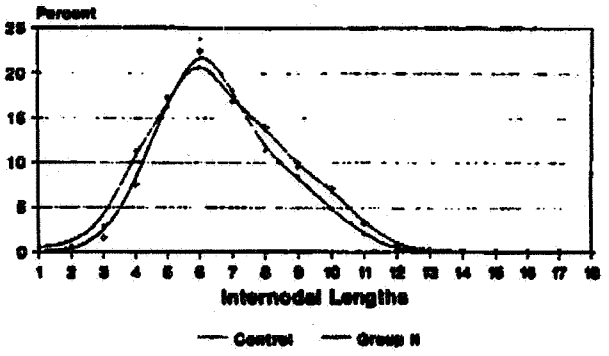


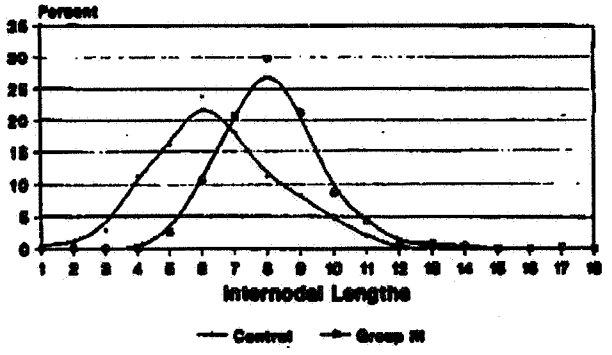


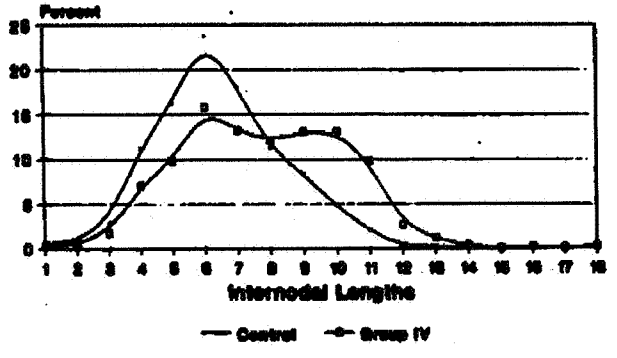




Female Rats







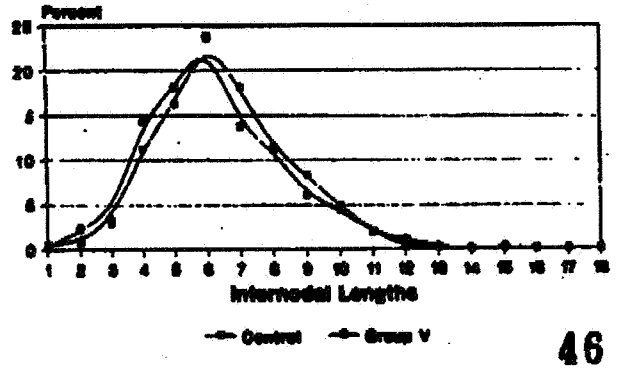


Figure 3C

010333

Teased Nerves
13-Month Sacrifice